Regulation of Hematopoiesis

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Normal hematopoiesis is a well-regulated process in which the generation of mature blood elements occurs from a primitive pluripotent stem cell in an ordered sequence of maturation and proliferation. Regulation occurs at the level of the structured microenvironment (stroma), via cell-cell interactions and by way of the generation of specific hormones and cytokines: erythropoietin, interleukin 3, granulocyte-monocyte colony-stimulating factor (GM-CSF), monocyte-macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), interleukin 5, interleukin 4, and other less well-defined factors, including the megakaryocyte growth factors. Understanding of this complex process has revealed insights into the pathophysiology of human disease and provided a theoretical framework for the therapeutic use of bone marrow transplantation and potential gene transfer therapy. Furthermore, ongoing clinical trials suggest that the hematopoietic growth factors may represent a significant new group of therapeutic reagents for patients with hematological and oncologic disease.

As opposed to most other organs in the body, the bone marrow in its native state continuously regenerates and constantly recapitulates its own maturational and developmental program. Interference in this process, whether iatrogenic, genetic, or environmental in origin, rapidly results in severe disease. It is also this aspect of normal bone marrow function which forms the basis for the acceptance of blood cell donations from a normal donor with relative impunity, permits the administration of high-dose chemotherapy, and also allows for the ultimate transfusion procedure; that is, a bone marrow transplant.

HISTORY AND TERMINOLOGY

Although the concept that the bone marrow might be able to regenerate itself even in a foreign host and therefore be used to treat patients with anemia occurred to clinicians as early as 1891 [1], the lack of a scientific basis for the physiology of the bone marrow led to its use in fashions destined to fail therapeutically—for example, as an oral agent. By 1949, however, Jacobson and colleagues had discovered that mice exposed to bone marrow ablative radiation doses could be protected from death by shielding the spleen from the radiation; they concluded that the beneficial effect of such manipulation was due to a splenic autograft [2]. Initially, workers in the field believed that the salutary autograft effect was solely a humoral one, enhancing the ability of the animal's own blood-forming organs to recover from the radiation insult. By 1956, however, further work involving marrow grafting from a donor to a host mouse led to the realization that

Abbreviations: ANLL: acute non-lymphocytic leukemia CFU: colony-forming unit of the spleen CML: chronic myelogenous leukemia G-CSF: granulocyte colony-stimulating factor GM-CSF: granulocyte-monocyte colony-stimulating factor M-CSF: monocyte-macrophage colony-stimulating factor NK: natural killer (cells)

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this protective effect was actually due to colonization of the host bone marrow by cells derived from the donor’s bone marrow and spleen, thus creating a “radiation chimera” [3,4]. Work over the next ten years concentrated on further investigation of the source of cells necessary to produce recovery from radiation injury [5]. Not surprisingly, syngeneic bone marrow was the most efficient. These studies established the marrow and spleen as a continuously regenerating source of the hematopoietic terminal elements: that is, the mature red blood cells, granulocytes, monocytes, lymphocytes, and platelets. In 1961, Till and McCulloch [6] investigated the physiology of marrow reconstitution when limiting numbers of donor bone marrow cells were given to an irradiated host recipient; they discovered that, in spleens removed from such mice after injection of donor cells, clonal colonies of myeloid-erythroid cells developed. This phenomenon was also observed in the bone marrow itself. These colonies were grossly visible in the spleen and were designated CFUs for “colony-forming unit of the spleen.” Cytogenetic analysis for clonality using specific radiation-induced cytogenetic markers demonstrated that each individual spleen colony was different in cellular origin from all the other spleen colonies but that all cells within a particular colony appeared to be derived from a single precursor (or progenitor) cell. When one examined mature blood and spleen cells from reconstituted animals, these radiation-induced cytogenetic markers of clonality were shared by both lymphocytes and myeloid elements [7,8]. By the mid-1960s, then, the basic paradigm of bone marrow development was established: progenitor cells (stem cells) must be present in small numbers in normal marrow and these cells could divide and either produce more progeny stem cells or differentiate further into a more mature cell, which in turn could give rise either to more of itself or to a yet more differentiated daughter cell. Furthermore, the most primitive, or totipotent stem cell, must be able to give rise to both lymphoid and myeloid elements, the latter including red cells, granulocytes, monocytes, and platelets.

As work progressed on understanding the cells that were involved in this reconstitution, the idea of humoral control of hematopoiesis was not entirely lost. It was clear, however, that further dissection of this normal physiology would have to move from in vivo studies to in vitro model systems. The laboratories of Sachs [9] and Metcalf [10] worked to establish such systems, first in “liquid culture” and then, in order to identify which cells gave rise to which other cells in a geographically limited fashion, a system of solid-phase culture. It became apparent that in these in vitro systems, a single bone marrow progenitor cell was able to give rise to more mature progeny. Indeed, when one plated bone marrow or spleen cells in solid medium (methylcellulose, agar, or plasma clot) small colonies of cells would develop, each apparently derived from a single clonal precursor. Some of these colonies included granulocytes and macrophages, some included only erythroid cells, and some included multiple cells of various mixed lineages. Each colony in the solid phase could be examined and counted, and this procedure gave rise to the nomenclature for progenitor cells. For example, the cell that gave rise to a colony composed of granulocytes and macrophages was called a “CFU-gm” for colony-forming unit, granulocytes and macrophages. Further painstaking work over the next 20 years led to the evolving schema of hematopoietic stem cell development, differentiation, and maturation that provided the framework for our understanding of both marrow transplantation and the control of normal and abnormal hematopoiesis (Fig. 1).

It should be noted that even with the current relatively sophisticated knowledge of this normal process, many aspects of it still remain a mystery. For example, evidence in
model systems (albeit circumstantial evidence) led to the idea that the true totipotent stem cell that can reinitiate the entire developmental process goes through a relatively early differentiation into a committed lymphoid versus a committed myeloid progenitor cell. The former is then responsible for the development of the B and T lymphocyte (and probably the natural killer [NK] cell) while the latter is responsible for myeloid, erythroid, and megakaryocytic lineages. Clinical observations, however, confused this issue in the late 1970s and early 1980s, when it was recognized that the disease, chronic myelogenous leukemia (CML), which had always been considered to derive from an aberrant myeloid stem cell with preservation of normal lymphopoiesis, could sometimes lead to a true acute lymphoblastic leukemia during blast crisis \[11,12\]. Moreover, during the stable phase of the disease, it was discovered that one could sometimes demonstrate characteristic involvement of the CML clone in the B lymphocytes and
occasionally the T lymphocytes (specifically, presence of a Philadelphia chromosome). This interplay of knowledge gained from clinical "experiments of nature" and from basic biological research is a constant theme in advances in the understanding of hematopoiesis.

The same attempts at developing an \textit{in vitro} model of hematopoiesis confirmed the hypothesis of earlier investigators that growth of a primitive stem cell into more mature progeny was dependent, not only on factors inherent in that cell itself, but also on humoral regulation \cite{13}. These secreted molecules (cytokines) may act over long distances as most hormones do (for example, production of erythropoietin by the kidney affecting marrow erythroid activity) or may act only over relatively short and geographically limited regions in the normal physiologic setting. As protein factors that affect the growth and differentiation of progenitor cells were discovered, they received a number of different names from different laboratories involved in their investigation. This semantic excess persists somewhat to the present day. Most of the factors, however, were named as "CSFs" for "colony-stimulating factors." Thus, the first growth inducer that was found to stimulate both macrophage and granulocyte colonies was called GM-CSF. It quickly became apparent that there was not only a profusion of names but that these names can be misleading. Thus, GM-CSF may affect proliferation not only of granulocyte-macrophage colonies but of a variety of other progenitor cells as well. Furthermore, the sources of these regulatory proteins may, in some cases, be legion. Although T lymphocytes are one of the principal sources, macrophages, fibroblasts, and endothelial cells are also critical.

With the identification of humoral factors capable of regulating these \textit{in vitro} systems, it became possible to begin attempts at obtaining such factors in highly purified form. Originally approached through classical techniques of protein chemistry, the rapid development of molecular cloning techniques led to an accelerated development of these factors, allowing for their study not just \textit{in vitro} but also \textit{in vivo} animal models and subsequently in man. Once isolated in purified form, it became apparent not only that these factors have multiple effects on multiple progenitor cells, but they also have important effects on mature "end-stage" cells such as granulocytes. While such end-stage cells are incapable of further division, the colony growth factors may lead to important alterations in the functional attributes of the cells. For example, GM-CSF affects migration and cytolytic pathways in the mature polymorphonuclear leukocyte.

Until relatively recently, then, the remarkable advances in our understanding of normal hematopoiesis have evolved by separating many of the individual components (various progenitor cells and growth factors) and intensely studying them in isolated form. \textit{In vivo}, however, the hematopoietic organs (particularly the bone marrow but, in the case of the lymphopoietic system, the spleen, lymph nodes, and thymus as well) have an extraordinary organized and quite specific structure \cite{14}. Progenitor cells and mature cells are geographically organized relative to each other and to the vasculature. Furthermore, there is an underlying framework on which hematopoiesis takes place. This framework is often referred to as the bone marrow "stroma." The stroma consists of endothelial cells, fibroblasts, adipocytes, macrophages, and probably other elements as well. The stromal elements produce many of the growth factors alluded to above. The extracellular matrix produced by these elements may also serve as an organizing foundation of glycosaminoglycans to concentrate and orient these locally acting hormones \cite{15}. 


In summary, continuous renewal of the hematopoietic system involves an orderly sequence of maturation and differentiation of cells presumably from a totipotent stem cell through stages of increasing commitment (and loss of the ability to "de-differentiate," at least in normal physiology) to progressively more mature progeny. Most of the early cells are capable of either replicating themselves or of pushing further toward a more differentiated cell. This process occurs in an organized structure (known as the stroma) and is influenced by a variety of geographically short-acting growth factors produced by stromal cells and other regulatory elements and by a few geographically distant hormones. Many of these growth factors have specific hierarchical effects and may influence both growth and differentiation as well as end-stage cell function.

HEMATOPOIETIC STEM CELLS

If examined closely, it is clear that the above statements, as outlined in Fig. 1, while summarizing our model of hemopoiesis, are actually quite glib. The proposed schema begs a number of very important questions. First, how many stem cells are needed to reproduce an entire bone marrow? Second, under normal circumstances, how many stem cells in an individual are actively participating in the production of end-stage mature functioning cells? Third, how does a stem cell “decide” whether to produce another stem cell of the same type when it divides or whether to produce a more mature daughter cell that has been pushed further along in the maturation sequence? These questions are important, not just for our understanding of the basic biology of this remarkable process, but also because they have very important implications for the therapeutic use of manipulations of the hematopoietic system (whether by marrow transplantation or the administration of humoral growth factors or cellular components to patients). Moreover, understanding the answers to these questions might lead to a marked improvement in our understanding of pathophysiological processes; that is, diseases of the hematopoietic system.

In order to address the question of how many stem cells are needed fully to re-populate the lympho-hematopoietic system, it is necessary to be able to identify the true totipotent stem cell. All such approaches are approximate, but the most successful has been to identify cell surface membrane glycoproteins that are relatively maturation stage-specific in their expression. Thus, there are cell surface proteins (against which monoclonal antibodies can be developed) which appear only on mature cells in the differentiation schema and others that appear on the more immature progenitor stem cells. Since no single cell surface protein has been described which uniquely distinguishes totipotent and other stem cells, a combination of such “markers” must be used to identify these cells. In man, the CD34 (MY10) marker combined with antibodies recognizing HLA-Dr as well as mature lineage-specific markers (for example, CD19 and CD3) have been most useful [16]. In murine models of hematopoiesis, recent elegant work utilizing the stem cell markers THY1 and SCA1 combined with lineage-specific markers for B cells (B220), granulocytes (GR1), myelomonocytic cells (MAC1), and T cells (CD4, CD8) has shown that a population of cells identified as having expression of SCA1 and low expression of THY1 but absence of all the mature lineage-specific markers identifies the hematopoietic stem cell capable of giving rise to CFUₕ [17]. Cells of this surface phenotype represent approximately 0.03 percent of the normal mouse bone marrow. Morphologically, these cells are medium-sized round cells and are generally in the resting phase of the mitotic cell cycle (G₀–G₁). If a mouse is
given lethal bone marrow irradiation, one CFU₄ splenic colony is observed for every ten intravenously transferred syngeneic stem cells. Given the estimated seeding efficiency of the spleen, this result implies a nearly one-to-one relationship of colony-forming units to cells injected. In further experiments designed to determine how many totipotent stem cells need to be injected to reconstitute the murine lympho-hematopoietic system, it was found that approximately 20 of these cells would lead to efficient reconstitution of 50 percent of mice.

In normal physiology, hundreds of stem cells might be undergoing maturation or, conceivably, only a few stem cells might be participating in such a process. Data from Lemischka and colleagues [18] using retroviral markers to distinguish different stem cells in murine reconstitution experiments, appear to indicate that, at any given time after a transplant, the bone marrow is generating all of its mature progeny from only one or at most two or three stem cells. Exactly which stem cell is being used may change somewhat over time, but these experiments further confirm how remarkably few stem cells are needed to generate the entire lympho-hematopoietic mass, which includes nearly four liters of marrow in addition to the mass of the spleen, lymph nodes, and peripheral blood circulating hematopoietic elements. Such studies also have profound implications for attempts at treatment of genetic disorders by "gene transfer" therapy.

Finally, there are two major mechanisms by which a stem cell could "decide" whether to become another stem cell or whether to differentiate further: either an essentially random ("stochastic") process or one carefully controlled by a variety of other regulatory elements. The controlling regulatory elements would include the humoral growth factors. Ogawa and colleagues have obtained elegant numerical data to suggest statistically that this process is, in fact, a stochastic one rather than a deterministic one. They hypothesize that random commitment takes place sequentially during differentiation of the stem cell [19,20]. Such a model of stochastic lineage selection remains consistent with the apparent induction of differentiation by growth factors in vitro and in vivo since it assigns to those factors the role of supporting a given population of progenitors that have been randomly triggered and allowing the death of populations of progenitors that are not being actively supported by the growth factor. This view contrasts with the alternate hypothesis that growth factors could induce differentiation in stem cells and thus provide a "determinism" to the process.

**GROWTH FACTORS IN HEMOPOIESIS**

One can conceptually divide the growth factors into those that support very early cells of hematopoietic lineage versus those that support the growth of late cells in the hematopoietic lineage [21–26]. The early-acting factors include interleukin 3, GM-CSF, and interleukin 4. Late-acting, and more lineage-specific, factors include erythropoietin, G-CSF, M-CSF, GM-CSF, interleukin 5, and interleukin 4. The fact that some of these factors have been included in both categories is not an error. Rather, as more information becomes available, it becomes clear that many of these factors can act at different stages of differentiation and even with variable effects at different stages of differentiation. In addition, many of these factors may be synergistic, one with another. There are also some growth factors whose existence is strongly suggested by bench and clinical research data but which have not yet been purified and/or cloned. Prominent among these are the megakaryocyte-specific factors [27,28].

One other aspect to the hematopoietic growth factors should be kept in mind as these
TABLE 1
Hematopoietic Growth Factors

| Growth Factor       | Origin                  | Function                                      |
|---------------------|-------------------------|-----------------------------------------------|
| M-CSF (CSF-1)       | Endothelial cells       | Stimulates macrophage differentiation         |
|                     | Fibroblasts             | Important for maintenance of mature monocytes |
|                     | Macrophages             |                                               |
|                     | Placenta                |                                               |
|                     | T cells                 |                                               |
| Erythropoietin      | Kidney (?endothelium)   | Stimulates erythropoiesis                     |
|                     | Liver                   | No effect on more primitive progenitors       |
| Interleukin 5 (IL-5)| T cells                 | Stimulates production of eosinophils          |
|                     |                         | Induces terminal B-cell differentiation       |
| G-CSF               | Endothelial cells       | Stimulates production of neutrophils          |
|                     | Macrophages             | Predominantly acts late in hierarchy of developement with strong maturational effects |
|                     | Epithelial cells        |                                               |
|                     | Fibroblasts             |                                               |
|                     | Neutrophils             |                                               |
| GM-CSF              | Endothelial cells       | Stimulates production of neutrophils, macrophages, eosinophils |
|                     | Fibroblasts             | Predominately induces proliferation and differentation of early myeloid progenitors |
|                     | Macrophages             |                                               |
|                     | T cells                 |                                               |
|                     | NK cells                |                                               |
| Interleukin 3 (IL-3) (multi-CSF)| T cells | Stimulates production of neutrophils, monocytes, eosinophils, basophils, and platelets |
|                     | NK cells                | Predominately induces proliferation and differentation of early myeloid progenitors |

agents enter clinical trials and possible clinical use in the future. Although many of these proteins were originally described and discovered because of their effect on hematopoiesis, they may have significant effects on the growth and perhaps even the differentiation of cells of other lineages. Indeed, some laboratories have been able to demonstrate receptors for the hematopoietic growth factors on solid tumor cell lines [29,30]. Moreover, as expected, these hematopoietic growth factors do not just affect erythroid, myeloid, and megakaryocytic lineages but also may affect the lymphoid lineages (T, B, and NK cells). From the biological point of view, it is not surprising that nature might remain relatively conservative in utilizing such growth-promoting factors, but this discovery may have important implications in terms of the use of these agents in patients with various kinds of malignancies and immune disorders.

The mechanism by which growth factors stimulate cells is another important consideration. In order for a secreted protein growth factor to affect the proliferation of a cell, the cell to be affected must have on its surface a specific growth factor receptor. Each of the different hematopoietic cytokines is presumed to have such a specific receptor. For some factors, the receptor is well characterized (for example, the C-FMS proto-oncogene product is the M-CSF receptor), while for others further work is needed. The production of a cytokine by a cell which is also expressing the receptor for that cytokine is known as "autocrine" stimulation. This process may be an important mechanism for the growth of hematopoietic-derived tumors.
In addition to the "positive" regulatory influences of growth factors on hematopoiesis, there must also be a variety of "negative" regulatory elements to prevent overindulgent progenitor cell activity. Candidates for this function include the natural killer lymphocyte [31], but understanding of this aspect of hematopoietic regulation is quite incomplete. Studies on both adult hematopoiesis and fetal ontogeny and those clinical circumstances that may mimic fetal ontogeny [32] may help to dissect this physiology.

**CLINICAL POTENTIAL FOR THE REGULATION OF HEMATOPOIESIS**

The potential circumstances in which alterations of abnormal hematopoiesis by therapeutic manipulation could result in successful disease treatment are legion. First, there are disorders in which lack of a specific bone marrow cytokine or hormone is an intrinsic part of the pathophysiology of the disorder. One of the best examples of this type is the anemia due to end-stage renal disease. In that case, production of erythropoietin is markedly decreased because of the loss of renal tissue. Therefore, replacement of erythropoietin results in reversal of the anemia. Whether there are also disease states that involve the specific absence of a given myelopoietic, lymphopoietic, or megakaryocytic growth factor as their prime etiology is unknown but, if so, then replacement of that growth factor should lead to reversal of the lesion. The second potential therapeutic use of such agents would be for disorders in which an adequate quantity of a given hematopoietic cell is present but in which function is abnormal and the abnormality of function occurs because of inadequate production of a specific growth factor. Again, it remains unclear whether any normal genetic or acquired disorder has such an abnormality as its primary lesion. Potential candidates, however, include the myelodysplastic syndromes, congenital granulocyte dysfunction syndromes, and genetic and acquired "lymphodysplastic" syndromes. The third area in which altering hematopoiesis by the use of humoral or cellular factors might prove of great clinical benefit is for those disorders in which the primary lesion may not be lack of the relevant factor but in which the use of pharmacologic doses of such factors may improve either (1) quantity of a given cell or cells in the blood and tissues or (2) function of a given cell in the blood or tissues. For example, it may be possible to improve one or both of these problems in acute leukemia, myeloproliferative and myelodysplastic disorders, aplastic anemia, lymphoproliferative disorders, the immune deficiency of severe burns, and possibly in a variety of infectious diseases, including HIV, mycobacterial, and parasitic infections. Finally, cells and factors that regulate hematopoiesis may have an extremely important use in a supportive role when combined with other therapies. The most obvious example is combining the use of hematopoietic growth factors with chemotherapeutic and/or radiotherapeutic treatment of cancer. Since the major side effect of these modalities is one of marrow suppression and resultant pancytopenia, the use of growth factors might enable either the administration of higher doses of primary therapy (it is hoped with greater success at killing tumor cells) or alleviation of severe side effects of currently successful therapy (for example, in successfully "cured" tumors such as lymphoma, leukemia, small-cell carcinoma of the lung, germ cell tumors, and all the disorders treated by marrow transplantation).

As with any new agent used in either physiologic or pharmacologic doses, attention to side effects may be the rate-limiting step in utilization of the agent. These include all the usual (often difficult to predict) potential side effects of any drug (for example, increased hypertension with erythropoietin, vascular leakage syndrome with interleukin 2, and bone pain with GM-CSF) but also side effects that are relatively unique to
hematopoietic growth factors. Evidence has been alluded to earlier to suggest that many acute non-lymphocytic leukemia (ANLL) cells are dependent on normal hematopoietic growth factors for their continued proliferation, and in some cases these ANLL cells may even act in an autocrine fashion by producing their own needed growth factors [33,34]. Indeed, certain oncogenes work by inducing endogenous production of growth factors (as postulated for the V-sis oncogene [35]) or by representing growth factor receptors themselves (C-FMS proto-oncogene product is the receptor for M-CSF [36]). Because of this basic science information, one of the potential worrisome side effects of hematopoietic growth factor use is, of course, the promotion of pre-leukemia or leukemia itself.

SUMMARY

Although the ultimate understanding of the physiology of hematopoiesis may require a body of knowledge comparable to that needed for understanding all of normal human development, there have nevertheless been remarkable advances in our comprehension of the cells and humoral factors involved in this unique process. No doubt, this new knowledge will allow us to define better a variety of primary disorders of hematopoiesis. Moreover, some of the growth factors identified are already in clinical practice, and others will soon enter that arena. The introduction of these agents will have a major effect on transfusion medicine, infectious diseases, and hematology-oncology.

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