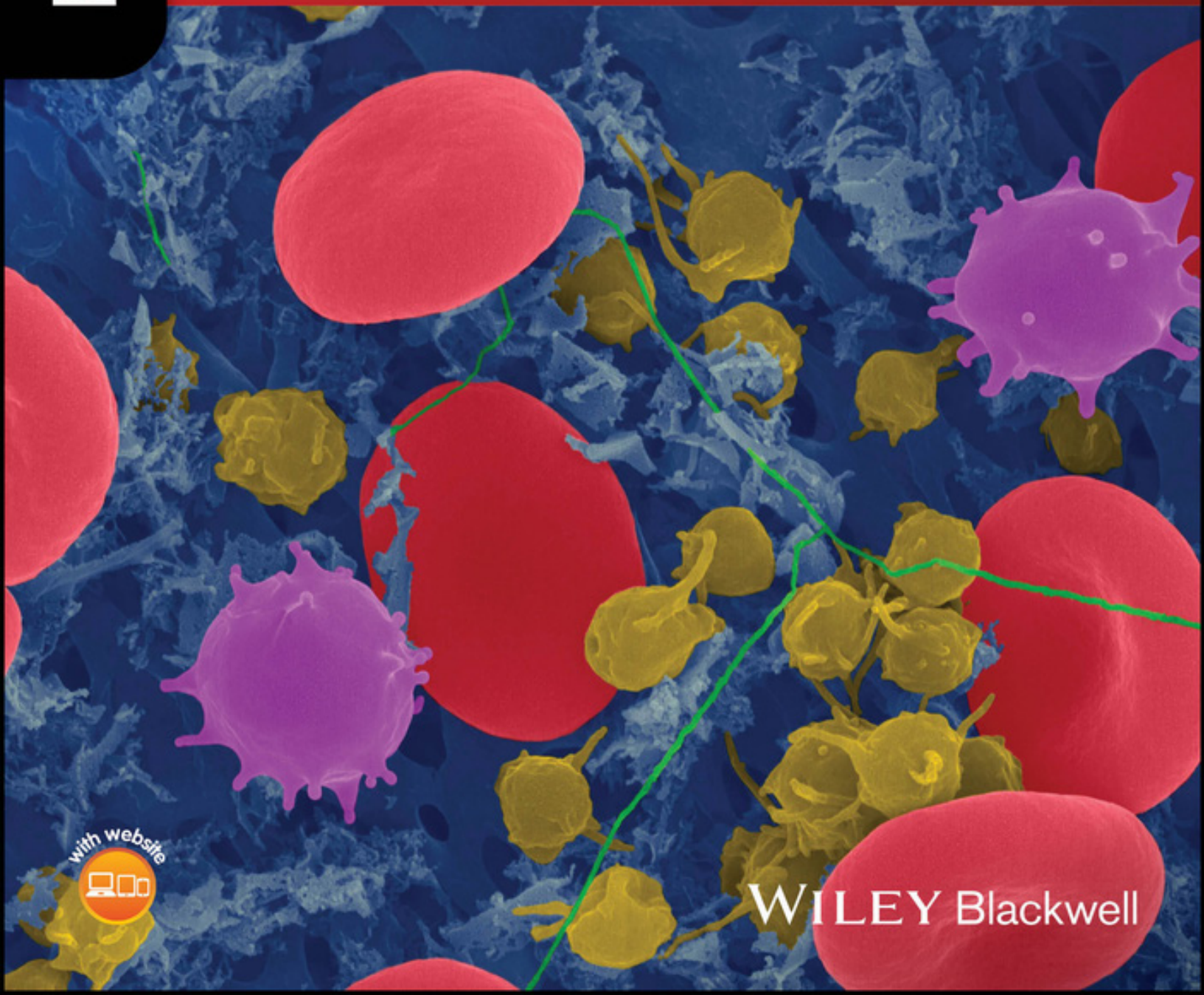
ESSENTIALS

HOFFBRAND'S ESSENTIAL HAEMATOLOGY

A. VICTOR HOFFBRAND
WITH PRATIMA CHOWDARY, GRAHAM COLLINS AND JUSTIN LOKE

NINTH EDITION



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Hoffbrand's Essential Haematology

Ninth Edition

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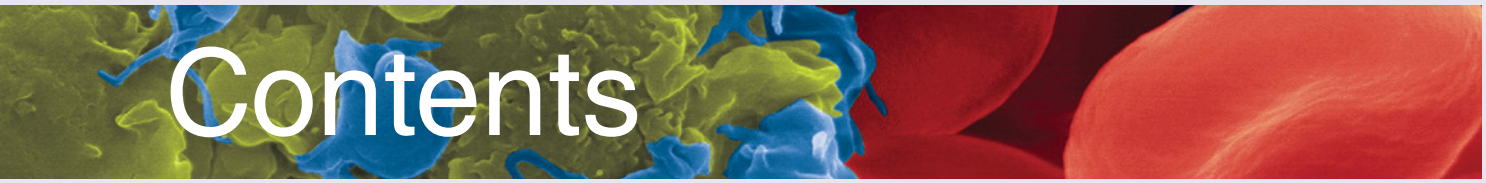

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Preface to the Ninth Edition

Advances in the understanding the pathogenesis of blood diseases and improvements in their treatment have continued apace in the 5 years since the eighth edition of *Hoffbrand's Essential Haematology* was published. Gene mutations are increasingly used to define and classify inherited and acquired haematological diseases, as a guide to therapy and to predict prognosis. Mutations underlying many rarer blood diseases have been identified, allowing appropriate panels of DNA probes to be established, facilitating the diagnosis of future cases. Many more drugs directed against specific sites in the cell signalling pathways have been approved.

The past five years have also seen substantial advances in immunological treatment for malignant haematological diseases. Mono- and bi-specific antibodies are increasingly incorporated into frontline therapy as well into treatment of relapsed disease. Chimeric antigen receptor (CAR)-T cells are challenging stem cell transplantation for potential cure for relapsed B-cell lymphoid neoplasms.

New drugs have also been introduced for treatment of benign (now termed in the United States 'classical') haematological diseases. These include mitapivat for pyruvate kinase deficiency, sutimlimab for cold agglutinin disease, luspatercept for anaemia in thalassaemia and myelodysplasia and pegcetacoplan for paroxysmal nocturnal haemoglobinuria. Drugs inhibiting prolyl hydroxylase in the hypoxia-inducible factor pathway are being developed to treat anaemia. They are already in illegal use for 'doping' of athletes to enhance their performance.

The fifth edition of the World Health Organisation (2022) Classification of the Haemato-lymphoid Tumours has been incorporated throughout this new edition and is given as an Appendix. The International Consensus Classification (ICC) of Myeloid Neoplasms, Acute Leukaemias and Mature Lymphoid Neoplasms was also published in 2022. It is beyond the scope of this book to compare and contrast the WHO and ICC classifications. Reference to the ICC classification are given in the Appendix.

David Steensma, the remarkable co-author of HEH8 stepped down for this new edition when he was appointed Global Head of Haematology at Novartis Institute for Biological Research. For the first time for *Essential Haematology*, there will be co-authorship by a specialist in the coagulation field. Professor Pratima Chowdary, Director of the Katherine Dormandy Haemophilia and Thrombosis Centre at the Royal Free Hospital, London, has ensured that the major section of the book dealing with bleeding and thrombotic disorders is authoritative and up to date. Graham Collins, Associate Professor of Haematology, Oxford Haematology and Cancer Centre has had the monumental task of updating the sections of HEH dealing with the lymphoid malignancies. Dr Justin Loke, AACR-CRUK Transatlantic Fellow, University of Birmingham, UK, now at the Dana-Farber Cancer Institute, Boston, USA, has undertaken the parallel task of updating the chapters dealing with the myeloid malignancies.

We are grateful to Dr Connor Sweeney, Professor Ashutosh Wechelaker and Professor Irene Roberts for their expert contributions to chapters 17 (acute lymphoblastic leukaemia), 23 (amyloid) and 34 (pregnancy and neonatal haematology), respectively. We are also grateful to Professor Barbara Bain who kindly checked the validity of our accompanying MCQs and to Dr Kirollos Kamel for his valuable contributions to chapters 30 and 31 (thrombosis and its management). We thank our publishers Wiley-Blackwell and especially Sophie Bradwell, Mandy Collison, Neelukiran Sekar and Kimberly Monroe-Hill for their unstinting help and support at all stages of production of this new edition. We also thank Jane Fallows for producing again such beautiful, clear diagrams.

Essential Haematology began life in 1980 as a textbook for medical students. We hope medical and other undergraduate students will continue to use it and share our excitement about one of the most advanced fields in medicine. With the vast expansion of knowledge of blood and its diseases over the past 44 years, the book has inevitably expanded. It is now also suitable for those beginning a career in haematology, for clinical and non-clinical scientists and nurses with an interest in blood and its diseases and for those working in closely related fields.

A. Victor Hoffbrand
London, 2024

About the Companion Website

Don't forget to visit the companion website for this book:

www.wiley.com/go/haematology9e 

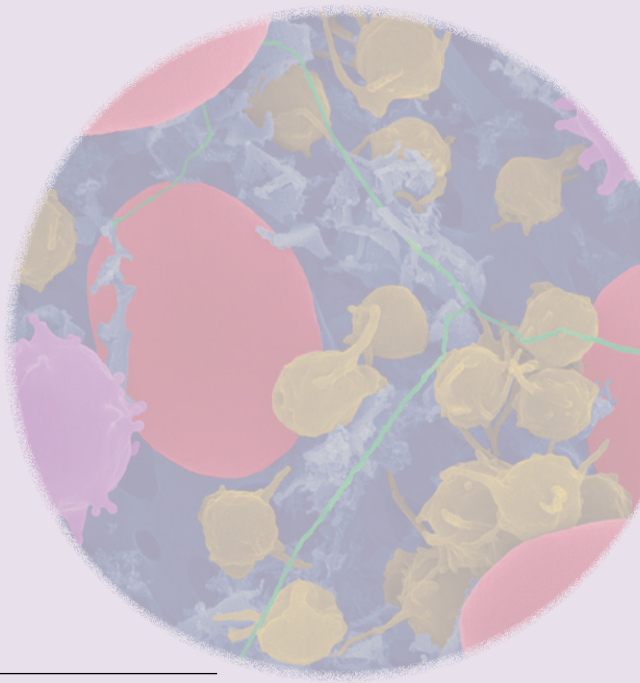
There you will find invaluable material designed to enhance your learning, including:

Multiple Choice Questions
Figures (PPT)
Tables (PDF)

CHAPTER 1

Haemopoiesis

Key topics



Hoffbrand's Essential Haematology, Ninth Edition. A. Victor Hoffbrand, Pratima Chowdary, Graham P. Collins, and Justin Loke.

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This chapter deals with the general aspects of blood cell formation (haemopoiesis). The processes that regulate haemopoiesis and the early stages of formation of red cells (erythropoiesis), granulocytes and monocytes (myelopoiesis) and platelets (thrombopoiesis) are also discussed.

Site of haemopoiesis

In the first few weeks of gestation, the embryonic yolk sac is a transient site of primitive haemopoiesis. Definitive haemopoiesis derives from a population of stem cells first observed in the aorta–gonads–mesonephros (AGM) region of the developing embryo. These common precursors of endothelial and haemopoietic cells are called haemangioblasts and seed the liver, spleen and bone marrow.

From 6 weeks until 6–7 months of foetal life, the liver and spleen are the major haemopoietic organs and continue to produce blood cells until about 2 weeks after birth (Table 1.1; see Fig. 7.1b). The placenta also contributes to foetal haemopoiesis. The bone marrow takes over as the most important site from 6 to 7 months of foetal life. During normal childhood and adult life, the marrow is the only source of new red cells, granulocytes, monocytes and platelets. The developing cells are situated outside the bone marrow sinuses; mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation.

In infancy all the bone marrow is haemopoietic, but during childhood and beyond there is progressive replacement of marrow throughout the long bones with fat cells, so that in adult life haemopoietic marrow is confined to the central skeleton and proximal ends of the femurs and humeri (Table 1.1). Even in these active haemopoietic areas, approximately 50% of the marrow consists of fat in the middle-aged adult (Fig. 1.1). The remaining fatty marrow is capable of reversion to haemopoiesis, and in many diseases there is also expansion of haemopoiesis down the long bones. Moreover, in certain disease states, the liver and spleen can resume their foetal haemopoietic role ('extramedullary haemopoiesis').

Table 1.1 Dominant sites of haemopoiesis at different stages of development.

Foetus	0–2 months (yolk sac)
	2–7 months (liver, spleen)
	5–9 months (bone marrow)
Infants	Bone marrow (practically all bones); dwindling contribution from liver/spleen that ceases in the first few months of life
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

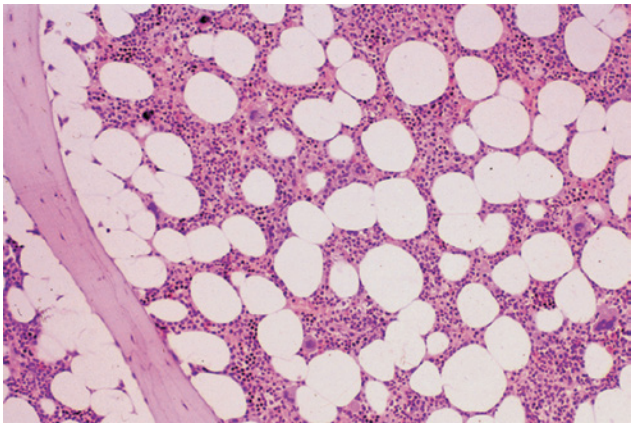

Figure 1.1 Normal bone marrow trephine biopsy (posterior iliac crest). Haematoxylin and eosin stain; approximately 50% of the intertrabecular tissue is haemopoietic tissue and 50% fat.

Haemopoietic stem and progenitor cells

Haemopoiesis starts with a pluripotent stem cell that can self-renew by asymmetrical cell division but also gives rise to the precursor of the separate cell lineages. The stem cells are able to repopulate a bone marrow from which all stem cells have been eliminated by lethal irradiation or chemotherapy. Self-renewal and repopulating ability define the **haemopoietic stem cell** (HSC). HSCs are rare perhaps 1 in every 20 million nucleated cells in bone marrow. Newer DNA sequencing techniques suggest that a typical adult has approximately 50 000 HSCs.

HSCs are heterogeneous, with some able to repopulate a bone marrow for more than 16 weeks, called **long-term HSCs**, while others, although able to produce all haemopoietic cell types, engraft only transiently for a few weeks and are called **short-term HSCs**. Although the exact cell surface marker phenotype of the HSC is still unknown, on immunological testing these cells are positive for the markers cluster of differentiation 34 (CD34), CD49f and CD90 and negative for CD38 and CD45RA and for cell lineage-defining markers (Lin). Morphologically, HSCs have the appearance of small- or medium-sized lymphocytes.

Cell differentiation occurs from the stem cells via committed **haemopoietic progenitors**, which are restricted in their developmental potential (Fig. 1.2). The existence of the separate progenitor cells can be demonstrated by *in vitro* culture techniques. Stem cells and very early progenitors are assayed by culture on bone marrow stroma as long-term culture-initiating cells, whereas later progenitors are generally assayed in semi-solid media. As examples, in the erythroid series progenitors can be identified in special cultures as burst-forming units (BFU-E, describing the 'burst' with which they form in culture) and

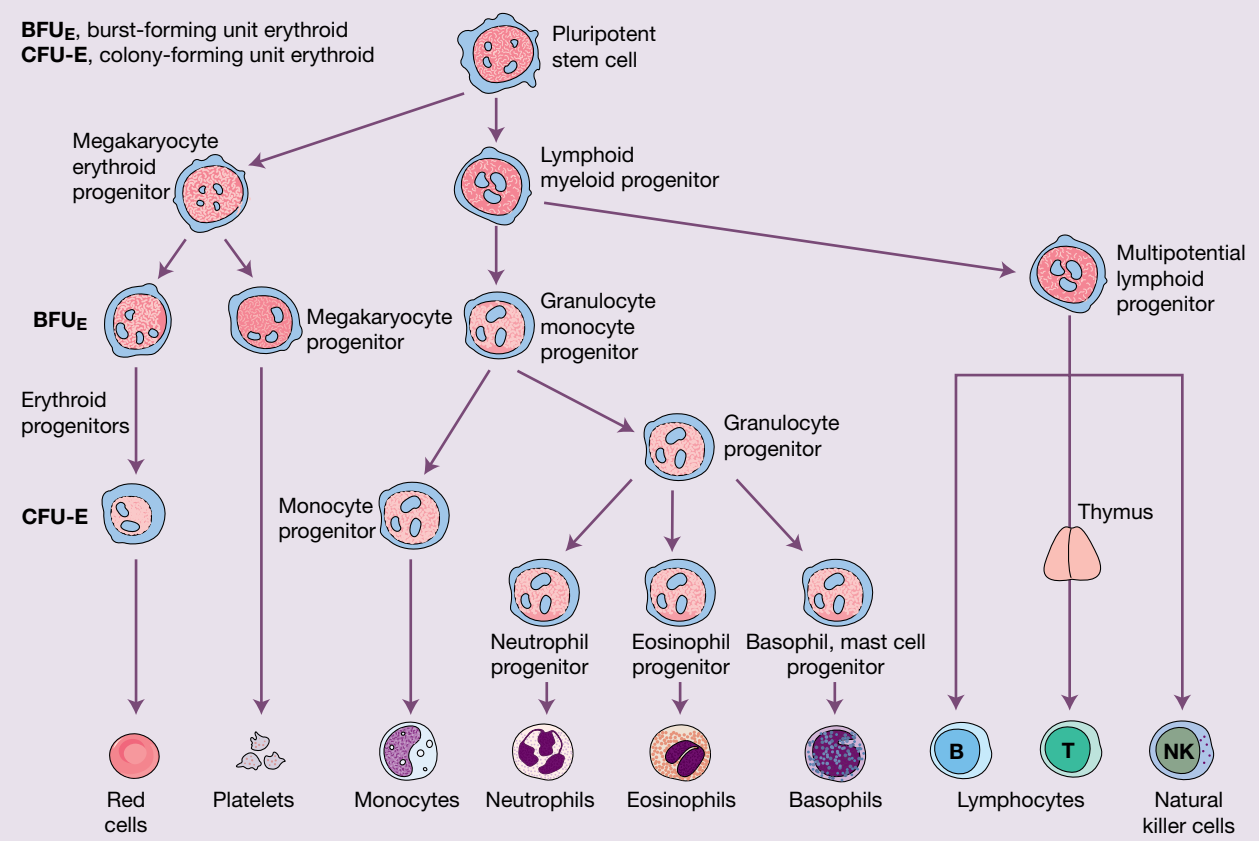

Figure 1.2 Diagrammatic representation of the bone marrow pluripotent stem cells (haemopoietic stem cells, HSC) and the cell lines that arise from them. A megakaryocytic/erythroid progenitor (MkEP) and a mixed lymphoid/myeloid progenitor are formed from the pluripotent stem cells. Each gives rise to more differentiated progenitors. BFU-E, burst-forming unit erythroid; CFU-E, colony-forming unit erythroid.

colony-forming units (CFU-E; Fig 1.2); the mixed granulocyte/monocyte progenitor is identified as a colony-forming unit-granulocyte/monocyte (CFU-GM) in culture. Megakaryocytes derive from a megakaryocyte progenitor, itself derived from an earlier mixed erythroid–megakaryocyte progenitor.

In the haemopoietic hierarchy, the pluripotent stem cell gives rise to a **mixed erythroid and megakaryocyte progenitor**, which then divides into separate erythroid and megakaryocyte progenitors. The pluripotent stem cell also gives rise to a **mixed lymphoid, granulocyte and monocyte progenitor**, which divides into a progenitor of granulocytes and monocytes and a mixed lymphoid progenitor, from which B- and T-cell lymphocytes and natural killer (NK) cells develop (Fig. 1.2). The spleen, lymph nodes and thymus are secondary sites of lymphocyte production (Chapter 9).

As the stem cell has the capability for **self-renewal** (Fig. 1.3), the marrow cellularity remains constant in a normal, healthy steady state. There is considerable amplification in the system: one stem cell is capable of producing about 10^6 mature blood cells after 20 cell divisions (Fig. 1.3). In humans, HSCs are capable of about 50 cell divisions (the 'Hayflick limit'), with progressive telomere shortening with each division affecting viability.

Under normal conditions most HSCs are dormant, with at most only a few percent active in cell cycle on any given day. Any given HSC enters the cell cycle approximately once every 3 months to 3 years in humans. By contrast, progenitor cells are much more numerous and highly proliferative. With ageing, the number of stem cells falls and the relative proportion giving rise to lymphoid rather than myeloid progenitors also falls. Stem cells also accumulate genetic mutations with age, an average of 8 exonic coding mutations by age 60 years (1.3 per decade). These, either passengers without oncogenic potential or drivers that cause clonal expansion, may be present in neoplasms arising from these stem cells (Chapters 11, 16).

The progenitor and precursor cells are capable of responding to haemopoietic growth factors with increased production of one or other cell line when the need arises. The development

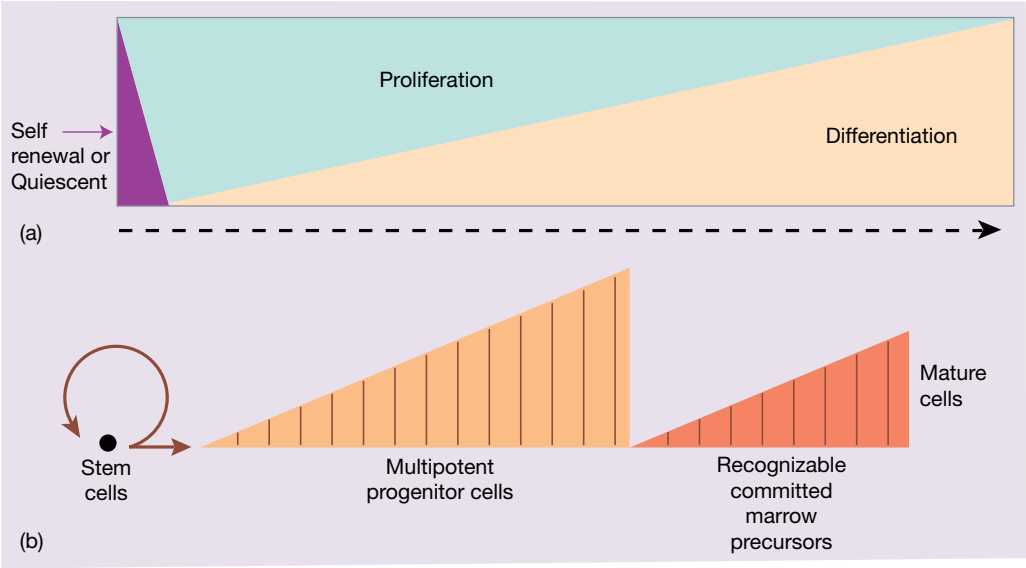

Figure 1.3 **(a)** Bone marrow cells are increasingly differentiated and lose the capacity for self-renewal as they mature. **(b)** A single stem cell gives rise, after multiple cell divisions (shown by vertical lines), to >10^6 mature cells.

of the mature cells (red cells, granulocytes, monocytes, megakaryocytes and lymphocytes) is considered further in other sections of this book.

Bone marrow stroma and niches

The bone marrow forms a suitable environment for stem cell survival, self-renewal and formation of differentiated progenitor cells. It is composed of various types of stromal cells and a microvascular network (Fig. 1.4). **The stromal cells include adipocytes, fibroblasts, macrophages, megakaryocytes, osteoblasts, osteoclasts, endothelial cells and mesenchymal stem cells (which have the capacity to self-renew and differentiate into osteocytes, adipocytes and chondrocytes).** The stromal cells secrete extracellular molecules such as collagen, glycoproteins (fibronectin and thrombospondin) and glycosaminoglycans (hyaluronic acid and chondroitin derivatives) to form an extracellular matrix.

The HSCs reside in two types of niche. These provide some of the growth factors, adhesion molecules and cytokines which support stem cells, maintaining their viability and reproduction, e.g. stem cell factor (SCF) expressed by stromal and endothelial cells binds to its receptor, KIT (CD117), on stem cells. The niches are either vascular, including arterioles and sinusoids that converge on a central vein, or endosteal with osteoblasts and osteoclasts closely associated with bone. Sympathetic nerves and non-myelinated Schwann cells are important regulators of stem cell quiescence or release.

Haemopoietic stem cells (as well as mesenchymal stem cells) traffic around the body. They are found in peripheral blood in low numbers. In order to exit the bone marrow, cells must cross the blood vessel endothelium, and this process of mobilization is enhanced for HSCs by the administration of growth factors such as granulocyte colony-stimulating factor (G-CSF). The reverse process, stem cell homing, depends on a chemokine gradient in which stromal-derived factor 1 (SDF-1), which binds to its receptor CXCR4 on HSC, is critical.

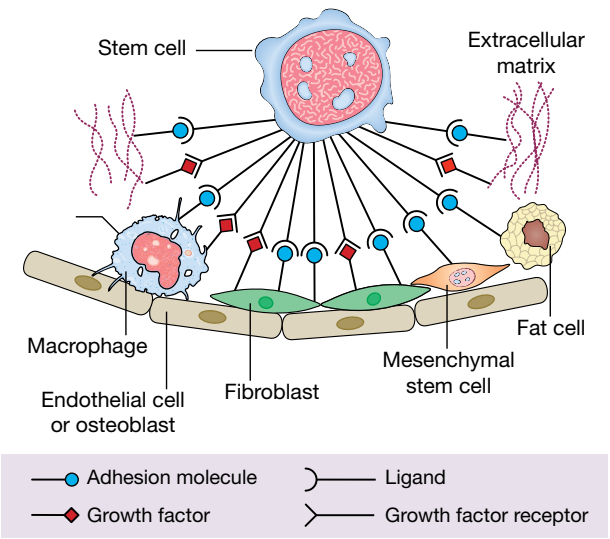

Figure 1.4 Haemopoiesis occurs in a suitable microenvironment ('niche') provided by a stromal matrix on which stem cells grow and divide. The niche may be vascular (lined by endothelium) or endosteal (lined by osteoblasts). There are specific recognition and adhesion sites; extracellular glycoproteins, e.g. fibronectin, collagen and other compounds, form a matrix and are involved in stem cell binding (see text).

The regulation of haemopoiesis

Transcription factors

Haemopoiesis starts with stem cell division in which one cell replaces the stem cell (*self-renewal*) and the other is committed to differentiation. These early committed progenitors express low levels of transcription factors that commit them to discrete cell lineages.

Transcription factors regulate gene expression by controlling the transcription of specific genes or gene families (Fig. 1.5). Typically, they contain at least two domains: a DNA-binding domain, such as a leucine zipper or helix–loop–helix motif which binds to a specific DNA sequence, and an activation domain, which contributes to the assembly of the transcription complex at a gene promoter. The transcription factors interact, so that reinforcement of one transcription programme may suppress that of another lineage

Which cell lineage is selected for differentiation depends on both chance and the external signals received by progenitor cells. Examples of transcription factors involved in haemopoiesis include RUNX1, GATA2 and MT2A in the earliest stages; GATA1, GATA2 and FOG1 in erythropoiesis and megakaryocytic differentiation; PU.1 and the CEBP family in granulopoiesis; PAX5 in B lymphocyte and NOTCH1 in T lymphocyte development. The transcription factors induce synthesis of proteins specific to a cell lineage. For example, GATA1 binds to specific motifs on the erythroid genes for globin and haem synthesis and so activates these genes. Mutation, deletion or translocation of transcription factor genes underlies many cases of haematological neoplasms (Chapter 11).

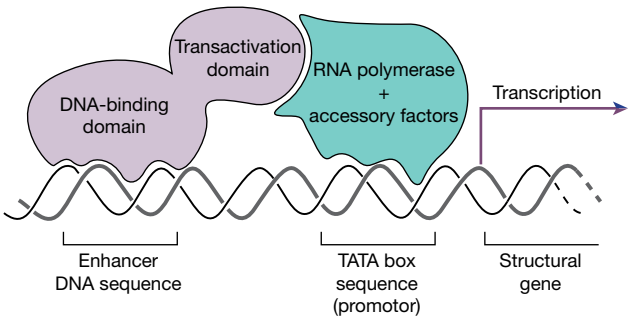

Figure 1.5 Model for control of gene expression by a transcription factor. The DNA-binding domain of a transcription factor binds a specific enhancer sequence adjacent to a structural gene. The transactivation domain then binds a molecule of RNA polymerase, thus augmenting its binding to the TATA box. The RNA polymerase now initiates transcription of the structural gene to form mRNA. Translation of the mRNA by the ribosomes generates the protein encoded by the gene. Transcription factors work in combination to both activate and repress the expression of a large number of genes.

Haemopoietic growth factors

The haemopoietic growth factors are a group of glycoproteins that regulate the proliferation and differentiation of haemopoietic progenitor cells and the function of mature blood cells. **They may act locally at the site where they are produced by cell–cell contact, e.g. SCF, or circulate in plasma, e.g. G-CSF or erythropoietin (EPO)**. They also bind to the extracellular matrix to form niches to which stem and progenitor cells adhere. The growth factors may cause cell proliferation, but can also stimulate differentiation and maturation, prevent apoptosis and affect the function of mature cells (Fig. 1.6).

The growth factors share a number of common properties (Table 1.2) and act at different stages of haemopoiesis (Table 1.3; Fig. 1.6). **Stromal cells are the major source of growth factors except for EPO, 90% of which is synthesized in the kidney, and thrombopoietin (TPO), made largely in**

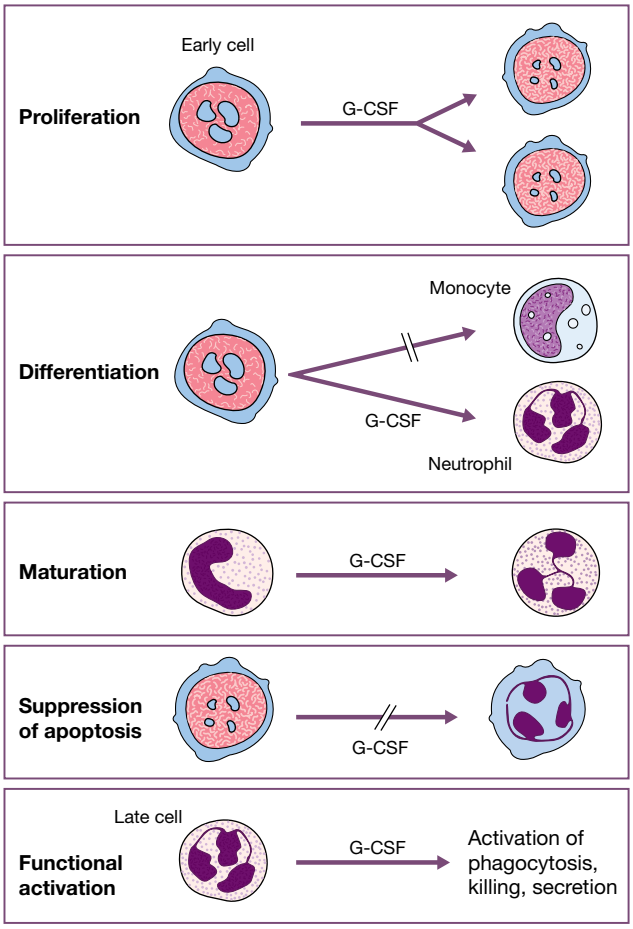

Figure 1.6 Growth factors may stimulate the proliferation of early bone marrow cells, direct differentiation to one or other cell type, stimulate cell maturation, suppress apoptosis or affect the function of mature non-dividing cells, as illustrated here for granulocyte colony-stimulating factor (G-CSF) for an early myeloid progenitor and a mature neutrophil.

Table 1.2 General characteristics of myeloid and lymphoid growth factors.

Glycoproteins that act at very low concentrations
Act hierarchically
Usually produced by many cell types
Usually affect more than one lineage
Usually active on stem/progenitor cells and on differentiated cells
Usually show synergistic or additive interactions with other growth factors
Often act on the neoplastic equivalent of a normal cell
Multiple actions: proliferation, differentiation, maturation, prevention of apoptosis, functional activation

Table 1.3 Haemopoietic growth factors (see also Fig. 1.7).

Act on stromal cells
IL-1, TNF
Act on pluripotential stem cells
SCF, TPO, FLT3-, NOTCH1
Act on multipotent lymphoid/myeloid progenitor cells
IL-3, IL-7, SCF, FLT3-L, TPO, GM-CSF
Act on lineage-committed progenitor cells
Granulocyte/monocyte production: IL-3, GM-CSF, G-CSF, M-CSF, IL-5 (eosinophil CSF)
Mast cell production: KIT-ligand
Red cell production:
IL-3, EPO
Platelet production: IL-3, TPO
Lymphocyte/NK cell production: IL-1, IL-2, IL-4, IL-7, IL-10, other ILs

CSF, colony-stimulating factor; EPO, erythropoietin; FLT3-L, FLT3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage/monocyte colony-stimulating factor; NK, natural killer; SCF, stem cell factor (also known as TAL1); TNF, tumour necrosis factor; TPO, thrombopoietin.

the liver. An important feature of growth factor action is that two or more factors may synergize in stimulating a particular cell to proliferate or differentiate. Moreover, the action of one growth factor on a cell may stimulate production of another growth factor or growth factor receptor.

SCF, TPO.NOTCH1 and FLT3 ligand act locally on the pluripotential stem cells and on myeloid/lymphoid progenitors (Fig. 1.7). Interleukin-3 (IL-3) has widespread activity on lymphoid/myeloid and megakaryocyte/erythroid progenitors. Granulocyte–macrophage colony-stimulating factor (GM-CSF), G-CSF and macrophage colony-stimulating factor (M-CSF) enhance neutrophil and macrophage/monocyte production, IL-5 eosinophil, KIT mast cell, TPO platelet and EPO red cell production. These lineage-specific growth factors also enhance the effects of SCF, FLT3-L and IL-3 on the survival and differentiation of early haemopoietic cells. Interleukin-7 is involved at all stages of lymphocyte production, and various other interleukins and toll-like receptor ligands (not shown) direct B and T lymphocyte and NK cell production (Fig. 1.7).

These factors maintain a pool of haemopoietic stem and progenitor cells on which later-acting factors, EPO, G-CSF, M-CSF, IL-5 and TPO, act to increase production of one or other cell lineage in response to the body's need. Granulocyte and monocyte formation, for example, can be stimulated by infection or inflammation through release of IL-1 and tumour necrosis factor (TNF), which then stimulate stromal cells to produce growth factors in an interacting network (Fig. 8.4). In contrast, cytokines, such as transforming growth factor-β (TGF-β) and γ-interferon (IFN-γ), can exert a negative effect on haemopoiesis and may have a role in the development of aplastic anaemia (p. 313).

Growth factor receptors and signal transduction

The biological effects of growth factors are mediated through specific receptors on target cells. Many receptors, such as the EPO receptor (EPO-R) and GM-CSF-R, are from the **haemopoietin receptor superfamily** which dimerize after binding their ligand.

Dimerization of the receptor leads to activation of a complex series of intracellular signal transduction pathways, of which the three major ones are the JAK/STAT (signal transducer and activator of transcription) pathway, the mitogen-activated protein (MAP) kinase and the phosphatidylinositol 3-kinase (PI3K) pathways (Fig. 1.8; see also Fig 9.4, Fig 15.2). The Janus-associated kinase (JAK) proteins are a family of four tyrosine-specific protein kinases that associate with the intracellular domains of the growth factor receptors (Fig. 1.8). A growth factor molecule binds simultaneously to the extracellular domains of two or three receptor molecules, resulting in their aggregation. Receptor aggregation induces activation of the JAKs, which then phosphorylate members of the STAT family of transcription factors. This results in their dimerization and translocation from the cell cytoplasm across the nuclear membrane to the cell nucleus. Within the nucleus STAT dimers activate the transcription of specific genes. A model for the control of gene expression by a transcription factor is shown in Fig. 1.5. The clinical importance of this pathway is revealed for example by the finding of an activating mutation of the *JAK2* gene as a cause of polycythaemia vera and related myeloproliferative neoplasms (p. 195).

JAK can also activate the MAPK pathway, which is regulated by RAS and controls proliferation. PI3 kinases phosphorylate

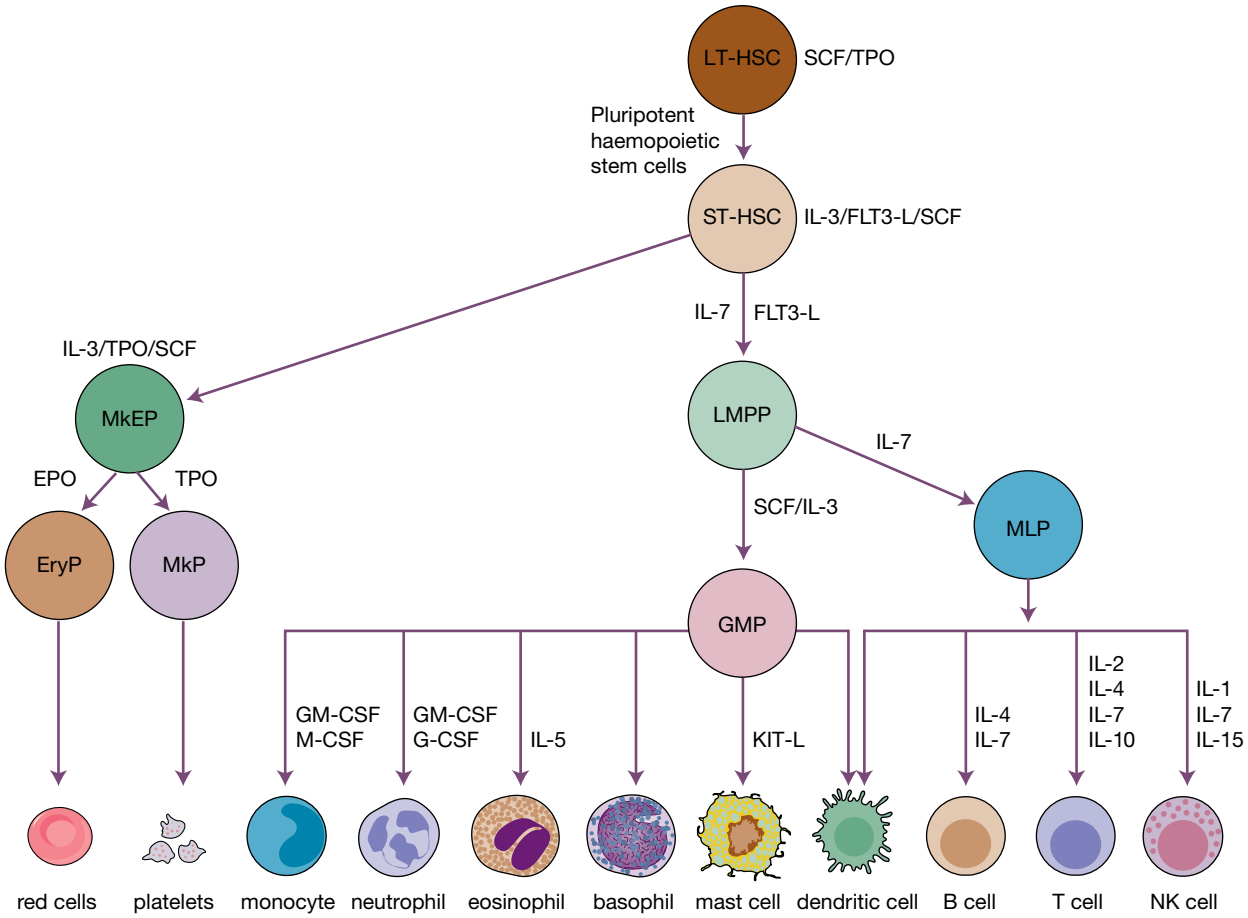

Figure 1.7 The role of growth factors in normal haemopoiesis. Multiple growth factors act on the earlier marrow stem and progenitor cells. EPO, erythropoietin; EryP, erythroid progenitor; FLT3-L, FLT3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; GMP, granulocyte–macrophage progenitor; HSC, haemopoietic stem cells; IL, interleukin; LMPP, lymphoid-primed multipotential progenitor; LT, long-term; M-CSF, macrophage/monocyte colony-stimulating factor; MkEP, megakaryocyte–erythroid progenitor; MkP, megakaryocyte progenitor; MLP, multipotential lymphoid progenitor; NK, natural killer; PSC, pluripotential stem cell; SCF, stem cell factor; ST, short-term; TLR, toll-like receptor; TPO, thrombopoietin. Source: Adapted from A.V. Hoffbrand *et al*. (2019) *Color Atlas of Clinical Hematology: Molecular and Cellular Basis of Disease,* 5th edn. Reproduced with permission of John Wiley & Sons.

inositol lipids, which have a wide range of downstream effects, including activation of AKT. This results in a block of apoptosis and other actions (Figs. 1.8, 15.2). Different domains of the intracellular receptor protein may signal for the different processes e.g. proliferation or suppression of apoptosis, mediated by growth factors.

A second, smaller group of growth factors, including SCF, FLT3L and M-CSF (Table 1.3), bind to receptors that have an extracellular immunoglobulin-like domain linked via a transmembrane bridge to a cytoplasmic tyrosine kinase domain. Growth factor binding results in dimerization of these receptors and consequent activation of the tyrosine kinase domain. Phosphorylation of tyrosine residues in the receptor itself generates binding sites for signalling proteins which initiate complex cascades of biochemical events, resulting in changes in gene expression, cell proliferation and prevention of apoptosis.

Adhesion molecules

Cell adhesion molecules (CAMs) are glycoprotein molecules which mediate the attachment of cells to each other, to the extracellular matrix and play roles in cell-cell synapse formation. They typically are composed of three domains: intracellular, transmembrane and extracellular. They are divided into four large families: integrins, immunoglobulin super family, selectins and cadherins. They function as 'molecular glue' maintaining tissue structure and function. The integrins are particularly

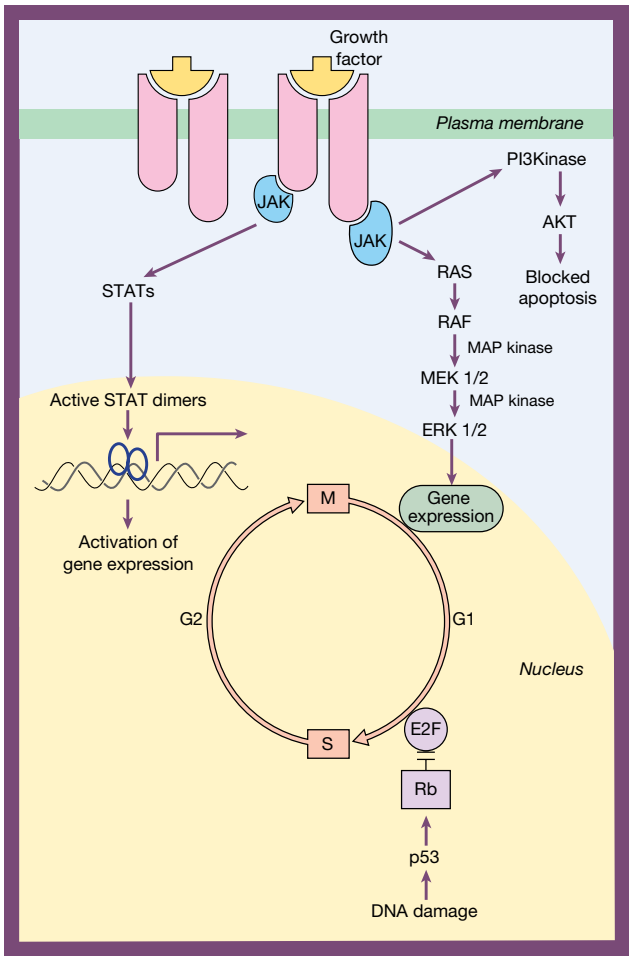

Figure 1.8 Control of haemopoiesis by growth factors. The factors act on cells expressing the corresponding receptors. Binding of a growth factor to its receptor activates the JAK/STAT, MAPK and phosphatidyl-inositol 3-kinase (PI3K) pathways (see also Fig. 15.2), which leads to transcriptional activation of specific genes. E2F is a transcription factor needed for cell transition from G1 to S phase. E2F is inhibited by the tumour suppressor gene Rb (retinoblastoma), which can be indirectly activated by p53. The synthesis and degradation of different cyclins stimulate the cell to pass through the different phases of the cell cycle. The growth factors may also suppress apoptosis by activating AKT (protein kinase B).

important in linking the extracellular environment including collagen, fibronectin and fibrinogen to intracellular signalling pathways. The selectins which include E (endothelial)-selectin, L (leucocyte)-selectin and P (platelet)-selectin are particularly important in the immune system in helping white cells in trafficking and homing.

In the bone marrow CAMs attach haemopoietic precursors, leucocytes and platelets to various components of the extracellular matrix, to endothelium, to other surfaces and to each other. The CAMs on the surface of leucocytes and platelets are termed receptors and these interact with proteins termed ligands on the surface of target cells, e.g. endothelium. The molecules are important in the development and maintenance of inflammatory as well as immune responses, and in platelet–vessel wall and leucocyte–vessel wall interactions. Glycoprotein IIb/IIIa, for example, is a CAM, also called integrin IIβ/IIIα and involved in platelet adhesion to vessel walls and to each other (Chapter 26).

The pattern of expression of adhesion molecules on tumour cells may determine their mode of spread and tissue localization e.g. the pattern of metastasis of carcinoma cells to specific visceral organs or bone or of non-Hodgkin lymphoma cells into a follicular or diffuse pattern. The adhesion molecules may also determine whether or not cells circulate in the bloodstream or remain fixed in tissues. They may also partly determine whether or not tumour cells are susceptible to the body's immune defences. Attempts to treat cancer and other diseases with drugs which inhibit specific adhesion molecules have so far been unsuccessful.

The cell cycle

The cell division cycle, generally known simply as the **cell cycle**, is a complex process that lies at the heart of haemopoiesis. Dysregulation of cell proliferation is also the key to the development of malignant disease. The duration of the cell cycle is variable between different tissues, but the basic principles remain constant. The cycle is divided into the mitotic phase (**M phase**), during which the cell physically divides, and **interphase**, during which the chromosomes are duplicated and cell growth occurs prior to division (Fig. 1.8). The M phase is further partitioned into classical **mitosis**, in which nuclear division is accomplished, and **cytokinesis**, in which cell fission occurs.

The interphase is divided into three main stages: a **G_1 phase**, in which the cell begins to commit to replication, an **S phase**, during which DNA content doubles and the chromosomes replicate, and the **G_2 phase**, in which the cell organelles are copied and cytoplasmic volume is increased. If cells rest prior to division, they enter a G_0 state where they can remain for long periods of time. The number of cells at each stage of the cell cycle can be assessed by exposing cells to a chemical or radiolabel that gets incorporated into newly generated DNA.

The cell cycle is controlled by two **checkpoints,** which act as brakes to coordinate the division process, at the end of the G_1 and G_2 phases. Two major classes of molecules control these checkpoints, **cyclin-dependent protein kinases** (Cdk), which phosphorylate downstream protein targets, and **cyclins**, which bind to Cdk and regulate their activity. An example of the importance of these systems is demonstrated by mantle cell lymphoma, which results from the constitutive activation of cyclin D1 as a result of a chromosomal translocation (p. 279).

Epigenetics

Epigenetics refers to changes in DNA and chromatin that affect gene expression other than those that affect DNA sequence (Fig. 16.1).

Cellular DNA is packaged by wrapping it around histones, a group of specialized nuclear proteins. The complex is tightly compacted as chromatin. In order for the DNA code to be read, transcription factors and other proteins need to physically attach to DNA. Histones act as custodians for this access and so for gene expression. Histones may be modified by methylation, acetylation and phosphorylation, which can result in increased or decreased gene expression and so changes in cell phenotype.

Epigenetics also includes changes to DNA itself, such as methylation of DNA bases. The methylation of cytosine residues to methylcytosine results in inhibition of gene transcription. The DNA methyltransferase genes *DNMT3A* and *B* are involved in this methylation. *TET1, 2, 3* and *IDH1* and *IDH2* are involved in the hydroxylation and breakdown of methylcytosine and restoration of gene expression (Fig. 16.1). These genes are frequently mutated in the myeloid malignancies, especially myelodysplastic syndromes and acute myeloid leukaemia (Chapters 13, 15 and 16).

Apoptosis

Apoptosis (programmed cell death) is a regulated process of physiological cell death in which individual cells are triggered to activate intracellular proteins that lead to the death of the cell. Morphologically it is characterized by cell shrinkage, condensation of the nuclear chromatin, fragmentation of the nucleus and cleavage of DNA at inter-nucleosomal sites. It is an important process for maintaining tissue homeostasis in haemopoiesis and lymphocyte development.

Apoptosis results from the action of intracellular cysteine proteases called **caspases**, which are activated following cleavage and lead to endonuclease digestion of DNA and disintegration of the cell skeleton (Fig. 1.9). There are two major pathways by which caspases can be activated. The first is by activation through membrane proteins such as Fas or TNF receptor via their intracellular death domain. An example of this mechanism is shown by activated cytotoxic T cells expressing Fas ligand, which induces apoptosis in target cells. The second pathway is via the release of cytochrome c from mitochondria. Cytochrome c binds to APAF-1, which then activates caspases. DNA damage induced by irradiation or chemotherapy may act through this pathway.

The protein p53 encoded by the *TP53* gene on chromosome 17 has an important role in sensing DNA damage. It activates apoptosis by raising the cell level of BAX, which then increases cytochrome c release (Fig. 1.9). p53 also shuts down the cell cycle to stop the damaged cell from dividing (Fig. 1.8). The cellular level of p53 is controlled by a second protein, MDM2. Following death, apoptotic cells display molecules that lead to their ingestion by macrophages. Loss of TP53 is a major mechanism by which malignant cells evade controls that would induce cell death.

As well as molecules that mediate apoptosis, there are several intracellular proteins that protect cells from apoptosis.

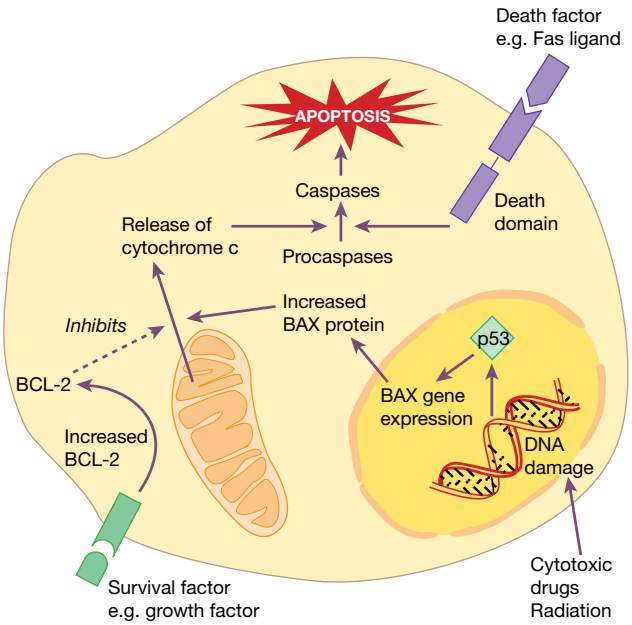

Figure 1.9 Representation of apoptosis. Apoptosis is initiated via two main stimuli: (i) signalling through cell membrane receptors such as FAS or tumour necrosis factor (TNF) receptor; or (ii) release of cytochrome c from mitochondria. Membrane receptors signal apoptosis through an intracellular death domain leading to activation of caspases which digest DNA. Cytochrome c binds to the cytoplasmic protein Apaf-1 leading to activation of caspases. The intracellular ratio of pro-apoptotic, e.g. BAX, or anti-apoptotic, e.g. BCL-2, members of the BCL-2 family may influence mitochondrial cytochrome c release. Growth factors raise the level of BCL-2, inhibiting cytochrome c release, whereas DNA damage, by activating p53, raises the level of BAX, which enhances cytochrome c release.

The best-characterized example is BCL-2. BCL-2 is the prototype of a family of related proteins, some of which are anti-apoptotic and some, like BAX, pro-apoptotic. The intracellular ratio of BAX and BCL-2 determines the relative susceptibility of cells to apoptosis, e.g. determines the lifespan of platelets, and may act through regulation of cytochrome c release from mitochondria.

Many of the genetic changes associated with malignant disease lead to a reduced rate of apoptosis and hence prolonged cell survival. The clearest example is the translocation of the *BCL2* gene to the immunoglobulin heavy chain locus in the t(14;18) translocation in follicular lymphoma (p. xxx). Overexpression of the BCL-2 protein makes the malignant B cells less susceptible to apoptosis. The drug venetoclax which inhibits BCL-2 is now widely used to treat both myeloid and lymphoid malignant diseases. Apoptosis is the normal fate for most B cells undergoing selection in the lymphoid germinal centres.

Several translocations leading to the generation of fusion proteins, such as t(9;22), t(11;14) and t(15;17), also result in inhibition of apoptosis (Chapter 11). In addition, genes encoding

proteins that are involved in mediating apoptosis following DNA damage, such as p53 and ATM, are also frequently mutated and therefore inactivated in haemopoietic malignancies.

Necrosis is death of cells and adjacent cells due to ischemia, chemical trauma or hyperthermia. The cells swell and the plasma membrane loses integrity. There is usually an inflammatory infiltrate in response to spillage of cell contents. Autophagy is the digestion of cell organelles by lysosomes. It may be involved in cell death, but in some situations also in maintaining cell survival by recycling nutrients.

SUMMARY

- Haemopoiesis (blood cell formation) arises from pluripotent stem cells in the bone marrow. Haemopoietic stem cells give rise to mixed and then single lineage progenitor and precursor cells which, after multiple cell divisions and differentiation, form red cells, granulocytes (neutrophils, eosinophils and basophils), monocytes, platelets, B and T lymphocytes and natural killer (NK) cells.
- Haemopoietic tissue occupies about 50% of the marrow space in normal adult marrow. Haemopoiesis in adults is confined to the central skeleton, but in infants and young children haemopoietic tissue extends down the long bones of the arms and legs.
- Stem cells reside in the bone marrow in osteoblastic or endothelial niches formed by stromal cells. They also circulate in the blood.
- Growth factors attach to specific cell surface receptors and produce a cascade of phosphorylation events in the cell nucleus.
- Transcription factors are molecules that bind to DNA and control the transcription of specific genes or gene families. They carry the message to those genes that are to be 'switched on or off', to stimulate cell division, differentiation or functional activity or to suppress apoptosis.
- Adhesion molecules are a large family of glycoproteins that mediate the attachment of marrow precursors and mature leucocytes and platelets to extracellular matrix, to endothelium and to each other.
- Epigenetics refers to changes in DNA and chromatin that affect gene expression other than those that affect DNA sequence. Histone modification and DNA (cytosine) methylation are two important examples relevant to haemopoiesis and haematological malignancies.
- Apoptosis is a physiological process of cell death resulting from activation of caspases. The intracellular ratio of pro-apoptotic proteins, e.g. BAX, to anti-apoptotic proteins, e.g. BCL-2, determines the cell susceptibility to apoptosis.

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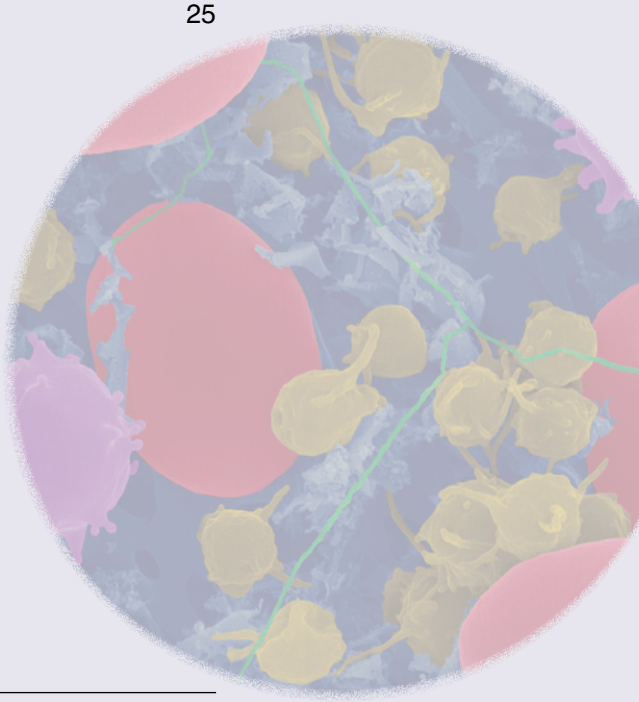
CHAPTER 2

Erythropoiesis and general aspects of anaemia

Key topics



Hoffbrand's Essential Haematology, Ninth Edition. A. Victor Hoffbrand, Pratima Chowdary, Graham P. Collins, and Justin Loke.

Companion website: www.wiley.com/go/haematology9e

Blood cells

All the circulating blood cells derive from pluripotential stem cells in the marrow. They divide into three main types. The most numerous are **red cells (erythrocytes)**, specialized for the carriage of oxygen from the lungs to the tissues and of carbon dioxide in the reverse direction (Table 2.1). They have a 4-month life span, whereas the smallest cells, **platelets** involved in haemostasis, circulate for only 10 days. **The white cells are made up of four main types of phagocyte: neutrophils, eosinophils, basophils and monocytes**, which protect against bacterial and fungal infections (Chapter 8); and of **lymphocytes**, which include **B cells**, involved in antibody production, **T cells** (CD4 helper and CD8 suppressor), concerned with the immune response and in protection against viruses and foreign cells, and **natural killer (NK) cells**, a subset of CD8 T cells (Chapter 9). White cells have a wide range of life span (Table 2.1).

The red cells and platelets are counted and their diameter and other parameters measured by an automated cell counter (Fig. 2.1). The counter also enumerates the different types of white cell by flow cytometry and detects abnormal cells.

Erythropoiesis

We each make approximately 10^{12} new erythrocytes each day by the complex and finely regulated process of erythropoiesis. This progresses from the stem cell through progenitor cells, the erythroid and megakaryocyte colony-forming unit (CFU_{MkE}), burst-forming unit erythroid (BFU_E) and erythroid CFU (CFU-E; Fig. 1.2) to the first recognizable erythrocyte precursor in the bone marrow, the pronormoblast (Fig. 2.2). This process occurs in an erythroid niche in which about 30 erythroid cells at various stages of development surround a central macrophage.

The pronormoblast is a large cell with dark blue cytoplasm, a central nucleus with nucleoli and slightly clumped chromatin (Fig. 2.2). It gives rise to a series of progressively smaller normoblasts by a number of cell divisions. These also contain

Table 2.1 The blood cells.

Cell	Diameter (μm)	Life span in blood	Number	Function
Red cells 	6–8	120 d	Male: $4.5–6.5 \times 10^{12}$/L Female: $3.9–5.6 \times 10^{12}$/L	Oxygen and carbon dioxide transport
Platelets 	0.5–3.0	10 d	$140–400 \times 10^9$/L	Haemostasis
Phagocytes				
Neutrophils 	12–15	6–10 h	$1.8–7.5 \times 10^9$/L	Protection from bacteria, fungi
Monocytes 	12–20	20-40 h	$0.2–0.8 \times 10^9$/L	Protection from bacteria, fungi
Eosinophils 	12–15	Days	$0.04–0.44 \times 10^9$/L	Protection against parasites
Basophils 	12–15	Days	$0.01–0.1 \times 10^9$/L	
Lymphocytes 	7–9 (resting) 12–20 (active)	Weeks or years	$1.5–3.5 \times 10^9$/L	B cells: immunoglobulin synthesis T cells: protection against viruses; immune functions
Natural killer cells 	10 (resting) 10–20 (active)	Hours or days	0.1–0.4	Protection against virus-infected and neoplastic cells

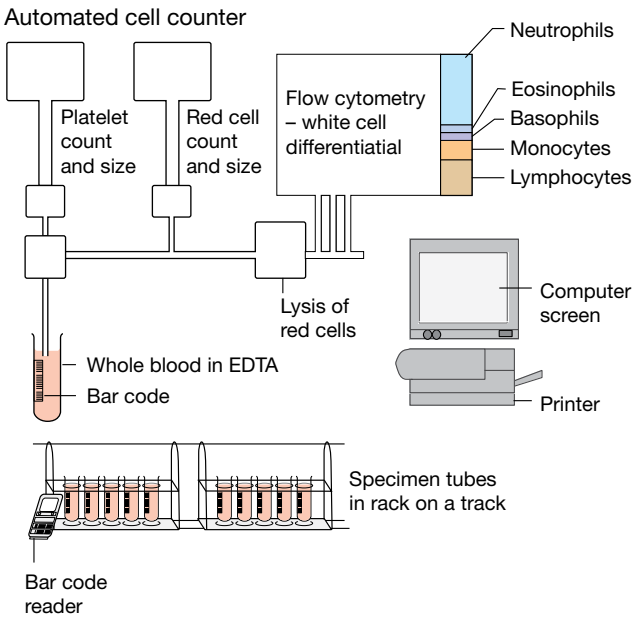


Figure 2.1 Automated blood cell counter. Source: A.B. Mehta, A.V. Hoffbrand (2014) *Haematology at a Glance*, 4th edn. Reproduced with permission of John Wiley & Sons.

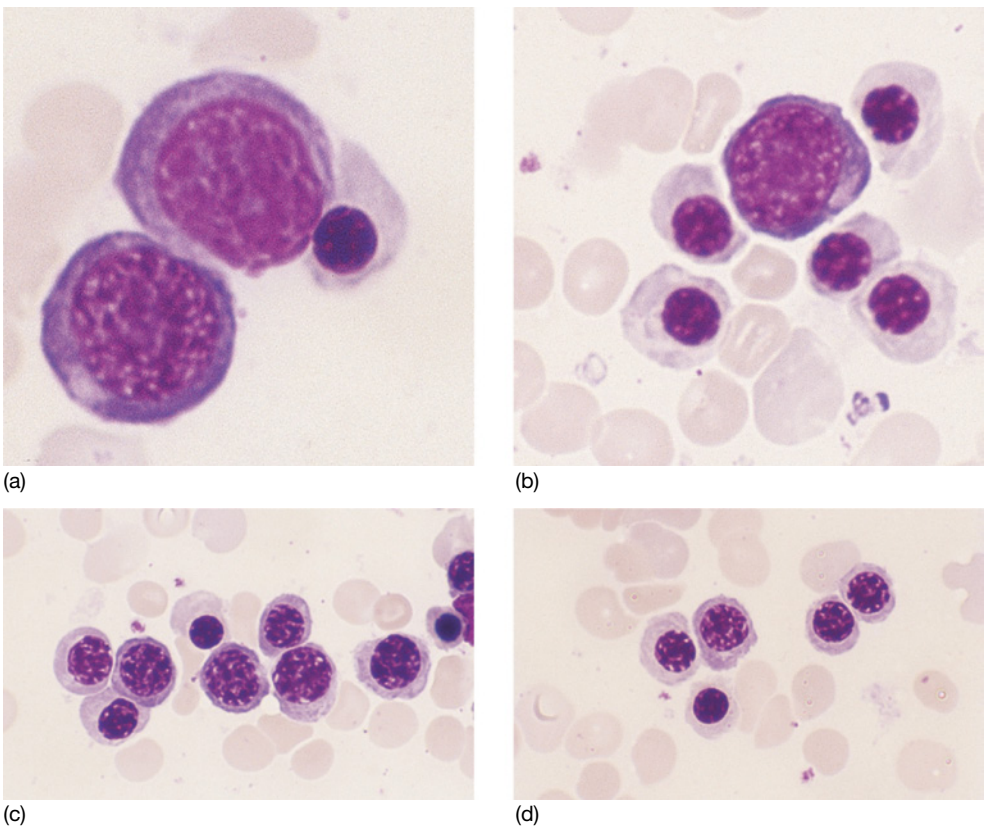


Figure 2.2 Erythroblasts (normoblasts) at varying stages of development. The earlier cells are larger, with more basophilic cytoplasm and a more open nuclear chromatin pattern **(a, b)**. The cytoplasm of the later cells is paler blue and more eosinophilic as a result of haemoglobin formation **(c, d)**.

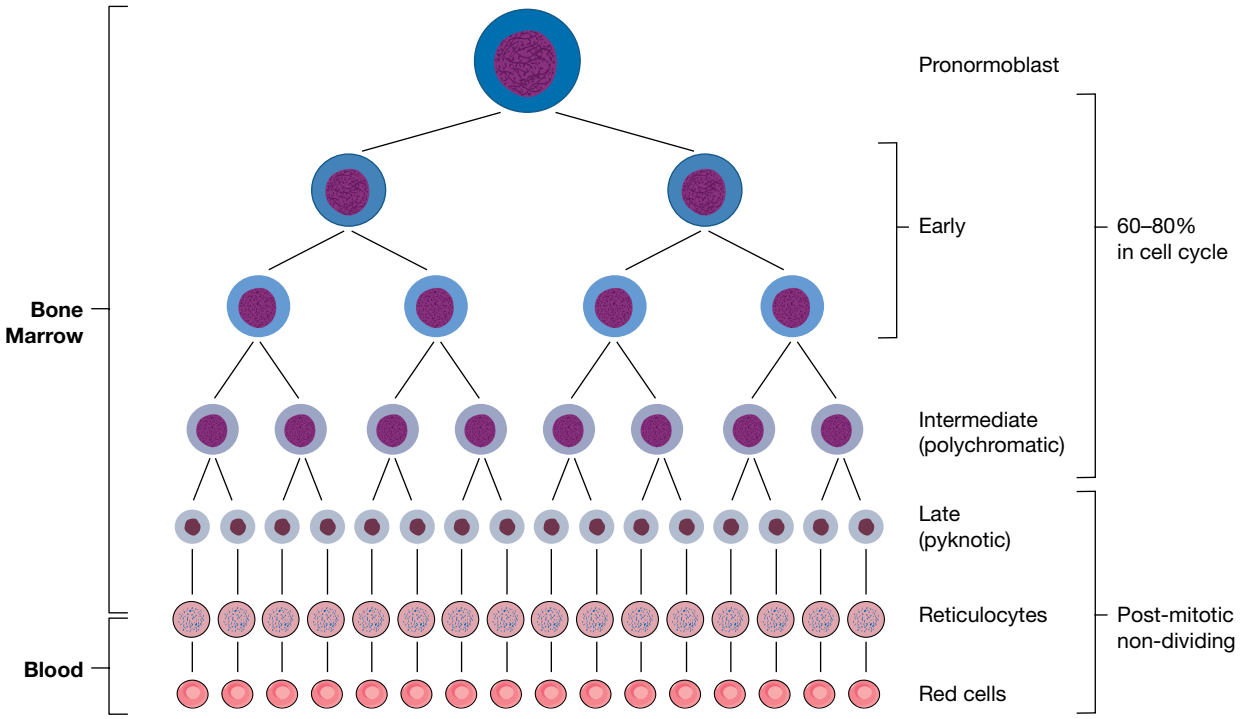
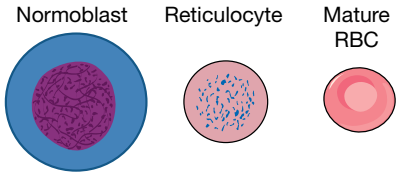


Figure 2.3 The amplification and maturation sequence in the development of mature red cells from the pronormoblast.

progressively more haemoglobin (which stains pink) in the cytoplasm; the cytoplasm also stains paler blue as it loses its RNA and protein synthetic apparatus, while nuclear chromatin becomes more condensed (Figs. 2.2 and 2.3). The nucleus is finally extruded from the late normoblast within the marrow and a reticulocyte results. This still contains some ribosomal RNA and so is still able to synthesize haemoglobin (Fig. 2.4).



Nuclear DNA	Yes	No	No
RNA in cytoplasm	Yes	Yes	No
In marrow	Yes	Yes	Yes
In blood	No	Yes	Yes

Figure 2.4 Comparison of the DNA and RNA content, and marrow and peripheral blood distribution, of the erythroblast (normoblast), reticulocyte and mature red blood cell (RBC).

The **reticulocyte** is slightly larger than a mature red cell. It circulates in the peripheral blood for 1–2 days before maturing, when RNA is completely lost. A completely pink-staining mature erythrocyte results, which is a non-nucleated biconcave disc (Fig. 2.4). One pronormoblast usually gives rise to 16 mature red cells (Fig. 2.3). Normoblasts are not present in normal human peripheral blood (Fig. 2.4). They appear in the blood if erythropoiesis is occurring outside the marrow (extramedullary erythropoiesis) and also with some marrow diseases.

Erythropoietin

Erythropoiesis is regulated by the hormone erythropoietin, a heavily glycosylated polypeptide. Ninety percent of the hormone is produced in the peritubular interstitial cells of the kidney and 10% in the liver and elsewhere. There are no preformed stores. The stimulus to erythropoietin production is the oxygen (O_2) tension in the tissues of the kidney (Fig. 2.5a). Erythropoietin production increases with decreased O_2 delivery to the kidney. This is caused most frequently by anaemia, but also occurs when haemoglobin for some metabolic or structural reason is unable to give up O_2 normally, when atmospheric O_2 is low or with defective cardiac or pulmonary function or damage to the renal circulation.

Hypoxia induces stabilization of the hypoxia-inducible factor (HIF-1α) which then forms a dimer with HIFβ, the dimer stimulating erythropoietin production. The dimer also

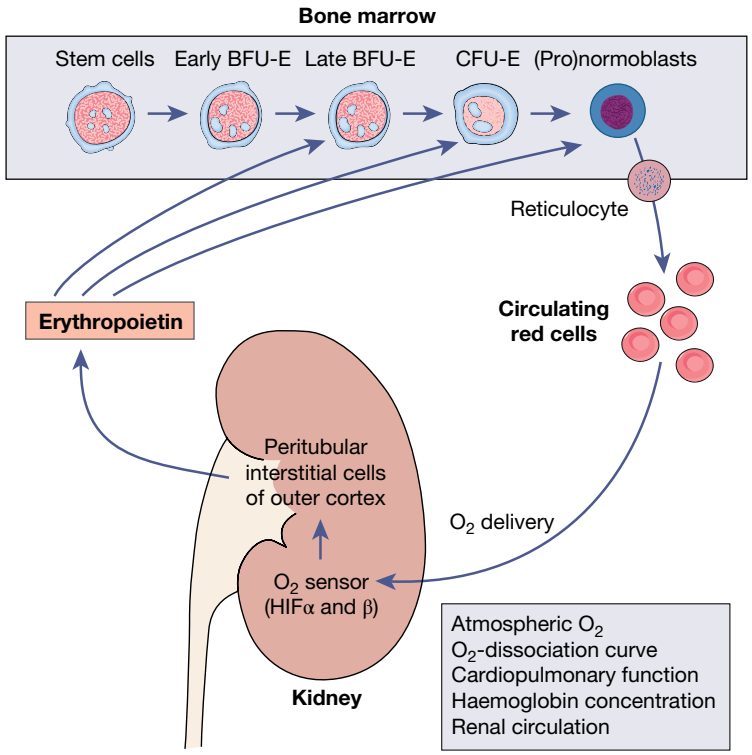


Figure 2.5a The production of erythropoietin by the kidney in response to its oxygen (O_2) supply. Erythropoietin stimulates erythropoiesis and so increases O_2 delivery. BFU_E, erythroid burst-forming unit; CFU-E, erythroid colony-forming unit.

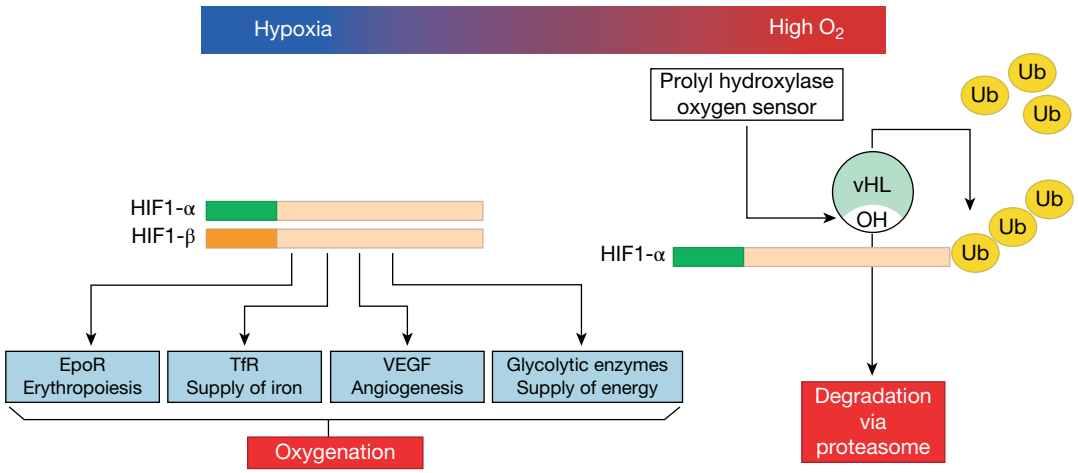


Figure 2.5b The oxygen sensor: hypoxia stabilises hypoxia inducible factor (HIF)α which then forms a dimer with HIFβ, which stimulates erythropoietin production. PHD2 (prolyl hydroxylase), the oxygen sensor, uses molecular oxygen to hydroxylate HIF-1α. This hydroxylation allows von Hippel-Lindau (vHL) binding to HIFα and stimulates its breakdown by ubiquitination. Source: D.R. Higgs *et al*. In A.V. Hoffbrand *et al*. (eds) (2016) *Postgraduate Haematology*, 7th edn. Reproduced with permission of John Wiley & Sons.

stimulates new vessel formation, glycolytic enzyme and transferrin receptor synthesis and increased iron absorption by reducing hepcidin synthesis. Prolyl hydroxylase (PHD2) is a key oxygen sensor. It uses molecular oxygen to hydroxylate HIFα. Hydroxylation allows the von Hippel-Lindau (vHL) protein to break down HIFα by ubiquitination (Fig. 2.5b). Mutations in the genes *vHL, PHD2* and *HIF2α* are rare causes of congenital polycythaemia (Chapter 15). Daprodustat,

roxadustat and vadadustat which inhibit PDH2 and raise endogenous erythropoietin production are in clinical trials for treating the anaemia of chronic renal failure and as a result of chemotherapy for cancer.

Erythropoietin stimulates erythropoiesis by increasing the number of progenitor cells committed to erythropoiesis.

The transcription factor GATA2 is involved in initiating erythroid differentiation from pluripotential stem cells. Subsequently the transcription factors GATA1 and FOG1 are activated by erythropoietin receptor stimulation and are important in enhancing expression of erythroid-specific genes, e.g. of globin, haem biosynthetic and red cell membrane proteins, and also enhancing expression of anti-apoptotic genes and of the transferrin receptor1 (CD71). Late BFU_E and CFU-E, which have erythropoietin receptors, are stimulated to proliferate, differentiate and produce haemoglobin. The proportion of erythroid cells in the marrow increases and, in the chronic state, there is anatomical expansion of erythropoiesis into fatty marrow and sometimes into extramedullary sites. In infants, the marrow cavity may expand into cortical bone, resulting in bone deformities with frontal bossing and protrusion of the maxilla (Chapter 7).

Conversely, increased O_2 supply to the tissues (because of an increased red cell mass or because haemoglobin is able to release its O_2 more readily than normal) reduces the erythropoietin drive. Plasma erythropoietin levels can be valuable in clinical diagnosis. They are high in anaemia, unless this is due to renal failure or if a tumour-secreting erythropoietin is present, but low in severe renal disease or polycythaemia vera (Fig. 2.6).

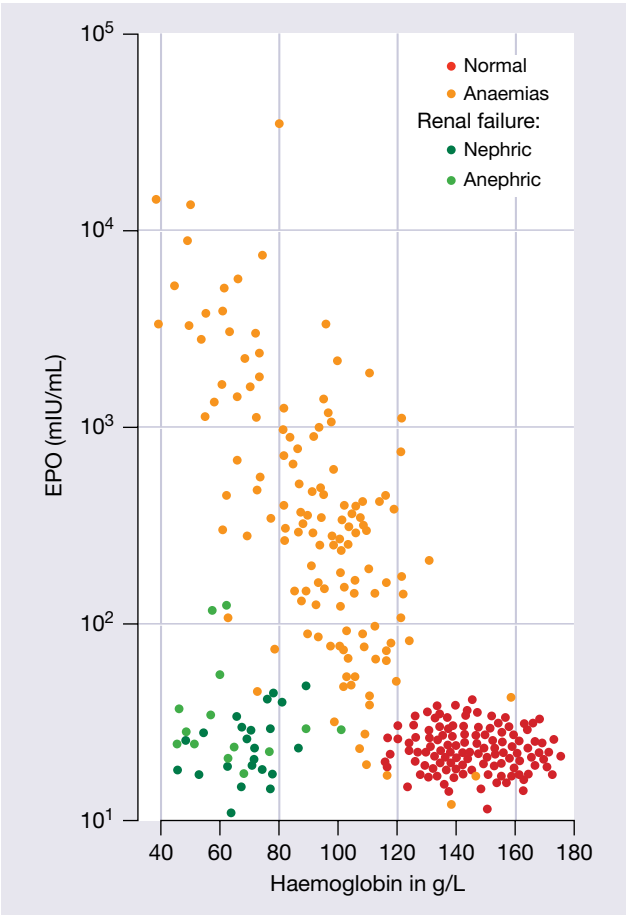


Figure 2.6 The relation between the concentration of erythropoietin (EPO) in plasma and haemoglobin concentration. Anaemias in this figure exclude conditions shown to be associated with impaired production of EPO. Source: Modified from M. Pippard *et al*. (1992) *Br. J. Haematol*. 82: 445. Reproduced with permission of John Wiley & Sons.

Indications for erythropoietin therapy

Recombinant erythropoietin is needed for treating anaemia resulting from renal disease or from various other causes. It is given subcutaneously either three times weekly, once every 1–2 weeks or every 4 weeks, depending on the indication and on the preparation used (erythropoietin alpha or beta; darbepoetin alpha, a heavily glycosylated longer-acting form; or Micera, the longest-acting preparation). The main indication is end-stage renal disease (with or without dialysis). The patients often also need oral or intravenous iron. Other indications are listed in Table 2.2. The haemoglobin level and quality of life may be improved. A low serum erythropoietin level prior to treatment is valuable in predicting an effective response. Side effects include a rise in blood pressure, thrombosis and local injection site reactions. Erythropoietin has been associated with progression of some tumours which express EPO receptors and so with reduced survival. It is only indicated as an alternative to blood transfusion in cancer patients with symptomatic anaemia where the benefits outweigh the risks of tumour progression and of venous thrombosis. Prolyl hydroxylase inhibitors (see above) are undergoing trials for treating anaemia in cancer patients.

The marrow requires many other substances for effective erythropoiesis. These include metals iron and cobalt, vitamins (vitamin B_{12}, folate, vitamin C, vitamin E, vitamin B_6, thiamine and riboflavin) and hormones androgens and thyroxine. Deficiency in any of these may be associated with anaemia.

Table 2.2 Clinical indications (in selected subjects) for erythropoietin.

Anaemia of chronic renal disease
Myelodysplastic syndrome
Anaemia associated with malignancy and chemotherapy
Anaemia of chronic diseases, e.g. rheumatoid arthritis
Anaemia of prematurity
Perioperative uses

Haemoglobin

Haemoglobin synthesis

Each molecule of normal adult **haemoglobin** A (Hb A, the dominant haemoglobin in blood after the age of 3–6 months) consists of four polypeptide chains, $\alpha_2\beta_2$, each with its own haem group. Normal adult blood also contains small quantities of two other haemoglobins: Hb F and Hb A_2. These also contain α chains, but with γ and δ chains, respectively, instead of β (Table 2.3). The synthesis of the various globin chains in the foetus and adult is discussed in Chapter 7.

Haem synthesis occurs largely in mitochondria by a series of biochemical reactions, commencing with the condensation of glycine and succinyl coenzyme A under the action of the key rate-limiting enzyme δ-aminolaevulinic acid synthase (ALAS) (Fig. 2.7). Pyridoxal phosphate (vitamin B_6) is a coenzyme for this reaction. The main sources of succinyl CoA are glutamine and glucose, which are converted to alpha-ketoglutarate, a succinate precursor inside the erythroid cells. Ultimately, protoporphyrin combines with iron in the ferrous (Fe^{2+}) state to form haem (Fig. 2.8). A tetramer of four globin chains, each with its own haem group in a 'pocket', is then formed to make up a haemoglobin molecule (Fig. 2.9). An enzyme eIF2alpha kinase, also known as haem-regulated inhibitor (HRI), senses intracellular haem concentration. If this is low as in iron deficiency, HRI phosphorylates its substrate eIF2alpha which then reduces globin synthesis by inhibiting its mRNA translation.

An adjacent gene *Nprl3* shares some enhancers with the α-globin gene and so the control of expression of the two genes is coupled. *Nprl3* provides negative regulation of mTORC1, a critical controller of cellular metabolism. Nprl3 is essential for optimal erythropoiesis and for responding to fluctuating nutrient (including iron) and growth factor concentrations.

Table 2.3 Normal haemoglobins in adult blood.

	Hb A	Hb F	Hb A_2
Structure	$\alpha_2\beta_2$	$\alpha_2\gamma_2$	$\alpha_2\delta_2$
Normal (%)	96–98	0.5–0.8	1.5–3.2

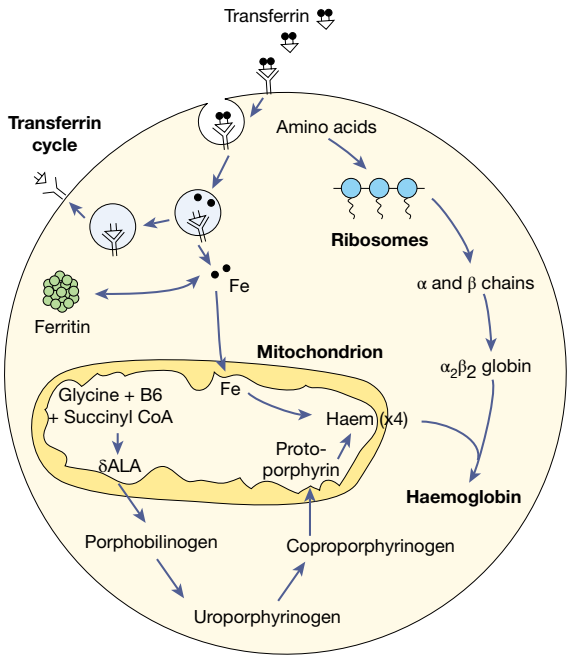


Figure 2.7 Haemoglobin synthesis in the developing red cell. The mitochondria are the main sites of protoporphyrin synthesis, iron (Fe) is supplied from circulating transferrin and globin chains are synthesized on ribosomes. δ-ALA, δ-aminolaevulinic acid; CoA, coenzyme A.

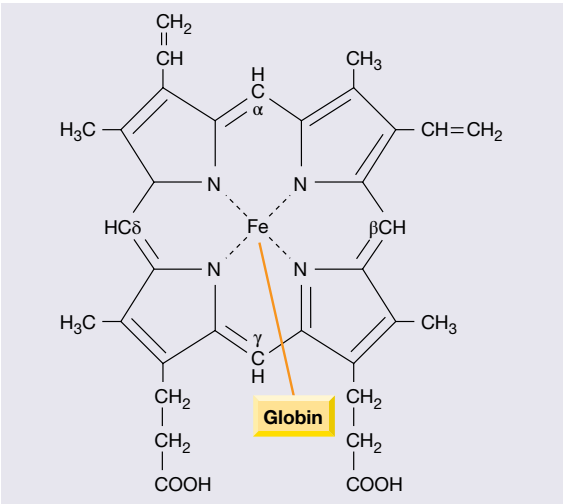


Figure 2.8 The structure of haem.

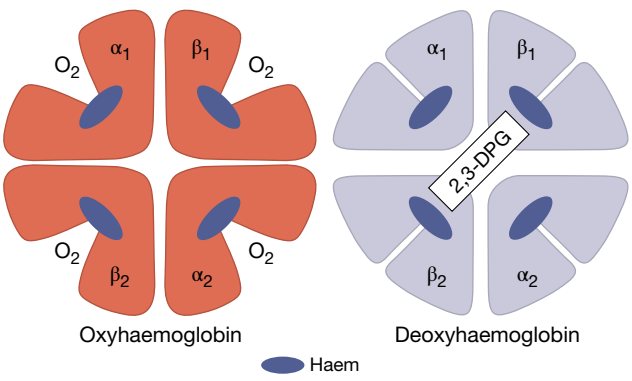


Figure 2.9 The oxygenated and deoxygenated haemoglobin molecule. α, β, globin chains of normal adult haemoglobin (Hb A); 2,3-DPG, 2,3-diphosphoglycerate.

Haemoglobin function

The red cells in systemic arterial blood carry O_2 from the lungs to the tissues and return in venous blood with CO_2 to the lungs. As the haemoglobin molecule loads and unloads O_2, the individual globin chains move on each other (Fig. 2.9). The $\alpha_1\beta_1$ and $\alpha_2\beta_2$ contacts stabilize the molecule. When O_2 is unloaded the β chains are pulled apart, permitting entry of the metabolite 2,3-diphosphoglycerate (2,3-DPG), resulting in a lower affinity of the molecule for O_2. This movement is responsible for the sigmoid form of the haemoglobin O_2 dissociation curve (Fig. 2.10). The P_{50} (the partial pressure of O_2 at which haemoglobin is half saturated with O_2) of normal blood is 26.6 mmHg. With increased affinity for O_2, the curve shifts to the left (the P_{50} falls), while with decreased affinity for O_2, the curve shifts to the right (the P_{50} rises).

Normally, *in vivo*, O_2 exchange operates between 95% saturation (arterial blood) with a mean arterial O_2 tension of 95 mmHg and 70% saturation (venous blood) with a mean venous O_2 tension of 40 mmHg (Fig. 2.10).

The normal position of the curve depends on the concentration of 2,3-DPG, H^+ ions and CO_2 in the red cell and on the structure of the haemoglobin molecule. High concentrations of 2,3-DPG, H^+ or of CO_2, and the presence of sickle haemoglobin (Hb S), shift the curve to the right (oxygen is given up more easily), whereas foetal haemoglobin (Hb F) – which is unable to bind 2,3-DPG – and certain rare abnormal haemoglobins associated with polycythaemia shift the curve to the left because they give up O_2 less readily than normal.

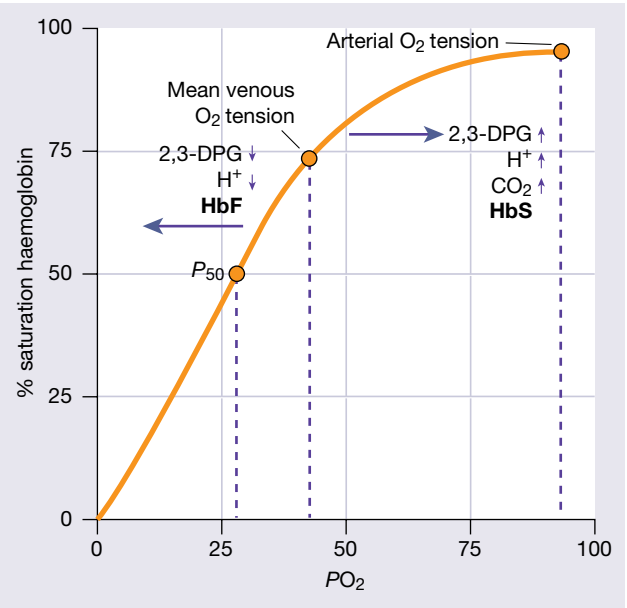


Figure 2.10 The haemoglobin oxygen (O2) dissociation curve. 2,3-DPG, 2,3-diphosphoglycerate.

Methaemoglobinaemia

This is a clinical state in which circulating haemoglobin is present with iron in the oxidized Fe^{3+} instead of the usual Fe^{2+} state. It may arise because of a hereditary deficiency of the enzyme methaemoglobin reductase or inheritance of a structurally abnormal haemoglobin (Hb M). Hb Ms contain an amino acid substitution affecting the haem pocket of the globin chain. Toxic methaemoglobinaemia and/or sulphaemoglobinaemia occurs when a drug or other toxic substance oxidizes haemoglobin. In all these states, the patient is likely to show cyanosis.

The red cell

In order to carry haemoglobin into close contact with the tissues and for successful gaseous exchange, the red cell, 8 µm in diameter, must pass repeatedly through the microcirculation, whose minimum diameter is 3.5 µm. It must maintain haemoglobin in a reduced (ferrous) state and maintain osmotic equilibrium despite the high concentration of protein (haemoglobin) in the cell. A single journey round the body takes 20 seconds and its total journey throughout its 120-day life span has been estimated to be 480 km (300 miles). To fulfil these functions, the cell is a flexible biconcave disc with an ability to generate energy as adenosine triphosphate (ATP) by the anaerobic glycolytic (Embden–Meyerhof) pathway (Fig. 2.11) and to generate reducing power both as nicotinamide adenine dinucleotide (NADH) by this pathway and as reduced nicotinamide adenine dinucleotide phosphate (NADPH) by the hexose monophosphate shunt (Fig. 2.11, Fig. 6.6).

Red cell metabolism

Embden–Meyerhof pathway

In this series of biochemical reactions, glucose that enters the red cell from plasma by facilitated transfer is metabolized to lactate (Fig. 2.11). For each molecule of glucose used, two molecules of ATP and thus two high-energy phosphate bonds are generated. This ATP provides energy for maintenance of red cell volume, shape and flexibility.

The Embden–Meyerhof pathway generates NADH, which is needed by the enzyme methaemoglobin reductase to reduce functionally dead methaemoglobin containing ferric iron, produced by oxidation of approximately 3% of haemoglobin each day, to functionally active haemoglobin containing ferrous ions. The Rapoport–Luebering shunt, or side-arm, of this pathway (Fig. 2.11) generates 2,3-DPG, important in the regulation of haemoglobin's oxygen affinity (Fig. 2.10).

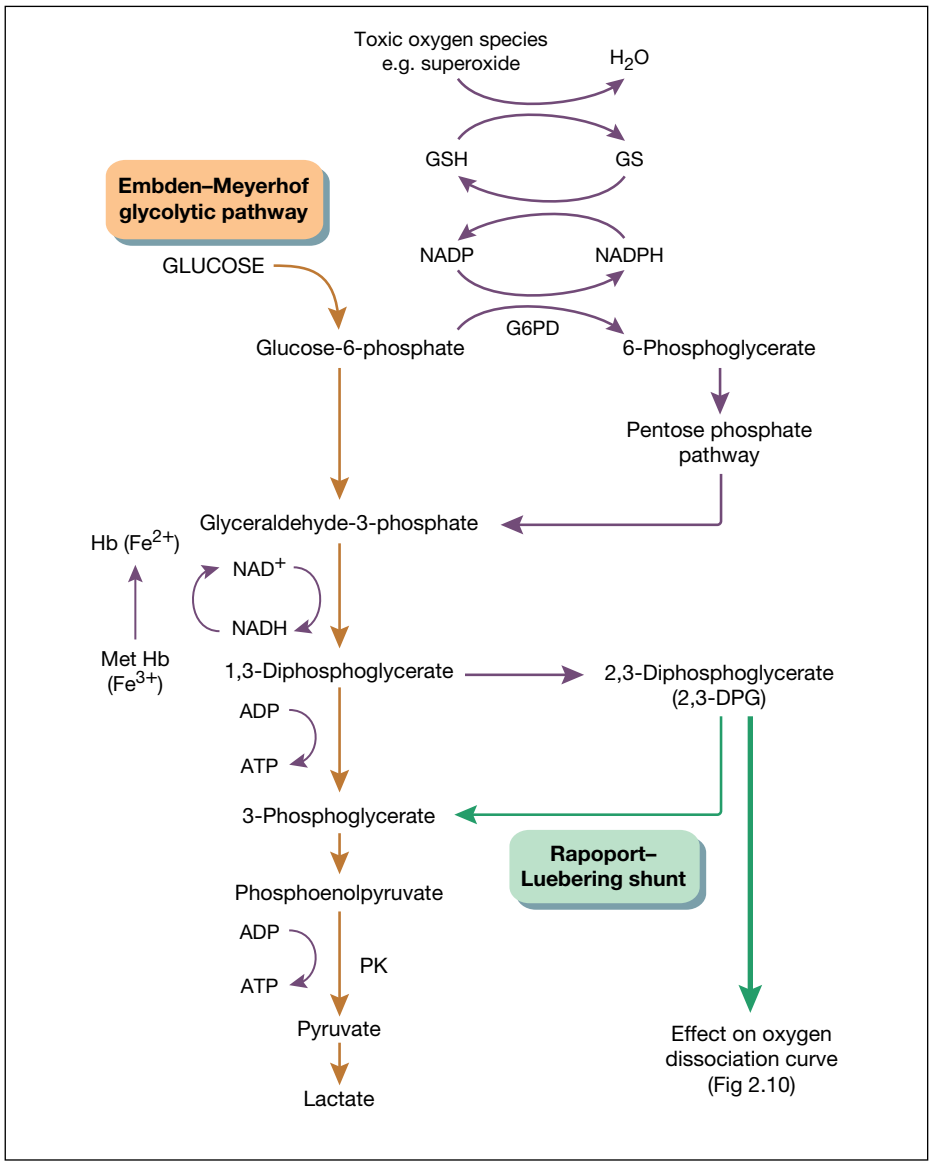


Figure 2.11 The anaerobic Embden–Meyerhof pathway generates energy as ATP and reducing power as NADH. The pentose-phosphate shunt pathway generates additional reducing power as NADPH. Further down the main pathway the Rapoport–Luebering shunt generates 2,3 DPG which effects oxygen binding and release by haemoglobin (Fig 2.10). GS, glutathione; GSH, reduced glutathione; G6PD, glucose-phosphate dehydrogenase; PK, pyruvate kinase; NAD, NADP, ADP, ATP see text.

Hexose monophosphate (pentose phosphate) shunt

Approximately 10% of glycolysis occurs by this oxidative pathway in which glucose-6-phosphate is converted to 6-phosphogluconate and so to a pentose-5-phosphate (Fig. 2.11, Fig. 6.6). NADPH is generated and is linked with glutathione, which maintains sulphydril (SH) groups intact in the cell, including those in haemoglobin and in the red cell membrane. In one of the most common inherited abnormalities of red cells, glucose-6-phosphate dehydrogenase (G6PD) deficiency, the red cells are extremely susceptible to oxidant stress (Chapter 6).

Red cell membrane

The red cell membrane comprises a lipid bilayer, membrane integral proteins and a membrane skeleton (Fig. 2.12). Approximately 50% of the membrane is protein, 20% phospholipids, 20% cholesterol molecules and up to 10% is carbohydrate. Carbohydrates occur only on the external surface, while proteins are either integral, penetrating the lipid bilayer, or form a skeleton on the inner surface of the membrane. Several red cell proteins have been numbered according to their mobility on polyacrylamide gel electrophoresis (PAGE), e.g. band 3, proteins 4.1, 4.2 (Fig. 2.12).

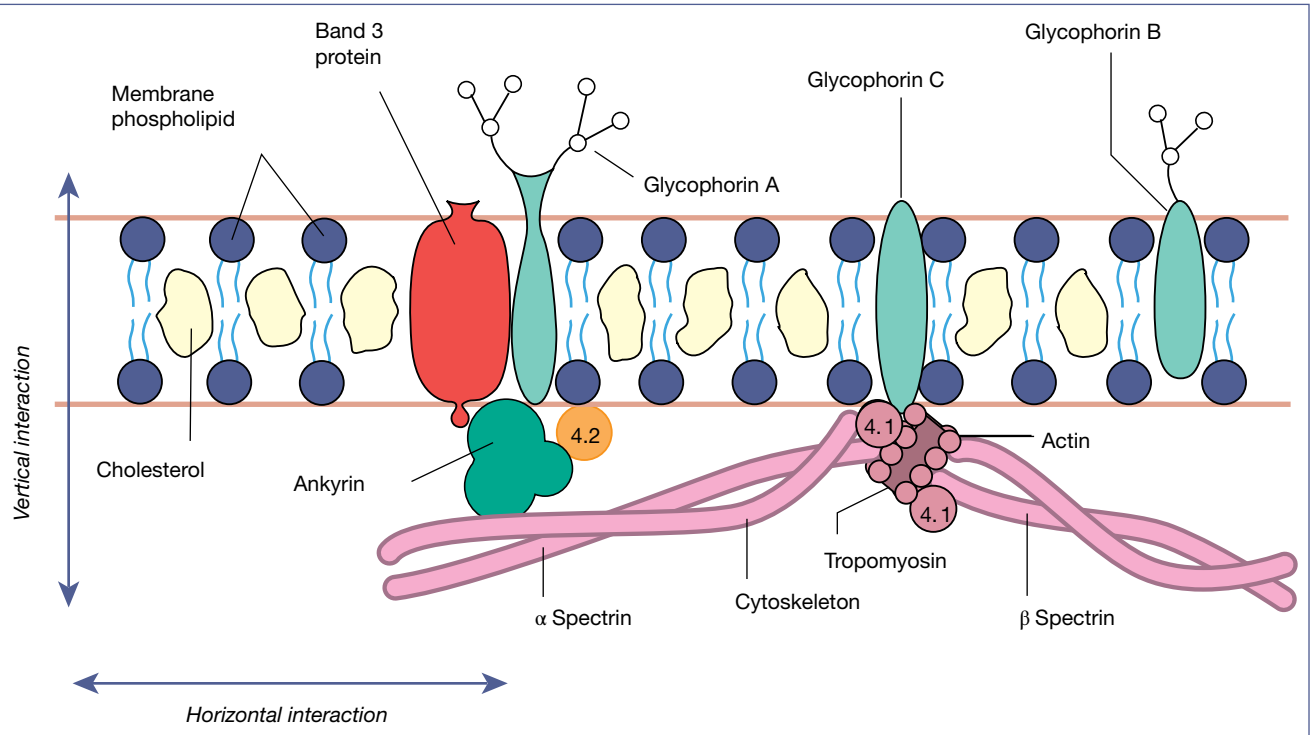


Figure 2.12 The structure of the red cell membrane. Some of the penetrating and integral proteins carry carbohydrate antigens; other antigens are attached directly to the lipid layer.

The membrane skeleton is formed by structural proteins that include α and β spectrin, ankyrin, protein 4.1 and actin. These proteins form a horizontal lattice important in maintaining the biconcave shape. Spectrin is the most abundant and consists of two chains, α and β, wound around each other to form heterodimers, which then self-associate head to head to form tetramers. These tetramers are linked at the tail end to actin and attached there to protein band 4.1. At the head end, the β spectrin chains attach to ankyrin, which connects them to band 3, the transmembrane protein that acts as an anion channel ('vertical connection'; Fig. 2.12). Protein 4.2 enhances this interaction.

Defects of the membrane proteins explain some of the abnormalities of shape of the red cell membrane, e.g. hereditary spherocytosis and elliptocytosis (Chapter 6), while alterations in lipid composition because of congenital or acquired abnormalities in plasma cholesterol or phospholipid may be associated with other membrane abnormalities (Fig. 2.16).

Anaemia

Anaemia is defined as a reduction in the haemoglobin concentration of the blood below normal for age and sex (Table 2.4). Although normal values can vary between laboratories, typical values would be less than 135 g/L in adult males and less than 115 g/L in adult females (Fig. 2.13). The World Health Organisation (WHO) defines anaemia as a haemoglobin level < 130 g/L for adult males, < 120 g/L for adult non-pregnant females and <110 g/L from the age of 6–59 months. Newborn infants have a high haemoglobin level; 140 g/L is taken as the lower limit at birth (Fig. 2.13). Anaemia in pregnancy and neonates is discussed in Chapter 34.

Alterations in total circulating plasma volume as well as in total circulating haemoglobin mass determine the haemoglobin concentration. Reduction in plasma volume as in dehydration may mask anaemia or even cause apparent (pseudo) polycythaemia (Chapter 15). Conversely, an increase in plasma volume as with splenomegaly or pregnancy may cause anaemia even with a normal total circulating red cell and haemoglobin mass.

After acute major blood loss, anaemia is not immediately apparent because the total blood volume is reduced. It takes up to a day for the plasma volume to be replaced and so for the degree of anaemia to become apparent. Regeneration of red cells and haemoglobin mass takes substantially longer. The initial clinical features of major blood loss are therefore a result of reduction in blood volume rather than of anaemia.

Global incidence

On the basis of the WHO definitions, anaemia was estimated in 2010 to occur in about 33% of the global population. Prevalence was greater in females than males at all ages and most frequent in children less than 5 years old. Anaemia was most frequent in

Table 2.4 Normal values for blood cells and haematinics.

	Males	Females
Haemoglobin (g/L)	135.0–175.0	115.0–155.0
Red cells (erythrocytes) ($\times 10^{12}$/L)	4.5–6.5	3.9–5.6
PCV (haematocrit) (%)	40–52	36–48
Mean cell volume (MCV) (fL)	80–95	
Mean cell haemoglobin (MCH) (pg)	27–34	
Reticulocyte count ($\times 10^9$/L)	50–150	
White cells (leucocytes)		
Total ($\times 10^9$/L)	4.0–11.0	
Neutrophils ($\times 10^9$/L)	1.8–7.5 (Caucasians 1.5–7.5 (Africa and Middle-East)	
Lymphocytes ($\times 10^9$/L)	1.5–3.5	
Monocytes ($\times 10^9$/L)	0.2–0.8	
Eosinophils ($\times 10^9$/L)	0.04–0.44	
Basophils ($\times 10^9$/L)	0.01–0.1	
Platelets ($\times 10^9$/L)	150–400	
Serum iron (μmol/L)	10–30	
Total iron-binding capacity (μmol/L)	40–75 (2.0–4.0 g/L as transferrin)	
Serum ferritin* (μg/L)	40–340	14–150
Serum vitamin B_{12}* (ng/L)	160–925 (20–680 pmol/L)	
Serum folate* (μg/L)	3.0–15.0 (4–30 nmol/L)	
Red cell folate** (μg/L)	160–640 (360–1460 nmol/L)	

PCV, packed cell volume.
* Normal ranges differ between laboratories.
** Normal ranges differ between different laboratories.

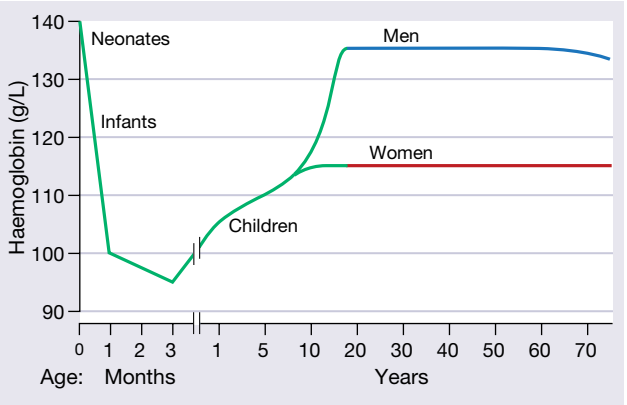


Figure 2.13 The lower limit of blood haemoglobin concentration in healthy men, women and children of various ages.

South Asia, and in Central, West and East Sub-Saharan Africa. The main causes are iron deficiency (caused by life-long poor diet combined with menstruation and/or repeated pregnancies, hookworm, schistosomiasis), the anaemia of inflammation (Chapter 3), sickle cell diseases, thalassaemia (Chapter 7), malaria (Chapter 32).

Clinical features of anaemia

The major adaptations to anaemia are in the cardiovascular system with increased cardiac stroke volume and tachycardia and in the haemoglobin O_2 dissociation curve. In some patients with quite severe anaemia, there may be no symptoms or signs, whereas others with mild anaemia may be severely incapacitated. The presence or absence of clinical features depends on:

1 ***Speed of onset*** Rapidly progressive anaemia causes more symptoms than anaemia of slow onset. This is because there is less time for adaptation in the cardiovascular system and in the O_2 dissociation curve of haemoglobin.
2 ***Severity*** Mild anaemia often produces no symptoms or signs, but these are usually present when the haemoglobin is less than 90 g/L. Even severe anaemia (haemoglobin concentration as low as 60 g/L) may produce remarkably few symptoms, when there is very gradual onset in young subjects who are otherwise healthy.
3 ***Age*** The elderly tolerate anaemia less well than the young because normal cardiovascular compensation is impaired.
4 ***Haemoglobin O_2 dissociation curve*** Anaemia, in general, is associated with a rise in 2,3-DPG in the red cells and a shift in the O_2 dissociation curve to the right, so that oxygen is given up more readily to tissues. This adaptation, which takes days to occur, is particularly marked in some anaemias that either raise 2,3-DPG directly, e.g. pyruvate kinase deficiency (Chapter 6), or that are associated with a low-affinity haemoglobin, e.g. Hb S (Fig. 2.10).

Symptoms

If the patient does have symptoms, these are usually shortness of breath, particularly on exertion, weakness, lethargy, palpitation and headaches. In older subjects, symptoms of cardiac failure, angina pectoris, intermittent claudication or confusion may be present. Visual disturbances because of retinal haemorrhages may complicate very severe anaemia, particularly of rapid onset (Fig. 2.14).

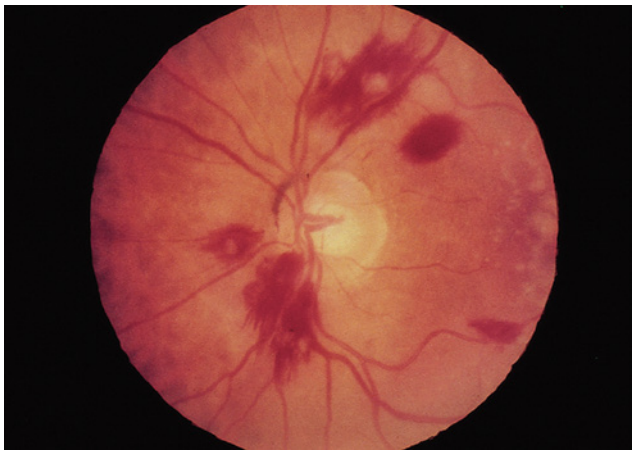


Figure 2.14 Retinal haemorrhages in a patient with severe anaemia (haemoglobin 25 g/L) caused by severe haemorrhage.

Signs

These may be divided into general and specific. General signs include pallor of mucous membranes or nail beds, which occurs if the haemoglobin level is less than 90 g/L (Fig. 2.15). Conversely, skin colour is not a reliable sign. A hyperdynamic circulation may be present with tachycardia, a bounding pulse, cardiomegaly and a systolic flow murmur. Particularly in the elderly, features of congestive heart failure may be present.

Specific signs are associated with particular types of anaemia, e.g. koilonychia (spoon nails) with iron deficiency, jaundice with haemolytic or megaloblastic anaemias, leg ulcers with sickle cell and other haemolytic anaemias, or bone deformities with thalassaemia major.

The association of features of anaemia with excess infections or spontaneous bruising suggests that neutropenia or thrombocytopenia may be present, possibly as a result of bone marrow failure.

Classification of anaemia

Red cell indices

The most useful classification is based on the red cell indices, especially MCV. This divides the anaemia into microcytic, normocytic and macrocytic (Table 2.5). As well as suggesting the nature of the primary defect, this classification may also indicate an underlying abnormality before overt anaemia has developed.

In two common physiological situations, the mean corpuscular volume (MCV) may be outside the normal adult range. In the newborn for a few weeks, the MCV is high, but in infancy it is low, e.g. 70 fL at 1 year of age and rises slowly throughout childhood to the normal adult range. In normal pregnancy there is a slight rise in MCV, even in the absence of other causes of macrocytosis, e.g. folate deficiency.

Other laboratory findings

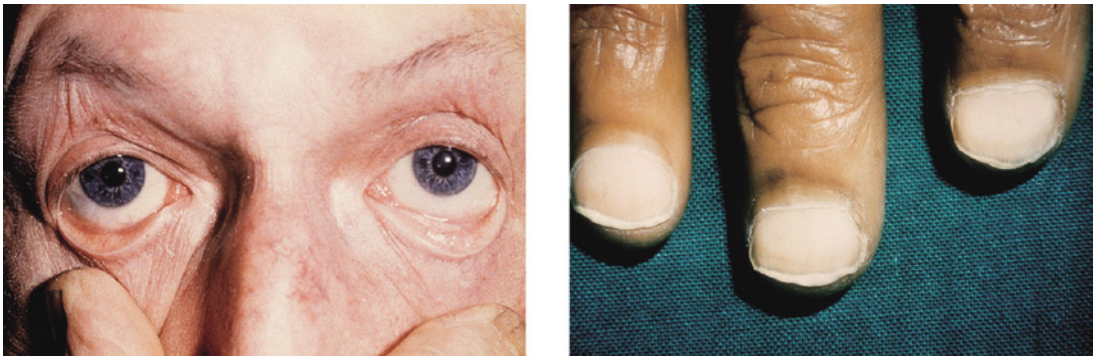
Although the red cell indices will indicate the type of anaemia, further useful information can be obtained from the initial blood sample.

Leucocyte and platelet counts

Measurement of these helps to distinguish 'pure' anaemia from 'pancytopenia' (subnormal levels of red cells, neutrophils and platelets), which suggests a more general marrow defect or destruction of cells, e.g. hypersplenism. In anaemias caused by haemolysis or haemorrhage, the neutrophil and platelet counts are often raised; in infections and leukaemias, the leucocyte count is also often raised, and there may be abnormal leucocytes or neutrophil precursors present.

Reticulocyte count

The normal percentage is 0.5–2.5%, and the absolute count $50–150 \times 10^9$/L (Table 2.4). This should rise in anaemia because of erythropoietin increase and be higher the more



(a) (b)

Figure 2.15 Pallor of the conjunctival mucosa **(a)** and of the nail bed **(b)** in two patients with severe anaemia (haemoglobin 60 g/L).