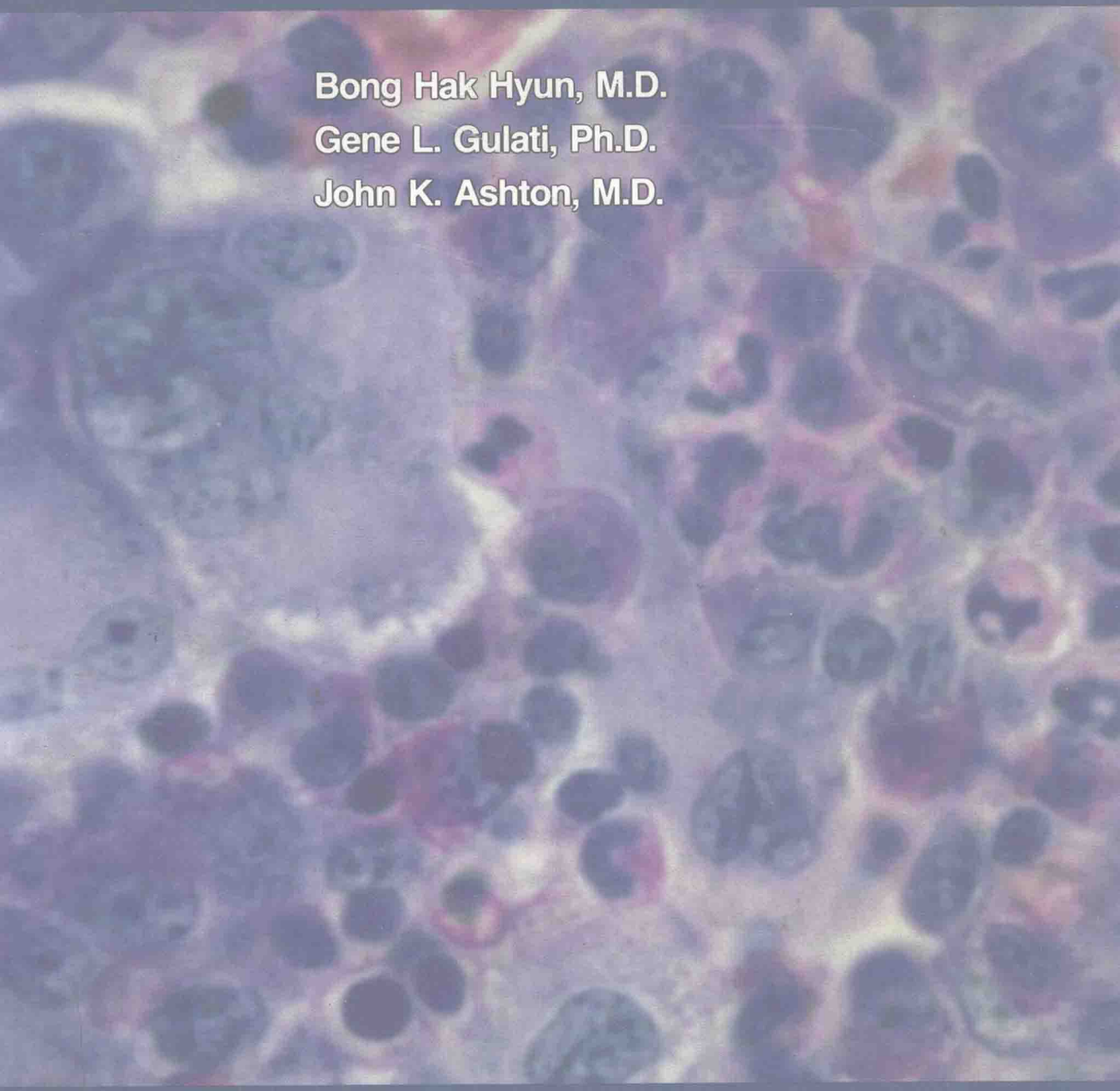
Color Atlas of Clinical Hematology



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Preface

Hematology is a field that has benefited from rapid and continuing advances in basic science and clinical applications. Nevertheless, in spite of constantly changing and expanding technology, the key to hematologic diagnosis remains most often the morphologic evaluation of the peripheral blood and bone marrow. New students and laboratory workers in hematology who will deal with these problems, whether at the bench or in the clinical setting, need to acquire the appropriate level of familiarity with normal and abnormal forms and with the range and variety of disease processes that may be reflected in hematologic reactions.

We have tried to make the coverage of topics in this atlas sufficiently comprehensive to be useful to students at various levels and those engaged in the practice of hematology. We have deliberately utilized a variety of preparations including smears and sections, special stains, histochemical and cytochemical techniques, and immunoperoxidase staining, and have stressed the importance of correlating findings in the peripheral blood with those in the bone marrow. Disorders of the spleen and lymph nodes, although closely related in many instances, are outside the scope of this work. The textual material is designed to supply basic information in a concise form; advanced students will want to supplement it with one or more of the large textbooks of hematology.

We owe a great debt of gratitude to those who have helped make this book possible. We wish to acknowledge the contributions of hematologic specimens made by Drs. Koichi Maeda, Robert W. McKenna, George C. Hoffman, Arkadi M. Rywlin, C. Francis Varga, Vincent Galdi and Stephen Van D. Chandler. The pictures have been selected from photomicrographs taken over a period of years by our residents, fellows and colleagues, too numerous to name. Mr. In Hwan Jo and Ms. Young Yea Ahn skillfully and painstakingly prepared the color prints. Mrs. Cres A. Martinez provided superior secretarial and organizational help. Mr. John Gardner and Ms. Lila G. Maron of Igaku-Shoin were unfailingly patient, encouraging and supportive.

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PART I

STRUCTURE, FUNCTION, TECHNIQUES, AND HEMATOLOGIC DISORDERS

Chapter 1

Hematopoiesis

Blood, defined as intravascular body fluid, consists of red cells (erythrocytes), white cells (leukocytes) and platelets (thrombocytes) suspended in plasma. Normally the red cells and platelets form relatively uniform populations, whereas the white cells comprise several distinct cell lines, namely, the granulocytes (neutrophils, eosinophils and basophils), lymphocytes and monocytes. Blood cells are released into the circulation after their production in the so-called hematopoietic sites in the body.

The process of blood cell production (hematopoiesis) begins early in embryonic life, being first evident in the yolk sac in the first lunar month. The cells produced by the yolk sac are primarily erythroid precursors containing embryonic hemoglobins. By the end of the second lunar month, the activity of the yolk sac ceases with the migration of hematopoietic stem cells to the liver, which starts producing different lines of blood cells. The hepatic hematopoietic activity is at its maximum during the second trimester of pregnancy, declining gradually thereafter to none or an insignificant level by the end of the antenatal period. The spleen's contribution to hematopoiesis is slight and is generally limited to the period of the third through the sixth lunar months. The bone marrow begins to produce blood cells in the fifth lunar month, at which time lymphoid tissues such as the thymus and lymph nodes also start producing lymphocytes. The bone marrow activity continually increases, and at the end of antenatal life, the bone marrow is 100% cellular. At birth and throughout postnatal life, the bone marrow remains the primary supplier of multipotent stem cells and the only site of hematopoiesis other than lymphoid tissues, which contribute lymphocytes and plasma cells. The liver and the spleen, however, maintain the potential for hematopoiesis throughout life. Although at birth the marrow of different bones is considered to be 100% hematopoietic (red), there are continual changes in the distribution of hematopoietic tissue within the bones throughout life. Fatty tissue (yellow marrow) begins to replace red marrow from the periphery of the body toward the axial skeleton, relatively rapidly in some bones (tibia, femur) and gradually in others (sternum, vertebrae and iliac bones). In the adult, most hematopoietic tissue is present in the vertebrae, ribs, sternum and the pelvic bones.

Mechanism of Hematopoiesis

The undifferentiated reticular cells (also known as uncommitted stem cells) in the bone marrow undergo proliferation (mitotic division) and differentiation for self-renewal and for the production of stem cells committed to generate blood cells (hematopoietic stem cells), lymphocytes (lymphoid stem cells), and the connective tissue cells including fibroblasts, osteoblasts, chondrocytes, lipocytes, and endothelial cells.

The hematopoietic stem cells (hemocytoblasts), though morphologically unrecognizable by current methodology, have been identified functionally and named the "colony forming units-spleen" (CFU-S) based on the assay system used. Further proliferation and differentiation of the multipotent hematopoietic stem cells is essential to self-replication and the development of morphologically recognizable unipotent progenitors committed to the production of red cells, white cells including granulocytes and monocytes, and platelets. Similarly, the lymphoid stem cells undergo proliferation and differentiation or maturation to renew their own population and to produce lymphocytes and plasma cells. The production of

differentiated and functionally mature blood cells from their stem cells occurs through various stages of development in a sequential fashion, as described below.

Erythropoiesis

The hematopoietic stem cell under the influence of a humoral factor, erythropoietin, differentiates first into a cell functionally designated as "erythroid colony forming cell" (E-CFC) and then into the pronormoblast (rubriblast), the earliest morphologically recognizable progenitor committed to produce red blood cells. Each pronormoblast undergoes a series of heteromorphogenic mitotic divisions through the stages of early and late basophilic normoblasts (prorubricytes), ultimately producing a total of 16 polychromatophilic normoblasts (rubricytes). With continued maturation without further division, each polychromatophilic normoblast differentiates sequentially into an orthochromic normoblast (metarubricyte), reticulocyte and mature erythrocyte. As the cell divides its size becomes smaller, and as the maturation proceeds, the nucleoli disappear and the nucleus becomes smaller, denser and pyknotic until it is extruded from the orthochromic normoblast. The extruded nucleus is phagocytosed and digested by macrophages. The cytoplasm undergoes changes in its staining characteristics from blue (due to RNA content) in the pronormoblast and basophilic normoblast stages to varying degrees of pink mixed with a bluish tinge in the polychromatophilic normoblast, orthochromic normoblast and reticulocyte, to completely pink in the mature erythrocyte, as a result of the accumulation of hemoglobin.

The synthesis of hemoglobin, though not evident until the polychromatophilic normoblast stage in Wright-stained blood films, probably starts in the late basophilic normoblast stage and continues through the stage of the reticulocyte. The production of reticulocytes from the pronormoblasts takes only about three to five days. The reticulocytes remain in the bone marrow for one or two days prior to being released into the circulation, where they lose their cytoplasmic reticulum and become mature red cells, also within one or two days. The mature red cells in the form of biconcave discs normally remain in the circulation for 120 days, carrying out their sole function of the transport and protection of the oxygen-carrying pigment, hemoglobin. The red cells are lost from the circulation by senescence. Under usual steady-state conditions the daily loss of about 1% of circulating red cells is balanced by the production and release of an equivalent number of new red cells. Among several factors (hormones, minerals and vitamins) known to influence the rate of red cell production is the humoral factor, erythropoietin, which is produced mainly but not exclusively by the kidneys. It is considered to be the major regulator of erythropoiesis. The serum erythropoietin level increases as a response to decrease in the oxygen tension of tissues.

Granulopoiesis and Production of Monocytes

The hematopoietic stem cell in the bone marrow under the influence of colony stimulating factor (CSF), a humoral factor apparently produced by monocyte-macrophages, differentiates first into a cell functionally defined as a "granulocyte-monocyte colony forming cell" (GM-CFC) and then into a cell morphologically identifiable as a myeloblast. Both of these cells, the GM-CFC and the myeloblast,

are committed to producing granulocytes and monocytes. Each myeloblast, presumably under the action of the CSF (also known as granulopoietin), divides and differentiates into two promyelocytes, each of which multiplies and differentiates into two myelocytes. At this stage two or three additional mitotic divisions result in self-replication of myelocytes, and the simultaneous development of specific granulation permits classification of myelocytes into three cell lines, neutrophilic, eosinophilic and basophilic. Further development of granulocytic cells occurs through progressive maturation only, leading to the formation of metamyelocytes, bands and mature segmented cells. Neutrophils, eosinophils and basophils are the mature end products of the maturation of neutrophilic, eosinophilic and basophilic myelocytes, respectively. With each mitotic division between promyelocyte and the late myelocyte stage, the cell size is reduced as are the nuclear size and the number of primary (azurophilic) granules. The promyelocyte is usually larger than the myeloblast and contains the most numerous primary granules, which represent membrane-bound organelles containing mainly lysosomal enzymes (acid hydrolases and peroxidase) with a small amount of muramidase. As the maturation sequence proceeds from the myeloblast to the myelocyte stage, the number of nucleoli decreases until their complete disappearance, the nuclear chromatin becomes progressively more clumped, and the cytoplasm loses its basophilia and acquires pink staining characteristics. The specific (secondary) granules seen in the myelocyte and later stages are also membrane-bound organelles and contain muramidase, collagenase, lactoferrin, phagocytin, alkaline phosphatase and several other enzymes. The maturation of myelocyte to metamyelocyte and band is characterized by progressive condensation of the chromatin and the indentation of the nucleus. Segmentation of the nucleus ultimately results in the formation of mature granulocytes. It takes about 14 days from the myeloblast stage to the release of granulocytes from the marrow into the circulation. Upon entering the circulation, the neutrophils move freely between a circulating granulocyte pool (CGP), which is reflected in the usual total and differential leukocyte count, and a marginal granulocyte pool (MGP), which is marginated along the walls of capillaries and venules. The MGP constitutes about 50% of the total body granulocyte pool and represents a large reserve readily available to the circulating blood upon demand. The estimated average time that neutrophils remain in the blood is six to eight hours, the total life span (in the blood and tissues) being nine to ten days.

There is experimental evidence to suggest that eosinophils may be derived from a progenitor (eosinophilic colony forming cell, EO-CFC) separate from the GM-CFC, under the influence also of a humoral factor (eosinophil colony stimulating factor, EO-CSF) distinct from CSF or granulopoietin. It has also been shown that the EO-CSF, unlike the CSF which is produced by monocyte-macrophages, is produced by lymphocytes when stimulated by pokeweed mitogen or mercaptoethanol.

Though the evidence is lacking, the possibility of a distinct progenitor for basophil production has also been suggested. The eosinophilic granules are similar to the neutrophilic granules in that they are also rich in peroxidase and in lysosomal enzymes with the exception of muramidase. The basophil granules are known to contain heparin, histamine and hyaluronic acid, but generally lack or may have minimal amounts of hydrolytic enzymes (acid phosphatase, alkaline phosphatase). The evidence for the presence of peroxidase in the basophils is controversial. Crystalloid protein structures (Charcot-Leyden crystals) have been shown by electron microscopy within the granules of eosinophils and after de-

granulation in the basophils. Eosinophils and basophils, like neutrophils, remain in the blood only for a few hours and then migrate to the tissues.

Neutrophils are attracted (chemotaxis) to the sites of tissue invasion by microorganisms. These cells are capable of phagocytosis, killing and digesting of bacteria and yeasts. The eosinophils, besides providing some defense against parasitic infestations, are involved in certain hypersensitivity reactions. They may also participate in the phagocytosis and destruction of microorganisms, but do so less readily than neutrophils. The function of basophils is less well understood. They are involved in immediate hypersensitivity reactions such as asthma as well as in delayed hypersensitivity reactions such as contact allergies.

As pointed out earlier, monocytes and neutrophils share a common progenitor, the GM-CFC. The production of monocytes from the GM-CFC proceeds sequentially through the stages of myeloblast, promonocyte and monocyte by way of several mitotic divisions and the process of maturation. The promonocyte, like the promyelocyte, is somewhat larger than the myeloblast. The monocyte, being only slightly smaller than the promonocyte, may also be larger than the myeloblast. As maturation proceeds from the myeloblast to the monocyte stage, the nuclear chromatin becomes progressively but only slightly denser, nucleoli decrease in number and ultimately disappear, and the staining characteristics of the cytoplasm change from blue to blue-gray and then to gray with fine azurophilic granules evident in the promonocyte and the monocyte stages. Upon release from the bone marrow, monocytes are also believed to be distributed between a circulating monocyte pool (CMP) and a marginal monocyte pool (MMP) in a ratio of 1:3.5. The monocytes circulate in the blood also for a short period (16–36 hours) and then migrate to various tissues, where they transform into macrophages or histiocytes. The life span of macrophages is perhaps in the range of several months or even a few years. Mononuclear phagocytes are involved in the disposal of microorganisms, senescent cells and foreign matter, besides participating in the immune response by mechanisms not yet fully elucidated. It is believed that they play a necessary role in the processing of antigens and their recognition by lymphocytes, and act as effector cells (''stimulated macrophages'') in certain immune reactions.

Whereas the exact mechanism regulating the production of granulocytes in vivo remains poorly understood, there is a widely held concept that GM-CSF, a glycoprotein derived from monocyte-macrophages, is the chief mediator of proliferation and differentiation of the cells of the granulocytic and monocytic series.

Lymphopoiesis and Production of Plasma Cells

Lymphoid stem cells in the bone marrow proliferate to self-replicate and to export cells to the thymus (central lymphoid organ) and the peripheral lymphoid tissues—lymph nodes, spleen, gut, tonsils, blood and lymph. The cells that pass through the thymus acquire certain antigenic characteristics and become prothymus cells (pro-T cells), which ultimately mature to become T cells with varied functional attributes, e.g., helper T cells and suppressor T cells. Some of the cells from the thymus also migrate to the peripheral lymphoid tissues, there to proliferate and populate these tissues with immunocompetent T cells. On the other hand, some of the lymphoid stem cells in the bone marrow divide and differentiate into pro-B cells and then into B cells in the marrow. Some of these lymphocytes