Concise Guide to Hematology
Dr. Schmaier dedicates this book to his life partner Linda and to Alec and Lauren who are contributing to the next generation of physicians.

Dr. Lazarus dedicates this book to his loving wife Joan and sons Jeffrey and Adam and spouses Jana and Sarah for their unwavering support.
List of Contributors, ix

Preface, xii

1 Introduction to Hematology, 1
   Alvin H. Schmaier

2 Hematopoiesis, 6
   Yuan Lin and Stanton L. Gerson

3 Red Blood Cell Biochemistry and Physiology, 16
   Kevin T. McDonagh

4 Anemia: Clinical Approach, 24
   Peter W. Marks

5 Iron Deficiency, 35
   Alice Ma

6 Vitamin-B₁₂ (Cobalamin) and Folate Deficiency, 44
   Aṣok C. Antony

7 Congenital Hemolytic Anemias, 62
   Archana M. Agarwal and Josef T. Prchal

8 Acquired Hemolytic Anemias, 75
   Scott D. Gitlin

9 Overview of Hemostasis, 91
   Alvin H. Schmaier

10 Approach to the Bleeding Patient, 103
    Alvin H. Schmaier
11 Congenital Bleeding Disorders, 112
   Anjali A. Sharathkumar and Steven W. Pipe

12 Acquired Bleeding Disorders, 131
   Howard A. Liebman

13 Platelet Function in Hemostasis and Inherited Disorders of Platelet
   Number and Function, 140
   A. Koneti Rao and David W. Essex

14 Acquired Thrombocytopenia, 154
   Theodore E. Warkentin and Andrew E. Warkentin

15 Thrombosis and Anticoagulation, 174
   Alvin H. Schmaier

16 Myeloid Cell Physiology and Disorders, 184
   Alvin H. Schmaier, Lilli M. Petruzzelli, Niels Borregaard and
   Laurence A. Boxer

17 Bone Marrow Structure and Diagnostic Testing, 197
   Howard J. Meyerson and Hillard M. Lazarus

18 Myeloproliferative Neoplasms and Myelodysplastic Syndromes, 220
   Gabriela Motyckova and Richard M. Stone

19 Acute Leukemia, 235
   Tsila Zuckerman and Jacob M. Rowe

20 Classification of Lymphoma, 251
   Yi-Hua Chen and Amy Chadburn

21 Clinical Evaluation and Management of Lymphoma, 268
   Makiko Ban-Hoefen, Jonathan W. Friedberg and Richard I. Fisher

22 Plasma Cell Disorders, 287
   Sumit Madan and Philip R. Greipp

23 Pediatric Hematology, 307
   Sanjay P. Ahuja

24 Blood Banking, 319
   Lawrence Tim Goodnough

25 Transfusion Therapy, 332
   Beth H. Shaz and Christopher D. Hillyer
26 Hematopoietic Cell Transplantation, 344
   Hillard M. Lazarus

27 Atlas of Hematology Slides, 363
   Alvin H. Schmaier

Index, 389
Contributors

Archana M. Agarwal MD
Assistant Professor, Pathology
University of Utah and ARUP Laboratories
Salt Lake City, UT, USA

Sanjay P. Ahuja MD, MS
Assistant Professor, Pediatric Hematology/Oncology
Director, Hemostasis and Thrombosis Center
Rainbow Babies and Children’s Hospital
Case Western Reserve University
Cleveland, OH, USA

Asok C. Antony MD, FACP
Professor of Medicine
Indiana University School of Medicine
Indianapolis, IN, USA

Makiko Ban-Hoefen MD
Hematology/Oncology Fellow
James P. Wilmot Cancer Center
University of Rochester Medical Center
Rochester, NY, USA

Niels Borregaard MD, PhD
Professor of Hematology and Internal Medicine
University of Copenhagen;
Department of Hematology
Rigshospitalet
Copenhagen, Denmark

Laurence A. Boxer MD
Henry and Mala Dorfman Family Professor of Pediatric Hematology/Oncology
Department of Pediatrics and Communicable Diseases
University of Michigan
Ann Arbor, MI, USA

Amy Chadburn MD
Professor of Pathology
Division of Hematopathology
Department of Pathology
Northwestern University, Feinberg School of Medicine
Chicago, IL, USA

Yi-Hua Chen MD
Assistant Professor of Pathology
Division of Hematopathology
Department of Pathology
Northwestern University, Feinberg School of Medicine
Chicago, IL, USA

David W. Essex MD
Associate Professor of Medicine
Sol Sherry Thrombosis Research Center and Division of Hematology
Temple University School of Medicine
Philadelphia, PA, USA

Richard I. Fisher MD
Director, James P. Wilmot Cancer Center
Samuel E. Durand Chair in Medicine
Professor of Medicine
University of Rochester Medical Center
Rochester, NY, USA

Jonathan W. Friedberg MD, MMSc
Professor of Medicine and Oncology
Chief, Hematology Oncology Division
James P. Wilmot Cancer Center
University of Rochester Medical Center
Rochester, NY, USA
Contributors

**Stanton L. Gerson MD**
Director, Case Comprehensive Cancer Center
Director, Seidman Cancer Center
Asa and Patricia Shiverik Professor of Hematology and Oncology
Case Western Reserve University
University Hospitals Case Medical Center
Cleveland, OH, USA

**Scott D. Gitlin MD, FACP**
Associate Professor of Internal Medicine
Division of Hematology/Oncology
University of Michigan
Ann Arbor VA Health System
Ann Arbor, MI, USA

**Lawrence Tim Goodnough MD**
Professor of Pathology and Medicine
Director of Transfusion Service
Department of Pathology
Stanford University Medical Center
Stanford, CA, USA

**Philip R. Greipp MD**
Professor of Medicine
Division of Hematology
Mayo Clinic
Rochester, MN, USA

**Christopher D. Hillyer MD**
President and Chief Executive Officer
New York Blood Center
Professor of Medicine
Department of Medicine
Weill Cornell Medical College
New York, NY, USA

**Hillard M. Lazarus MD, FACP**
Professor of Medicine
Case Western Reserve University
University Hospitals Case Medical Center
Cleveland, OH, USA

**Howard A. Liebman MD**
Professor of Medicine and Pathology
Jane Anne Nohl Division of Hematology
Department of Medicine
University of Southern California-Keck School of Medicine
Los Angeles, CA, USA

**Yuan Lin PhD**
Division of Hematology and Oncology
Case Western Reserve University
University Hospitals Case Medical Center
Seidman Cancer Center
Cleveland, OH, USA

**Alice Ma MD**
Associate Professor of Medicine
Department of Medicine
Division of Hematology/Oncology
University of North Carolina
Chapel Hill, NC, USA

**Sumit Madan MD**
Research Fellow
Division of Hematology
Mayo Clinic
Rochester, MN, USA

**Peter W. Marks MD, PhD**
Associate Professor of Medicine
Department of Internal Medicine/Hematology
Yale University School of Medicine
New Haven, CT, USA

**Kevin T. McDonagh MD**
Markey Foundation Chair
Chief, Division of Hematology, Oncology and Blood and Marrow Transplantation
University of Kentucky College of Medicine
Lexington, KY, USA

**Howard J. Meyerson MD**
Director of Hematopathology and Flow Cytometry
University Hospitals Case Medical Center
Associate Professor of Pathology
Case Western Reserve University
Cleveland, OH, USA

**Gabriela Motyckova MD, PhD**
Hematology/Oncology Fellow
Dana-Farber Cancer Institute
Boston, MA, USA

**Lilli M. Petruzzelli**
Sr. Medical Director, Oncology Translational Medicine
Novartis Institutes for BioMedical Research, Inc.
Cambridge, MA, USA
Contributors

Steven W. Pipe MD
Associate Professor of Pediatrics and Pathology
Laurence A. Boxer Research Professor of Pediatrics and Communicable Diseases
Director, Division of Pediatric Hematology and Oncology
Pediatric Medical Director, Hemophilia and Coagulation Disorders Program
Director, Special Coagulation Laboratory
C.S. Mott Children’s Hospital
University of Michigan
Ann Arbor, MI, USA

Josef T. Prchal MD
Professor of Medicine, Genetics and Pathology
University of Utah and ARUP Laboratories; Huntsman Cancer Hospital
George E. Waheln VA Medical Center
Salt Lake City, UT, USA

A. Koneti Rao MBBS, FACP
Sol Sherry Professor of Medicine
Chief, Hematology Section
Co-Director, Sol Sherry Thrombosis Research Center
Temple University School of Medicine
Philadelphia, PA, USA

Jacob M. Rowe MD
Professor of Hematology
Director, Department of Hematology and Bone Marrow Transplantation
Rambam Medical Center and Bruce Rappaport Faculty of Medicine—Israel Institute of Technology
Haifa, Israel

Alvin H. Schmaier MD
Robert W. Kellermeyer Professor of Hematology/Oncology
Case Western Reserve University
University Hospitals Case Medical Center
Cleveland, OH, USA

Anjali A. Sharathkumar MD, MS
Medical Director of Hemophilia and Thrombophilia Program
Assistant Professor of Pediatrics
Children’s Memorial Hospital
Northwestern University, Feinberg School of Medicine
Chicago, IL, USA

Beth H. Shaz MD
Chief Medical Officer
New York Blood Center
New York, NY, USA;
Clinical Associate Professor
Department of Pathology and Laboratory Medicine
Emory University School of Medicine
Atlanta, GA, USA

Richard M. Stone MD
Professor of Medicine
Dana-Farber Cancer Institute
Boston, MA, USA

Andrew E. Warkentin BHSc(Hon)
Medical Student
Faculty of Medicine
University of Toronto
Toronto, ON, Canada

Theodore E. Warkentin MD,
BSc(Med), FRCP(C), FACP
Professor, Department of Pathology and Molecular Medicine, and Department of Medicine
Michael G. DeGroote School of Medicine
McMaster University
Hamilton, ON, Canada

Tsila Zuckerman MD
Director, Bone Marrow Transplantation Unit
Department of Hematology and Bone Marrow Transplantation
Rambam Medical Center and Bruce Rappaport Faculty of Medicine—Israel Institute of Technology
Haifa, Israel
This volume originally arose from the need to redesign the hematology course curriculum for second-year medical students at the University of Michigan. The first version was published as *Hematology for Medical Students* in 2003. I was pleased to hear favorable comments by medical students and also some colleagues in the field who used the text for their own medical school courses. In preparing for the second version, we were very pleased that Wiley-Blackwell took an interest in having a book like this prepared for an American audience. In re-designing the text, we realized that its appeal is greater than just medical students. Residents in pediatrics, internal medicine, and pathology found the first version of the text useful and our fellows in hematology and oncology at the University of Michigan and Case Western Reserve University also used it as a handy quick review of basic hematology and hematologic malignancy. Thus, the name was changed to *Concise Guide to Hematology*. In preparing the second version I recruited Dr. Hillard Lazarus as a co-editor. Hillard has a terrific background in classic hematology and is an expert in hematologic malignancies. He also is a critical reader and writer and I have appreciated his efforts in helping me to make this edition possible. Together, we agreed to recruit experts in the various fields of hematology as authors of the chapters. We personally thank the authors for the quality of their contributions to this volume. Lastly, we thank the key personnel at Wiley-Blackwell, Maria Khan, Deirdre Barry, Jennifer Seward, and Cathryn Gates for their focus and contributions to the preparation of this text.

*Alvin H. Schmaier*
*Cleveland, OH, USA*
*July, 2011*
CHAPTER 1

Introduction to Hematology

Alvin H. Schmaier

Case Western Reserve University, University Hospitals Case Medical Center, Cleveland, OH, USA

I. Introduction

Hematology is the study of the normal and pathologic aspects of blood and blood elements. Blood is a unique fluid comprised of many cellular elements as well as a liquid portion consisting of proteins, amino acids, carbohydrates, lipids and elements. The hematopoietic system is characterized by turnover and replenishment throughout life. The pluripotent hematopoietic stem cell (HSC) is the progenitor of the cells in blood. The cellular elements that arise from this stem cell that circulates in blood include red blood cells, white blood cells, and platelets. Normal white blood cells in the peripheral circulation include neutrophils, monocytes, eosinophils, basophils and lymphocytes. Since the HSC also gives rise to cells of the lymphoid system, the study of hematology also includes the lymph nodes and lymph tissue. There is no specific organ for hematologic disorders and its diseases arise within the bone marrow, lymph nodes, or the intravascular compartment. The intravascular compartment where these cells circulate includes the endothelial cell lining of blood vessels and the proteins in the blood plasma. The circulating cell–endothelial cell interface and the rheologic aspects of blood coursing through the intravascular compartment also influence “hematology” and its many parts.

This text has been structured to introduce the trainee to the area of hematology. Since the vast majority of medical students and residents do not become hematologists, there are certain essential items that all trainees must learn about this area of medicine. The trainee will learn the physician’s approach to anemia and red blood cell disorders and be able to fully evaluate a complete blood count (CBC). Screening tests for bleeding disorders for the diagnosis of an individual who has a defect in the proteins or cellular elements that prevent bleeding will be addressed. The trainee also will be exposed to the clinical, biologic, and genetic risk factors that contribute to thrombosis. Finally,
the student will be introduced to those white cell disorders that are diagnosed and treated by non-hematologists and the uncommon but serious white blood cell disorders where a hematology consultation is needed.

II. Origins of hematopoietic cells

Hematopoiesis begins early in embryonic development. The HSC and the blood vessel lining cells or endothelial cells are thought to be derived from the same precursor cell in the aorto-gonad mesonephros (AGM) system. The common precursor to the HSC and the endothelial cell is the hematoblast. It has been proposed that this cell has the capacity to differentiate into both cell classes. The HSC is present in small numbers and retains its ability to differentiate into all blood cells as well as proliferate. In the earliest stages of embryogenesis, these cells circulate through the embryo to supply oxygen and deliver nutrients. The stem cells that arise from the AGM later in embryogenesis give rise to the blood system that seeds the liver and then the bone marrow. These cells demonstrate the ability to “travel” from the time they leave the yolk sac to populate tissues and still circulate in small numbers even in adults, a property exploited in clinical hematopoietic cell transplantation. These cells regress in the liver, kidney, and spleen, but in times of stress, they can resume blood product production as seen in myeloproliferative disorders and myelofibrosis. Under the influence of specific growth and transcription factors, cells become committed to specific lineages.

A. The myeloid system

Cells of this group arise in the central marrow cavity (called the “medullary” cavity). Myeloid lineage blood cells arising elsewhere in the body are designated as “extramedullary” in origin. The myeloid system consists of the following cells: red blood cells (erythrocytes), white blood cells (neutrophils, monocytes, eosinophils, basophils) and platelets (thrombocytes). Neutrophils, eosinophils and basophils have been collectively called “granulocytes” because the presence and nature of their cytoplasmic granules define their function; however, when physicians use the term “granulocytes”, they are often referring just to neutrophils.

1. Erythrocytes (red blood cell, RBC)

An erythrocyte is a specialized anucleated cell that packages hemoglobin, the protein that is a respiratory gas transport vehicle that carries oxygen from the lungs to and carbon dioxide from tissues and back to the lungs to dispel. Erythrocytes undergo erythropoiesis whereby they mature from an early progenitor cell to the non-nucleated, biconcave disk, the erythrocyte, that with the absence of its nucleus and the flexibility of its membrane is able to bend to traverse 2–3 micron capillaries. It is regulated by the growth factor, erythropoietin. The process of erythropoiesis takes 4 days to produce a non-nucleated biconcave disk that enters the circulation with residual RNA in its cytoplasm. A new RBC in the circulation is slightly bigger than older cells.
The reticulocyte count as identified by a special stain represents the percentage of early RBC of the total number of RBC in the circulation. Red blood cell RNA remains in the erythrocyte about 1 day, so a normal “reticulocyte count” is <2%. The red cell life span is 120 days, and normally there are about 5 million RBC/µL in whole blood in adult males and 4.5 million RBC/µL in adult females. Old RBCs lose their energy-producing (ATP) capacity, develop stiff membranes, and are removed from circulation by the macrophages of the mononuclear–phagocytic system of the spleen. Their hemoglobin is normally retained in the reticuloendothelial (RE) system but can be lost when there is brisk shortened red blood cell survival, i.e., hemolysis.

2. Neutrophils
Neutrophils are also referred to as polymorphonuclear neutrophils, PMN or polys, segmented neutrophils, or segs (Atlas Figure 2; see also Chapter 3). The neutrophil contains a nucleus that is usually a 3–4 lobed or “segmented” structure that stains a bluish color with Wright–Giemsa stain. An early form of a neutrophil is a “band” that shows an unsegmented nucleus. A neutrophil normally takes 12–13 days to be produced in bone marrow. Its life span in the circulation is about 12 hours and they can live in tissues for several days. The marrow pool of mature neutrophils is 30–40 times that seen in the circulation. In the circulation, half are “marginated” or adherent to the endothelial cells and half flow of the blood stream. Margination of neutrophils allows them to serve as a “reserve” to be released in time of stress such as infection. Only one half of the neutrophils that circulate are reflected in the “white blood cell count” (WBC). In the adult, neutrophils constitute 50–80% of the total WBC analyzed (4000–10,000/µL). Neutrophils exit the circulation via diapedesis into tissue through the capillary junctions in response to chemotactic stimuli. Their functions are to phagocytize and digest bacteria, cellular debris, and dead tissue. Both neutrophils and monocytes are part of the body’s innate immunity in contrast to adaptive or learned immunity of lymphocytes (see below; see also Chapter 16).

3. Monocytes
Monocytes are large, mononuclear cells with an indented (kidney-shaped) nucleus that form the circulating component of the mononuclear phagocyte system (Atlas Figure 3). The nucleolus in mature monocytes circulating in the peripheral circulation is usually not identified on blood by light microscopy. Monocytes spend 1–3 days in bone marrow and 8–72 hours in the peripheral blood. They have a similar functional role to neutrophils in host defense against organisms. Once they traverse into tissues, they can differentiate into macrophages that can survive in tissues for long periods (up to 80 days). Macrophages are tissue-resident as opposed to circulating monocytes. Macrophages are characterized and named for their tissue origin: alveolar macrophages in lung, Kupffer cells in liver, splenic macrophages, and oligodendrocytes/glial cells in brain. They function to phagocytize pathogens, cellular debris and dead tissue.
4. Eosinophils
Eosinophils are characterized by their prominent orange-reddish (refractile) granules seen on Wright–Giemsa stain (Atlas Figure 6). Eosinophils usually have bilobed nuclei. Eosinophils increase in reaction to foreign protein and thus are seen in parasitic infection (especially larva of roundworms, helminths), allergic conditions, cancer and certain drugs. Granules contain several proteins, most notably major basic protein (MBP). Normally eosinophils constitute 0–2% of WBC differential cell count.

5. Basophils
Basophils are equally colorful with very dark, bluish prominent granules following Wright–Giemsa stain (Atlas Figure 7). Granules contain: histamine, heparin, and hyaluronic acid. Histamine release (basophil degranulation) is part of the allergic reaction. Normally basophils are 0–1% of WBC differential blood count. They are often increased in patients with chronic myelogenous leukemia and other myeloproliferative disorders. Mast cells which are tissue basophils also have prominent granules and play a role in host defenses against parasites.

6. Platelets (thrombocytes)
Platelets bud off from the cytoplasm of the bone marrow megakaryocytes. The “mega” karyocyte in the bone marrow is recognized by its large size. Uniquely, the cell doubles its nuclear and cytoplasmic material but does not divide. Megakaryocyte growth and platelet segmentation is regulated by thrombopoietin. Platelets are anucleated cell fragments that contain remnant mRNA. They have a 7–10 day half-life and their first 1–2 days are spent in the spleen. Platelets can be entrapped by an enlarged spleen as seen in congestive and inflammatory disorders. They play a central role in hemostasis as they contain many hemostatic cofactors and inhibitors in their granules. They also have a role in inflammation since they contain many growth factors. At the megakaryocyte level, plasma proteins can be adsorbed and packaged into platelet granules (see Chapter 13).

B. Mononuclear phagocytic system
The mononuclear phagocyte system consists of circulating monocytes derived from the myeloid progenitor cell in the bone marrow that migrate from the circulation into tissues and differentiate into macrophages. The mononuclear phagocytic system is also called the reticuloendothelial (RE) system. These cells are found in bone marrow, thymus, lymph nodes, spleen, serosal surfaces, adrenal cortex, Peyer’s patches, and Waldeyer’s ring. They function as a “clean-up system” for circulating debris, microorganisms and aged, defective or antibody-coated RBC.

C. Lymphocyte system
Lymphocytes are mostly in lymph nodes, but are also a large blood and bone marrow component. As already mentioned above, they are part of our
adaptive immunity system. The major lymphocyte subsets are B and T cells. NK (natural killer) cells are a specialized lymphoid population. All cells arise in the bone marrow, but T cells mature in the thymus and B cells mature in the lymph nodes, spleen or other lymphoid tissue, e.g., Peyer’s patches in the gut and Waldeyer’s ring in the throat. Immunosurface markers are used to classify lymphocytes. B cells are identified by CD19 and CD20. T cells are identified by CD3, CD4 or CD8. NK cells comprise 10% of circulating lymphocytes and are identified by the CD3–CD56+ phenotype.

III. The physical states of blood

(i) Blood is a suspension of cells in a solute of water, water-soluble proteins, and electrolytes.

(ii) The viscosity of blood = 1.1–1.2 centipoise. The viscosity of blood is highly influenced by red blood cell and protein concentration. Increased viscosity can occur from an elevation in the cellular components as is seen in polycythemia (increased numbers of red blood cells) and protein as seen in disorders such as multiple myeloma (elevated IgG levels) and Waldenström’s macroglobulinemia (elevated IgM levels). Red cell size (smaller size increases viscosity) and the speed of blood flow in a given vessel also influence viscosity (viscosity in the aorta is much less than in a small arteriole).

(iii) Blood volume averages 70 mL/kg of body weight; thus the 70 kg adult has roughly 5 liters of blood. The blood volume of an individual (man, dog, etc.) is approximately 7% of the total body weight. Children may have a slightly higher % (~10%) blood volume to total body weight.

(iv) Cellular composition of blood averages 38–42% in women, 40–44% in men; the percent volume contributed by red blood cells is called the "hematocrit" or packed cell volume.

(v) Plasma is anticoagulated blood (i.e., blood where the calcium chloride has been chelated [i.e., bound] and not available for interaction with proteins) from which the cellular components (red cells, white cells, and platelets) have been removed by centrifugation. It contains the blood coagulation proteins. Serum is the liquid in blood that has been collected without an anticoagulant. Many of the proteins have "clotted" and form a precipitate along with the cellular components of the blood. It is usually yellow in color unless the red blood cells lyse (hemolyze) releasing free hemoglobin that gives a red color in visible light. Plasma coagulation studies can only be performed on blood that has been obtained with a proper anticoagulant (usually sodium citrate in clinical medicine) and the plasma separated from the blood cells.
I. Introduction

Hematopoiesis is the process of the development of blood cell lineages throughout life. Hematopoiesis is necessary to replenish dying cells with new blood cells. The key role of hematopoietic cells in maintaining hematopoietic homeostasis, host immunity and tissue oxygenation requires that they are highly regulated.

II. Definitions

1. The hematopoietic system: the hematopoietic system includes the elements of the blood, marrow, lymph nodes, endothelial cells, thymus and spleen that are involved in the production of all blood lineages. This system further includes cytokine-producing cells and stromal elements of the bone marrow and spleen. In human physiology, the hematopoietic system supplies various cells in the body with oxygen, contributes to the formation of blood clots when needed, and provides protection against infection and pathogens.

2. Blood cells: Blood cells include red blood cells (erythrocytes, RBCs), white blood cells (leukocytes) and platelets which provide a variety of functions within the body. RBCs carry oxygen, platelets contribute to hemostasis, thrombosis and the inflammatory response, and white blood cells are involved in immunity.

3. Hematopoietic homeostasis: Hematopoiesis is in a delicate state of homeostasis—the process of maintaining balanced production to offset ongoing destruction of blood cells. Some cell lineages such as neutrophils only survive for several hours after release from the bone marrow into the circulation. RBCs can survive longer, lasting 60 to 120 days, and terminally differentiated lymphocytes, plasma cells, may survive for up to 20 to 30
years. Hematopoietic cell production is regulated by cytokines and growth factors and monitored by tissue sensors (tissue oxygenation for red blood cells for example). The specific regulators and sensors for all hematopoietic elements, however, have not been clearly elucidated.

III. Mature hematopoietic cells and their functions

Mature RBCs carry hemoglobin bound oxygen to tissues and release that oxygen under the hypoxic conditions of tissues. Hemoglobin delivery of oxygen is dependent on pH and a number of other metabolic functions that alter the confirmation of the tetramer and the cooperative discharge of oxygen. RBCs also transport and release CO₂ generated from body metabolism in tissues to be expelled from the body via the lungs (see Chapter 3).

Platelets contain a high concentration of proactive inflammatory, hemostatic cofactors, proangiogenic proteins, inhibitors of blood coagulation, inflammation, and fibrinolysis inside their granules. These components are actively secreted on or about the activated platelet surface when an appropriate stimulus arises (see Chapter 13).

White blood cells consist of many cell types including granulocytes (neutrophils, eosinophils and basophils), monocytes, lymphocytes and dendritic cells. Neutrophils are the most common cells.

Neutrophils migrate into tissues in response to inflammation or infection where they may ingest or phagocytose particles and bacteria. These cells contain oxidases and myeloperoxidase within granules that can be activated to produce superoxide to kill ingested bacteria (see Chapter 16).

Eosinophils, basophils and mast cells respond to IgE to produce acute allergic responses. Mast cells are specialized long-lived tissue resident cells similar to basophils that are the initiators of the allergic response. They secrete histamine and vasoactive proteins and recruit eosinophils and basophils in response to antigens bound to IgE. Activated eosinophils express IgE receptors and amplify the allergic response. Eosinophils also contain granules with specialized proteins important for the immune reaction to parasites. Basophils also have IgE receptors and granules containing histamine and mediate allergic inflammation. These cells are present in low numbers in the peripheral blood.

Monocytes enter tissues and become resident macrophages in the lung, liver or other tissues where they also participate in the inflammatory and immune response. These cells produce a wide variety of small chemicals, such as chemokines (small peptide chemicals) and cytokines, for chemotraction and immune modulation of all immune cells. In disease states, these cells can form clusters that result in granulomas and mediate chronic inflammation. Monocytes can also be antigen-presenting cells for the lymphoid cell population, inducing an immune response.

Dendritic cells are important mediators of innate and adaptive immune responses and are the main antigen presenting cell in the body. They have
been suggested to derive from both common myeloid progenitor and common lymphoid progenitor.

B lymphocytes produce antibodies in response to stimulation. The initial response results in secretion of IgM immunoglobulin. The binding specificity towards the antigen is often modest at the initiation of an immune response. Upon antigen exposure, B cells migrate to specific regions within lymph nodes termed germinal centers where the cells proliferate and generate daughter cells with higher affinity for antigen through a mutational process referred to as somatic hypermutation. During this process the cells may switch to produce a different antibody isotype, usually IgG to carry out specific effector functions. B cells mature into long-lived plasma cells and memory B cells to maintain immunologic memory.

T lymphocytes produce cells with cytotoxic, helper or suppressor functions that mediate responses to viral infections or inflammatory conditions. The cytotoxic cells are particularly important to rid the body of virally infected cells. T cells produce cytokines and chemokines that modulate most immune responses including granuloma formation and are required for B-cell antibody production.

IV. Hematopoiesis during development and in adult

1. Hematopoiesis during development

The earliest forms of blood cells are observed in the yolk sac. These cells emanate from a primitive precursor population and produce both cells with oxygen-carrying capacity and a small number of primitive lymphocytes. More definitive hematopoiesis takes place later in development in fetal liver, and during the third trimester, production is transferred to the bone marrow in the developing embryo.

RBC production is unique in development because of the complex evolution in the hemoglobin locus resulting in a structured sequence of distinct hemoglobins produced during fetal life. Because of the oxygen requirements in the fetus and the absence of direct air exchange in the lung, different hemoglobins are produced during gestation. Most significant is fetal hemoglobin or hemoglobin F, a unique tetramer that disappears normally within a few months after birth. It is the main type of hemoglobin in the fetus. It has greater oxygen-affinity than adult hemoglobin A, allowing for the extraction of oxygen from the maternal blood stream. A small percentage of adult hemoglobin or hemoglobin A appears late in gestation and becomes the dominant form within 6 months of birth, reflecting the change in oxygen requirements after birth. Interestingly, fetal hemoglobin ameliorates the disease manifestations of homozygous hemoglobin S, the cause of sickle cell anemia. For this reason, erythropoietin and hydroxyurea (hydroxyurea), which promotes the generation of hemoglobin F, are used to treat sickle cell anemia.
2. Hematopoiesis in adult

In adults, hematopoiesis mainly occurs in bone marrow and thymus. Myelopoiesis (non-lymphoid) and lymphopoiesis diverge during the early stage of differentiation. The hematopoietic stem cell’s first lineage commitment is to differentiate to a common myeloid progenitor or a common lymphoid progenitor (Figure 2.1). The common myeloid progenitor produces megakaryocytic, erythroid (RBC), granulocytic and monocytic lineages. The granulocytes, the neutrophils, eosinophils and basophils, are the most phylogenetically related. Monocytes arise from a common granulocyte-monocyte progenitor.

Erythropoiesis is the process of generating RBC. Thrombopoiesis refers to the formation of platelets from their precursor megakaryocytes. Erythrocytes and megakaryocytes both develop from a common precursor cell. RBC production is stimulated by the growth factor, erythropoietin. Megakaryocytes are unusual in that the cell undergoes nuclear division without cytoplasmic division; the generating cell contains a high amount of DNA content, 32n–64n, compared to 2n of normal diploid cell. Each megakaryocyte can generate large numbers of platelets by “budding” off pieces of cytoplasm. The process is stimulated by thrombopoietin (TPO), a cytokine hormone mainly produced by liver and kidney.
Since leukemias often recapitulate the normal developmental process, it is not uncommon to encounter a leukemia with both granulocyte and macrophage differentiation capable of recapitulating the common granulocyte-macrophage progenitor or less often a leukemia demonstrating erythrocyte and megakaryocyte differentiation simulating the common megakaryocyte-erythrocyte progenitor.

The common lymphoid progenitor cell differentiates into B-cell, T-cell and natural killer cells. B-lymphoid development remains localized in the bone marrow, whereas developing T cells emigrate from the bone marrow to the thymus to undergo terminal differentiation. B and T-lymphoid development requires rearrangement of the DNA in the maturing cells; the immunoglobulin locus for B cells and T-cell receptor locus for T cells. DNA recombination in the developing lymphocytes randomly combines variable, diversity and joining gene segments (VDJ) to generate antibody and T-cell receptor proteins with tremendous diversity ($>1 \times 10^7$) to match potential antigens from a wide variety of infectious or noxious agents. The DNA rearrangement process is intimately related to T and B-cell survival and maturation as lack of effective DNA recombination results in cell death. B-lymphopoiesis occurs under the influence of IL-7. The effects of IL-15 and IL-2 are important later in lymphopoiesis.

**V. Hematopoietic stem and progenitor cells and their functions**

1. **Hematopoietic stem and progenitor cells**

An HSC represents approximately one in 10,000 hematopoietic cells in the bone marrow space. During the normal hematopoietic steady state, the HSC is quiescent. When an activation signal reaches the HSC microenvironment, usually due to proliferative stress, the HSC enters into a proliferation state. It may then undergo asymmetric replication, producing another HSC capable of self-renewal and a “daughter” progenitor cell, which begins to differentiate, rapidly divide, and proliferate into mature blood cells (Figure 2.1). The differentiation of HSCs into progenitor cells is accompanied by changes in cell surface molecule expression. As HSCs lose their quiescent state, they acquire growth factor receptors and increase the production of certain messenger RNAs. This process occurs by altering transcription factor-mediated expression of genes for differentiation and lineage commitment.

When HSCs leave the bone marrow niche (a specialized regulatory environment), they usually lose the HSC phenotype and irreversibly begin lineage commitment. However, some can lodge in another niche and resume the quiescent HSC phenotype. A single HSC may produce more than 1 million progeny over the course of this continuous process of proliferation and differentiation.

Hematopoietic progenitor cells, such as common myeloid or lymphoid progenitors, are all differentiated from HSCs. Traditional understanding of
hematopoiesis is that the process is unidirectional. Once a progenitor cell has committed to a lineage, there is no turning back, although some researchers argue that hematopoietic progenitor cells are more versatile.

2. Phenotypic and functional analysis of hematopoietic stem and progenitor cells
With the discovery and utilization of surface markers to distinguish hematopoietic cells, a hierarchical map of hematopoiesis was quickly established. The expression of specific surface marker, which is often identified and given a CD (cluster of differentiation) number, defines a stage of hematopoietic stem and progenitor cells. A short list of unique markers to identify and provide lineage specificity to human hematopoietic stem and progenitor cells are given in Table 2.1.

In vitro assays provide clear identification of hematopoietic precursor populations. Samples of blood or bone marrow can be grown artificially in vitro in methylcellulose with appropriate cytokines to produce cell type specific colonies, which represent progeny from a specific progenitor cell (Figure 2.2a). For instance, cells grown in the presence of erythropoietin results in CFU-E (colony forming unit-erythroid) or BFU-E (burst forming unit-erythroid) colonies indicative of erythroid precursors. These colonies contain hemoglobin-producing cells devoid of other lineages. Cells grown in cultures containing G-CSF (granulocyte colony-stimulating factor) or GM-CSF (granulocyte–macrophage colony-stimulating factor) with or without other early phase cytokines such as stem cell factor (SCF), Flt3 or IL-3 result in granulocytic or monocytic colonies, respectively. In some instances these growth factors plus thrombopoietin are used to produce megakaryocytic colonies. Likewise exposure to IL-7 will produce B lymphocytes and growth with IL-2 will stimulate T-lymphocyte colonies.

Another in vitro assay is the long-term culture initiating cell assay (LT-CIC), which reflects an earlier progenitor cell. This in vitro test, in addition to those described above, only assays hematopoietic progenitors. In order to assess the production of long-term stem cells in vivo, bone marrow cell transplantation is needed. One approach is to inject CD34+ human bone marrow cells into

<table>
<thead>
<tr>
<th>Table 2.1</th>
<th>Surface markers to identify hematopoietic stem and progenitor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stem and progenitor cells</strong></td>
<td><strong>Identified surface markers</strong></td>
</tr>
<tr>
<td>HSC</td>
<td>CD 34, CD133,</td>
</tr>
<tr>
<td>Common myeloid progenitor</td>
<td>CD33, CD13, CD117</td>
</tr>
<tr>
<td>Common lymphoid progenitor</td>
<td>CD135</td>
</tr>
<tr>
<td>Megakaryocytic progenitor</td>
<td>CD41/CD61</td>
</tr>
<tr>
<td>Erythroid progenitor</td>
<td>Glycophorin A (CD235A)</td>
</tr>
<tr>
<td>T-lymphoid progenitor</td>
<td>CD3, CD7, TDT</td>
</tr>
<tr>
<td>B-lymphoid progenitor</td>
<td>CD19, CD79a, TDT</td>
</tr>
</tbody>
</table>
NOD-SCID (non-obese diabetic severe combined immunodeficiency) mice. These immunodeficient mice cannot reject foreign cells and allow human progenitor cells to grow in the murine bone marrow. The “gold standard” for true long-term hematopoietic stem cells is the serial transplantation assay (Figure 2.2b).

VI. Hematopoietic microenvironment

Hematopoietic microenvironments have important roles in regulating HSC activity and the process of hematopoiesis. There are two types of microenvironments in the bone marrow: one is the endosteal microenvironment, which has been considered as the main HSC niche, while the other is the sinusoidal microenvironment, a zone around the endothelial cell network within the bone marrow. The endosteum is a thin layer of connective tissue which lines
the inner surface of the bony trabeculae within the medullary cavity of long bones.

The microenvironment responds to the cellular density, local tissue hypoxia and systemic hypoxemia, inflammatory signals, and other stress response factors. The microscopic spaces within the microenvironment provide an important recess or niche within the marrow that nurtures HSCs and their progenies for highly regulated homeostatic hematopoiesis. The bone marrow microenvironment is relatively radioresistant and can reconstitute after ablative radiation therapy or chemotherapy.

Multipotent mesenchymal stromal cells (MSC) fibroblasts and adipose cells play important roles in the microenvironment of the bone marrow by providing hematopoietic growth factors and a nurturing space (niche) for hematopoiesis to take place. In addition, T cells also support and regulate hematopoiesis by providing specific cytokines and chemokines.

VII. Regulation of hematopoiesis

The bone marrow microenvironment provides one level of regulation of hematopoiesis. Another form of regulation is cell-to-cell contact mediated by cell adhesion molecules, such as integrins. Integrins are cell-surface molecules that impact cell-to-cell and cell-to-stromal adhesion and bi-directional signaling that stimulate hematopoietic progenitors towards lineage-specific differentiation. Additionally, circulating and locally produced cytokines provide important lineage-specific stimuli.

Early progenitor-stimulating cytokines include stem cell factor (SCF), thrombopoietin, IL-6, IL-3, and FLT3 ligand. These cytokines stimulate HSC to begin the proliferation process.

G-CSF and GM-CSF provide stimulation for myelopoiesis. G-CSF is used in clinical medicine to elevate neutrophil counts in patients receiving chemotherapy or who have developed neutropenia. M-CSF (macrophage colony-stimulating factor) stimulates macrophage proliferation. These hematopoietic growth factors are made by lymphocytes.

Erythropoietin stimulates erythroid differentiation, resulting in the production of mature RBCs. Erythropoietin is made in the kidney as a result of sensing hypoxemia by the periglomerular cells (see Chapter 3). Renal failure is associated with anemia because of the reduced concentrations of erythropoietin produced. Clinically, genetically engineered erythropoietin is effective in resolving the anemia in patients with renal disease.

Thrombopoietin as well as erythropoietin stimulate the production of megakaryocytes and their terminal differentiation and fragmentation into platelets. Anemia, a disease with decreased RBC number, stimulates the production of erythropoietin from the kidney, thrombocytopenia stimulates thrombopoietin production from the liver, and neutropenia results in increased G-CSF. These cytokines act as an autocrine regulatory loop for blood cells. Along with other hematopoietic growth factors, they are critical for hematopoiesis maintenance.
VIII. Abnormal hematopoiesis

1. Cytopenias

Cytopenias, or low blood cell numbers, represent a severe disruption of hematopoiesis. Under normal homeostasis, hematopoiesis would be maintained through all types of stress with induction of proliferation and stimulation of progenitor pools by cytokines. Over-production likewise would result in a feedback loop leading to reduced blood cell production. Cytopenias may occur when an abnormal immune response destroys progenitor cells or peripheral blood cells. Reduced cell numbers also may occur when there is specific decreased production of the cell lineage or multiple cell lineages due to a primary defect in the progenitor or a toxin (e.g., medication) interfering with cell production.

Common examples include immune thrombocytopenia as a result of the immunoglobulins targeting early megakaryocytes and platelets (see Chapter 14). The etiology is the production of auto-antibodies from a dysregulated B and T-cell population either idiopathic, after viral infection, or associated with lymphoma or chronic lymphocytic leukemia. Drug exposure can also cause idiopathic immune-mediated thrombocytopenia. Granulocytopenia can be caused by an antibody response to drugs with cross-reactivity towards drug metabolite bound to the surface of myelocytes or granulocytes. Likewise, anemia can be caused by antibodies against RBCs (see Chapter 8). These antibodies are autoimmune, producing a positive Coombs test (positive test to detect antibodies on RBCs also referred to as the direct antiglobulin test). Some RBC antibodies are produced during drug treatment, viral infection, or blood cell exposure during pregnancy. Other common causes of cytopenias include acute viral infections that may destroy progenitor cells. Specific examples include parvovirus infection targeting of RBC progenitors, HIV infection which targets lymphocytes or CMV which attacks leukocytes. Additional causes of cytopenias include rapid tissue margination or sequestration of cells in response to infection or exhaustion of the production pools due to rapid destruction. Blood loss or bleeding, or the cessation of production of cells during an acute illness due to a tumor necrosis factor (TNF)-alpha-mediated stress response may also result in low cell numbers. The anemia of chronic disease appears to be a chronic response of the marrow to inflammation by the cytokine hepcidin (see Chapter 5) and results in an anemia that resolves when the chronic disease dissipates.

Finally, chemotherapy and radiation therapy are common causes of iatrogenic cytopenias in cancer patients and those receiving these treatments for autoimmune diseases. These agents destroy the proliferative potential of HSCs resulting in loss of differentiation of lineage-specific cells. Over time, the remaining HSC pool usually recovers and hematopoiesis is restored and cell lineages repopulate.

In the presence of cytopenias, cytokines may be useful as therapeutic agents. Erythropoietin is given to promote erythropoiesis and is especially...
useful in renal failure because of the reduced levels of erythropoietin produced. G-CSF is given to promote granulopoiesis, either after chemotherapy, due to marrow failure, or during acute infection. Romiplostim, an injectable peptide thrombopoietin analog, or eltrombopag, an oral thrombopoietin mimic by targeting the TPO receptor, are given to promote platelet production in chronic idiopathic immune thrombocytopenic purpura. Presently, there is no clinically useful cytokine as yet available to stimulate lymphopoiesis.

2. Marrow failure states
Acquired marrow failure may result from damage to the microenvironment, HSC, or from cells that are responsible for producing the critical chemokines and cytokines that regulate hematopoiesis. They may also result from mutations in the HSC that renders them ineffective in cell proliferation. Such a situation could result from an exogenous stimulus including a viral infection, be the result of an autoantibody directed against a HSC, or represent genomic instability of the stem cell lineage, resulting in loss of hematopoietic function. Aplastic anemia, myelodysplastic syndrome, and paroxysmal nocturnal hemoglobinurea are examples of marrow failure states (Chapters 8, 18 and 19).

3. Leukemias
All leukemias arise from HSCs and their early progenies. Acute leukemias are characterized by cells that fail to differentiate. Specific chromosomal abnormalities giving rise to altered gene expression are commonly observed in acute leukemias. Most leukemias have many additional mutations that appear to convert the cell with the chromosomal translocation into a leukemic state, e.g., increased cell numbers due to a failure to differentiate, lack of programmed cell death, or increased proliferation. Leukemia-initiating cells or leukemia-sustaining cells are a minor population within the leukemic cell population. These cells share many features of HSC, but seem to be the more resilient with the greatest proliferative potential and the greatest resistance to common chemotherapeutic agents. The relationship between the leukemia-initiating cell and the onset of the chromosomal and other genetic changes is not clear. Treatment of leukemias tends to target a proliferative fraction with cell cycle specific and other DNA damaging agents.

IX. Conclusions
In this chapter, we present general concepts involving hematopoiesis. HSCs are the most studied adult stem cells. However, there are still large numbers of questions which remain to be answered. For example, what are the unique surface markers for a homogenous population of HSC? Only by identifying a more homogenous population can we accurately reveal their unique phenotype.
CHAPTER 3

3 Red Blood Cell Biochemistry and Physiology

Kevin T. McDonagh

University of Kentucky College of Medicine, Lexington, KY, USA

Understanding the factors that regulate RBC growth and development and the genetic and biochemical basis of RBC physiology is critical for an informed approach to the diagnosis and treatment of anemia.

I. Red blood cell development

A. Early development

Red blood cells (RBC) are normally produced in the bone marrow. The process of RBC development is called erythropoiesis. RBC are derived from pluripotent hematopoietic stem cells (HSCs), and share a common precursor (or progenitor cell) with other myeloid lineage cells including megakaryocytes, granulocytes, monocytes/macrophages, eosinophils, and basophils. HSC arise from developing vasculature early in embryological development. Thus, inherited or acquired abnormalities in hematopoietic stem cells or myeloid progenitor cells may be associated with functional or quantitative defects in multiple types of blood cells.

B. Regulation of growth

The growth and maturation of RBCs from the HSC and myeloid progenitor cells is regulated by a complex interplay between genetically defined developmental programs and external signals generated by remote and/or neighboring cells.

1. Hematopoietic growth factors are an important class of external signals used to regulate hematopoiesis. Multiple subtypes have been identified and characterized.

2. Erythropoietin (EPO) is the most important growth factor regulating erythropoiesis.

   (a) EPO is produced in the kidney by peritubular cells that sense tissue oxygen content. When oxygen delivery to the kidney falls (due to