Section I General Hematology and Hematopathology

Chapter 1

Development of Hematopoiesis in the Fetus, at Birth, and after Birth

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Overview

Hematopoiesis is a complex process encompassing the continuous generation of specialized, mature blood cells from pluripotent hematopoietic stem cells (HSCs). The hematopoietic system is not fully developed at birth. The proportion of bone marrow (BM) components and normal hematologic values for neonates, infants, older children, and adults are different as a result of the unique characteristics of embryonal and fetal development of the hematopoietic system, which continues to evolve after birth [1]. Knowledge of these differences is essential to distinguish normal development from a pathologic process when evaluating blood and BM in pediatric patients.

Development of Hematopoiesis

Embryonic and Fetal Hematopoiesis

In humans, hematopoiesis (Fig. 1.1) begins in the yolk sac with the generation of angioblastic foci or "blood islands" during the third week of gestation [1]. The blood islands contain primitive erythroblasts. The erythroblasts are large and nucleated, containing embryonic hemoglobin [1,2]. Embryonic erythropoiesis in the yolk sac is followed by the appearance of non-erythroid progenitors in both the yolk sac and aorta-gonadal-mesonephros (AGM) region (four to five weeks) [1–3]. The AGM region contains pluripotent HSCs [1]. At the sixth week of gestation, the HSCs from the AGM and yolk sac colonize the fetal liver, spleen, and BM [1,2].

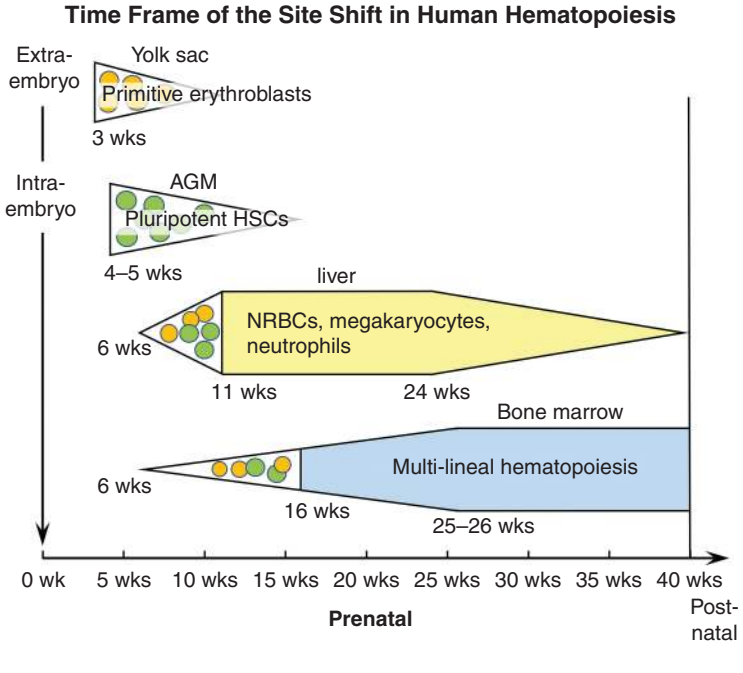

Figure 1.1 Schematic representation of developmental time frame of the site shift in human hematopoiesis.

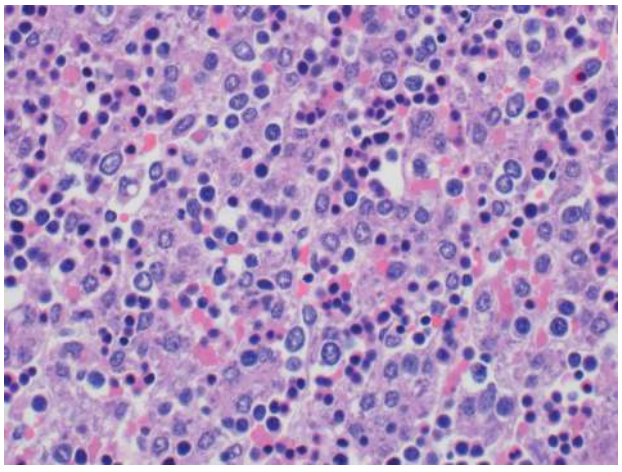

Figure 1.2 Fetal liver, 18 weeks of gestation, showing numerous erythroid progenitors (H&E, 400x). NRBC, nucleated red blood cell.

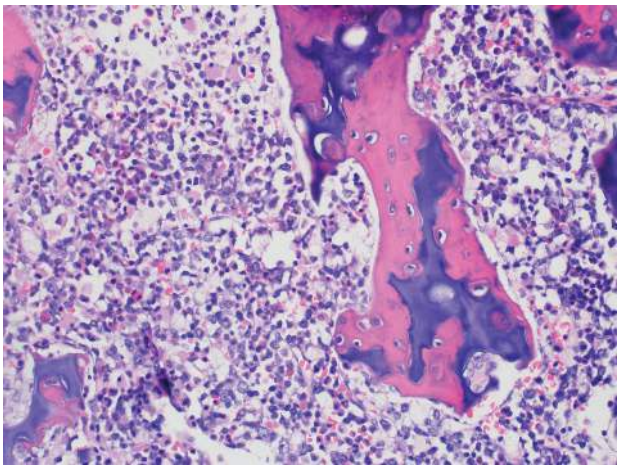

Figure 1.3 Bone marrow biopsy showing >90% cellularity in a 14-day-old neonate (H&E, 400x).

The fetal liver becomes the major site of hematopoiesis between 11 and 24 weeks of gestation [1]. The early stage of fetal hepatic hematopoiesis consists of predominant erythroid progenitors. Unlike yolk sac erythropoiesis, hepatic erythropoiesis is definitive and resembles that found in postnatal life; for example, it generates nucleated red blood cells (NRBCs) (Fig. 1.2) [1]. Megakaryocytes appear during the 12th week of gestation, and mature neutrophils appear after the 16th week. The spleen does not normally function as an active site of hematopoiesis.

The BM starts hematopoiesis during the 16th week of gestation but does not become a primary hematopoiesis site until the 25th or 26th week of gestation [1]. Fetal BM hematopoiesis is multi-lineal and generates definitive NRBCs containing fetal hemoglobin (HbF) and hemoglobin A (HbA), as well as myeloid and lymphoid progenitors [1].

Neonatal and Childhood Hematopoiesis

In a full-term infant, hepatic hematopoiesis has ceased except in scattered small foci that become inactive soon after birth. The BM becomes the primary site of hematopoiesis. In neonates (Fig. 1.3) and young children (see Fig. 1.4), hematopoiesis takes place throughout the entire BM space, including the long bones [2,4]. BM cavities are filled with hematopoietic elements and fat cells. Fat cells gradually increase as age increases, beginning with the digits and advancing toward the axial skeleton [4].

Adult Hematopoiesis

Hematopoiesis is limited to the BM of the flat bones (skull, ribs, sternum, vertebrae, scapulae, clavicles, pelvis, upper half of the sacrum) and the proximal portions of the long bones (femur, humerus). The remaining BM cavity is occupied by fat cells [4]. As age increases, fat cells increase. In the elderly, fatty tissue fills most of the BM space. However, under stressful conditions, hematopoietic elements can replace fat cells [1,4].

The iliac crest is the standard site for BM biopsy in all age groups because it is a non-weight-bearing structure and there are no close vital organs. The iliac crest can retain hematopoietic activity into the ninth decade of life and beyond.

Hematopoietic Regulation

Hematopoiesis is a complex, dynamic process. The growth, differentiation, and maturation of hematopoietic cells occur in a sequential order and are regulated by cell-to-cell interaction and cytokine activities in the BM microenvironment.

Cytokines are cell-signaling molecules that aid cell-to-cell communication and regulate hematopoiesis. Cytokines exist in peptides, proteins, and glycoproteins. They are produced by a broad range of cells. Cytokines act on primitive stem cells and lineage-committed progenitor cells [4]. This process is mediated through specific receptors that transmit a sequence of intracellular signals. Cytokines may act locally at their production site or travel

in the blood. Particular cytokines can elicit more than one activity due to actions on different target cells. Not all cytokines stimulate the growth and differentiation of BM cells. Some cytokines inhibit hematopoiesis [1,2,4].

The stimulatory cytokines are produced by both BM stromal cells and non-stromal cells [2]. Stromal cells generate stem cell factors, Fms-like tyrosine kinase 3 ligand, interleukin (IL)-6 and IL-11, granulocyte colony-stimulating factor, and monocyte colony-stimulating factor [2,4]. Non-stromal cells produce IL-1 (by monocytes, granulocytes), IL-3 (by T-cells), IL-5 (by T-cells), granulocyte-monocyte colony-stimulating factor (by T-cells), erythropoietin (by renal peritubular cells), and thrombopoietin (by hepatocytes) [2]. These molecules primarily act synergistically to regulate the self-renewal of hematopoietic stem cells, the proliferation and differentiation of lineage-committed progenitor cells, and the function of mature hematopoietic cells [4].

The inhibitory cytokines are manufactured by BM stromal cells and macrophages. Examples include tumor necrosis factor, transforming growth factor β, interferon gamma (IFNγ), macrophage inflammatory protein 1α, tetrapeptides, and pentapeptides [2,4]. These pro-inflammatory cytokines contribute to the marrow suppression seen in chronic inflammatory conditions [2].

Pathologic conditions are usually caused by imbalances in hematopoietic cytokine production, such as anemia of renal failure due to erythropoietin deficiency, aplastic anemia due to IFNγ excess, and thrombocytopenia of hepatic failure due to thrombopoietin deficiency [2].

Adhesion molecules also participate in hematopoiesis regulation. This process is mediated by binding of the adhesion molecules with their receptors present on the surface of target cells. As a result, these adhesion molecules promote the attachment of various hematopoietic cells to each other, to stromal cells, and to the extracellular matrix, affecting the generation, differentiation, and function of hematopoietic cells and regulating the retention and release of hematopoietic cells in the BM [4]. The important adhesion molecules include adhesion molecules of the immunoglobulin family, integrins, and selectin [4].

At the gene level, the *GATA1* and *PU.1* genes primarily regulate primitive erythroid and myeloid development [5]. The *RUNX1* and *GATA2* genes are regulators for myelopoiesis [5,6]. The *RUNX1* gene also regulates lymphoid and erythroid development. The *HOX* gene family is expressed in hematopoietic stem cells and progenitors and is involved in the regulation of early development, as well as lineage and stage differentiation [7]. The *Cyclin D3* gene is engaged in the regulation of erythroid number and size [6].

Normal Bone Marrow in Children

The BM is a functionally dynamic structure, and if the need for leukocyte, erythrocyte, or platelet production increases, hematopoiesis expands, and the fat is replaced by red BM elements. In young children, an increase in hematopoiesis is accommodated by a reduction in sinusoids.

The BM is located between the bone trabeculae. The different hematopoietic components are not randomly distributed. The early myeloid progenitors are localized in the paratrabecular areas. Normally, there are no more than two or three layers of maturing myeloid elements. With maturation, the cells migrate to the intertrabecular space. The erythroid progenitors mature and differentiate in erythroblastic islands (a central macrophage surrounded by erythroblasts). As the erythroblasts become more differentiated, the erythroid islands migrate toward sinusoids. Megakaryocytes reside near marrow sinusoids, allowing for platelets to shed directly into the circulation [8].

The cellularity and composition of the BM are dependent on age (Table 1.1) [8]. At birth, the BM is almost devoid of fat and contains only hematopoietic elements. With advances in age, the red hematopoietic marrow is gradually replaced by fat. The BM cellularity is ≥80% in normal infants and approximately 60% during the first five years of life; it remains relatively constant,

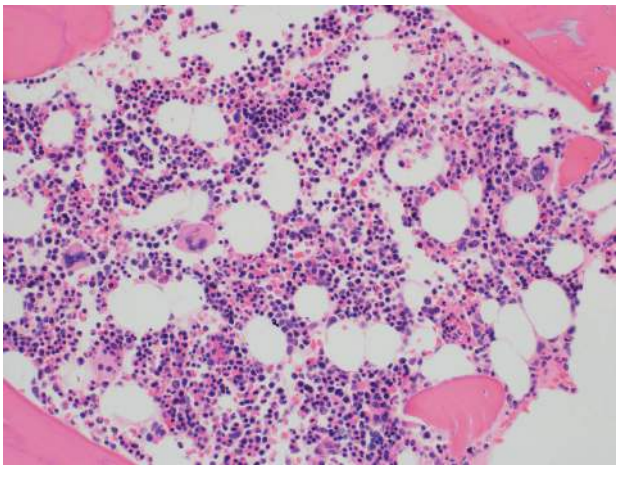

Figure 1.4 Bone marrow biopsy revealing 60% cellularity in a 17-year-old male (H&E, 400x).

Table 1.1 Bone Marrow Cellularity and Cellular Composition in Normal Children of Various Ages

Age	Cellularity	Main cellular composition				
		Myeloid lineage (M)	Erythroid lineage (E)	Megakaryocytes	Lymphocytes	Others
Newborn (right after birth)	90–100%	↑, left shift				<5% blasts
Neonate (≤30 days)	90%	↓ in first 2 weeks	↓	Monolobated small forms	Gradually ↑, most B-cells	
Infant (1 month to <24 months)	80–90%	Reach a steady level (30–35%)	Initial ↓, ↑ in the 2nd month, stabilization at 3–4 months	Monolobated small forms	About 50% after the 1st month, high number of B-cells and hematogones	Absent iron store, M:E = 5–12:1
2–5 years	60–80%	↑	↑		↓ B-cells, ↓ hematogones, slightly ↑ T-cells	Detectable stainable iron after age 4–5 years
6–12 years	50–70%				<20% lymphocytes, T-cells > B-cells	Iron store at adult level M:E = 3–4:1
>12 years	40–60%				<20% lymphocytes, T-cells > B-cells	Iron store at adult level M:E = 3–4:1

comparable with that of other age groups, after that (Fig. 1.4) [8].

At birth, the BM has a predominance of myeloid progenitors and a relatively low number of erythroid progenitors and lymphocytes. During the first month of life, myeloid and erythroid progenitors start to decrease, and lymphocytes increase. Myeloid components reach a steady level after the first month. Erythroid elements become stabilized at four months of life. The number of megakaryocytes in children is comparable with that of adults. Another significant difference in the BM composition between children and adults is the presence of a higher number of lymphocytes, with a higher ratio of B-lymphocytes:T-lymphocytes, in young children compared with adults. The number of B-cells gradually decreases with the increase in T-lymphocytes after the age of four years [8].

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Chapter 2

Normal Anatomy and Function of Lymph Nodes

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Overview

The immune system, a host defense system, recognizes foreign antigens and defends against invading microorganisms. It consists of primary (central) and secondary (peripheral) lymphoid organs. Primary lymphoid organs include the bone marrow and thymus, which contain lymphoid precursors and support initial antigen-independent differentiation from the immature stage to the mature stage. Secondary lymphoid organs comprise the lymph nodes, spleen, and sites of mucosa-associated lymphoid tissue, including the tonsils, appendix, and Peyer patches of the gastrointestinal tract, where antigen-dependent proliferation and differentiation of B- and T-lymphocytes take place [1].

Gross Anatomy

The lymph node is a bean-shaped structure. There are about 450 lymph nodes throughout the body [1]. They are usually <1 cm in diameter, except in early childhood (2–10 years), when they may be larger [2]. They are connected to one another by lymph vessels. Clusters of lymph nodes are found in the neck, axilla, mediastinum, retroperitoneum, and inguinal regions; they drain organs/tissues in contact with the external environment.

Structure and Function

The basic architectural structure of a lymph node includes the capsule, cortex, paracortex, medulla, sinuses (subcapsular, cortical, and medullary), and vasculature (Fig. 2.1).

Cortex

The cortex is the major component of the lymph node; it is located deep to the capsule and subcapsular sinus. Primary and secondary lymphoid follicles are the major components of the cortex. The cortex is a primarily B-cell–dependent compartment and plays an important role in humoral immunity.

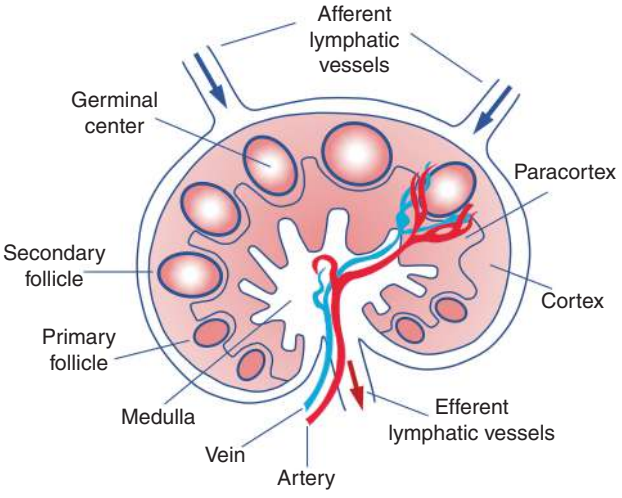

Figure 2.1 Schematic representation of a lymph node. The cortex contains predominantly B-cells, forming primary and secondary follicles. The paracortex contains mainly T-cells and dendritic cells. The medulla contains mostly B-cells and plasma cells. The lymphocytes enter the lymph node through afferent lymphatic vessels. The lymphatic vasculature and blood vasculature are connected through a conduit system, and both ultimately drain into the medullary sinus and then to an efferent lymphatic vessel.

Primary (resting) follicles are round aggregates of small, dark-staining, inactive (naive) B-lymphocytes without a germinal center (GC) (Fig. 2.2), usually near the capsule and within a network of follicular dendritic cells (FDCs).

Secondary follicles consist of a GC surrounded by small B-cells that form a darker corona (mantle zone) in each follicle (Fig. 2.3). GCs are composed of small and large cleaved lymphocytes (centrocytes), large noncleaved lymphocytes, and a minor population of small non-cleaved lymphocytes (centroblasts). Occasional T-cells, plasma cells, FDCs, and tingible-body macrophages are normally present [3]. Infrequently, the lymphoid follicles

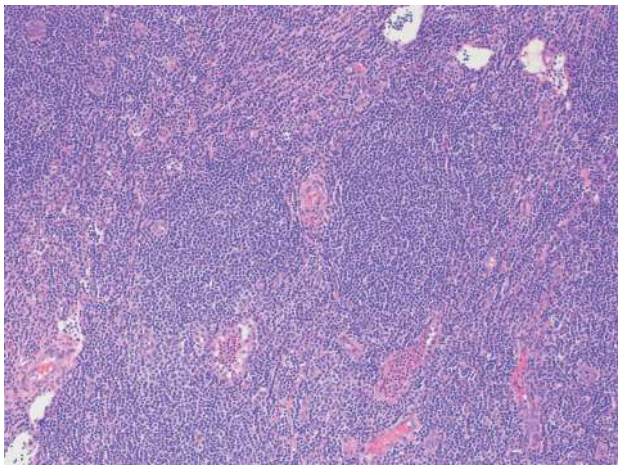

Figure 2.2 Primary follicles with round aggregates of small, dark-staining, inactive B-lymphocytes without germinal center (H&E, 100x).

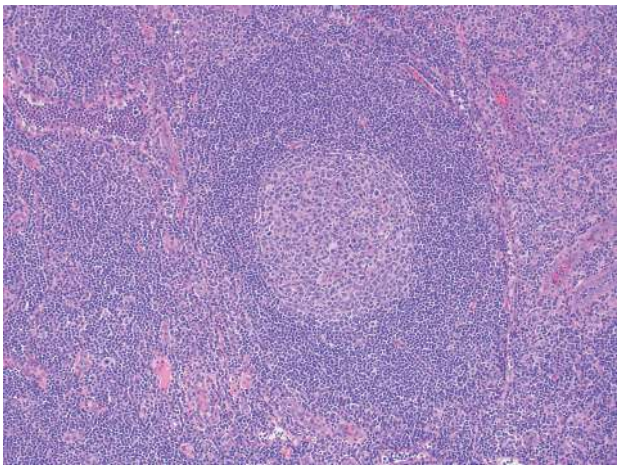

Figure 2.3 A secondary follicle with a germinal center surrounded by a darker mantle zone (H&E, 100x).

exhibit a well-developed marginal zone that surrounds the mantle zone and is composed of small lymphocytes with slightly irregular nuclei and clear cytoplasm.

GCs are dynamic structures and are formed by the proliferation of naive B-cells. The naive B-cells enter the lymph node through the high endothelial venules (HEVs) located in the paracortex, where they are directed to the follicles in response to CXCL13. The CXCL13 molecule is expressed by FDCs and is crucial for the follicular clustering of B-cells and for attracting follicular B-helper T-cells (Tfh cells), a distinctive CD4+ T-cell subset [4]. Following antigen stimulation, B-cells travel to the border of the primary follicles to meet antigen-specific CD4+ Tfh cells, which promote B-cell proliferation and differentiation and initiate the formation of the GC [2].

In GCs, B-cell clonal expansion and diversification of the B-cell receptor repertoire occur mainly in centroblasts [5] through somatic hypermutation of variable regions of immunoglobulin genes. The resulting B-cells then become centrocytes. Both centroblasts and centrocytes are BCL2–; therefore, they are programmed to die unless they are rescued by high-affinity interaction between their antigen receptor and a given antigen [2]. GC B-cells express CD10 and BCL6. BCL6 is a nuclear transcriptional factor that regulates the proliferation and differentiation of GC B-cells.

Centrocytes expressing surface immunoglobulin (Ig) with a high affinity toward antigen are rescued from apoptosis and differentiate into memory and antibody-producing B-cells. The antibody-producing B-cells migrate to the medulla, where they become plasma cells that leave the lymph node and migrate to the bone marrow. The late centrocytes express IRF4/MUM1 [2].

Paracortex

The paracortex is the area between the cortex and medulla, containing predominantly small T-lymphocytes (CD4+ helper/inducer, CD8+ cytotoxic/suppressor) and dendritic cells (DCs) of the interdigitating dendritic subtype. The ratio of CD4 to CD8 is 2–10:1, which is reversed in viral infection [3]. Many T-cells in the paracortex are CD45RO+ (with a memory function, a short life span, and the capacity to elaborate cytokines) or CD45RA+ (with a long life span and an ability to elaborate interleukin [IL]-2) [3]. HEVs are characteristically located in this region. The paracortex also contains B-cells and foci of plasmacytoid dendritic cells (PDCs), which are medium-sized, round cells with vesicular nuclei and well-defined cytoplasmic borders [2,3].

The paracortex is the region where the adaptive immune response begins. Like B-cells looking for their cognate antigens, the circulating naive T-cells migrate to the paracortex via the HEVs as a result of homing cytokines secreted by HEVs, stromal cells, and DCs [2]. The DCs, antigen-presenting cells, present antigens to the naive T-cells (CD4+ and/or CD8+) to become effector T-cells that either leave the lymph node or stay within the lymph node as memory T-cells or Tfh cells that promote the terminal differentiation of B-cells [2,4].

Table 2.1 Major Cell Elements and Their Immunophenotype in Normal Lymph Node

Cell type	Main location	Immunophenotype
Germinal center (GC) B-cell (centroblast, centrocyte)	Secondary follicles	CD10+, BCL6+, CD20+, CD38+, CD27+, BCL2–, IgD– Late centrocytes: IRF4/MUM1+
Mantle-type (naive) B-cell	Primary and secondary follicles	IgM+, IgD+, CD23+, CD38+, BCL2+, CD20+, CXCL13+, CD10–, CD27–
Marginal zone B-cell	secondary follicles	CD20+, CD5–, CD10–
Plasma cell	GC, medulla	CD138+, CD38+, CD20–, surface K/λ–, cytoplasmic K/λ+
GC T-cell (follicular B-helper T-cell [Tfh cell])	GC	CD4+, CD40 L+, CXCR5+, ICOS+, PD1+, IL-21+, CD57+, BCL6+
Helper T-cell	Paracortex, medulla	CD4+
Cytotoxic and suppressor T-cell	Paracortex, medulla	CD8+
Follicular dendritic cell	Primary and secondary follicles	CD21+, CD23+, CD35+, VCAM-1+, ICAM-1+, CXCL13+, S100–, lysozyme–
Interdigitating dendritic cell	Paracortex	S100+, CD21–, CD35-, lysozyme±, CD68±
Macrophage	GC, medulla	CD68+, lysozyme+, CD4+, S100–
Plasmacytoid dendritic cell	Paracortex, medulla	CD123+, CD68+, lysozyme+

Medulla

The medulla is located between the paracortex and the hilum of the lymph node and is composed of medullary sinuses and medullary cords. The medullary cords contain small B- and T-lymphocytes, plasma cells, and occasional immunoblasts or mast cells [3]. The medullary sinuses are composed of macrophages, lymphocytes, and circulating granulocytes [3]. A paired arteriole and venule run along the central axis of each medullary cord. The medullary region also contains monocytoid B-cells and PDCs.

Table 2.1 lists the major cell elements and their immunophenotype.

Lymph Node Sinuses

Lymph node sinuses are channels lined with endothelial cells that carry lymph from the afferent lymphatics to the efferent lymph vessels at the hilum. There are subcapsular, cortical, and medullary sinuses. Afferent lymphatics drain into the subcapsular sinus running around the periphery of the lymph node, and from this sinus, the cortical sinuses pass down toward the medulla. Within the medulla, the medullary sinuses converge upon the efferent lymphatic vessel at the hilum. Sinus macrophages capture exogenous antigens that enter the lymph node through the lymph and present them to B- and T-cells to generate the body's immune reaction. The cortical and medullary sinuses are the major gateways for lymphocytes and plasma cells exiting the lymph node [1].

Vasculature

In addition to the afferent and efferent lymphatic vessels, the vascular structure of the lymph node includes arteries, capillaries, post-capillary HEVs, and veins [3]. One or more small arterial vessels enter the node via the hilum and then divide into branches in the medulla; these branches give rise to arterioles in the cortex. The arterioles subsequently ramify into capillaries, which empty into the post-capillary HEVs. The HEVs have a cuboidal endothelium bearing a specialized lymphocyte-homing receptor that is recognized by circulating lymphocytes, thus facilitating the passage of lymphocytes from the blood and into the lymph node. The HEVs eventually lead to veins that pass through the lymph node cortex and medulla and exit at the hilum [3].

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Chapter 3

Anemia

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Overview

Anemia is defined as a hemoglobin (Hb) level or red blood cell (RBC) mass of less than the 5th percentile for age [1]. Approximately 20% of American children have anemia [1]. Most children with anemia are asymptomatic, and the condition is often discovered incidentally on routine laboratory testing.

Hemoglobin is a tetrameric protein composed of two pairs of globin chains complexed with four heme groups and is responsible for the transportation of oxygen of RBCs [2,3]. Normal adult RBCs contain 97% hemoglobin A (HbA; $\alpha_2\beta_2$), 2% HbA_2 ($\alpha_2\delta_2$), and 1% fetal Hb (HbF; $\alpha_2\gamma_2$). At birth, HbF is predominant. Within the first year of life, it is largely replaced by HbA [3].

Causes of anemia vary by age [1]. In children, it is usually due to reduced RBC production (e.g., defects in Hb synthesis, erythroid nuclear maturation) or elevated RBC turnover (blood loss, hemolysis).

The classification of anemia is variable. The morphologic classification is based on the size of RBCs, as measured by the mean corpuscular volume (MCV): microcytic anemia (MCV < 80 μm^3 [80 fL]), normocytic anemia (80–100 μm^3 [80–100 fL]), or macrocytic anemia (>100 μm^3 [100 fL]) [1]. The RBC distribution width (RDW) measures the size variance of RBCs. A normal RDW suggests uniform RBC size, whereas a high RDW (>14%) implies divergent sizes [1]. The classification can also be based on defects of erythrocyte production versus destruction or genetic versus acquired etiologies [2,4]. In the following discussion, the most common types of anemia are addressed, with additional types reviewed in the chapter on bone marrow (BM) failure syndromes (Chapter 4).

Constitutional Anemia and Hemoglobinopathy

Thalassemias

Definition

Thalassemias are a heterogeneous group of disorders caused by inherited mutations that result in a decrease in the synthesis of hemoglobin chains but a normal hemoglobin structure. *Beta-thalassemia* refers to decreased β-chains; *α-thalassemia* refers to reduced α-chains; and *δβ-*, *δ-*, and *γδβ-thalassemias* similarly correspond to decreased synthesis of the aforementioned chains [2,5–7].

Clinical Features

Thalassemias occur in Mediterranean, African, and Asian populations due to evolutionary protection against malaria [5,6]. Anemia is the main manifestation. Clinical classification is based on the severity of anemia and associated symptoms: thalassemia major is severe and transfusion-dependent, thalassemia intermedia is less severe and non–transfusion-dependent, and thalassemia minor is a carrier state with hematological abnormalities but no symptoms (Table 3.1) [3,5].

Pathogenesis

In β-thalassemia, almost 200 mutations affecting the β-chain genes on chromosome 11 have been identified, consisting primarily of point mutations leading to reduced (β+) or absent (β0) chain synthesis [3,6,7]. As a result, α-chains are unpaired or paired with δ-chains or γ-chains, producing HbA2 (α2δ2) or HbF (α2γ2), respectively [6]. Unpaired α-chains precipitate in red cells, causing membrane damage, apoptosis, and hemolysis [5].

Table 3.1 Clinical and Genetic Features of α- and β-Thalassemia

Phenotype	Number of defective genes	Genotype	HbA ($\alpha_2\beta_2$)	HbA2 ($\alpha_2\delta_2$)	HbF ($\alpha_2\gamma_2$)	Other features
Normal		αα/αα, ββ	97%	2%	1%	
β-Thalassemia						
Major	2 (homozygous)	$\beta^0\beta^0$	0	Rest	>95%	• Mediterranean descent common • Symptoms occur at 3 months to 1 year old • Severe microcytic anemia, hemolysis
		Severe $\beta^0\beta^+$, $\beta^+\beta^+$	↓↓	≥2%	30–90%	
Minor/trait	1 (heterozygous)	β^0/β or β^+/β	↓ (usually >90%)	↑	Normal or↑	• Asymptomatic with mild or no anemia, microcytosis
Intermediate	2 or 1	Milder $\beta^0\beta^+$, $\beta^+\beta^+$, or severe β^0/β, β^+/β		3.5–7%	1–3%	• Moderate to severe anemia
α-Thalassemia						
Silent carrier	1	−α/αα				Normal
Trait/minor	2	−α/−α or −−/αα		Normal or ↓		• Asian and African descent common • Asymptomatic • Mild or no anemia, microcytosis • Hb Bart: 5–10% (neonate), 0% (≥1 year)
HbH disease /major	3	−α/−−	↓	↓	Not ↑	• Asian descent common • Chronic moderate hemolytic anemia • Normal life span • Heinz body formation • HbH: 5–30% (≥1 year) • Hb Bart: 20–40% (neonate), 4.8% (thereafter), and variable (adults)
Hydrops fetalis	4 (homozygous α^0-thalassemia)	−−/−−	0	0	0	• Southeast Asian descent common • Fetal or neonatal death, severe anemia • Hb Bart: >80%