



Joanna Zjawinska. *Harlequin*. Oil on canvas, 32" × 37". Courtesy of the Hanson Gallery, New Orleans, Louisiana.

Continued research may help to define how stem cells may contribute to hematopoiesis and related processes.

Hematopoietic Stem Cells and Hematopoiesis

Clayton Smith, MD

Background: *The highly orchestrated process of blood cell development and homeostasis is termed "hematopoiesis." Understanding the biology of hematopoietic stem cells as well as hematopoiesis is important to developing improved treatments for hematologic malignancies, congenital disorders, chemotherapy-related cytopenias, and blood and marrow transplants.*

Methods: *The author reviews the current state of the art regarding hematopoietic stem cells and hematopoiesis.*

Results: *Several new concepts, including stem cell plasticity, suggest the possibility that stem cells may have the ability to differentiate into other tissues in addition to blood cells.*

Conclusions: *While much is known about hematopoietic stem cells and hematopoiesis, much remains to be clarified about the environmental and genetic processes that govern the growth and development of the blood system. In addition, careful studies remain to be conducted to determine whether hematopoietic stem cells can differentiate into extra-hematopoietic tissues.*

Introduction

Each day the human body produces billions of new white blood cells, red blood cells, and platelets to replace blood cells lost to normal cell turnover processes as well as to illness or trauma. A variety of homeosta-

tic mechanisms allow blood cell production to respond quickly to stresses such as bleeding or infection and then return to normal levels when the stress is resolved. The highly orchestrated process of blood cell production and homeostasis is termed *hematopoiesis*. An understanding of the principal mechanisms in hematopoiesis, as well as our current understanding of the processes central to hematopoiesis, is important to the practice of oncology for a variety of reasons. Disorders of hematopoiesis underlie a number of hematologic malignancies and other disorders such as leukemia, aplastic anemia, lymphoma, myelodysplasia, myeloproliferative disorders, and inborn errors of metabolism. Chemotherapy-induced cytopenia is one of the primary causes of morbidity and mortality in the treatment of cancer.

From the Blood and Marrow Transplant Program at the H. Lee Moffitt Cancer Center, Tampa, Florida.

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Address reprint requests to Clayton Smith, MD, MCC 2020, 12902 Magnolia Drive, Tampa, FL 33612. E-mail: smithca@moffitt.usf.edu

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Studying the biology of hematopoiesis has identified several growth factors, including G-CSF and GM-CSF, that may shorten the period of posttreatment neutropenia and may improve treatment outcomes. Understanding the biology of hematopoiesis and the regeneration of immunity may result in decreased morbidity, mortality, and expense of autologous and allogeneic blood and marrow transplants. Delineating the processes that control hematopoiesis may also point the way to developing conditions that expand the numbers of blood cells available for transplant. This could lead to safer and simpler transplants for patients with a variety of cancers and other diseases.

Hematopoietic Stem Cells and Progenitors

All of the mature blood cells in the body are generated from a relatively small number of hematopoietic stem cells (HSCs) and progenitors.^{1,2} Murine models, particularly short- and long-term transplant studies, have provided a number of insights into the biology of HSCs and progenitors.^{3,4} The results of these studies have demonstrated that HSCs are able to generate every lineage found in the hematopoietic system including red blood cells, platelets, and a variety of lymphoid and myeloid cells.^{1,4} Some of the most important lymphoid cells include natural killer (NK) cells, T cells, and B cells, while important myeloid cells include granulocytes, monocytes, macrophages, microglial cells, and dendritic cells.⁵ Each of these cell types can be generated from a single HSC, and each HSC has an enormous capacity to generate large numbers of these cells over many years and perhaps even decades. In the mouse, a single HSC can reconstitute the entire hematopoietic system for the natural lifespan of the animal.⁶ Murine HSCs are rare and are present at a frequency of 1/10,000 to 1/1,000,000 cells in the bone marrow depending on the species, age, and technical aspects of the model. While HSCs are primarily found in the bone marrow, they are present in a variety of other tissues including peripheral blood and umbilical cord blood, and are found at low numbers in the liver, spleen, and perhaps many organs.⁷ These HSCs may have somewhat different properties, but they all have the ability to generate all the different blood lineages in large numbers for a prolonged period of time.

Phenotypically, murine HSCs are small cells with minimal cytoplasm, and they express high levels of the multidrug resistant (MDR) proteins and high levels of aldehyde dehydrogenase (ALDH).^{4,8} These cells tend not express surface markers seen on mature HSCs but can express low levels of the thy-1 surface protein and relatively high levels of the sca-1 surface marker.^{1,9}

HSCs generate the multiple hematopoietic lineages through a successive series of intermediate progenitors. These include common lymphoid progenitors (CLPs), which can generate only B, T, and NK cells, and common myeloid progenitors (CMPs), which can generate only red cells, platelets, granulocytes, and monocytes.^{10,11} Downstream of the CLPs and CMPs are more mature progenitors that are further restricted in the number and type of lineages that they can generate.¹⁰ Ultimately, terminally differentiated cells are produced that cannot divide and undergo apoptosis after a period of time ranging from hours (for neutrophils) to decades (for some lymphocytes). When a bone marrow or blood stem cell transplant is performed, it appears that progenitors contribute to engraftment for only a short period of time, while long-term blood production is derived primarily from HSCs.¹² A summary of the process of blood development is presented in Fig 1.

Defining HSCs and progenitors in humans and studying their biology are more difficult than in mice because the laboratory tests of human HSCs and progenitors have significant limitations and there are no simple ways of performing highly controlled long-term transplant experiments in humans. Consequently, most of what is known about human progenitors and HSCs is based on the results of (1) *in vitro* studies, (2) studies involving the transplantation of human cells into immunodeficient animals such as NOD/SCID (non-obese diabetic/severe combined immunodeficient) mice or fetal sheep, and (3) primate and other large ani-

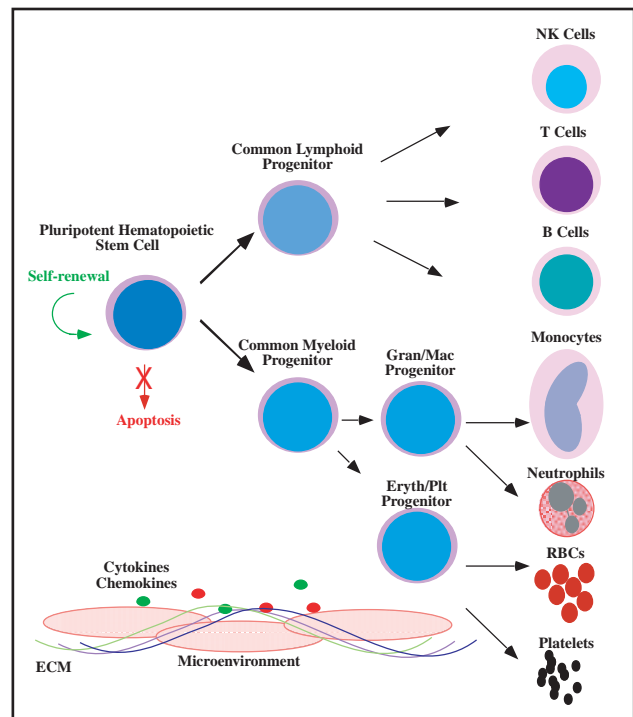


Fig 1. — A summary of the process of blood development. ECM = extracellular matrix.

mal transplant studies.¹³⁻¹⁶ Based on these indirect in vitro and in vivo models, the biology of the human hematopoietic system has become partially defined and appears, for the most part, to parallel the biology of the murine system. Primitive human HSCs can generate all the cell lineages for long periods of time and most likely sustain long-term hematopoiesis. Progenitors such as CMPs generate only limited numbers and types of cells and primarily contribute to short-term engraftment.^{17,18} Currently, the phenotype of human HSCs and progenitors is not completely defined. Most studies suggest that human HSCs and progenitors are small quiescent cells that express the surface glycoprotein CD34.^{16,19} In addition, the cells express high levels of MDR activity, lack expression of lineage commitment markers, and express low levels of thy-1. Human CD34+ cells that express high levels of CD34 and low or absent levels of a variety of cell surface markers, including CD33, CD38, thy-1, and CD71, appear to be enriched for primitive progenitor and HSC activity, while more mature progenitors express one or more of these markers.^{20,23} Based on these findings, a number of technologies have been developed to isolate CD34-expressing cells for transplant purposes as a way of eliminating allogeneic T cells that could cause graft-vs-host disease (GVHD) as well as depleting contaminating autologous tumor cells.²⁴ Despite this, recent studies indicate that some important HSCs and progenitors may exist that do not express CD34.²⁵⁻²⁸ Consequently, a number of efforts are underway to more effectively refine the description and biology of human HSCs and progenitors. As an example, our group has demonstrated that HSCs and progenitors can be isolated using a fluorescent substrate for ALDH.²⁹ This approach has a number of potential advantages over CD34 selection methods and may become more widely used over the next several years.

Hematopoiesis

The process of hematopoiesis involves a complex interplay between the intrinsic genetic processes of blood cells and their environment. This interplay determines whether HSCs, progenitors, and mature blood cells remain quiescent, proliferate, differentiate, self-renew, or undergo apoptosis.³⁰⁻³² All of the genetic and environmental mechanisms that govern blood production operate by affecting the relative balance of these fundamental cellular processes. Under normal conditions, the majority of HSCs and many progenitors are quiescent in the G₀ phase of the cell cycle; however, many of the more mature progenitors are proliferating and producing mature offspring.³³ In the absence of any stresses, this is balanced by the rate of apoptosis in progenitors and mature cells.³¹ In the event of a stress such as bleeding or infection, several

processes occur. Stored pools of cells in the marrow or adherent to the endothelium are quickly released into the circulation in order to localize to the site of injury.³⁴ Fewer progenitors and mature cells undergo apoptosis.^{35,36} In addition, quiescent progenitors and HSCs are stimulated by a variety of growth factors to proliferate and differentiate into mature white cells, red blood cells, and platelets. When the bleeding, infection, or other underlying stress ceases and the demand for blood cells returns to normal, the anti-apoptotic and proliferative processes wind down, blood cells are redistributed back to their storage sites, and the kinetics of hematopoiesis return to baseline levels. This process repeats itself innumerable times during the lifespan of an individual and is seen in an exaggerated form following chemotherapy or bone marrow transplantation.

Probably the best characterized environmental regulators of hematopoiesis are cytokines.³⁷ Cytokines are a broad family of proteins that mediate positive and negative effects on cellular quiescence, apoptosis, proliferation, and differentiation. In general, cytokines function by engaging a specific receptor and activating a variety of signaling pathways. This includes activation of a tyrosine kinases such as focal adhesion kinase, pp60src, and c-Abl, MAP kinases, jun Kinase (JNK), and protein kinase C (PKC).³⁸ Mediators of cell growth and differentiation such as c-src, phosphoinositides, protein kinase C, and growth factor-mediated signaling pathways are also modulated by cytokines. Cytokines including interleukin-3 and GM-CSF induce cell proliferation, while other cytokines including flt-3 ligand and kit ligand protect cells from apoptosis and sensitize them to the effects of growth promoting cytokines.³⁹⁻⁴¹ Cytokines may also facilitate the interactions between stem cells and elements in the microenvironment including extracellular matrix (ECM) components.⁴² Regulators of HSCs including transforming growth factor-beta (TGF- β) and tumor necrosis factor-alpha (TNF- α) modulate cell cycle activity and engraftment.⁴³ Newly discovered cytokines including Wnt and the notch ligand family may also have important effects on stem cell biology.^{44,45} Some cytokines, including TNF- α , may be either inhibitory or activating depending on their concentration and other ongoing physiologic processes.⁴⁶ Several known cytokines, such as kit ligand, exist in either a soluble or membrane-bound form and have different activities depending on whether they are bound or soluble and on the environmental context in which they are acting.⁴¹ Hematopoietic regulatory cytokines are produced through both autocrine and paracrine mechanisms and in many cases are produced by nonhematopoietic cells including bone marrow stroma and endothelium.

Chemokines are another class of compounds that are important regulators of hematopoiesis.⁴⁷⁻⁴⁹ These molecules regulate blood cell trafficking and homing to sites of need and may also be negative and positive growth regulators.⁵⁰ Chemokines are composed of a large family of proteins that mediate a variety of processes including inflammation, leukocyte migration and development, angiogenesis, and tumor cell growth and metastasis. Chemokines bind to one or more of a large family of structurally related guanine protein-coupled transmembrane receptors. In hematopoiesis, chemokines can inhibit progenitor growth, regulate migration of hematopoietic progenitors, and mediate T-cell development in the thymus. For example, the chemokine SDF-1 (which binds the receptor CXCR4) is essential for trafficking of hematopoietic cells in the developing embryo, mediating homing of HSCs and progenitors to the bone marrow following transplantation and, in stem cell mobilization, for collecting peripheral blood stem cells for transplant purposes.⁴⁷ A number of other chemokines likely play important roles in hematopoiesis and are under active investigation.

Other important environmental regulators of hematopoiesis include the ECM components, other hematopoietic and nonhematopoietic cells, nutrients and vitamins, and a variety of physiologic processes. HSCs and progenitors bind tightly to a number of ECM components including heparin sulfates, chemokines, collagens, laminin, thrombospondin-1, fibronectin, and others. These molecules provide a scaffold for colocalizing progenitors and HSCs with a wide array of positive and negative cytokines and other growth regulators. In addition, ECM and stromal components may directly mediate signaling to HSCs to activate growth, protect cells from apoptosis, or modulate responses to positive and negative regulatory factors. The adhesion molecules on HSCs and progenitors that mediate binding to these ECM components include integrins, selectins, and mucins. Adherence of cells to microenvironmental elements can trigger a variety of signaling pathways and can lead to changes in intracellular ions such as proton (pH), calcium, and the small GTPase Rho as well as lipid mediators such as phosphoinositides, diacylglycerol, and arachidonic acid metabolites.⁵¹ Adhesion may also regulate expression of immediate-early genes such as c-fos and key cell cycle events such as kinase activity of cyclin-cdk complexes and phosphorylation of the retinoblastoma (Rb) protein.⁵² Cell adhesion may potentiate the responses to growth factors and by modulating the downstream components of growth factor signaling cascades including PI 3 kinase, AKT, and p70rsk.⁵³ Hematopoietic and nonhematopoietic cells that may regulate hematopoiesis include NK cells, T cells,

macrophages, fibroblasts, osteoblasts, adipocytes, and perhaps even neurons.^{54,55} These cells may produce important growth factors, facilitate engraftment, or induce apoptosis. A number of nutrients, trace elements, and vitamins (eg, zinc, selenium, copper, vitamins A, D, and E) are also critical to hematopoiesis. Retinoids and particularly retinoid antagonists play important roles in differentiation at even low concentrations. A variety of physiologic processes can also affect hematopoiesis including the shear stress mediated by the movement of fluids across the cells, mechanical stretching and compression forces acting on the cells, oxygen concentration, the three dimensional microenvironment, and the redox state of the microenvironment.

In addition to this wide array of environmental factors that regulate hematopoiesis, a number of intrinsic genetic events are critical to hematopoiesis as well. The Rb family, the E2Fs, cyclins, SCL, Hox, and other gene families appear to regulate proliferation and self-renewal of early hematopoietic cells.⁵⁶⁻⁵⁹ The bcl family and others regulate apoptosis in hematopoietic cells. A variety of genes including the C/EBP, MyD, PaxB, and Ikaros appear to play critical roles in hematopoietic cell differentiation and lineage commitment.⁶⁰⁻⁶² The development of informative molecular biology techniques for examining gene expression in small numbers of isolated cells is ushering in a new understanding of the role that these genes play in hematopoiesis.^{63,64} In particular, defining how these genes interact with the environmental factors that govern hematopoiesis is an area of intense research.⁶⁵ The majority of evidence currently suggests that many genetic events are preprogrammed to occur in a certain sequence and timing. In this model, termed the stochastic model, the developmental fate of cells is predetermined by intrinsic genetic processes as occurs in embryogenesis. The environmental signals then act upon cells to amplify or modulate the genetic effects. For example, a lymphoid cell is genetically programmed to undergo IgG VDJ gene rearrangement; this cell then proliferates into a large number of cells under the influence of cytokines such as interleukin-7. Another model, termed the instructional model, hypothesizes that the environment can play a primary role in determining the fate of HSCs and progenitors and can direct the cells toward any of the various lineages and developmental outcomes.⁶⁶ In this model, the environment can direct an HSC or multipotential progenitor down a particular developmental pathway by inducing the appropriate genetic changes. As discussed below, the role that the environment may play in directing the developmental pathway of HSCs and progenitors is currently the subject of debate in the field of hematopoiesis.

Evolving Conception of Stem Cells

Over the last several decades that HSCs have been studied, it has been assumed that their progeny were restricted to the hematopoietic system and that hematopoiesis was a one-way hierarchical process (Fig 1). In the last few years, however, a series of studies have raised the possibility that HSCs and their progeny may be more plastic than originally assumed. Some studies have now demonstrated that cells believed to be developmentally restricted to a particular lineage could be put into an environment where they could recover their ability to commit to other lineages (Fig 2).^{67,68} For example, cells progenitors can be grown in the laboratory to generate relatively mature B cells.⁶² Traditionally, it was expected that these cells could not generate other lymphoid cells and certainly not other myeloid cells. Surprisingly, it was found that the relatively mature B cells could be placed into a culture condition that rescued their ability to generate a variety of other cells including myeloid cells (Fig 2). This raises the possibility that progenitors and even relatively mature cells could be “reprogrammed” to recover some of their stem cell properties in the right conditions, a process termed plasticity. This has generated much excitement since, if the critical “reprogramming” signals could be defined, it might allow scientists and clinicians

to rebuild custom hematopoietic and immune systems for individual conditions and clinical settings.

Even more surprising than these observations is a series of reports in the last few years suggesting that HSCs may have sufficient plasticity as to be able to generate nonhematopoietic cells including hepatocytes, muscle cells, epidermal cells, islet cells, neurons, myocardium, and other lineages under the right environmental conditions (Fig 2).⁶⁹⁻⁷³ In these studies, bone marrow cells from mice, rats, and even humans appear to have generated the various tissues after transplant. Several reports have also described stem cells in muscle, brain, and other tissues that may be able to generate hematopoietic cells.^{74,75} While the possibility of stem cell plasticity is exciting, the study of stem cell plasticity is in its infancy; it is far from clear whether HSCs and other stem cells can in fact generate other tissues at all or whether this can occur in clinically relevant frequencies.^{76,77}

A number of technical issues confound the study of stem cell plasticity and can lead to misinterpretation of the results.⁷⁸⁻⁸⁰ These include the fact that almost all experiments have used heterogeneous populations of cells, so it is not clear if a single cell is generating multiple tissue types. In addition, even small numbers of contaminating nonhematopoietic stem cells residing in the bone, such as mesenchymal stem cells, can expand and give the appearance that the nonhematopoietic tissue was derived from an HSC. No reports have yet demonstrated that the nonhematopoietic cells are functional, so it is possible that these cells may localize to tissues but not participate in any physiologically meaningful processes. Recent reports have demonstrated that donor cells can fuse with recipient nonhematopoietic cells and transfer genetic markers, giving the mistaken appearance that the transplanted hematopoietic cells generated other types of tissue.^{81,82} Also, it appears that prolonged manipulation of the donor cells outside the body prior to transplant may induce genetic mutations that can lead to apparent plasticity. These concerns may be addressed in the next few years by performing carefully controlled studies using single-cell transplants and clonal analysis of the hematopoietic and nonhematopoietic tissue.⁸³ If these reports confirm that stem cells are in fact plastic, it opens the door to a variety of exciting applications in treating diseases and aging.

Another form of stem cell plasticity is found within embryonic stem (ES) cells and other types of pluripotent stem cells (Fig 2).⁸⁴⁻⁸⁶ ES cell lines have been generated using tissue engineering techniques from blastocysts of mice, primates, and humans.⁸⁷ ES cells can be maintained for long periods of time in culture and, in

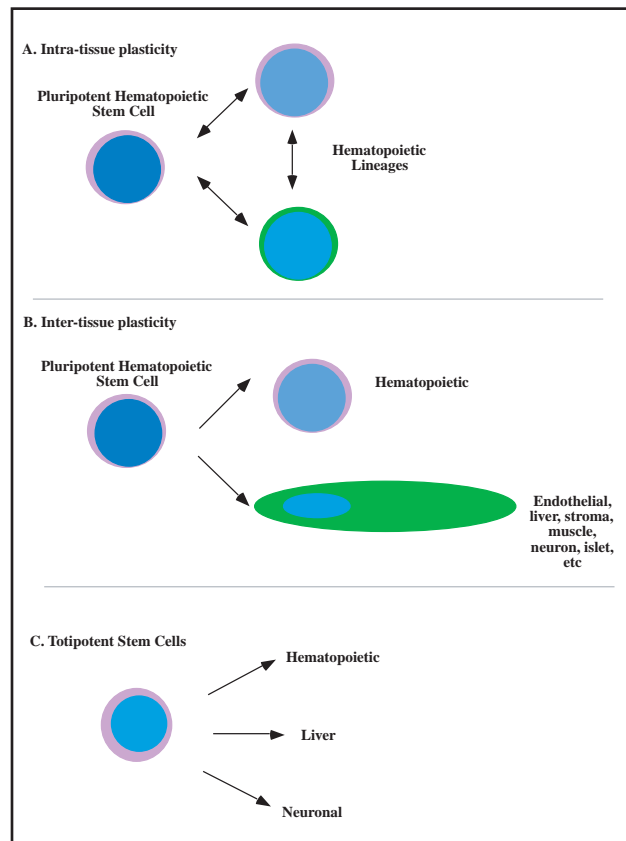


Fig 2. — Schema of intra-tissue plasticity (A), inter-tissue plasticity (B) and totipotent stem cells (C).

the right conditions, can be induced to differentiate into a variety of cell types including hematopoietic cells.⁸⁸ In mice, ES cells that have been differentiated into hematopoietic cells ultimately may be able to reconstitute some degree of hematopoiesis following transplant.⁸⁹ Using ES cells as a transplant source is still fraught with a variety of technical challenges including limited cell numbers and the possibility that ES cells may form tumors after transplant. In addition, there are significant ethical challenges to developing and using ES cells. Consequently, other sources of totipotent stem cells have been sought in adult tissue. Recently, a murine cell with ES-type properties, termed a multipotential adult progenitor cell (MAPC), was identified in bone marrow that was able to differentiate into a variety of tissues including mesenchymal cells, visceral mesoderm, neuroectoderm, and endoderm.⁹⁰ MAPCs were also able to engraft and differentiate into hematopoietic cells after transplantation into recipient mice. If these findings are confirmed, MAPCs may represent another type of stem cell that can be used for hematopoietic reconstitution. These cells may address many of the ethical and technical limitations of ES cells and are potentially an interesting resource.

Conclusions

A variety of mechanisms control hematopoiesis. The possibility of stem cell plasticity is tantalizing, but much work remains in order to define how stem cell plasticity may contribute to hematopoiesis and related processes. Ultimately, if stem cell plasticity is proven to be a real and practical phenomenon, it would provide the basis for exciting approaches to treating a variety of malignant, inflammatory, and degenerative diseases.

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