

Somatic stem cells of the blood: Haematopoietic stem cells

■ Elaine Dzierzak

- Erasmus University Medical Center,
Department of Cell Biology
- Rotterdam, The Netherlands

Introduction

The stem cells that form the adult blood, haematopoietic stem cells (HSC), are the most widely studied and clinically applied cell differentiation and transplantation system. Research in this field has been ongoing for several decades and has contributed to improved cell replacement therapies for blood related genetic deficiencies and malignancies. Current challenges in this field include: 1) *Ex vivo* expansion of HSCs for clinical use in autologous (self-derived) and allogenic (non-self) transplantations; 2) Knowledge of the stem cell molecular program for efficient manipulation of normal and leukaemic stem cells; 3) Stimulation of HSC generation from embryonic stem (ES)/precursor cells.

Current status

Enormous numbers of mature blood cells such as red blood cells, macrophages, lymphocytes and platelets are produced daily from HSCs through an extensive cellular differentiation hierarchy. HSCs are stringently defined by their ability to generate all blood lineages for long periods of time after transplantation into haematopoietic deficient recipients. Transplantation of HSCs was the first applied human stem cell replacement therapy.⁽¹⁾ Hundreds of thousands of patients have been successfully transplanted with donor HSCs from bone marrow, the adult tissue that harbours them. More recently, umbilical cord blood has also been successfully used for blood replacement therapy.⁽¹⁾

Graft-versus-host disease is much less frequent in patients receiving umbilical cord blood stem cell transplants, and these cells are easily accessible. However, the numbers of HSCs obtained from these tissues are limiting. Thus, as the best characterized cell differentiation system with clinical relevance, HSCs have been the focus of intense fundamental research.

Cellular, molecular and developmental research approaches have increased our understanding of the processes by which HSCs are generated, maintained, expanded and differentiated.^(2, 3) For example, the differentiation of HSCs into erythrocytes (red blood cells), granulocytes, macrophages and lymphocytes (white blood cells) is the result of stimulation by haematopoietic growth factors. Such growth factors have been identified and are routinely used in the clinic to stimulate haematopoiesis and to mobilise HSCs into the circulation. However, much less is known about self-renewal factors: Self-renewal is the process by which the HSC divides to produce two progeny, one progeny that differentiates into blood while the other cell retains stem cell potential. To date, no growth factors or growth factor combinations have been identified that faithfully promote the expansion of HSCs (i.e. the production of two stem cell progeny).

Since HSCs normally reside in particular environments within the body, the non-haematopoietic cells in these regions are of interest. These non-haematopoietic cells are derived from mesenchymal stem cells that differentiate into osteocytes (bone), adipocytes (fat), chondrocytes (cartilage), smooth muscle and vascular cells (blood vessels). These so-called stromal cells provide the signals necessary for the maintenance of HSCs throughout adult life. It is thought that there are only a defined number of these environ-

1. Hakim Nadey S and Paplois Vasilos E. (2003) History of Organ and Cell Transplantation, *Imperial College Press*, pp 304. and *Leukemia and Lymphoma Society*

mental niches and that HSC numbers are controlled by the numbers of, and factors produced within, these niches. Stromal cell lines from the bone marrow have been generated for the *ex vivo* laboratory study of the molecular interactions between HSCs and their surrounding environment. Knowledge of the molecular programs of HSCs, as well as the stromal cells within the niches, is of great importance for improvement of blood therapies.

Developmental environments and factors are a current intense focus of haematopoietic research.^(2,3) It is thought that HSCs are generated only during embryonic development and that no further generation occurs in the adult. Recent cell transplantation research in animal models has identified the originating source of HSCs as the cells of the developing embryonic blood vessels, such as the dorsal aorta and the umbilical artery. Several other tissues including the yolk sac and placenta may also contribute to the HSC pool found in the adult. HSC generation in the human embryo appears to be very similar to that of the mouse embryo.⁽³⁾ Within the first four weeks of conception, HSCs are found in the dorsal aorta, and slightly later in the yolk sac. They appear to be at least as potent as adult bone marrow and cord blood HSCs. Molecular manipulation of the genetic and epigenetic programs that direct the generation and expansion of HSCs in development should provide further important insights into the regulation of HSCs and may therefore lead to improved blood replacement therapies.

- HSCs produce all adult blood cells.
- HSCs are produced in multiple sites in the embryo and are thought to contribute to the adult blood system.
- Stromal cell niches support the growth of HSCs through the production of factors.
- Genetic and epigenetic programming of HSCs is beginning to be explored.

Prospects

Health related prospects for the use of information from HSC research include the ability to *de novo* produce HSCs in the adult from cells

of other lineages. Clearly, the demonstrated first development of HSCs from the embryonic vasculature implicates a particular molecular state (genetic and epigenetic) in the generation process. Elucidation of the program of these embryonic precursors, early HSCs and the cells of the embryonic stromal environment and comparisons with the programs of HSCs and cells of the stromal environment of the adult could suggest signalling pathways for stimulation or inhibition through small targeted drugs. Already known developmental signalling pathways such as the Notch, Hedgehog and Wnt pathways have been shown to affect haematopoietic cell growth. Thus, further cellular and molecular insights are yet gained into formation and expansion of HSCs during embryonic stages, and their potential contribution to the adult haematopoietic system. Interestingly, it is as yet unknown in mammals whether the HSCs generated during embryonic stages do indeed migrate and colonize the adult bone marrow to provide life-long haematopoiesis. It remains possible that rare endothelial cells in parts of the adult vasculature, perhaps the abundant vessels of the human placenta retain potential to generate haematopoietic cells. If this is the case, prospects for further expansion and induction of these vascular cells for therapeutic application are encouraging.

The availability of unlimited numbers of ES cells for differentiation to desired lineages has raised the possibility for use of ES cells in blood replacement therapies as an alternative to adult bone marrow or umbilical cord blood haematopoietic stem cells.⁽³⁾ Additionally, universal donor strains of ES cell derived HSCs would alleviate the problems inherent in obtaining closely matched donor stem cells. Since the first demonstration of haematopoietic differentiation of mouse ES cells over 20 years ago, many insights into the embryonic development of the mouse haematopoietic system have been applied to ES cell haematopoietic differentiation. Through the dedicated efforts of a few laboratories, the improvement of ES cell culture conditions has resulted in an enhanced ability to produce cells of the erythroid lineage (precursors of red blood cells), myeloid lineage (precursors of white blood cells with non-specific, non-adaptive, immune functions) and lymphoid lineage (precursors of B and T lymphocytes of the specific adaptive immune system), and to identify a common vascular and haematopoietic precursor.⁽⁴⁾

2. Dzierzak E. (2005) The emergence of definitive hematopoietic stem cells in the mammal. *Current Opinion in Hematology*, 12:197-2002.

3. Hematopoietic stem cell development: Review Issue (2005 Ed: Yoder MC. *Experimental Hematology*, 33.

Additionally, the haematopoietic differentiation of human ES cells has been achieved.⁽⁵⁾ These studies have furthered our understanding of some of the genetic programs directing the differentiation of the developmentally early haematopoietic cells.

Most interestingly, many advances have been made recently in the treatment of blood malignancies/leukaemias with the drug Gleevec. However, while this drug affects the cells that form in large part the leukaemia, small populations of leukaemia-inducing cells survive, and in time lead to the reappearance of the leukaemia. These “leukaemia stem cells” are interesting targets for novel drug treatments, which could be revealed through knowledge of the genetic and epigenetic programs in normal and leukaemic HSCs.

Realistic prospects or outcomes of research are:

- Stimulation of the developmental reprogram directing *de novo* HSC generation from vascular precursors
- ES cell derived HSCs in unlimited numbers and for universal engraftment in blood replacement therapies
- Targeted drug treatment of “leukaemia stem cells” for elimination of the persistent self-renewing cell source of leukaemia

Problems, concerns and open questions

Disappointingly, to date, haematopoietic differentiation of mouse and human ES cells has not achieved the production of potent transplantable adult HSCs.⁽⁵⁾ More studies of the *in vivo* development of HSCs will be necessary to provide clues concerning the directed differentiation of ES cells to HSCs. If the simultaneous differentiation of surrounding developing embryonic tissues is required for HSC induction (as appears to be the case in the mid-gestation dorsal aorta), multidimensional ES cell differentiation culture systems will need to be developed in which other lineages of cells are also induced. Additional studies will add insight into how HSCs can be generated from ES cells by manipulating developmental programs and additionally provide

new opportunities for obtaining large numbers of HSCs from other easily accessible tissues, such as the placenta. Moreover, these studies will further our understanding of how HSCs are generated from the embryonic vasculature.

- HSC generation from ES cells and/or vascular cells will most likely need a complex environment

Conclusions

The haematopoietic system is a dynamic, highly proliferative and complex differentiation system with many levels of regulation. Faulty regulation of HSC self-renewal can lead to 1) over proliferation, as in leukaemia or 2) lack of stem cell maintenance. An increasing knowledge of the process of self-renewal, as well as the developmental signals and environment leading to the generation of the HSCs, is essential for directing the generation and expansion of HSCs from adult and/or embryonic tissue sources or ES cells for the treatment of blood related diseases.

4. Passegué E, Jamieson CHM, Ailles LE, Weissman IL (2003) Normal and leukemic hematopoiesis: Are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? *PNAS* 100(1):11842-11849.

5. Wang L, Li L, Shojaei F, Levac K, Cerdan C, Menendez P, Martin T, Rouleau A, Bhatia M. (2004) Endothelial and hematopoietic cell fate of human embryonic stem cells originates from primitive endothelium with hemangioblastic properties. *Immunity*, 21:31-41.