HEMATOLOGY

Beck editor
Second Edition

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edited by William S. Beck

second edition

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PREFACE TO SECOND EDITION

Only a few years have passed since publication of the first edition but new and significant information has been erupting as if from dragon seed. Yet there is comfort in the fact that new insights are replacing old complexities and ambiguities on every side.

Among the lectures most extensively revised in this edition are those on the RES, hemoglobinopathies, thalassemias, hemolytic anemias, and newer aspects of the protein interactions in blood coagulation. Coverage of the last topic will appear rigorous and over-detailed in some eyes. But this important subject is clearly on the move. Without any doubt it is destined in the years ahead to yield important new advances.

The general outline of the syllabus remains as before. These are lectures--we resolutely call them that rather than chapters--which may be read by the student in his own time, both as preparation for and reminder of discussions in lecture hall and laboratory. There is no intention here to preempt the lecturer. However, experience in the hematology courses at the Harvard Medical School and the Harvard-M.I.T. Program in Health Sciences and Technology suggests that the lecture hour is now best used for illustration, expansion, or other forms of fugue and counterpoint.

Again we have utilized an outline format, which seems to help with the organization of ideas. Though one is hardly likely to find literary quality in these pages, we have tried to make this edition somewhat more readable and less telegraphic than its predecessor. Decisions on what stays and what goes have not been easy--and in view of the moderate increase in pages it may be that the decisions made were insufficiently stringent. The editor herewith pledges devout frugality in future editions.

Emphasis remains on physiology and pathophysiology. This has required systematic consideration of clinical abnormalities, which in the field of hematology are often brilliantly evocative exemplars of basic mechanisms. Therapy is mentioned but its discussions are intended on the whole to make pathophysiologic points. Topics are treated in a traditional sequence--red cells, then white cells, then clotting--and we assume that lectures will be read in that sequence. However, cross-references are supplied for those who read the lectures in some other sequence.

I must note again that authors of many of these lectures are indebted to those who lectured in former years. Rather than adopt complex rules of authorship, we have chosen to acknowledge these fine predecessors in this preface. Beyond those mentioned in the introduction to the first edition, I wish now to acknowledge the stimulating past contributions of Robert W. Colman, Daniel Deykin, Irving Umansky, Herman A. Godwin, and Alan C. Aisenberg. To each goes my sincere thanks. I must also give recognition to the capable efforts of Mary Ochs who prepared these pages for the camera and provided a sharp editorial eye.

My colleagues and I enjoy compiling our thoughts each year for our students in this course. These acts of celebration always call to mind the extraordinary fecundity of contemporary biological thought and the ingenuity of its students. Hematology is endlessly fascinating and this is a great time in which to be teaching it.

William S. Beck

February 1977

FROM INTRODUCTION TO FIRST EDITION

In the present Harvard Medical School curriculum, hematology is one of a dozen blocks within the large one-year Pathophysiology course, which begins in the middle of the first year. For many years we have furnished our students in this course with syllabus materials and lecture notes. These notes have long been known as "the camel," which, as everyone knows, is an animal that looks as though it had been put together by a committee. When responsibility for the course came to me, the scanty lecture notes took the form of severe and often uninformative outlines. Students complained of the burdens of note-taking and some brought tape recorders. Accordingly, lecturers were asked to flesh out their outlines and our camel grew. It became apparent, in time, that the value of the syllabus would be enhanced by careful editing. The result is the present volume.

The vast expansion of knowledge in the various branches of pathophysiology poses an increasingly difficult problem for those who would define the content of a core curriculum and establish standards and priorities for what is to be taught. It is the editor's view that this difficult cause can only be furthered by the thoughtful preparation of texts such as this. For the very act of editing such a volume necessitates choices and permits correlations and overviews that are never quite possible when many and diverse individuals are responsible for a course of instruction.

The major goal of this endeavor is to improve the quality and usefulness of these notes as teaching instruments. It is our intention to revise this small volume frequently so it will retain the freshness and currency that characterized the informal syllabus materials of previous years.

Some believe it would now be appropriate to abandon

formal lectures. I see much merit in that proposal. Surely, medical students are capable of handling reading assignments. Surely, they deserve exemption from having read to them lectures that they could as well read themselves. Still there is cause for regret about a step that would deny students an hour or two with colleagues who rate high as teachers, scientists, and personalities. How to make the best use of these hours will be the subject of experimentation and innovation in the years ahead.

Meanwhile, we hope that this brief volume will be useful to our students, both in the second year hematology course and in later clinical years. We hope, too, that students and physicians beyond Harvard Medical School may find this a helpful review of hematology, one that may serve as a compendium and guide to the several large new textbooks of hematology. In a sense, this volume is a successor to Ham's famous *Syllabus* and its revision by Page and Culver. Unlike the present volume, however, the earlier syllabuses placed major emphasis on laboratory diagnosis.

I wish, in closing, to acknowledge a group of fine young collagues who taught for a time in this course and then went to positions of leadership elsewhere. I refer to Neil Abramson, Richard H. Aster, and Richard A. Cooper. Each left his mark on this course and on the lecture notes prepared by successors. The same may be said for Louis K. Diamond, who for many years was a mainstay of the hematology course.

William S. Beck

February 1973

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LECTURE 1

ERYTHROPOIESIS AND INTRODUCTION TO THE ANEMIAS

William S. Beck

I. BIOLOGY OF HEMATOPOIESIS

A. Ontogeny

In the third week of human embryogenesis, mesenchymal cells in the yolk sac form clusters called blood islands. Peripheral cells of the islands join to form a primitive vascular system. Simultaneously, central cells of the islands differentiate into elements that become detached and are carried off by the mounting stream of primitive plasma. These are the yolk sac stem cells. Some differentiate into primitive erythroblasts, the earliest hemoglobin-synthesizing cells. Unlike pronormoblasts of adult bone marrow, they do not mature into erythrocytes. In the third month of embryonic life, yolk sac stem cells migrate to the liver, which then becomes the chief site of blood cell formation. Additional contributions are then made by the spleen, lymph nodes, and thymus. Hematopoiesis may continue in the liver until after birth. However, bone marrow hematopoiesis begins in the fourth lunar month and by the end of gestation is the major source of blood cells. The terms medullary and extramedullary hematopoiesis denote blood cell production, respectively, by bone marrow and by tissues other than bone marrow. At birth, medullary hematopoiesis occurs in almost every bone. Flat bones (sternum, ribs, skull, vertebrae, and innominates) retain most of their hematopoietic activity throughout life, but hematopoiesis progressively diminishes within the shafts of long bones. In the adult, it is limited to the ends of these bones. At times of increased demand for blood cells, active marrow reappears in these sites. With the exception of lymphocyte production, hematopoiesis in the adult occurs exclusively in bone marrow. As noted below, even lymphocytes de-

rive from medullary precursors. Some extramedullary hematopoiesis persists at birth, but it rapidly diminishes to resume only under abnormal circumstances. In such instances, liver and spleen are the major loci of extramedullary hematopoiesis. Curiously, the thymus never resumes this embryonic function.

B. Phylogeny

Much has been learned of the physiology of blood from studies of the evolution of the hematopoietic system. Amphioxus and other primitive chordates lack blood cells. In some invertebrates, hemoglobin occurs in solution in plasma. The mature red cells of reptiles, birds, and fish contain nuclei and metabolically active ribosomes and mitochondria. The locus of adult blood-forming tissue varies in different species. For example, it is kidney in amphibia and teleosts; gonads in some fishes; liver in turtles; and tissues around the heart in sturgeon and paddlefish.

C. Mammalian bone marrow

The fine structure of bone marrow was not elucidated until recently. Nutrient arteries enter marrow cavity through bone foramina. Arteries branch into distibuting arterioles that give rise to an endosteal bed of sinusoids. From the bed, sinuses travel in a radial direction toward the central longitudinal veins lying in the long axis of the bone. Hematopoietic tissue lies between the sinuses. Erythropoiesis, granulopoiesis (or myelopoiesis), and thrombopoiesis take place extravascularly in the marrow stroma outside the sinusoids. Rates of hematopoietic activity and blood supply are related. Sinusoidal walls have three layers: endothelial cells, basement membrane, and adventitial cells. Endothelial and adventitial cells are both mono-

nuclear reticulum cells that are capable of phagocytosis (as will be discussed in Lecture 2). Blood is present within the sinusoids, but intrasinusoidal materials (both diffusible and particulate) have free access to extrasinusoidal areas through gaps in the walls. Hematopoietic cells, having undergone maturation outside the sinusoids, gain access to the sinusoids at a critical moment in the maturation sequence. The mechanism of this event, known as release of blood cells from marrow into blood, is poorly understood.

II. STEM CELLS

Much evidence exists that the marrow of adult mammals contains pluripotent stem cells that give rise to the several lines of differentiated blood cells--erythrocytes, granulocytes, thrombocytes (platelets), and also to lymphoid cells. In the past, semantic confusion has arisen from the fact that the term "stem cell" is defined in at least three ways.

A. Morphologic definition

One definition of the stem cell is as a morphologic entity. However, stem cells are so few in number they were not identified until recently. They were finally revealed, to the disappointment of some, as small mononuclear cells resembling lymphocytes. They are mobile cells and are normally present in the blood (about 1-5 per 10^5 nucleated cells). At certain times, e.g., after whole body irradiation, during antigenic stimulation, the number in blood increases. As noted above waves of migration also occur during embryonic life.

B. Kinetic definition

The stem cell is also definable as a kinetic entity. The stem cell pool is recognized by its ability (1) to be self-maintaining and (2) to give

rise to further differentiated cells. Our knowledge of bone marrow function implies that such cells must exist. In kinetic terms, any cell with these properties is a stem cell, even if it is already partially differentiated.

To obviate confusion between the kinetic and morphologic definitions of stem cell, the noncommittal term α cell is preferred in kinetic discussions in place of stem cell. An α cell is any cell that can replace itself and give rise to a more differentiated cell. The latter is termed an n cell. Discussions of bone marrow kinetics ordinarily deal with the behavior of compartments of cells. A compartment is defined as any distinct class of cells, whether the distinction is based on function, morphology, developmental stage, or other properties. The definition of the α cell just given is perhaps more accurately applied to the α cell compartment, for it is the compartment that renews itself and gives rise to further differentiated cells. This is the case because indeterminacy surrounds the behavior of individual α cells, which may behave asymmetrically ($\alpha \rightarrow \alpha, n$) or symmetrically ($\alpha \rightarrow 2\alpha$, or $\alpha \rightarrow 2n$). Thus, the net behavior of a population of α cells involves statistical considerations since experimental difficulties obscure the behavior of any single stem cell.

C. Operational definition

This definition regards the stem cell as a colony-forming unit, or CFU, in the assay system devised by Till and McCulloch. In this method, suspensions of marrow cells are injected intravenously into lethally irradiated mice in which the spleen and marrow have been reduced to stroma and are hematologically empty. After 10 days, discrete macroscopic colonies are observed in the animal's spleen,

which has become a "home" for the wandering injected stem cells. Single stem cells lodge in the spleen stroma and there, under the peculiarly specific influence of the so-called hematopoietic inductive microenvironment (HIM), proliferate and differentiate into large colonies. Initially, stem cells proliferate actively and produce minute clonal colonies of undifferentiated stem cells. After 5 days, specific differentiation takes place and discrete colonies are seen macroscopically on the surface of the spleen and within its parenchyma. Chromosome studies indicate that each colony is derived from a single stem cell. In view of this, enumeration provides a valid quantitative assay of the number of CFU's. In this method stem cells are detected indirectly by observing the results of their proliferation and differentiation; hence "CFU" is a noncommital or operational term applicable to colony-forming units from injected suspensions of marrow, spleen, or diverse materials.

Most spleen colonies (60% of them) contain only erythroid cells. About 20% contain only myeloid cells (neutrophilic); 15% are megakaryocytic. After 10 days, colonies may include more than one cell line--and more CFU's. This is evidence that the marrow stem cell gives rise to each of the principle cell lines. Some believe it also gives rise to other marrow elements, including lipocytes, fibrocytes, chondrocytes and osteocytes.

A second assay system for stem cell colonies employs tissue culture methods. Such techniques were unreliable until it was found that media must be semisolid and contain a stimulating factor. The first colonies obtained in culture consisted of myeloid cells and depended on a stimulating factor called colony-stimulating activity, or CSA, which

is readily provided by "feeder cells." A layer of neonatal kidney cells, for example, produces a factor that disposes marrow stem cells to form myeloid clones in 7-10 days. For human cell cultures, the best source of CSA is blood leukocytes. When leukocytes are fractionated, the richest subcellular source of CSA is the cell membrane fraction. CSA exists in several molecular forms that have been designated (with their mol. wts.): CSA I (60-70,000); CSA II (35,000); CSA III (15,000); and CSA IV (1,300). Forms I, II, and III are glycoproteins; IV is a hydrophobic peptide. Myeloid colonies in culture differ from spleen colonies, e.g., culture colonies contain fewer cells (20-50,000) than spleen colonies ($> 10^6$). Their progenitors are termed, respectively, CFU-C and CFU-S. This methodology then led to discovery of the CFU-E, a colony-forming unit, committed to erythropoiesis, and the BFU-E, a "burst"-forming unit, also committed to erythropoiesis and apparently a precursor of the CFU-E.

Known progenitors of hematopoietic cells and their lineages are summarized in Table 1-1 and Fig. 1-1.

Table 1-1. Known Hematopoietic Progenitor Cells

Term	Required stimulus	Detected by	Postulated role
CFU-S	Mouse tissues	Spleen colony assay	Pluripotent stem cell
CFU-C	Feeder cell layer; urine, tissue extracts, leukocyte extracts, etc.	Colony formation in agar medium	Committed progenitor of granulopoiesis
BFU-E	Erythropoietin	Colony formation in agar culture	Committed progenitor of erythropoiesis (early)
CFU-E	Erythropoietin	Colony formation in agar culture	Committed progenitor of erythropoiesis (late)

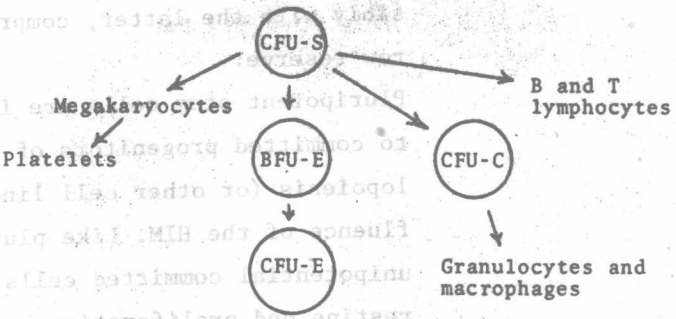

Fig. 1-1. *Postulated lineages and relationships of hematopoietic progenitor cells.* (Adapted from J. E. Till et al, *Fed Proc.* 34, 2279, 1975)

Although each type of the CFU is a distinct progenitor cell, only the CFU-S is a truly pluripotent stem cell. The CFU-C, BFU-E, and CFU-E are unipotential near-descendants already committed to differentiation along a specific line. They require only a stimulus to launch their maturation via blastic transformation and further differentiation. The stimulus is a humoral "poietin." Erythropoietin, the specific stimulus for stem cells committed to erythropoiesis, will be discussed later in this lecture. Mounting evidence supports the existence of a leukopoietin and a thrombopoietin responsible for the differentiation of stem cells committed to granulopoiesis and thrombopoiesis, respectively. These will be discussed in Lectures 16 and 25. Presumably leukopoietin and CSA are identical.

Stem cells have been profitably studied by "suicide" techniques in which actively dividing cells take up enough tritiated thymidine (^{3}H-TdR) to cause radiation-induced "suicide." In such experiments most pluripotential CFU-S's are spared. Thus it is believed that pluripotent stem cells are in two functional states: a majority that are at rest, and a small minority that are actively proliferating