SECTION IV

Blood components

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CHAPTER 13

Red blood cell production and kinetics

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Introduction

The main function of red blood cells, the erythrocytes, is to transport oxygen from the lungs to the other tissues of the body. Oxygen delivery is finely controlled by the number of erythrocytes circulating in the blood, which is a function of the rate of senescent erythrocyte removal and the rate of new erythrocyte (reticulocyte) entry. Circulating erythrocytes are maintained in an extremely narrow range because the normal bone marrow produces almost the same number of new erythrocytes each day as is lost through senescence. This daily turnover of approximately 1% of circulating erythrocytes represents 200–250 billion erythrocytes in a healthy adult. When increased numbers of erythrocytes are lost, such as with bleeding or hemolysis, the production of new erythrocytes increases rapidly, replacing the lost erythrocytes and reestablishing the steady-state number of erythrocytes. The rapid expansion of erythrocyte production in response to bleeding or hemolysis is so well regulated that rebound polycythemia does not occur. This exquisitely controlled production of erythrocytes is mediated through a negative feedback mechanism that involves renal oxygen supply and utilization, the hormone erythropoietin (EPO) that is produced in the kidneys, and the erythroid progenitor cells in the bone marrow that depend upon EPO to survive. Normal red blood cell production also depends upon adequate supplies of specific nutrients, among which iron, folate, and vitamin B_{12} are the most important. Disorders of the hematopoietic system or other diseases such as those associated with chronic inflammation inhibit the erythropoietic process.

Erythropoiesis

Erythropoiesis: a component of hematopoiesis

Erythropoiesis, the process of erythrocyte production, is part of the larger process by which a pluripotent hematopoietic stem cell (HSC) proliferates and differentiates into all of the cell types of the blood and immune systems, including platelets, granulocytes, monocytes and macrophages, T lymphocytes and B lymphocytes, as well as erythrocytes. Thus, normally regulated hematopoiesis is required for effective hemostasis, inflammation, immune responses, and tissue oxygenation. Current concepts of hematopoiesis are derived mainly from studies of mice and humans. These studies have included direct morphologic and immunologic analyses of cells in hematopoietic tissues, in vitro culture of hematopoietic cells, transplantation studies with hematopoietic cells, and genetic studies of mice with natural mutations, transgene expressions, or targeted gene knockouts.

Labeled endothelial cells in the ventral part of the aorta in developing mice transform into HSCs[1] by a mechanism that does not require mitosis.[2] Among the various functions of blood cells, tissue oxygenation by the erythrocytes is the first required during embryonic development and the most tightly regulated in postnatal life. Erythropoiesis has two sequential but overlapping phases during development. In the first or primitive phase, erythrocytes are produced in "blood islands" of the yolk sac during weeks 3–6 of human gestation, with primitive erythrocytes comprising the large majority of circulating erythrocytes at 8 weeks but declining to undetectable levels by 12 weeks of gestation.[3] In the subsequent definitive erythropoiesis phase, erythrocytes are produced mainly in the human fetal liver from 6 to 22 weeks of gestation, and mostly in the bone marrow at later times.[3] Definitive erythroid cells arise from HSCs that are first detected in the aortogonadomesonephros (AGM) region of the mesoderm,[4] circulate, seed the fetal liver, and then migrate from the fetal liver to the developing bone, where they initiate marrow hematopoiesis.[5–7] The hemoglobin of the primitive erythrocytes contains embryonic ε- and ζ-globins, whereas the hemoglobin of the definitive erythrocytes contains adult α-globin and either fetal γ-globin from mid-gestation through the first few postnatal months or mainly adult β-globin after the first few postnatal months.[8]

Stages of erythropoiesis

Erythroid progenitor cells arise from HSCs that commit to differentiation and are termed *multipotent progenitors* (MPPs). MPPs proliferate and undergo a series of decisions based on specific transcription factor activities that determine their progeny's fate in terms of blood cell lineage (see Figure 13.1). The myeloid transcription factors PU.1 and GATA1 direct differentiation toward the nonlymphoid lineages, and, if the activity of the GATA1 transcription factor is increased, differentiation toward the bipotent megakaryocytic-erythroid progenitor (MEP) is promoted.[9,10] MEP fate, in turn, is determined by the activities of two other competing

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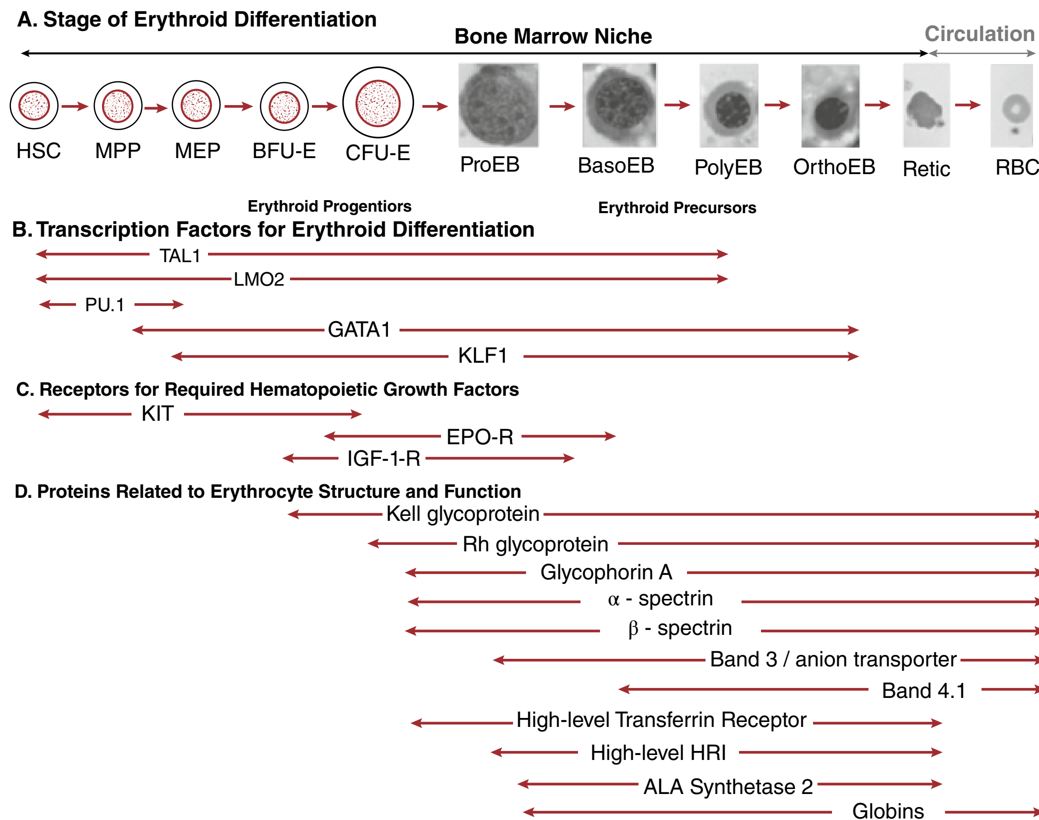

Figure 13.1 Cellular events in erythroid differentiation. (A) Stages of differentiation: hematopoietic stem cells (HSCs), burst-forming units-erythroid (BFU-Es), colony-forming units-erythroid (CFU-Es), proerythroblasts (ProEBs), basophilic erythroblasts (BasoEBs), polychromatophilic erythroblasts (PolyEBs), orthochromatic erythroblasts (OrthoEBs), reticulocytes (Retics), and erythrocytes (RBCs). (B) Transcription factors: basic helix–loop–helix factor (TAL1), Lim domain partner of TAL1 (LMO2), factor binding purine-rich GAGGAA sequence (PU.1), zinc finger factor binding GATA-containing sequences (GATA1), and Krüppel-like factor (KLF1). (C) Growth factor receptors for stem cell factor (KIT), erythropoietin (EPO-R), and insulin-like growth factor-1 (IGF-1-R). (D) Proteins related to erythrocyte structure and function and heme-regulated inhibitor (HRI; eIF2α kinase). Periods of expression for erythroid-specific forms of proteins are shown. Transferrin receptors are present in all stages, but the period of high-level expression that characterizes hemoglobin-producing erythroblast is shown. Band 4.1 shown for the spliced form found in circulating erythrocytes. Content of each protein may vary during the period shown. Source: Based on Koury.[76]

transcription factors: erythroid Krüppel-like factor-1 (KLF1), which promotes erythroid differentiation, and FLI1, which promotes megakaryocytic differentiation.[9,11]

In Figure 13.1, the hematopoietic stages committed solely to erythroid differentiation begin with the burst-forming units-erythroid (BFU-Es),[12] which produce large colonies or multiple colonies of human erythroblasts after 2–3 weeks in tissue culture. BFU-Es can circulate in the blood, but after they differentiate in marrow to the next defined stage, the colony-forming units-erythroid (CFU-Es),[12] they associate with a macrophage, forming an erythroblastic island (EBI), the basic unit of terminal mammalian erythropoiesis.[13] Coordinated KLF1 activity in both the central macrophage and the erythroid cells[14,15] of an EBI directs the development of as many as 30 or more adherent erythroblasts at various stages of differentiation from CFU-Es through enucleating orthochromatic erythroblasts. BFU-Es and CFU-Es were originally defined by in vitro colony-forming activity and erythroblasts by their morphological appearances in Giemsa-stained films of aspirated marrows. More recent studies using flow cytometry have enabled the identification and isolation of all stages of human erythroid progenitors BFU-Es, CFU-Es and their erythroblast progeny, the proerythroblasts (ProEBs), basophilic erythroblasts (BasoEBs), polychromatophilic erythroblasts (PolyEBs), and orthochromatic erythroblasts (OrthoEBs) based on the surface expressions of CD34, CD36, endoglin (CD105), and the transferrin receptor (CD71) for the progenitors; or glycophorin A, anion transporter (Band 3), and α4 integrin for the precursors.[16]

OrthoEBs enucleate within the bone marrow forming reticulocytes, very irregularly shaped cells containing hemoglobin and residual organelles (the "reticulum") that allow them to be distinguished from the mature erythrocytes. The extruded nucleus with its thin shell of hemoglobin-containing cytoplasm, termed a *pyrenocyte*, is rapidly phagocytosed by the central macrophage, which degrades the nucleus and hemoglobin and recycles the nucleosides and iron.[17] The final stage of differentiation, the erythrocyte, is achieved after the reticulocytes have entered the circulation, lost their residual internal organelles via autophagy,[18] and remodeled their irregular shapes by shedding of exosomes[19,20] and forming uniform biconcave disks. Reticulocyte maturation to an erythrocyte occurs within 1–2 days after entering the circulation, but mature erythrocytes continue to shed microvesicles until they are removed 110–120 days later as senescent cells.[21]

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Intracellular requirements for normal erythroid differentiation

A series of intracellular and extracellular events are needed for successful completion of the erythroid differentiation scheme as shown in Figure 13.1. The intracellular events include the expression of (1) hematopoietic and erythroid-specific transcription factors; (2) specific microRNAs and long, noncoding RNAs involved in the differentiation process; (3) proteins involved in the proliferation and differentiation of the erythroid cells; and (4) proteins such as hemoglobin, intrinsic membrane, and membrane skeleton proteins that comprise the mature erythrocyte.

GATA1, KLF1, and the transcription factor complex of TAL1/SCL, LMO2, and LDB1 are essential for erythropoiesis from the pre-EPO-dependent stages through late erythroblast stages.[11,22,23] In addition to regulating expression of erythroid-specific genes such as those encoding the EPO receptor, globins, and glycophorins, these transcription factors also regulate long, noncoding RNAs that can influence other erythroid gene expressions in the later stages of differentiation, such as the gene encoding Band 3 and the anion transporter.[24] The expression of transcription factors and other crucial erythroid proteins, in turn, are partially controlled by specific microRNAs, which regulate mRNA stability and translation at all stages of erythroid differentiation.[23,25] In fact, post-transcriptional regulation of protein synthesis by microRNAs allows the control of the reticulocyte maturation process that occurs days after the erythroid cell has lost its nucleus.[26]

During the terminal stages of erythropoiesis in the EBI, the erythroblasts undergo progressive decreases in size, nuclear condensation, and subsequent enucleation. Decreased cell size between the ProEB and OrthoEB stages is achieved by a shortened duration of the G1 phase of the cell cycle, resulting in less protein accumulation between cytokineses.[27,28] These terminal erythroblast divisions are regulated by cyclin D3, a G1-phase cyclin,[29] and direct contact with the central macrophage shortens the G1 phase of the erythroblasts.[30] During these more rapid cell divisions, the ratio of heterochromatin to euchromatin increases with a progressive condensation and reduction in nuclear size[31] that are associated with histone deacetylation[32] and DNA demethylation in the mouse.[33] In humans, nuclear condensation occurs earlier (around the BasoEB stage) and involves the nuclear lamins.[34,35] In the formation of the reticulocyte and pyrenocyte, the condensed erythroblast nucleus is extruded by an active process similar to cytokinesis that requires filamentous actin,[36] nonmuscle myosin IIB,[37] and tropomodulin 1 (Tmod1).[34] KLF1 expression in the central macrophage regulates the phagocytosis of the pyrenocyte and subsequent degradation of its DNA and hemoglobin.[14,17]

Hemoglobin, the predominant protein of erythrocytes, is synthesized in a highly regulated process that begins in the BasoEBs and continues through the reticulocyte stage. Extremely large quantities of heme are produced without intracellular accumulations of iron or protoporphyrin. As iron accumulates in the basophilic erythroblast, it upregulates heme synthesis through a 5′-iron-responsive element (IRE) in erythroid-specific 5-aminolevulinic acid synthase (ALAS2, the first step of heme synthesis) mRNA that controls translation;[38] GATA1 activity induces heme synthetic enzymes and protein kinase A phosphorylates and activates ferrochelatase, which catalyzes the last step of iron incorporation into protoporphyrin;[39] and the amino acids glycine and glutamine (as source of succinyl CoA) provide the substrates for porphyrin synthesis.[40] Similarly, heme is incorporated into globin chains without accumulating intracellular excesses of globin chains or heme, and 2α-hemoglobin and 2β-hemoglobin chains are assembled into hemoglobin A tetramers without accumulating unpaired hemoglobin chains.[41]

Multiple layers of regulation that are specific to erythroid cells control heme and hemoglobin synthesis including (1) heme regulation of erythroblast GATA1 activity[42] and iron acquisition from endocytosed transferrin receptors;[38] (2) heme upregulation of erythroblast protein synthesis through inactivation of heme-regulated eIF2α kinase (heme-regulated inhibitor [HRI]), which in the absence of heme phosphorylates the translation initiation factor eIF2α, thereby inhibiting its ability to initiate general mRNA translation;[43] and (3) heme induction of rRNA and ribosomal proteins.[42] In the regulation of hemoglobin production, heme derepresses β-globin transcription by binding and enhancing the degradation of BACH1, a transcription repressor at the locus control region (LCR). With the loss of BACH1, the NFE2–Mafk transcription factor complex binds and activates β-globin transcription,[44,45] which combined with GATA1 and KLF1 activities leads to coordinated α-globin and β-globin transcriptions.[46] While β-globin transcription is upregulated, heme indirectly downregulates γ-globin transcription through increased translation of its major target gene, translation of activating transcription factor-4 (ATF4), which in turn induces BCL11A that inhibits γ-globin transcription.[47] Finally, α-hemoglobin-stabilizing protein (AHSP) regulates intracellular free α-globin chain content by coordination of heme insertion, appropriate folding, and assembly of α-globin chains into hemoglobin.[48]

In the terminal stages of erythroid differentiation, the plasma membrane and associated membrane skeleton undergo large changes in their composition. From the CFU-E through reticulocyte stages, several patterns of intrinsic membrane protein expression are found: (1) from a baseline of little or no expression, large increases occur in proteins that are major components of erythrocyte membranes, such as glycophorin A, glucose transporter 1 (GLUT1), and Band 3; (2) more gradual increases from low baseline levels occur in glycophorin C, and RhAG, RhD, and Lutheran antigens; (3) from a stable baseline, late declines of moderate degree occur in Kell antigen and transferrin receptor 1 (CD71); and (4) prominent declines occur in adhesion proteins such as CD36 and CD44, and integrin components α4, α5, and β1.[16] Most of the membrane skeletal proteins, including α- and β-spectrins, ankyrin, adducin, Band 4.1, Band 4.9, and tropomodulin, increase, whereas actin declines slowly during terminal erythroid differentiation.[16] This pattern of accumulation of membrane skeletal proteins during terminal erythropoiesis is mainly related to the accumulation pattern of Band 3, to which the membrane skeleton is bound,[49] and mRNA splicing of the skeletal proteins, such as Band 4.1.[50] In addition to regulating cellular structure, alternative splicing of transcripts plays a role in the regulating cell cycle and chromatin function during terminal erythropoiesis.[51] Once hemoglobin synthesis has been completed during normal steady-state erythropoiesis, the transferrin receptor 1 (CD71) is completely lost during reticulocyte maturation through the exosomal pathway.[52]

Extracellular requirements for erythroid differentiation

The extracellular requirements for erythroid differentiation include (1) stromal cell and matrix support within the marrow, (2) required hematopoietic growth factors, and (3) nutrients required for progenitor cell proliferation and differentiation. HSCs and BFU-Es can circulate in the blood, but to complete differentiation they must

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adhere to and be retained in specific areas in marrow termed *niches*. HSCs home to and are retained in the marrow by cytokines and chemokines that are produced by mesenchymal stem cells. The most prominent marrow cytokine is secreted and membrane-bound KIT ligand (SCF), which binds its receptor, KIT, on HSCs. The most prevalent marrow chemokine is stromal-cell-derived factor 1 (CXCL12), which binds its receptor, CXCR4, on HSCs.[53] In the marrow, HSCs differentiate through the MPP and MEP stages to reach the BFU-E stage. The marrow matrix protein laminin binds the p67 nonintegrin receptor on circulating BFU-Es, thereby promoting their retention and proliferation in the marrow.[54] When BFU-Es differentiate to CFU-Es, they associate with stromal macrophages forming the EBIs. At least five interacting surface membrane protein pairs mediate macrophage–erythroid interactions in EBIs: (1) macrophage vascular cell adhesion molecule 1 (VCAM1) binds erythroblast α4β1 integrin, (2) macrophage α_V integrin binds erythroblast interstitial cell adhesion molecule-4 (ICAM4/LW), (3) erythroblast–macrophage protein (EMP) on both macrophages and erythroblasts binds with itself on the other cell type, (4) macrophage CD169–Siglec1 binds erythroblast sialylated glycoproteins, and (5) macrophage hemoglobin–haptoglobin receptor (CD163) binds an unknown erythroblast ligand.[13]

In Figure 13.1, receptors for the hematopoietic growth factors necessary for normal erythropoiesis are shown for the period when they are required. The principal growth factor regulating erythropoiesis is EPO, which is discussed in detail in the "Erythropoietin" section. Prior to EPO dependence, specific growth factors maintain progenitor cell survival and proliferation, with the most prominent being SCF and insulin-like growth factor-1 (IGF1) supplied by the marrow environment.[55] CFU-Es and ProEBs lose SCF and IGF-1 responsiveness, respectively, while they are dependent on EPO for survival. However, in contrast to normal erythropoiesis, during periods of hypoxic stress, CFU-Es and ProEBs can expand their numbers greatly without any further differentiation. The two main extracellular mediators of this expansion are (1) glucocorticoids,[56,57] which are produced in the adrenals and appear to induce a protein in erythroid progenitors that binds the mRNAs that direct terminal erythroid differentiation;[58] and (2) bone morphogenetic protein 4 (BMP4),[59] a member of the transforming growth factor-β family of cytokines that can be produced by the central macrophage of the erythroblastic islands.[60] A major intracellular mediator of the erythroblast expansion in response to hypoxia is the upregulation of *Bmi-1* and *BMI-1* expressions in mice and humans, respectively.[61,62]

Included among the vitamins and minerals that cause anemia during deficiency states are copper; cobalt; vitamins A, C, and E; pyridoxine; riboflavin; and nicotinic acid.[63] However, the most common nutritional deficiencies that cause anemia are those of folate, vitamin B_{12}, and iron. The roles of these three nutrients are described in the "Nutritional Requirements for Erythropoiesis" section.

Erythropoietin

Regulation of EPO production by tissue hypoxia

EPO, a glycoprotein hormone, is a major component of the oxygenation–EPO negative feedback mechanism shown in Figure 13.2. The major determinant of oxygen delivery from the lungs to the peripheral tissues is the number of circulating erythrocytes. With anemia, when erythrocyte numbers are decreased, oxygen delivery decreases and the peripheral tissues become hypoxic. All tissues experience hypoxia during anemia, but those organs that respond with EPO production are the kidneys and, to a much lesser extent, the liver.[64] The kidney cells that produce EPO are a subset of interstitial fibroblasts located adjacent to proximal tubules, with EPO-producing cells in small foci of the inner cortex in slight anemia, larger areas within the inner half of the cortex in moderate anemia, and distributed throughout the cortex in severe anemia.[65,66] These progressive increases in the areas of EPO production in the kidney correspond to increasing areas of cortical hypoxia, which are a function of oxygen supply from the blood and local oxygen tissue utilization, which is mainly determined by the metabolic demands of the tubular epithelium. Rapid increases in EPO production after blood loss or hemolysis are not due to increased production by each EPO-producing cell but rather to recruitment to active EPO production of increased numbers of cells with the potential to produce EPO.[65] With a linear decrease in hematocrit, the number of cells actively producing EPO and the resultant plasma EPO levels increase exponentially,[65,66] as was originally reported for plasma EPO levels in most clinical anemias, except for those involving patients with renal disease, chronic inflammation, or malignancies.[67–69]

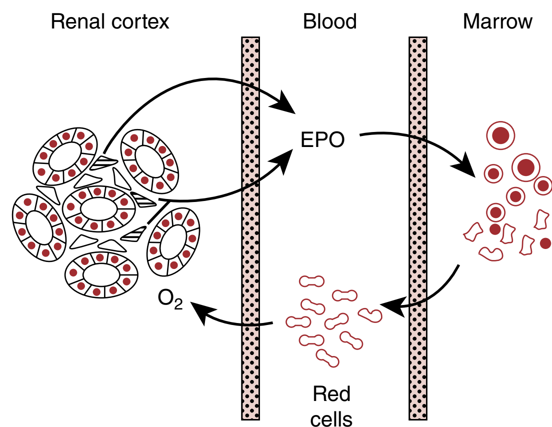

Figure 13.2 Oxygenation–erythropoietin (EPO) negative feedback mechanism. Circulating erythrocyte numbers determine the amount of oxygen (O_2) delivered from the lungs to other tissues. A specific subset of renal cortical interstitial cells (hatched) produces EPO when hypoxic. EPO is promptly secreted into the blood and prevents programmed death (apoptosis) of marrow erythroid progenitor cells. Erythroid progenitors that survive the EPO-dependent period of differentiation develop into reticulocytes (irregularly shaped, anucleate cells in marrow and blood) and subsequently mature into biconcave erythrocytes. Increased erythrocyte numbers resulting from increased plasma EPO deliver more oxygen to the kidneys, relieve renal hypoxia, and thus decrease EPO production.

Hypoxia sensing by EPO-producing cells involves hypoxia-inducible transcription factors (HIFs), a multicomponent complex that binds hypoxia-inducible transcription enhancer elements of various genes, including *EPO*, *VEGF*, and genes encoding several glycolytic enzymes.[70,71] Under normoxic conditions, the steady-state HIF-α component of the complex does not accumulate intracellularly because it is rapidly degraded by the ubiquitin–proteasome pathway (Figure 13.3).[72] However, when a cell with EPO-producing capacity experiences hypoxia, the degradation of HIF-α decreases and intracellular levels promptly increase. Polyubiquitination of HIF-α depends upon the von Hippel–Lindau protein (pVHL) interacting with those HIF-α molecules that have hydroxylation of two specific proline residues (Figure 13.3).[73–75] These prolyl hydroxylations are directly linked to the oxygenation because they are catalyzed by prolyl

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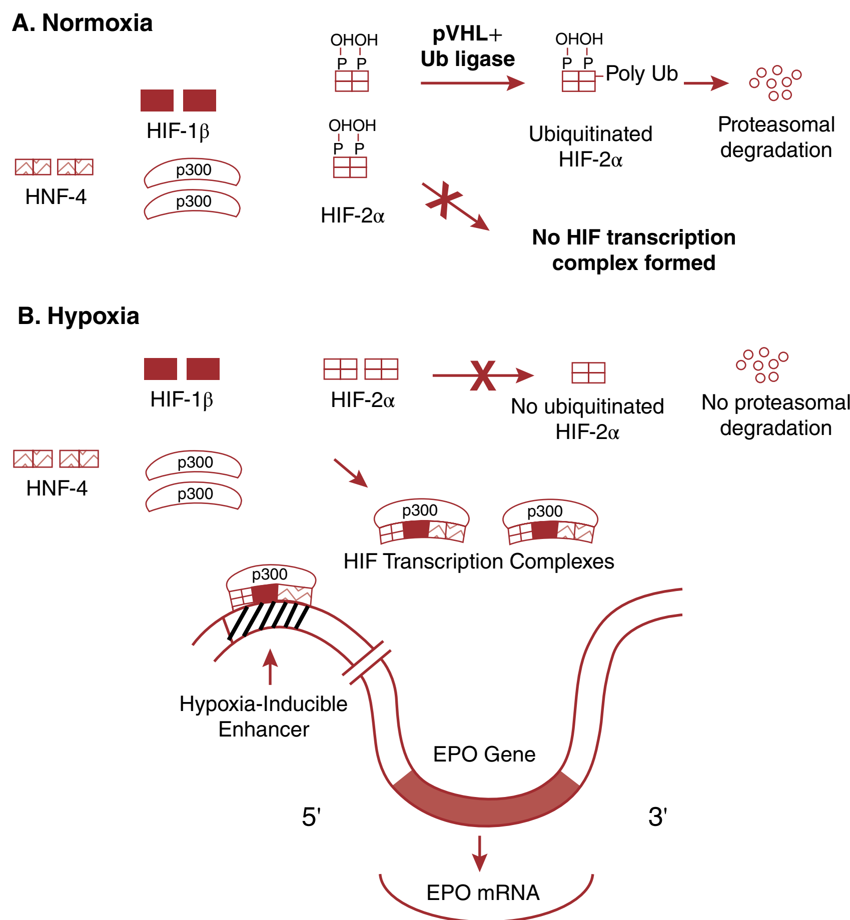

Figure 13.3 Induction of erythropoietin (EPO) gene transcription by hypoxia in renal cortical fibroblasts. (A) In cells capable of producing EPO, two components of hypoxia-inducible factor (HIF-2α and HIF-1β) are constitutively produced under normoxic conditions. However, the molecular oxygen present in the EPO-producing cells under normoxic conditions is used in the hydroxylation of two prolines in HIF-2α in a reaction mediated by specific HIF-prolylhydroxylases (HIF-PHDs). The prolyl hydroxylations (P-OH) lead to recognition by von Hippel–Lindau protein (pVHL), which targets HIF-2α for polyubiquitination (Poly Ub) by ubiquitin ligase. The polyubiquitinated HIF-2α is rapidly degraded by proteasomes. (B) When renal EPO-producing cells are hypoxic, HIF-2α is not hydroxylated and accumulates because it is not degraded by the ubiquitin–proteasomal pathway. HIF-2α forms heterodimers with HIF-1β and associates with two other components of the HIF transcription complex, hepatocyte nuclear factor-4 (HNF4) and p300. The HIF transcription complex binds to a hypoxia-inducible enhancer located 6–14 kilobase pairs upstream of the *EPO* coding sequences, and thereby increases *EPO* transcription and accumulation of EPO messenger RNAs. EPO mRNA is promptly translated, and EPO is secreted into the blood such that increases in circulating EPO can be detected within two hours of experiencing hypoxia.

hydroxylases (HIF-PHDs) that contain nonheme iron at their active sites and have molecular oxygen as a substrate. The transcription complex containing HIF-2α regulates renal *EPO* transcription through an enhancer that is located 6–14 kbp upstream of the *EPO* coding region.[71] Once hypoxia reaches the threshold that triggers *EPO* transcription, the resultant EPO messenger RNA is translated into the EPO glycoprotein, which is immediately secreted.[65] When an individual cell is triggered to produce EPO, it does so in an all-or-none manner.[65,76] Thus, EPO concentrations in the blood increase sharply within two hours after loss of blood, hemolysis, or a sudden decrease in atmospheric oxygen.

Effects of erythropoietin on erythroid progenitor cells

In the marrow, EPO binds to transmembrane glycoprotein erythropoietin receptors (EPO-Rs), which are first displayed on the surface of erythroid progenitor cells before the CFU-E stage and persist until the late basophilic erythroblast stage (Figure 13.1).[76] The binding of EPO to EPO-Rs leads to three major events: (1) homodimerization and conformational alterations of EPO-Rs, (2) initiation of intracellular signaling by the EPO-Rs, and (3) endocytosis of the EPO–EPO-R complexes, which are degraded via lysosomal activity.[77,78] Dimerization and structural changes of EPO-Rs after EPO binding induce both signaling and endocytosis. The endocytosis and intracellular degradation of the EPO–EPO-R complexes appear to be the normal mechanism for the clearance of EPO from the blood.[79] EPO-Rs have no intrinsic enzyme activity, but they interact with several signal transduction pathways through Janus tyrosine kinase-2 (JAK2). JAK2 physically associates with the cytoplasmic portion of EPO-Rs, chaperones EPO-Rs to the surface of the erythroid cell, and is activated by the conformational changes in the EPO-Rs produced by the binding of EPO.[80,81] Activated JAK2 phosphorylates itself and EPO-Rs as well as initiates signal transduction pathways that include signal transduction and activator of transcription-5 (STAT5), RAS–RAF–MAP kinases, and phosphoinositol–3 kinase/AKT kinase (protein kinase B).[82,83]

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Although the mechanisms linking EPO-R signaling to the biological effects of EPO have not been determined, EPO prevents the apoptotic death of erythroid progenitor cells in CFU-E through early BasoEB stages.[84–87] During EPO dependence, individual erythroid cells at the same stage of differentiation can display wide variation in their degree of dependence on EPO for survival.[88] Such variable susceptibility to apoptosis among EPO-dependent progenitors appears to be due to expression levels of FAS, a membrane protein of the tumor necrosis factor (TNF) family, which triggers apoptosis when it binds FAS ligand.[89] EPO, in turn, acts to decrease FAS expression in erythroid progenitors. FAS ligand, which binds and activates FAS, is produced in a constitutive manner in the marrow, mainly by mature erythroblasts in humans.[90] Thus, within the EBI, a negative feedback loop from the terminally differentiating erythroblasts can modulate the rate of CFU-E–ProEB apoptosis and indirectly control rates of erythrocyte production.[89] By a more indirect mechanism, EPO signaling also appears to protect late-stage erythroblasts from apoptosis, including in the post-EPO-dependent period, by inducing large amounts of the antiapoptotic protein BCL-X_L.[91,92]

Erythrocyte production kinetics based on EPO levels

A model that incorporates varying plasma EPO levels and heterogeneity in EPO dependence among the EPO-dependent progenitors has been proposed to explain various physiologic and pathologic rates of erythrocyte production.[93] In an expanded version of this model, erythroid progenitors enter the EPO-dependent period of differentiation, left of the dotted line in Figure 13.4, extending from the CFU-E through the early BasoEB stage and encompassing three generations of cells. The proportion of total cells that survive in a generation is shown under the population. The surviving cells are represented by circles, each of which contains a large black dot representing an intact nucleus. The cells lost to apoptosis are shown by circles containing an *X*. The number of surviving cells in a generation results in twice that number for the total cells in the subsequent generation. Most cells reaching the CFU-E stage need more EPO than the low levels found in normal plasma to sustain them and their progeny through the EPO-dependent period of differentiation. As a result, approximately 200–250 billion erythrocytes produced daily by a normal, healthy adult are the descendants of a minority of all the potential erythroid progenitor cells that could be generated during the EPO-dependent period (Figure 13.4A). When blood loss, hemolysis, or decreased atmospheric oxygen is encountered, plasma EPO increases, allowing the survival of many EPO-dependent progenitors that would die by apoptosis under normal conditions (Figure 13.4B). This enhanced survival increases reticulocyte production within a few days after encountering blood loss, hemolysis, or decreased atmospheric oxygen. The increased reticulocytosis leads to increasing erythrocyte numbers until oxygen delivery recovers to normal, accompanied by declining plasma EPO levels until normal levels are achieved. In pathologic states of chronically decreased oxygen delivery, such as lung disease or cardiac diseases with right-to-left shunts, the persistently increased EPO levels (and increased glucocorticoids and BMP4) allow greater-than-normal survival of EPO-dependent cells such that the total number of erythrocytes is maintained in the polycythemic range. Likewise, the acquired somatic mutation of JAK2 (V617F) that is associated with hyperactivity of EPO-R signaling most commonly results in polycythemia vera.[94]

When plasma EPO levels fall below normal, many erythroid progenitor cells that would survive the EPO-dependent period of differentiation under normal conditions die by apoptosis resulting in anemia from decreased reticulocyte production (Figure 13.4C). Renal disease is the major cause of decreased EPO. In mouse models of renal disease, EPO-producing renal cortical fibroblasts are transformed by TNFα signaling through NFκB into proliferating myofibroblasts that do not produce EPO.[95] Other clinical diseases noted to have decreased EPO levels are inflammatory disorders[68] and malignancies,[69] which are associated with increased inflammatory cytokines including TNFα, indicating that decreases in plasma EPO contribute to the anemia of chronic inflammation.

Nutritional requirements for erythropoiesis

Although erythropoiesis is finely regulated by the oxygenation–EPO feedback mechanism shown in Figure 13.2, the erythropoietic process is frequently limited by an insufficient supply of folate, vitamin B_{12}, or iron. Folate and vitamin B_{12} (cobalamin) are required for the synthesis of DNA, and the daily production of very large numbers of erythrocytes results in a large DNA synthesis requirement. Although iron is also needed by all proliferating cell populations, the erythroblasts need much more iron than any other cell type because they produce hemoglobin. Through the hypoxia-feedback mechanism, these nutrition-related anemias are associated with increased EPO levels,[67] but the increase in EPO is more limited in iron deficiency, as described in the "Iron Deficiency and Development of Microcytic Anemia" section, and increased EPO can only partially compensate for the decreased erythropoiesis caused by a specific nutrient deficiency. Administration of the deficient nutrient, however, results in the resolution of anemia in each of the deficiency states.

Deficiencies of folate or cobalamin and development of macrocytic anemia

After reduction to tetrahydrofolate (THF), folate functions as a carrier of one-carbon molecules and becomes a cofactor in the synthesis of three deoxyribonucleosides (dGTP, dATP, and TTP) that are required for DNA synthesis.[96] In two separate reactions, formyltetrahydrofolate (CHO-THF) provides two of the carbons in the synthesis of the purine precursor of adenosyl and guanosyl deoxyribonucleosides; in both of these reactions, 10-CHO-THF is converted to THF. In a third reaction, methylenetetrahydrofolate (CH2-THF) provides a methylene group and reducing equivalents in the methylation of deoxyuridylate to form thymidylate; in the process, CH2-THF is converted to dihydrofolate (DHF). To regenerate THF, the active one-carbon acceptor–donor form, DHF must be reduced by dihydrofolate reductase (DHFR).

Thus, drugs that inhibit DHFR such as methotrexate or trimethoprim–sulfamethoxazole cause a deficiency of THF, the functional form of folate. Cobalamin is a cofactor in the conversion of methyltetrahydrofolate (CH3-THF) to THF. CH3-THF, the most prevalent form of folate in plasma, is imported into cells and retained there by addition of polyglutamates. Cobalamin deficiency results in the trapping of folate in the CH3-THF form, from which it cannot be converted to THF and subsequently to the CHO-THF and CH2-THF forms required for deoxyribonucleoside synthesis.[97,98] Furthermore, CH3-THF is the poorest THF form for polyglutamation, resulting in generalized loss of intracellular folate.[96]

Folate deficiency, cobalamin deficiency, or drugs that inhibit DHFR will decrease intracellular levels of folate coenzymes needed for de novo synthesis of all the deoxynucleosides used in DNA synthesis, except for deoxycytidine. An inadequate supply of deoxynucleosides

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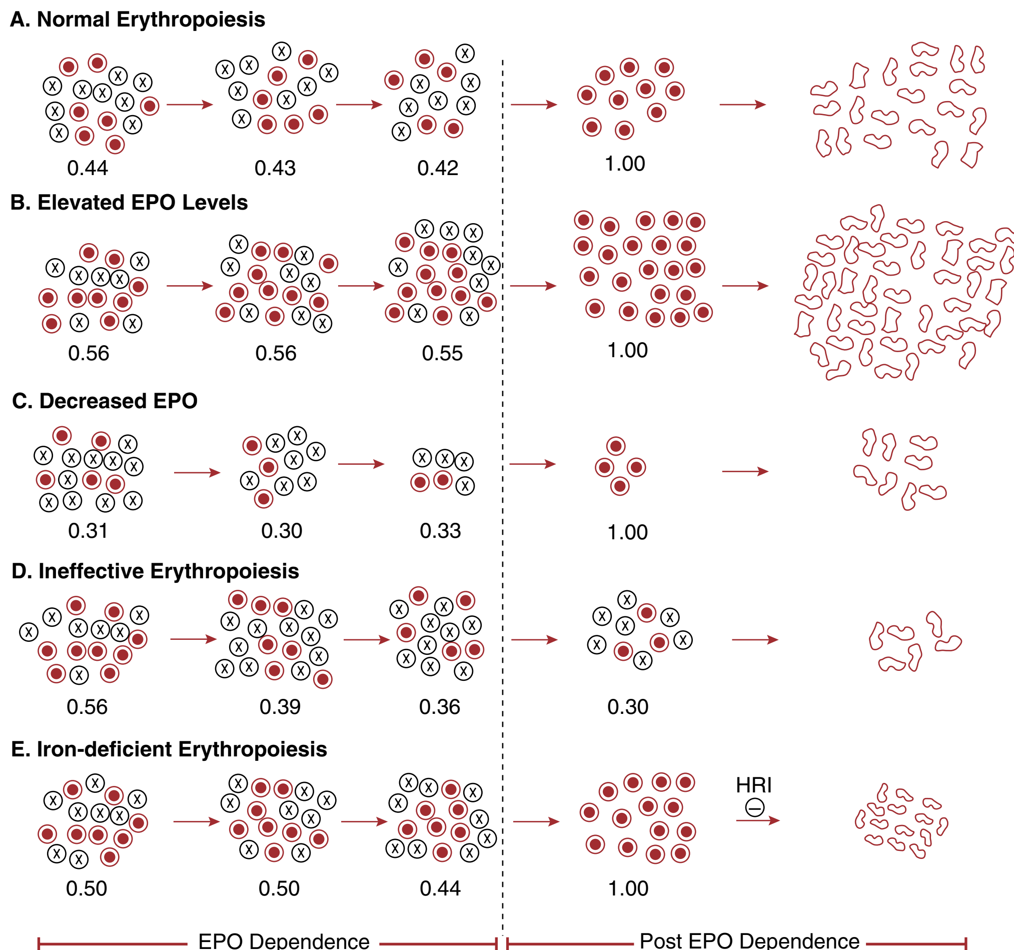

Figure 13.4 Model of erythropoiesis based on suppression of apoptosis by EPO and heterogeneity in EPO dependence among erythroid cells. From CFU-E through early basophilic erythroblast stages, erythroid progenitor/precursor cells depend on EPO for survival. The EPO-dependent period is left of the dotted line and encompasses three generations and two cell divisions. Each division is represented by an arrow. In the post-EPO-dependent period, to the right of the dotted line, two cell divisions occur. Surviving cells in each generation are shown as *circles* containing *large black dots* representing intact nuclei. Cells succumbing to apoptosis are shown as *circles* containing *X*s. The proportion of the total cells that survive is shown below each generation. The number of surviving cells in a generation results in twice that number of total cells in the subsequent generation. The final populations of cells shown on the right represent the anucleate, irregular reticulocytes. (A) Normal erythropoiesis, with average survival rates of 43% in the EPO-dependent generations, produces 200–250 billion reticulocytes daily. (B) Elevated EPO levels as found after acute blood loss or hemolysis increase survival rates to 56% in the EPO-dependent generation, and reticulocyte production rate more than doubles. (C) Decreased EPO levels, as found in renal failure, decrease survival rates to 32% in the EPO-dependent generation, and reticulocyte production is less than one-half of normal. (D) Ineffective erythropoiesis increases rates of apoptosis due to a pathologic process such as folate or vitamin B_{12} deficiency or β-thalassemia. High EPO levels in response to decreased erythrocyte production expand surviving cells in the early EPO-dependent generations, but the increased rates of apoptosis in the late EPO-dependent and post-EPO-dependent stages decrease daily reticulocyte production to less than one-third of normal. (E) Iron-deficient erythropoiesis with moderately elevated EPO activity for the degree of anemia slightly increases the survival rate during the EPO-dependent period, but in the post-EPO-dependent period, when hemoglobin is synthesized, heme-regulated inhibitor (HRI) prevents apoptosis by inhibiting general protein synthesis while enhancing the production of the mediator of stress erythropoiesis, the transcription factor ATF4. The inhibited protein synthesis decreases reticulocyte production rate, size, and hemoglobin content.

causes the accumulation of erythroid progenitors in the S phase of the cell cycle, which is followed by the induction of apoptosis.[99] Erythroid cells at the end of the EPO-dependent stage and the beginning of the period of hemoglobin synthesis appear to be most susceptible to this apoptosis. EPO-induced expansion of the EPO-dependent population at the CFU-E and proerythroblast stages leads to the presence of even greater numbers of these progenitor cells that subsequently undergo apoptosis just as they are beginning to produce hemoglobin.[100] The resultant clinical disease is megaloblastic anemia, which is characterized by ineffective erythropoiesis and macrocytic erythrocytes (Figure 13.4D). In ineffective erythropoiesis, progenitor cells in the EPO-dependent period expand due to increased EPO levels. The number of reticulocytes formed, however, is less than normal because of the increased rates of pathologic apoptosis in the EPO-dependent and post-EPO-dependent periods of differentiation.

Although the degree of ineffective erythropoiesis is prominent in megaloblastic anemia, the same process of inhibited DNA synthesis but with less apparent apoptosis is common in many macrocytic anemias. Cell size reductions during normal terminal erythroid differentiation result from shortening of the G1 phase of cell cycle while the lengths of S and G2/M phases remain unaffected.[27] Terminally differentiating erythroblasts with delayed or prolonged

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cell cycle durations produce larger-than-normal erythrocytes because they accumulate larger amounts of protein during the protracted periods between cell divisions.[41] With folic acid fortification of grain products, which began in the United States in 1998, folate deficiency related to dietary intake has been largely eliminated,[101] except for those with malabsorption disorders.[102] However, cobalamin deficiency and drugs that interfere with folate metabolism such as methotrexate, trimethoprim–sulfamethoxazole, and anticonvulsants remain clinically relevant causes of macrocytic anemias.[103] Drugs that directly inhibit DNA synthesis such as antivirals (azidothymidine or zidovudine), immunosuppressives (azathioprine), and ribonucleotide reductase inhibitors (hydroxyurea) are major causes of macrocytic anemia.[103] In addition, several inherited and acquired marrow failure syndromes that cause macrocytic anemia have either directly or indirectly inhibited DNA synthesis and increased apoptotic loss of erythroid progenitors.[41] Those with direct DNA synthesis inhibition include Fanconi anemia, in which increased DNA crosslinking requires more DNA repair before cell division is completed,[104] and dyskeratosis congenita anemia, in which chromosomal telomeres cannot be maintained.[105] In addition to direct DNA damage, the induction of p53 in these erythroblasts contributes to both delayed cell cycle and apoptosis.[106] Other marrow failure diseases show indirect inhibition of DNA synthesis, such as Diamond–Blackfan anemia[107,108] and 5q-myelodysplastic syndrome anemia,[109] in which impaired ribosomal biogenesis and/or function leads to secondary inhibition of DNA synthesis and accompanying apoptosis by p53 induction.[108]

Iron deficiency and development of microcytic anemia

In addition to its oxygen transport function in hemoglobin, iron has essential roles in heme as part of myoglobin, mitochondrial cytochromes, and peroxidases. Among many nonheme enzymes, iron is required by four enzymatic processes described in other sections of this chapter: prolyl hydroxylation in HIF-α stability, mitochondrial ferrochelatase insertion of iron into protoporphyrin, ribonucleotide reductase in deoxynucleoside synthesis, and aconitase in glucose metabolism. Two-thirds of the body's iron is in the hemoglobin of circulating erythrocytes, and iron deficiency most commonly arises from blood loss. Two milliliters of blood contains about 1 mg of iron, which is approximately the amount absorbed daily by the duodenum, balancing the 1 mg normally lost through shedding of gastrointestinal tract and skin cells. Erythroid progenitor cells are the greatest consumers of iron in the body, using about 25 mg daily under normal conditions, with most of the iron that is supplied to erythroid cells being recycled from macrophages that phagocytose senescent erythrocytes and degrade their hemoglobin. When erythropoietic demands are increased after bleeding or hemolysis, duodenal iron absorption is increased indirectly by erythroferrone, a hormone produced by erythroblasts that decreases hepatic production of hepcidin.[110] Hepcidin, a 25-amino-acid hormone produced in the liver, is induced by increased plasma iron and by inflammatory cytokines.[111] Hepcidin binds and downregulates surface expression of ferroportin, the cellular iron exporter of iron for all cells, including duodenal enterocytes, which are responsible for iron absorption.[112] The increased absorption mediated by erythroferrone is limited, however, and chronic blood loss of as little as 5 mL per day may cause iron deficiency.[113]

Specific regulators of iron and heme metabolism protect cells from the toxic effects of iron while assuring that crucial cellular processes that rely on iron are sustained in nonerythroid cells during iron deficiency. Therefore, as iron deficiency develops, erythropoietic utilization of iron becomes restricted and anemia develops. In iron-deficient cells, iron regulatory proteins (IRP1 and IRP2) bind to iron responsive elements (IREs) in 5′- and 3′-untranslated regions (UTRs) of mRNAs controlling production of proteins involved in cellular iron import, export, and storage.[114,115] Under iron-replete conditions, IRP1 functions as the enzyme aconitase with an iron–sulfur cluster in its active site; under iron-deficient conditions, IRP1 lacks the iron–sulfur cluster and binds IREs. IRP2 is rapidly degraded under iron-replete conditions but is stable and binds IREs during iron deficiency. IRP binding of IREs in the 5′-UTR of mRNAs inhibits their translation, decreasing their production. Two important examples are mRNAs for ferroportin and ferritin, the intracellular storage protein, both of which decrease during iron deficiency, allowing the maintenance of normal intracellular iron levels. In contrast, IRP binding of IREs in the 3′-UTR of mRNAs stabilizes them and enhances their translation. An example is transferrin receptor mRNAs, where IRP binding increases transferrin receptor production, thereby increasing cellular iron importation.

Although translation of ferroportin mRNA is controlled by the 5′-IRE in most cells, alternative splicing in duodenal enterocytes and erythroid precursor cells produces ferroportin mRNAs without 5′-IREs.[116,117] Thus, during iron deficiency, ferroportin expression is sustained in these two cell types, allowing uncompromised iron exportation into the plasma from duodenum and diminished accumulation within erythroid precursors and erythrocytes.[118,119] During iron deficiency, IRPs increase binding to 5′-IREs of two key mRNAs involved in erythropoiesis. IRP1 binds a 5′-IRE in HIF-2α mRNAs, leading to decreased translation of HIF-2α messages in the renal cortical fibroblasts that are capable of producing EPO.[120–122] The decreased intracellular HIF-2α protein results in less EPO production despite the hypoxia of the renal cortex from the decreased numbers of circulating erythrocytes. As a result, renal EPO production may be relatively diminished in the anemia of iron deficiency when compared to other anemias of similar severity. In erythroid progenitors, HRI-mediated increases in ATF4 and its target *GRB10* can dampen EPO signaling.[43] The decreased EPO activity in iron deficiency, relative to anemia from blood loss or hemolysis, leads to relatively increased apoptosis of erythroid cells in the EPO-dependent stages that immediately precede the stages that synthesize hemoglobin (compare Figures 13.4B and 13.4E).

In the later hemoglobin-producing stages of iron-deficient erythropoiesis, IRP1 binds a 5′-IRE in mRNAs encoding ALAS2, which is the rate-controlling enzyme in porphyrin synthesis.[38] The resultant decreases in ALAS2 lead to less accumulations of protoporphyrin and heme in the erythroblasts. The decreased heme in erythroblasts increases HRI activity, which inhibits protein synthesis in general and globin syntheses in particular.[43] The combined effects of relatively decreased EPO activity and HRI-mediated restriction of protein synthesis in iron deficiency result in a slower rate of completion of the terminal stages of erythroblasts, with decreased rates of red blood cell production resulting in hypochromic, microcytic anemia as shown in Figure 13.4E.

In addition to iron deficiency anemia, HRI plays a role in other microcytic anemias in which heme production is limited. Inherited disorders of ALAS2 cause sideroblastic anemia as iron accumulates in mitochondria when porphyrin synthesis does not provide sufficient protoporphyrin IX for heme formation. Likewise, mutations in ferrochelatase decrease intracellular heme.

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Mice deficient in HRI that become iron-deficient or have impaired porphyrin synthesis die from anemia when excess globin chains that cannot form hemoglobin without heme precipitate, denature, and cause oxidative damage resulting in apoptosis of erythroblasts.[123,124] Thus, HRI rescues iron-deficient erythroblasts from the thalassemia-like phenotype of oxidative damage from excess globin chains by restricting globin chain synthesis when heme synthesis is insufficient.

Thalassemia and the development of ineffective erythropoiesis and microcytosis

Thalassemia is the other major type of microcytic anemia. When thalassemia is severe, it is treated with chronic red cell transfusions. Thalassemias are caused by mutations that decrease the synthesis of either α- or β-globin with intracellular accumulations of the excess unpaired α- or β-globin chains.[125] Compared to the excess β-globin chains in α-thalassemia, which form tetramers of hemoglobin H, the excess α-globin chains in β-thalassemia are relatively insoluble. Excess free α-globin chains in β-thalassemia are partially decreased by accumulation of γ-globin chains producing fetal hemoglobin, binding to AHSP, ubiquitination–proteasomal degradation, and autophagy of aggregated α-globins.[126] If unpaired globin chains are not removed by these intracellular adaptations, they can precipitate and denature, leading to the formation of methemoglobin and hemichromes that bind, oxidize, and disrupt the function of erythroid membrane and membrane skeletal proteins.[127] When the cytoplasmic domain of Band 3 is affected by this oxidative damage, it leads to aggregation, deposition of anti-Band 3 IgG, complement fixation, and phosphatidylserine externalization that in turn targets the cells for erythrophagocytosis.[128,129] The decreased solubility of unpaired free α-globins in β-thalassemias results in ineffective erythropoiesis due to intramedullary apoptosis of erythroblasts, whereas α-thalassemias have relatively less erythroblast apoptosis but more erythrocyte hemolysis.[130] Apoptosis in the β-thalassemias affects the late stages of erythroblast differentiation, and, because the mitigation of the EPO response due to IRP activity in iron deficiency does not occur in thalassemia, erythroid progenitors and early-stage erythroblasts expand in response to increased EPO. These early-stage erythroid populations expand extensively, with the degree of expansion directly related to the rate of apoptosis in the late-stage erythroblast populations.[131] The large expansion of erythroblast populations in the more severe cases of β-thalassemia increases erythroferrone, which increases iron absorption and results in iron overload that complicates and limits transfusion therapy in these patients.[110]

Depending upon the severity of thalassemia, the oxidative stress due to denatured globin chains, heme, and nonheme iron can overwhelm the erythroblast's normal antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, and toxic oxygen species scavengers, such as reduced glutathione and peroxiredoxin.[132] In these oxidation-stressed thalassemic erythroblasts, HRI has also been found to have an antioxidant effect via increased Atf4, which induces expression of antioxidant genes, including heme oxygenase-1 (HO1), the first step in the degradation of heme.[133] In mice, HRI deficiency converts the moderate anemia of β-thalassemia intermedia into an embryonic lethal anemia with extensive accumulations of precipitated and denatured α-globin chains.[124] The antioxidant activity of HRI is accompanied by its general restriction of protein synthesis so that the phenotype in thalassemias is a microcytic, hypochromic anemia.

Anemia of chronic inflammation

Diseases that can secondarily decrease erythropoiesis include those that directly displace the EBIs in the bone marrow, such as metastatic neoplasms, lymphoid neoplasms, and myelofibrosis.[134,135] However, the most common cause of secondary inhibition of erythropoiesis is anemia of chronic inflammation (ACI), which occurs in patients with chronic infections, neoplasms, and inflammatory diseases. ACI has multiple components in common with the various mechanisms shown in Figure 13.4. These components include direct and indirect inhibitory effects of specific inflammatory cytokines on erythropoietic cells and their hematopoietic progenitors. Inflammatory cytokines with recognized inhibitory mechanisms include interleukin-1 (IL1), IL6, TNFα, and interferon-γ (IFNγ).[136] Direct inhibition of cell survival and growth by IFN-γ involves the induction of PU.1 in MEPs which suppresses erythroid differentiation and promotes megakaryocytic differentiation. In the subsequent stages of EPO dependence, IFN-γ enhances the expression of members of the apoptosis-inducing TNF receptor family, including receptors for TNFα, FAS, TNF-related apoptosis-inducing ligand (TRAIL), TNF-like weak inducer of apoptosis (TWEAK), and receptor-binding cancer antigen expressed on SiSo cells (RCAS1).[137–139] The EPO-dependent stages are also affected indirectly by decreased EPO production that is induced by TNFα. Although the concentrations of TNFα to which the EPO-producing fibroblasts in the renal cortices are exposed are lower than when the inflammation is within the renal tissue, plasma EPO levels are lower in ACI than in other anemic states without inflammation.[68,69]

The later stages of erythropoiesis when hemoglobin is produced have relatively restricted iron supplies due to IL6 and members of the bone morphogenetic protein (BMP) family that induce transcription of hepcidin in the liver.[111] Experimental models show that hepcidin induction can be mediated by IL6 signaling through the JAK2–STAT3 pathway[140–142] or by bacterial endotoxin signaling through the BMP–Smad1/5/8 signaling pathway.[143] Hepcidin downregulates ferroportin on all cells, but its effects on three specific types of cells are most important for the inhibition of erythropoiesis in ACI. In macrophages, decreased ferroportin greatly diminishes the recycling of iron recovered from phagocytosed senescent erythrocytes. This sequestration of iron in macrophages is partially offset in ACI erythroblasts because hepcidin-induced decreases in erythroid cell ferroportin limit their iron export compared to erythroid cells in iron deficiency.[118] In severe cases of ACI, the downregulation of ferroportin on duodenal enterocytes restricts iron absorption, and eventually iron deficiency can develop. When iron deficiency complicates ACI, HRI activity causes the usually normocytic anemia to become microcytic. Most of the studies have focused on the acute phase of inflammation. The long-term consequences and the development of an EPO-refractory anemia remain unclear.

Erythropoietic therapies

Enhancement of erythropoiesis to reduce the frequency of RBC transfusion may be achieved by increasing erythropoietic progenitors, supplying exogenous stimulators of erythropoiesis, increasing endogenous production of erythropoietic stimulators, and reducing effects of negative regulators of erythropoiesis. In aplastic anemia, decreased numbers of HSCs and MPPs result in decreased erythroid progenitors that descend from them. Immune suppression with cyclosporine plus antithymocyte globulin and stem cell stimulation with the thrombopoietin mimetic, eltrombopag, separately or in combination are effective in these patients.[144] In Diamond–Blackfan anemia (DBA), decreased progenitor cells are confined to the erythroid lineage and

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are the result of mainly mutant genes encoding ribosomal proteins. The majority of DBA patients respond, at least initially, to glucocorticoids hormones, prednisone or prednisolone, with expansion of erythroid progenitors.[145] In chronic, low-risk variants of myelodysplastic syndrome, the abnormal erythroid lineage involvement extends through the late-stage erythroblasts. About half of transfusion-dependent patients with the del(5q) karyotype can become RBC transfusion free with the immunomodulator, lenalidomide.[146] For those patients without del(5q) karyotype and low endogenous EPO levels, erythropoietic stimulating agents, recombinant human erythropoietin and darbepoetin, are effective alone and in combination with granulocyte-stimulating factor.[147] In patients with low-risk MDS[148] or beta-thalassemia,[149] luspatercept, which binds transforming growth factor-beta superfamily ligands including activin and growth-and-differentiation factor 11, increases erythropoiesis and reduces RBC transfusion requirements, but long-term effects are unknown. Patients with renal disease have EPO deficiency that is treated effectively with recombinant human erythropoietin rhEPO[150,151] or its modifications including hyperglycosylated rhEPO (darbepoetin)[152] and polyethylene glycol-conjugated rhEPO (CERA).[153] However, correction to or near normal ranges of hemoglobin or hematocrit is associated with increased thrombotic vascular events. HIF prolyl hydroxylase inhibitors stabilize HIF-2α and, thereby, increase endogenous *EPO* transcription, plasma EPO levels, and increase erythropoiesis in renal disease patients with anemia.[154–157] However, possible adverse long-term events of increased HIF such as VEGF-induced vessel growth in diabetic retinopathy or malignancies remain unknown.

Summary and outlook

Erythropoiesis, a component of hematopoiesis, is required for normal maintenance of red blood cell numbers and responds with increased production rates following blood loss or hemolysis. In the erythroid differentiation process, the rate of erythrocyte production is regulated largely by EPO, which is produced in the renal cortex in response to the tissue hypoxia that results from decreased oxygen delivery in anemic states. The oxygen–EPO feedback mechanism finely controls rates of erythrocyte production that never overshoot and result in polycythemia. This feedback mechanism, however, responds promptly to physiologic changes such as blood loss, hemolysis, or changes in atmospheric oxygen. Chronic underproduction of erythrocytes results in anemia, and effective treatments may prevent or reduce red blood cell transfusions for those with underproduction anemias. Use of recombinant EPO is routine for patients with the anemia of renal disease, an EPO deficiency state. Limited responses to EPO in patients with anemias due to malignancy or myelodysplasia, combined with an increased potential for thrombotic and cardiovascular complications of EPO therapy in general, have resulted in more restricted use of recombinant EPO or its modified forms in clinical practice. New therapeutic agents that increase endogenous EPO production or decrease the activity of negative erythropoietic regulators may help treat underproduction anemias. Deficiencies of folate and vitamin B12, which are required for the extensive cellular proliferation needed to produce erythrocytes, and iron, which is required to produce the major protein of erythrocytes, hemoglobin, are remediable causes of anemia. Chronic inflammation, another very common cause of anemia, is most improved by treating the primary disease, but new agents may enhance erythropoiesis in those cases in which treatment of the primary disease may be limited.

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A full reference list for this chapter is available at: www.wiley.com/go/simon/Rossi6

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