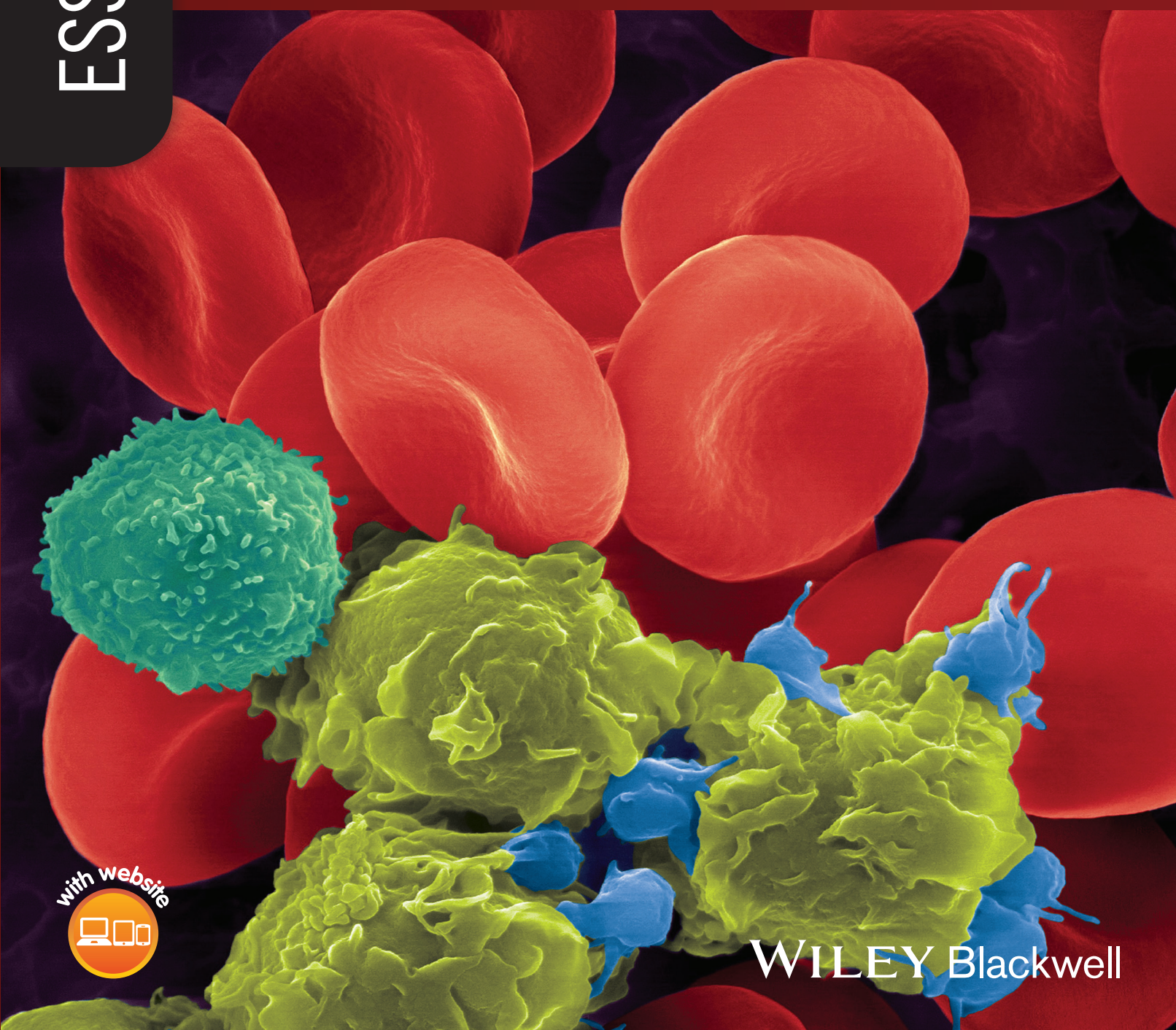
ESSENTIALS

HOFFBRAND'S ESSENTIAL HAEMATOLOGY

A. VICTOR HOFFBRAND | DAVID P. STEENSMA

EIGHTH EDITION



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Hoffbrand's Essential Haematology

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Contents

Preface to the Eighth Edition

It is only three years since the 7th edition of this book appeared, but the rapid advances in knowledge of the pathogenesis of blood diseases and in their treatment have necessitated the early publication of this 8th edition. The application of next generation sequencing has revealed the driving mutations underlying many of the neoplastic haematological diseases. Advances in genomic, immunological and immunohistochemical techniques have improved their classification and also enabled highly sensitive tracking of response to therapy. The detection and quantification of minimal residual disease is increasingly recognized as important in planning protocols for treatment of haematological malignancies and for deciding when to end therapy or to intensify it.

The latest (2016) World Health Organization classification of the haematological neoplastic diseases, which relies on much new cytogenetic and molecular genetic information, has been incorporated into the relevant chapters of this new edition. Preclinical clonal abnormalities and their significance in relation to future overt haematological or systemic diseases are being increasingly discovered and are discussed here.

Treatment has also changed substantially. This is partly due to the introduction of many new drugs, often targeted at the signalling pathway, which has been aberrantly activated by a specific mutation. The range of monoclonal antibodies used in therapy, some with conjugated toxins, has also increased. Chimeric antigen receptor (CAR)-T cells are now licensed for treatment of patients with relapsed and refractory B-cell malignancies and are in clinical trials for a wide range of other haematological malignancies. Gene therapy has become a reality for haemophilia and thalassaemia major, and is now also in trials for sickle cell anaemia. Drugs which promote effective erythropoiesis are proving beneficial for alleviating anaemia and transfusion dependence in myelodysplastic syndromes and thalassaemia. The treatment by anticoagulation of venous thrombosis and atrial fibrillation has undergone a major shift from warfarin to the new direct-acting oral anticoagulant drugs for the majority of patients. New text, figures and tables have been added throughout the book to incorporate and illustrate these major advances.

Professor Paul Moss co-authored four previous editions of *Essential Haematology*. We are immensely grateful for his outstanding expert input over many years in keeping its content up to date in a rapidly changing field and maintaining the style of the book. For this 8th edition, David Steensma, from Harvard Medical School and the Dana-Farber Cancer Institute in Boston, has joined as co-author. David is internationally known for his research in the myeloid malignancies and as a writer and teacher within the broader field of haematology.

Jean Connors, also Professor of Haematology at Harvard Medical School and Consultant Haematologist at Brigham and Women's Hospital and the Dana-Farber Cancer Institute Hospital, has substantially helped to update the five chapters dealing with platelets, blood coagulation and their disorders. We are grateful to Jean for her expert knowledge in revising these chapters, where considerable changes in practice have taken place in the last few years. We also thank Dr Keith Gomez of the Royal Free Hospital for his help in updating of the section of the multiple-choice questions relating to Chapters 24-28 which accompany the electronic version of the printed book.

We hope *Essential Haematology* will continue to be used worldwide by medical students and as a primer for those entering haematology as a speciality. The book is also aimed at clinical and non-clinical scientists, nurses and others with a special interest in the blood and its diseases. We thank our publishers Wiley-Blackwell and in particular Jennifer Seward, Nick Morgan and Magenta Styles for their tremendous support in producing this new 8th edition and Jane Fallows for her expert drawing of new figures. We hope *Essential Haematology* will continue internationally to provide a stimulating and comprehensive introduction to one of the most exciting and advanced fields in medicine.

Victor Hoffbrand
David Steensma
2019

Preface to the First Edition

The major changes that have occurred in all fields of medicine over the last decade have been accompanied by an increased understanding of the biochemical, physiological and immunological processes involved in normal blood cell formation and function and the disturbances that may occur in different diseases. At the same time, the range of treatment available for patients with diseases of the blood and blood-forming organs has widened and improved substantially as understanding of the disease processes has increased and new drugs and means of support care have been introduced.

We hope the present book will enable the medical student of the 1980s to grasp the essential features of modern clinical and laboratory haematology and to achieve an understanding of how many of the manifestations of blood diseases can be explained with this new knowledge of the disease processes.

We would like to thank many colleagues and assistants who have helped with the preparation of the book. In particular, Dr H.G. Prentice cared for the patients whose haematological responses are illustrated in Figs 5.3 and 7.8 and Dr J. McLaughlin supplied Fig. 8.6. Dr S. Knowles reviewed critically the final manuscript and made many helpful suggestions. Any remaining errors are, however, our own. We also thank Mr J.B. Irwin and R.W. McPhee who drew many excellent diagrams, Mr Cedric Gilson for expert photomicrography, Mrs T. Charalambos, Mrs B. Elliot, Mrs M. Evans and Miss J. Allaway for typing the manuscript, and Mr Tony Russell of Blackwell Scientific Publications for his invaluable help and patience.

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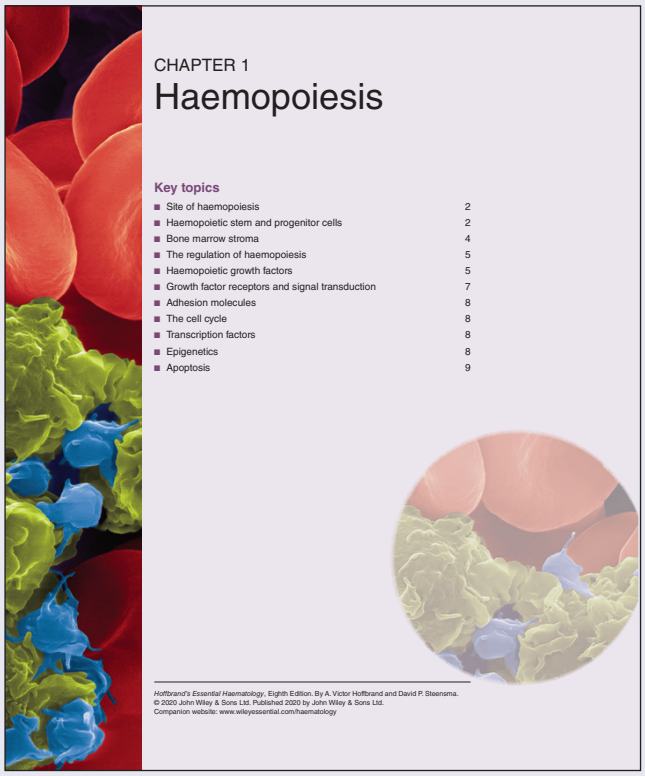

CHAPTER 1

Haemopoiesis

Key topics

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◀ Every chapter begins with a list of **Key topics** of the chapter.

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 (see p. 248). Over-expression of the BCL2 protein makes the malignant B cells less susceptible to apoptosis. 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(1;14) and t(15;17), also result in inhibition of apoptosis (see 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 ischaemia, 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 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 and circulate in the blood.
- Growth factors attach to specific cell receptors and produce a cascade of phosphorylation events to the cell nucleus. Transcription factors carry the message to those genes that are to be 'switched on', 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, endothelium and 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.
- Transcription factors are molecules that bind to DNA and control the transcription of specific genes or gene families.
- 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. BCL2) determines the cell susceptibility to apoptosis.

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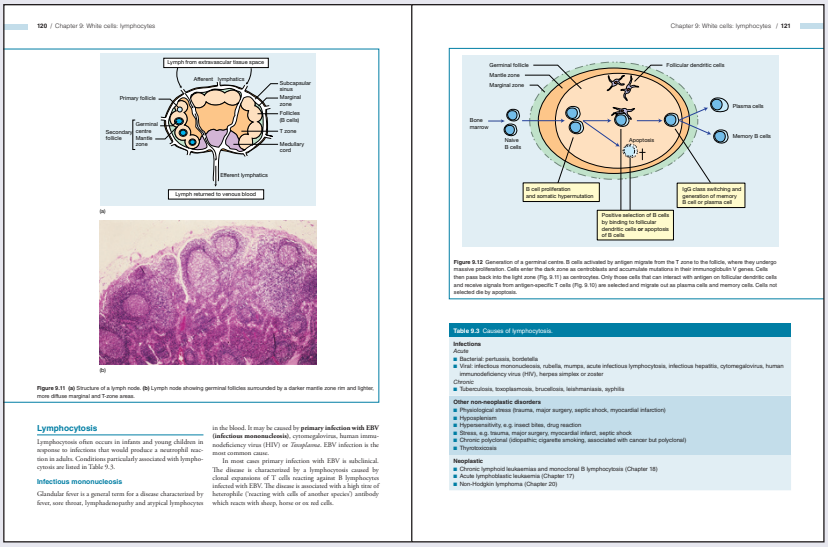

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Figure 9.11 **(a)** Structure of a lymph node. **(b)** Lymph node showing germinal follicles surrounded by a darker mantle zone rim and lighter, more diffuse marginal and T-zone areas.

Lymphocytosis

Lymphocytosis often occurs in infants and young children in response to infections that would produce a neutrophil reaction in adults. Conditions particularly associated with lymphocytosis are listed in Table 9.3.

Infectious mononucleosis

Glandular fever is a general term for a disease characterized by fever, sore throat, lymphadenopathy and atypical lymphocytes in the blood. It may be caused by **primary infection with EBV (infectious mononucleosis)**, cytomegalovirus, human immunodeficiency virus (HIV) or *Toxoplasma*. EBV infection is the most common cause.

In most cases primary infection with EBV is subclinical. The disease is characterized by a lymphocytosis caused by clonal expansions of T cells reacting against B lymphocytes infected with EBV. The disease is associated with a high titre of heterophile ('reacting with cells of another species') antibody which reacts with sheep, horse or ox red cells.

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Figure 9.12 Generation of a germinal centre. B cells activated by antigen migrate from the T zone to the follicle, where they undergo massive proliferation. Cells enter the dark zone as centroblasts and accumulate mutations in their immunoglobulin V genes. Cells then pass back into the light zone (Fig. 9.11) as centrocytes. Only those cells that can interact with antigen on follicular dendritic cells and receive signals from antigen-specific T cells (Fig. 9.10) are selected and migrate out as plasma cells and memory cells. Cells not selected die by apoptosis.

Table 9.3 Causes of lymphocytosis.

Infections

Acute
- Bacterial: pertussis, bordetella
- Viral: infectious mononucleosis, rubella, mumps, acute infectious lymphocytosis, infectious hepatitis, cytomegalovirus, human immunodeficiency virus (HIV), herpes simplex or zoster

Chronic
- Tuberculosis, toxoplasmosis, brucellosis, leishmaniasis, syphilis

Other non-neoplastic disorders
- Physiological stress (trauma, major surgery, septic shock, myocardial infarction)
- Hyposplenism
- Hypersensitivity, e.g. insect bites, drug reaction
- Stress, e.g. trauma, major surgery, myocardial infarct, septic shock
- Chronic polyclonal (idiopathic cigarette smoking, associated with cancer but polyclonal)
- Thyrotoxicosis

Neoplastic
- Chronic lymphoid leukaemias and monoclonal B lymphocytosis (Chapter 18)
- Acute lymphoblastic leukaemia (Chapter 17)
- Non-Hodgkin lymphoma (Chapter 20)

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5–7 days each week; vitamin C can be given to further increase iron excretion. Most iron is lost in the urine, but up to one-third is also excreted in the stools. Because of the difficult administration, lack of patient adherence is a major problem. It may be given on one or more each days each week in combination with daily deferiprone (or deferasirox) and can be used intravenously in combination with oral deferiprone in patients with severe iron overload at risk of dying from cardiac failure. Side-effects are particularly frequent if high doses are used in children and in adults without heavy iron overload. These include high tone deafness, retinal damage, bone abnormalities and growth retardation. Patients receiving deferoxamine should have auditory and fundoscopic examinations at regular intervals.

All three chelators can be given in children. Deferasirox is most frequently used and a liquid formulation of deferiprone and a sprinkle form of deferasirox are available.

Chelation is typically started in thalassaemia major after 10–15 units have been transfused or the serum ferritin is >800–1000 μg/L. In other conditions such as myelodysplastic syndromes, there is controversy about when to initiate chelation and hepatic and cardiac T2* MRI may help guide this decision.

Chelation is given to keep the cardiac T2* test at >20 msecs, liver to <7 mg/g dry weight and serum ferritin level at less than 1000–1500 μg/L, when the body iron stores are approximately 5–10 times normal. MRI assesses cardiac and liver iron accurately and should be repeated annually or more frequently if there is definite cardiac or liver damage (Fig. 4.5). Serum ferritin is useful in monitoring changes in iron stores, but as it is an acute phase reactant it may be elevated in the presence of recent infection or physiological stress such as surgery, and this may falsely suggest inadequate chelation.

Serial tests of heart, liver and endocrine function are also needed to monitor therapy.

Life expectancy has improved dramatically for thalassaemia major patients since the introduction of iron chelation. Chelation may even reverse liver, endocrine and cardiac damage in cases where this has developed before chelation is started or is due to inadequate chelation therapy.

SUMMARY

- Iron overload is caused by excessive absorption of iron from food (genetic haemochromatosis) or by repeated blood transfusions in patients with refractory anaemias. Each unit of blood contains 200–250 mg of iron.
- Excess iron absorbed from the gastrointestinal tract in genetic haemochromatosis accumulates in the parenchymal cells of the liver, the endocrine organs and, in severe cases, the heart.
- Genetic haemochromatosis is usually caused by homozygous mutation of the *HFE* gene causing C282Y protein change and a low serum hepcidin level. Rarer forms exist caused by mutations of other genes coding for proteins involved in iron regulation (hemojuvelin, hepcidin, transferrin receptor 2 and ferroportin). Repeated venesections are used to reduce the body iron burden.
- Transfusional iron overload most frequently occurs in thalassaemia major, but also in other transfusion-dependent refractory anaemias (e.g. some cases of myelodysplastic syndromes, sickle cell anaemia, primary myelofibrosis, red cell aplasia and aplastic anaemia).
- Transfusional iron overload causes damage to the liver, endocrine organs and heart, with iron accumulation also in macrophages of the reticuloendothelial system.
- Cardiac failure or arrhythmia caused by cardiac siderosis, best detected by MRI, is the most frequent cause of death from transfusional iron overload.
- Treatment is with iron chelating drugs: deferiprone and deferasirox, which are active orally, or deferoxamine, given subcutaneously or intravenously.
- Life expectancy has improved dramatically in thalassaemia major as a result of iron chelation therapy and the use of T2*MRI to accurately measure cardiac and liver iron.

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indicates that you can find accompanying multiple-choice questions and answers on the book's companion website.

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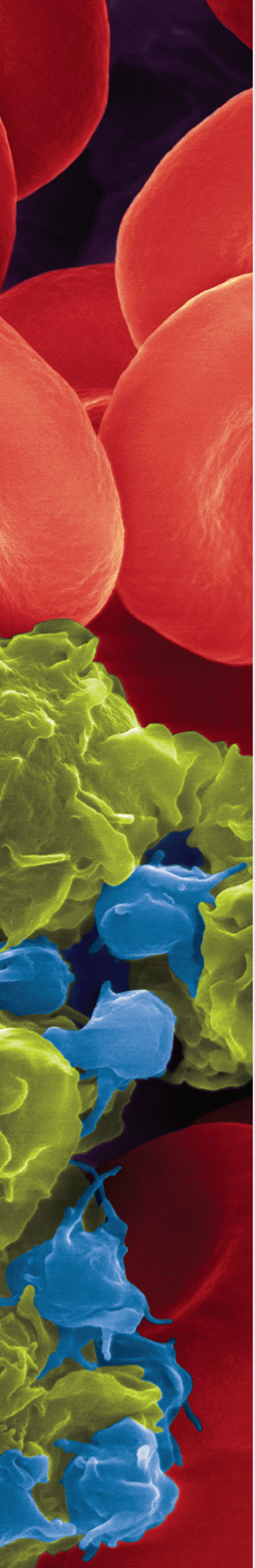
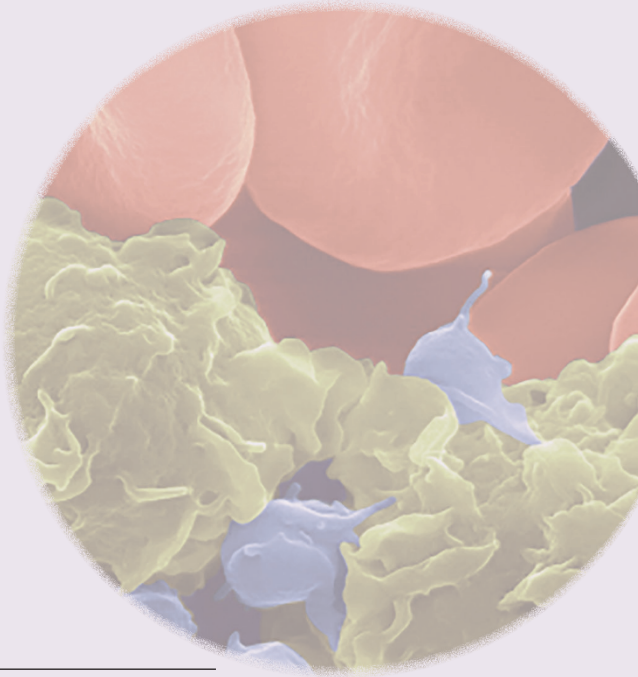


CHAPTER 1

Haemopoiesis

Key topics



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This first chapter is concerned 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 haemopoiesis called 'primitive haemopoiesis'. However, 'definitive haemopoiesis' derives from a population of stem cells first observed on the aorta-gonads-mesonephros (AGM) region of the developing embryo. These common precursors of endothelial and haemopoietic cells are called haemangioblasts and are believed to seed the liver, spleen and bone marrow.

From 6 weeks until 6–7 months of fetal 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 fetal haemopoiesis. The bone marrow is the most important site from 6–7 months of fetal life. During normal childhood and adult life, the marrow is the only source of new blood cells. 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 fatty replacement of marrow throughout the long bones, 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 fetal haemopoietic role ('extramedullary haemopoiesis').

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

Fetus	0–2 months (yolk sac)
	2–7 months (liver, spleen)
	5–9 months (bone marrow)
Infants	Bone marrow (practically all bones); dwindling post-parturition 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

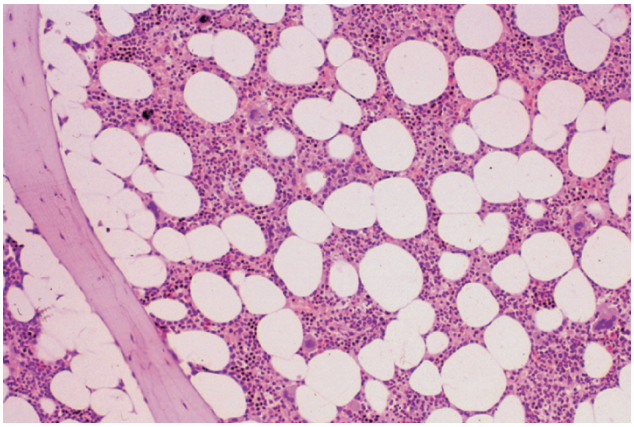

Figure 1.1 Normal bone marrow trephine biopsy (posterior iliac crest). Haematoxylin and eosin stain; approximately 50% of the intertrabecular tissue is haemopoietic tissue and 50% is fat.

Haemopoietic stem and progenitor cells

Haemopoiesis starts with a pluripotential stem cell that can self-renew by asymmetrical cell division, but also gives rise to the separate cell lineages. These 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 marker Cluster of Differentiation 34 ($CD34^+$) and negative for $CD38^-$ and for cell lineage-defining markers (Lin^-). Morphologically, HSCs have the appearance of a small or medium-sized lymphocyte (see Fig. 23.3). The cells reside adjacent to osteoblasts or to endothelial cells of sinusoidal vessels in endosteal or vascular 'niches', where they are surrounded by stromal cells, with which they interact in numerous ways. The niches also contain sympathetic nerve endings.

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 late 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

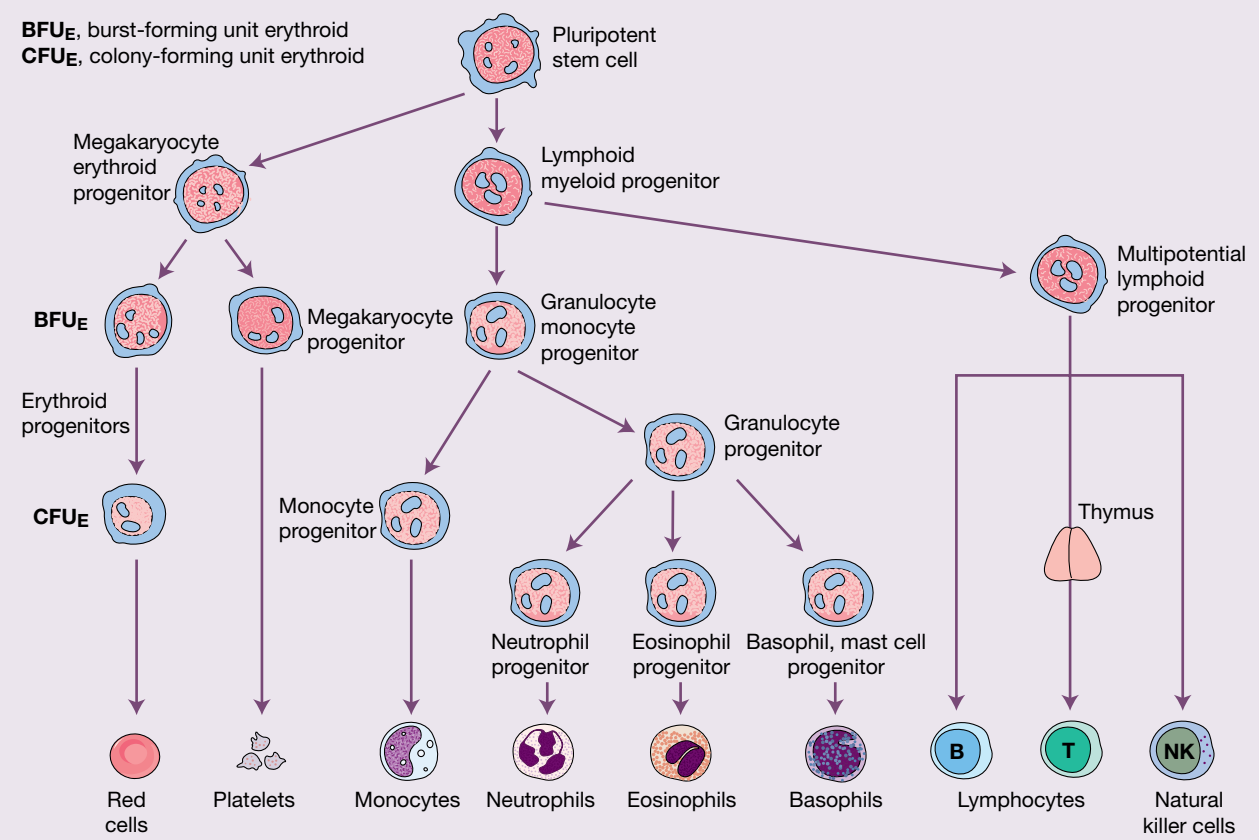

Figure 1.2 Diagrammatic representation of the bone marrow pluripotent stem cells (haemopoietic stem cells, HSC) and the cell lines that arise from them. A megakaryocytic/erythroid progenitor (MkEP) and a mixed lymphoid/myeloid progenitor are formed from the pluripotent stem cells. Each gives rise to more differentiated progenitors. The MkEP divides into erythroid and megakaryocyte progenitors. The mixed lymphoid progenitor gives rise to B and T lymphocytes and to natural killer cells. A granulocyte/monocyte progenitor gives rise to progenitors for monocytes, neutrophils, eosinophils, basophils and mast cells. The erythroid progenitors are also termed BFU-E and CFU-E. BFU-E, burst-forming unit erythroid; CFU-E, colony-forming unit erythroid.

(BFU-E, describing the 'burst' with which they form in culture) and 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 form from the CFU-Meg.

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 (see Chapter 9).

The stem cell has the capability for **self-renewal** (Fig. 1.3), so that 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 actively in cell cycle on any given day. In humans it has been estimated that any given HSC enters the cell cycle approximately once every 3 months to 3 years. 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 falls too. Stem cells also accumulate genetic mutations with age, an average of 8 exonic coding mutations by age 60 years (1.3 per decade), and these, either passengers without oncogenic potential or drivers that cause clonal expansion, may be present in neoplasms arising from these stem cells (see Chapter 11).

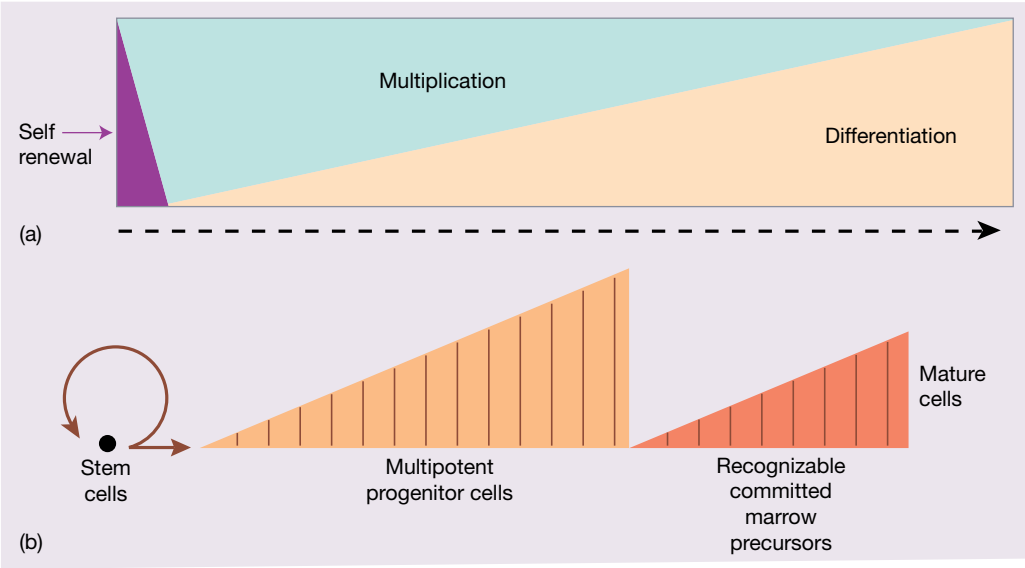

Figure 1.3 **(a)** Bone marrow cells are increasingly differentiated and lose the capacity for self-renewal as they mature. **(b)** A single stem cell gives rise, after multiple cell divisions (shown by vertical lines), to >10^6 mature cells.

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

Bone marrow stroma

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 mesenchymal stem cells, adipocytes**. **fibroblasts, osteoblasts, endothelial cells and macrophages**, and they secrete extracellular molecules such as collagen, glycoproteins (fibronectin and thrombospondin) and glycosaminoglycans (hyaluronic acid and chondroitin derivatives) to form an extracellular matrix. In addition, stromal cells secrete several growth factors necessary for stem cell survival.

Mesenchymal stem cells are critical in stromal cell formation. Together with osteoblasts or endothelial cells, they form niches and provide some of the growth factors, adhesion molecules and cytokines which support stem cells, maintaining their viability and reproduction. For example, stem cell factor (SCF) and the protein Jagged1 expressed by stromal cells bind to their respective receptors, KIT (CD117) and NOTCH1, on stem cells. NOTCH1 then becomes a transcription factor involved in the cell cycle.

Stem cells are able to traffic around the body and 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 by the administration of growth factors such as granulocyte colony-stimulating factor (G-CSF; see p. 100). The reverse process of stem cell **homing** appears to depend on a chemokine gradient in which the stromal-derived factor 1 (SDF-1) which binds to its receptor CXCR4 on HSC is critical.

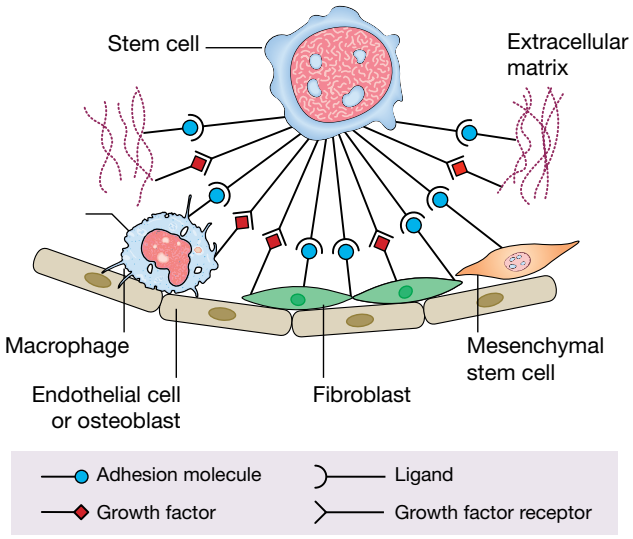

Figure 1.4 Haemopoiesis occurs in a suitable microenvironment ('niche') provided by a stromal matrix on which stem cells grow and divide. The niche may be vascular (lined by endothelium) or endosteal (lined by osteoblasts). There are specific recognition and adhesion sites; extracellular glycoproteins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

The regulation of haemopoiesis

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 may commit them to discrete cell lineages. Which cell lineage is selected for differentiation may depend both on chance and on the external signals received by progenitor cells. Several transcription factors (see p. 8) regulate the survival of stem cells (e.g. SCL, GATA2, NOTCH1), whereas others are involved in differentiation along the major cell lineages. For instance, PU.1 and the CEBP family of transcription factors commit cells to the myeloid lineage, whereas GATA2 and then GATA1 and FOG1 have essential roles in erythropoietic and megakaryocytic differentiation. These transcription factors interact, so that reinforcement of one transcription programme may suppress that of another lineage. The transcription factors induce synthesis of proteins specific to a cell lineage. For example, the erythroid-specific genes for globin and haem synthesis have binding motifs for GATA1.

Haemopoietic growth factors

The haemopoietic growth factors are a group of glycoprotein hormones 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. 1.5).

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 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 and FLT3 ligand act locally on the pluripotential stem cells and on myeloid /lymphoid progenitors (Fig. 1.6). 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

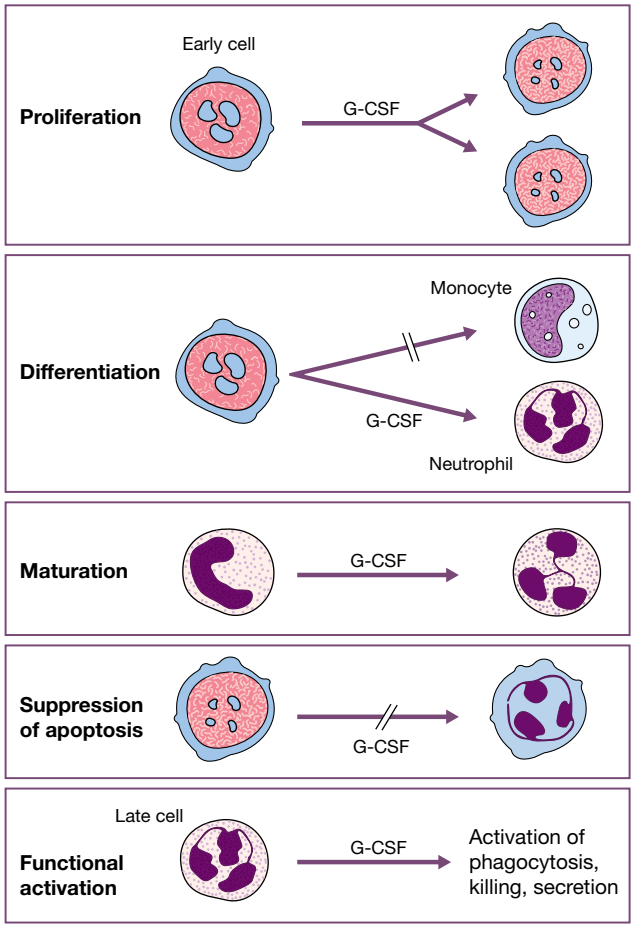

Figure 1.5 Growth factors may stimulate the proliferation of early bone marrow cells, direct differentiation to one or other cell type, stimulate cell maturation, suppress apoptosis or affect the function of mature non-dividing cells, as illustrated here for granulocyte colony-stimulating factor (G-CSF) for an early myeloid progenitor and a 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, functional activation, prevention of apoptosis of progenitor cells

Table 1.3 Haemopoietic growth factors (see also Fig. 1.6).

Act on stromal cells
IL-1, TNF
Act on pluripotential stem cells
SCF, TPO, FLT3-L
Act on multipotential 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.

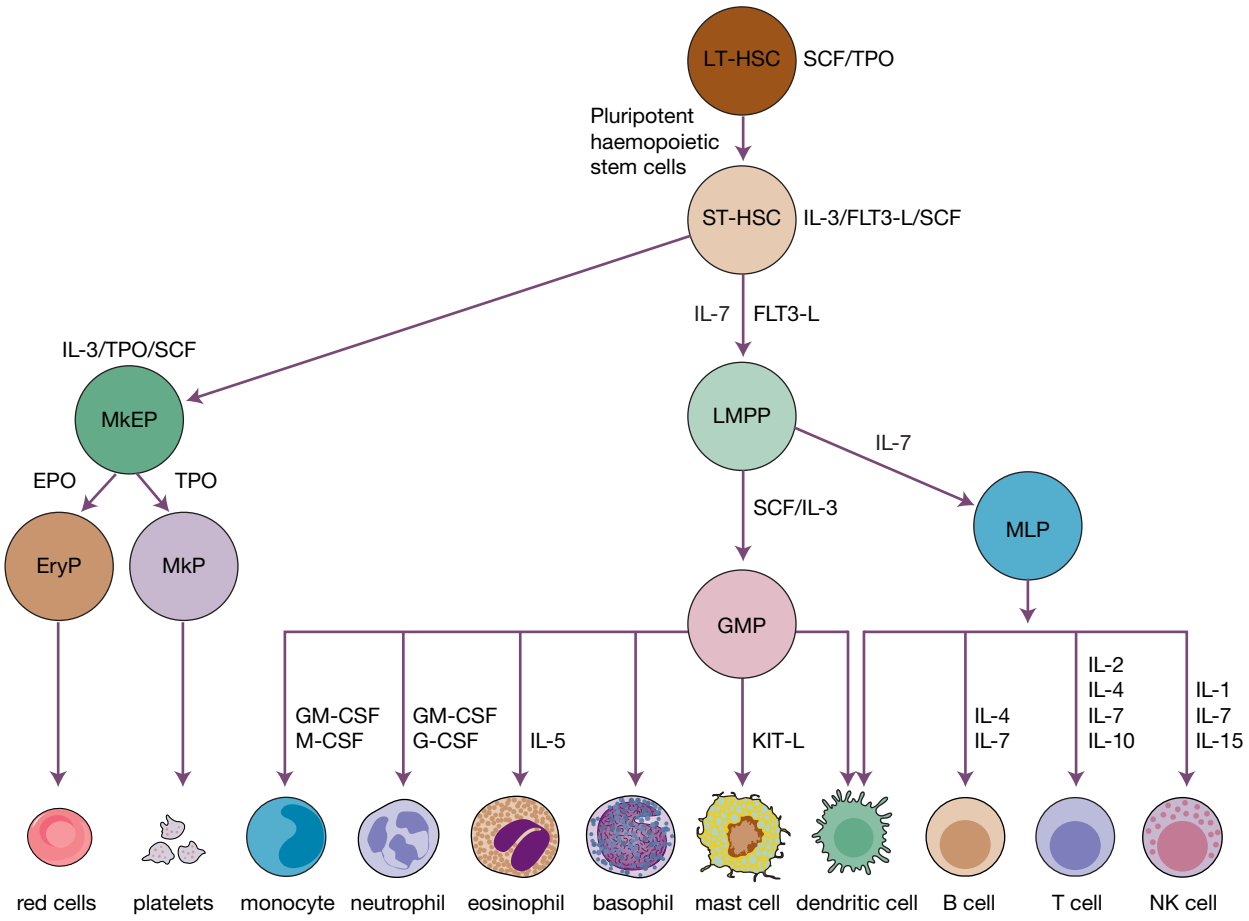

Figure 1.6 The role of growth factors in normal haemopoiesis. Multiple growth factors act on the earlier marrow stem and progenitor cells. EPO, erythropoietin; EryP, erythroid progenitor; FLT3-L, FLT3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; GMP, granulocyte–macrophage progenitor; HSC, haemopoietic stem cells; IL, interleukin; LMPP, lymphoid-primed multipotential progenitor; M-CSF, macrophage/monocyte colony-stimulating factor; MkEP, megakaryocyte–erythroid progenitor; MkP, megakaryocyte progenitor; MLP, multipotential lymphoid progenitor; ST, short-term; LT, long-term; NK, natural killer; PSC, pluripotential stem cell; SCF, stem cell factor; 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.

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 1.6).

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 (see 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 (see p. 276).

Growth factor receptors and signal transduction

The biological effects of growth factors are mediated through specific receptors on target cells. Many receptors, such as EPO receptor (EPO-R), 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, the mitogen-activated protein (MAP) kinase and the phosphatidylinositol 3 (PI3) kinase pathways (Fig. 1.7; see also Fig. 9.4 and 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. 1.7). 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 signal transducer and activator of transcription (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. 1.8. The clinical importance of this pathway is revealed for example by the finding of an activating mutation of the *JAK2* gene as the cause of polycythaemia vera and related myeloproliferative neoplasms (see p. 183).

JAK can also activate the MAPK pathway, which is regulated by RAS and controls proliferation. PI3 kinases phosphorylate inositol lipids, which have a wide range of downstream effects, including activation of AKT leading to block of apoptosis and other actions (Fig. 1.7; see Fig. 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.

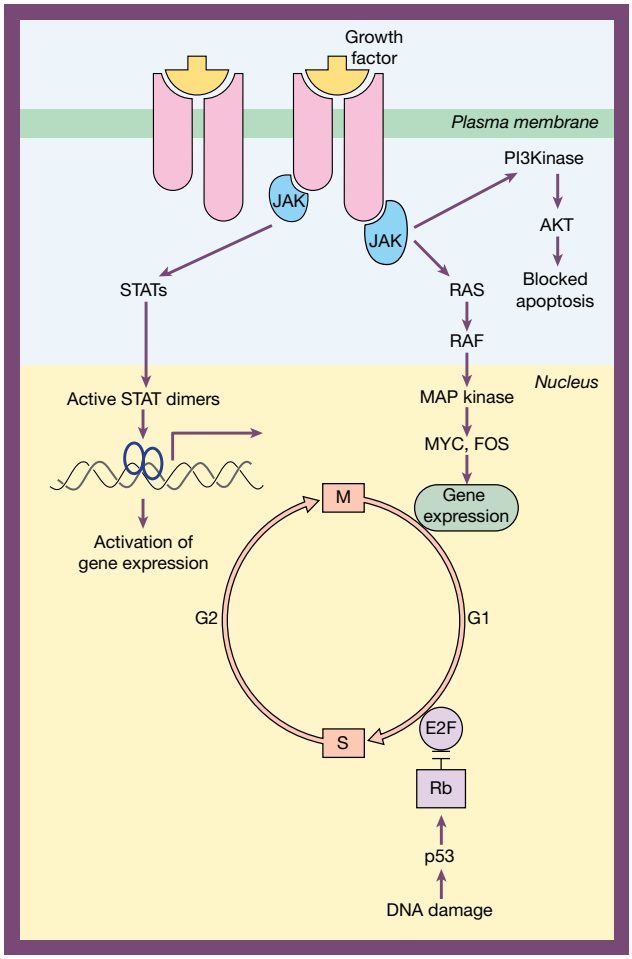

Figure 1.7 Control of haemopoiesis by growth factors. The factors act on cells expressing the corresponding receptors. Binding of a growth factor to its receptor activates the JAK/STAT, MAPK and phosphatidyl-inositol 3-kinase (PI3K) pathways (see Fig. 15.2), which leads to transcriptional activation 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).

A second, smaller group of growth factors, including SCF, FLT-3L 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.

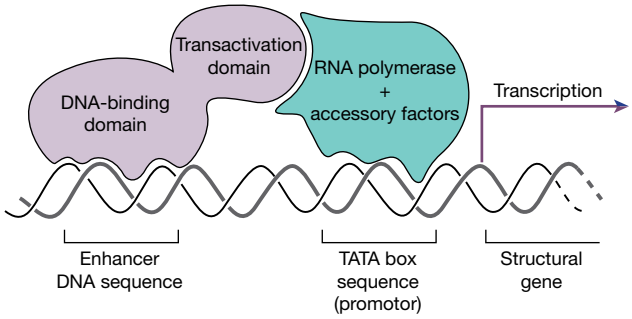

Figure 1.8 Model for control of gene expression by a transcription factor. The DNA-binding domain of a transcription factor binds a specific enhancer sequence adjacent to a structural gene. The transactivation domain then binds a molecule of RNA polymerase, thus augmenting its binding to the TATA box. 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.

Adhesion molecules

A large family of glycoprotein molecules termed adhesion molecules mediate the attachment of marrow precursors, leucocytes and platelets to various components of the extracellular matrix, to endothelium, to other surfaces and to each other. The adhesion molecules on the surface of leucocytes are termed receptors and these interact with proteins termed ligands on the surface of target cells, e.g. endothelium. The adhesion molecules are important in the development and maintenance of inflammatory and immune responses, and in platelet–vessel wall and leucocyte–vessel wall interactions Glycoprotein IIb/IIIa, for example, is an adhesion molecule, also called integrin IIbeta/IIIalpha, involved in platelet adhesion to vessel walls and to each other (Chapter 24).

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 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.

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. 1.7). The M phase is further partitioned into classical **mitosis**, in which nuclear division is accomplished, and **cytokinesis**, in which cell fission occurs.

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 or by flow cytometry.

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 (see p. 249).

Transcription factors

Transcription factors regulate gene expression by controlling the transcription of specific genes or gene families (Fig. 1.8). 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 assembly of the transcription complex at a gene promoter. Examples of transcription factors involved in haemopoiesis include GATA1, GATA2 and FOG1 in erythropoiesis, PU.1 in granulopoiesis, PAX5 in B lymphocyte and NOTCH in T lymphocyte development. Mutation, deletion or translocation of transcription factor genes underlies many cases of haematological neoplasms (see Chapter 11).

Epigenetics

Epigenetics refers to changes in DNA and chromatin that affect gene expression other than those that affect DNA sequence (see 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, which regulates gene expression in normal and tumour tissues. The methylation of cytosine residues to methyl cytosine results in inhibition of gene transcription. The DNA methyltransferase genes *DNMT3A* and *B* are involved in the methylation, and *TET1,2,3* and *IDH1* and *IDH2* in the hydroxylation and breakdown of methylcytosine and restoration of gene expression (see Fig. 16.1). These genes are frequently mutated in the myeloid malignancies, especially myelodysplastic syndromes and acute myeloid leukaemia (see 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 internucleosomal 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 signalling 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. 1.7). 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. The best-characterized example is BCL2. BCL2 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 BCL2 determines the relative susceptibility of cells to apoptosis (e.g. determines the lifespan of platelets)

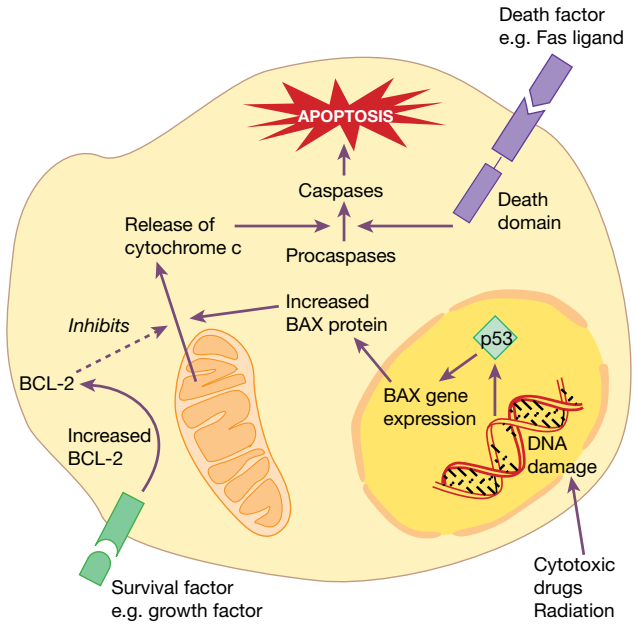

Figure 1.9 Representation of apoptosis. Apoptosis is initiated via two main stimuli: (i) signalling through cell membrane receptors such as FAS or tumour necrosis factor (TNF) receptor; or (ii) release of cytochrome c from mitochondria. Membrane receptors signal apoptosis through an intracellular death 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.

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 (see p. 248). Over-expression of the BCL2 protein makes the malignant B cells less susceptible to apoptosis. 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 (see 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 ischaemia, 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 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 and circulate in the blood.
- Growth factors attach to specific cell receptors and produce a cascade of phosphorylation events to the cell nucleus. Transcription factors carry the message to those genes that are to be 'switched on', 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, endothelium and 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.
- Transcription factors are molecules that bind to DNA and control the transcription of specific genes or gene families.
- 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. BCL2) determines the cell susceptibility to apoptosis.

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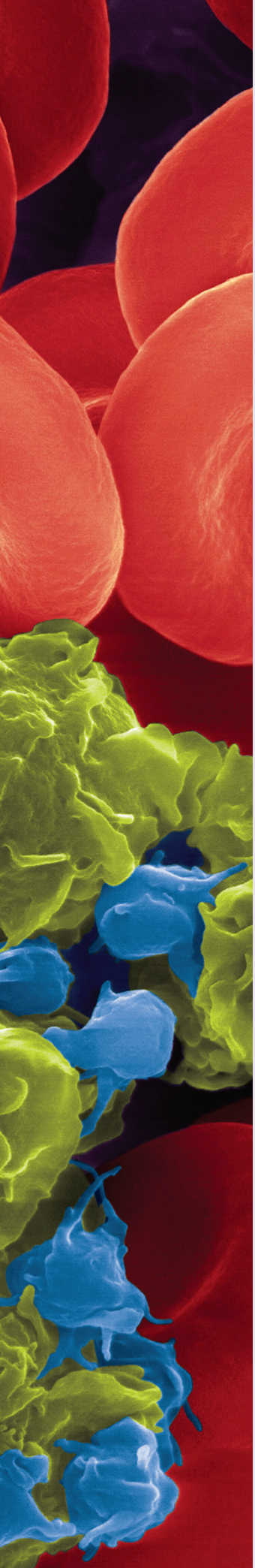
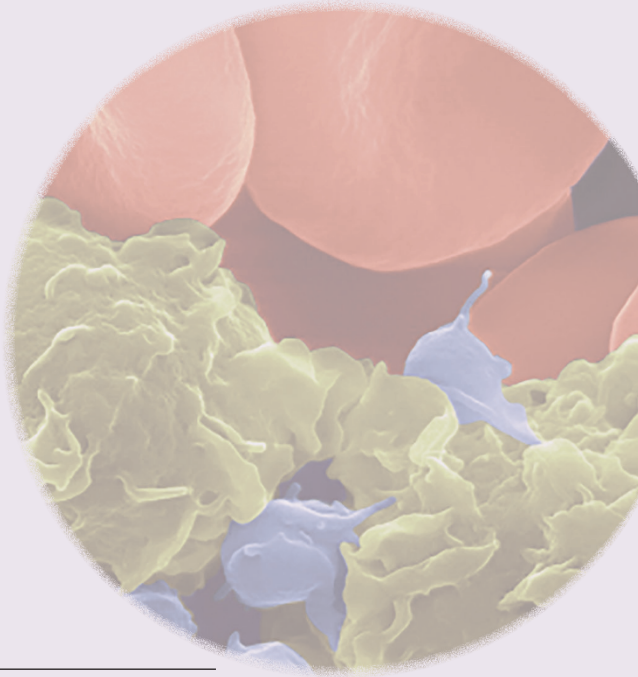


CHAPTER 2

Erythropoiesis and general aspects of anaemia

Key topics



Hoffbrand's Essential Haematology, Eighth Edition. By A. Victor Hoffbrand and David P. Steensma.

Companion website: www.wileyessential.com/haematology

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**, which are 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 lifespan, whereas the smallest cells, **platelets** involved in haemostasis, circulate for only 10 days. **The white cells are made up of four types of phagocyte: neutrophils, eosinophils, basophils and monocytes**, which protect against bacterial and fungal infections (see 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 other foreign cells, and natural killer cells, a subset of CD8 T cells (see Chapter 9). White cells have a wide range of lifespan (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.

We each make approximately 10^{12} new erythrocytes (red cells) each day by the complex and finely regulated process of erythropoiesis. Erythropoiesis passes from the stem cell through the progenitor cells, colony-forming unit (CFU), erythroid and megakaryocyte (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. They also contain progressively more haemoglobin (which stains pink) in the cytoplasm; the cytoplasm 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, which still contains some ribosomal RNA and is still able to synthesize haemoglobin (Fig. 2.4).

Table 2.1 The blood cells.

Cell	Diameter (μm)	Lifespan in blood	Number	Function
Red cells 	6–8	120 days	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 days	$140–400 \times 10^9$/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  B T	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

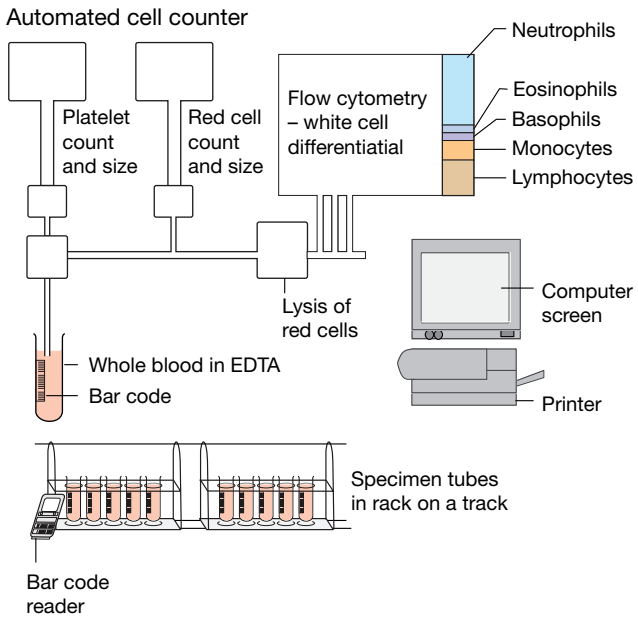


Figure 2.1 Automated blood cell counter. Source: A.B. Mehta, A.V. Hoffbrand (2014) *Haematology at a Glance*, 4th edn. Reproduced with permission of John Wiley & Sons.

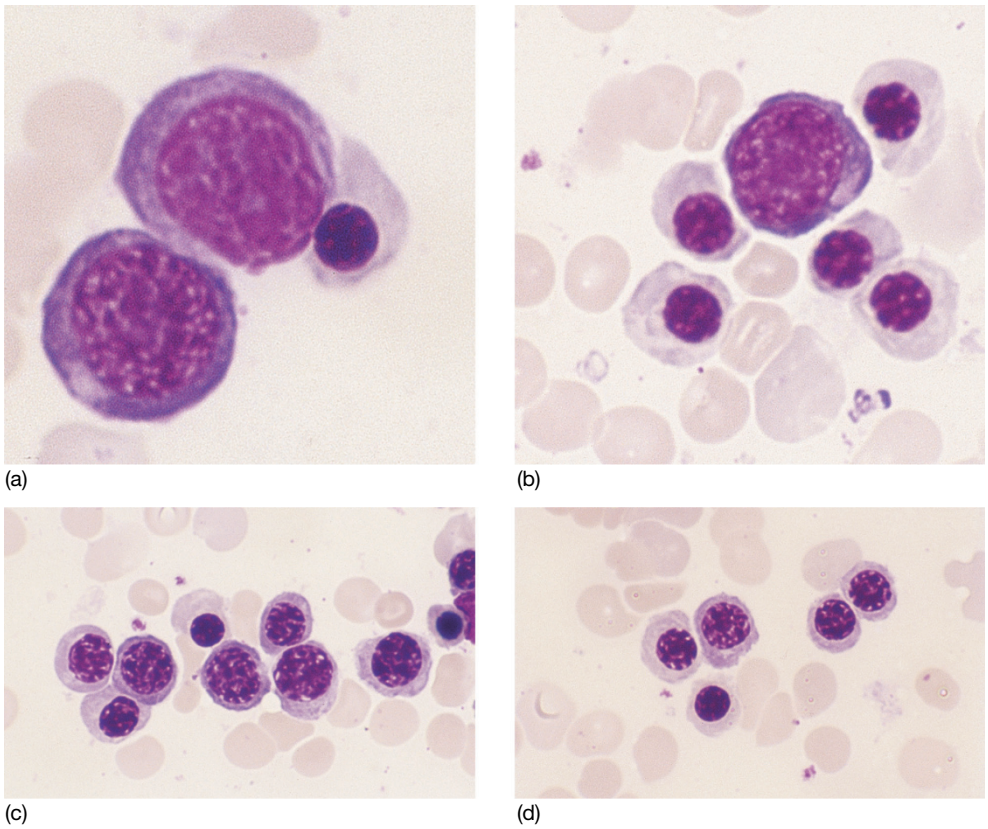


Figure 2.2 Erythroblasts (normoblasts) at varying stages of development. The earlier cells are larger, with more basophilic cytoplasm and a more open nuclear chromatin pattern. The cytoplasm of the later cells is more eosinophilic as a result of haemoglobin formation.

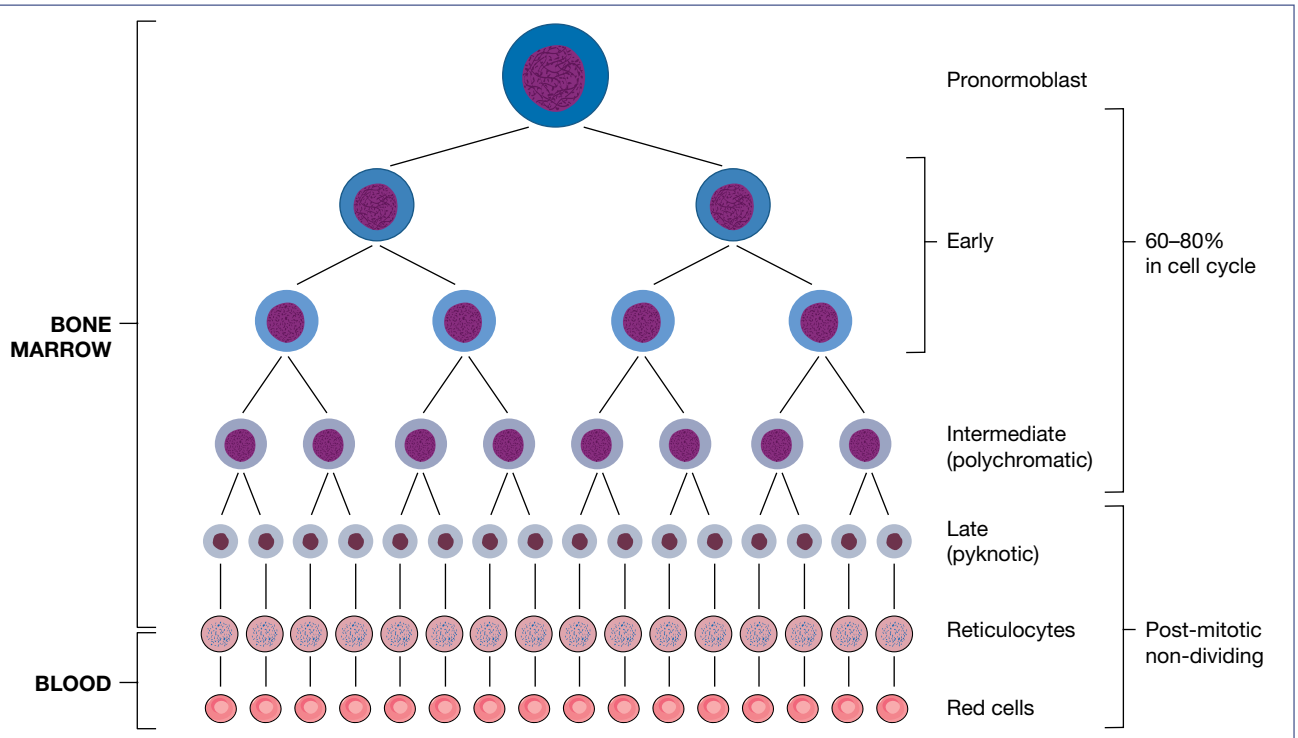
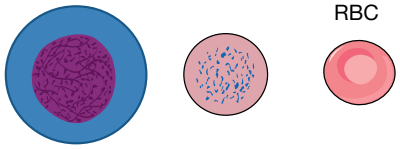


Figure 2.3 The amplification and maturation sequence in the development of mature red cells from the pronormoblast.

This cell is slightly larger than a mature red cell, and 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 (see Fig. 24.3). One pronormoblast usually gives rise to 16 mature red cells (Fig. 2.3). Nucleated red cells (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.



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).

Erythropoietin

Erythropoiesis is regulated by the hormone erythropoietin, a heavily glycosylated polypeptide. Normally, 90% 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 and the stimulus to erythropoietin production is the oxygen (O_2) tension in the tissues of the kidney (Fig. 2.5). Hypoxia induces synthesis of hypoxia-inducible factors (HIF-1α and β), which stimulate erythropoietin production and also new vessel formation and transferrin receptor synthesis, and reduce hepcidin synthesis, increasing iron absorption. Von Hippel-Lindau (VHL) protein breaks down HIFs and PHD2 hydroxylates HIF-1α, allowing VHL binding (Fig. 2.5). Mutations in the genes for these proteins may cause polycythaemia (see Chapter 15).

Erythropoietin production therefore increases in anaemia, and also when haemoglobin for some metabolic or structural reason is unable to give up O_2 normally, when atmospheric O_2 is low or when defective cardiac or pulmonary function or damage to the renal circulation affects O_2 delivery to the kidney.

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

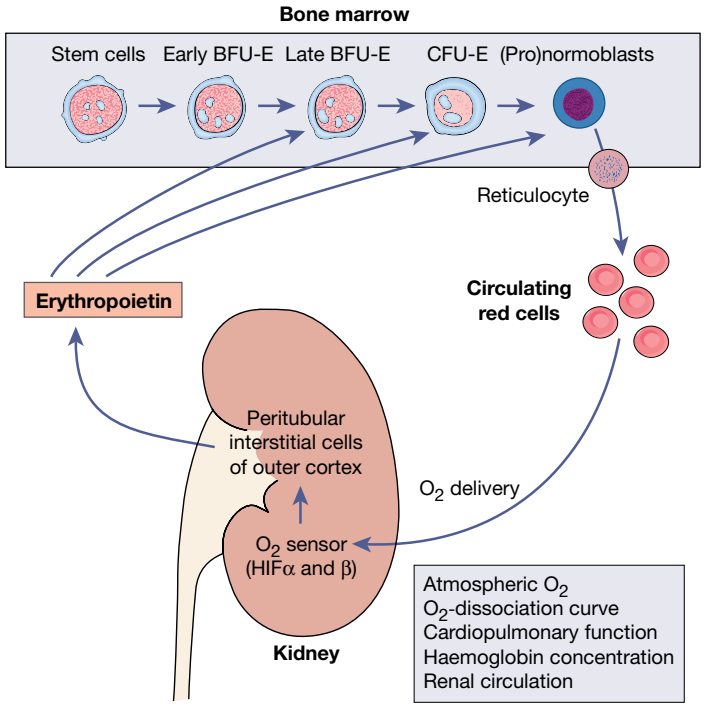


Figure 2.5 The production of erythropoietin by the kidney in response to its oxygen (O_2) supply. Erythropoietin stimulates erythropoiesis and so increases O_2 delivery. BFU_E, erythroid burst-forming unit; CFU_E, erythroid colony-forming unit. Hypoxia induces hypoxia inducible factors (HIFs) α and β, which stimulate erythropoietin production. Von-Hippel–Lindau (VHL) protein breaks down HIFs. PHD2 (prolyl hydroxylase) hydroxylates HIF-1α, allowing VHL binding to HIFs. Mutations in VHL, PHD2 or HIF-1α underlie congenital polycythaemia (see p. 189).

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. globin, haem biosynthetic and red cell membrane proteins) and also enhancing expression of anti-apoptotic genes and of the transferrin receptor (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 (see p. 85).

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).

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. It has been associated with progression of some tumours which express EPO receptors.

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

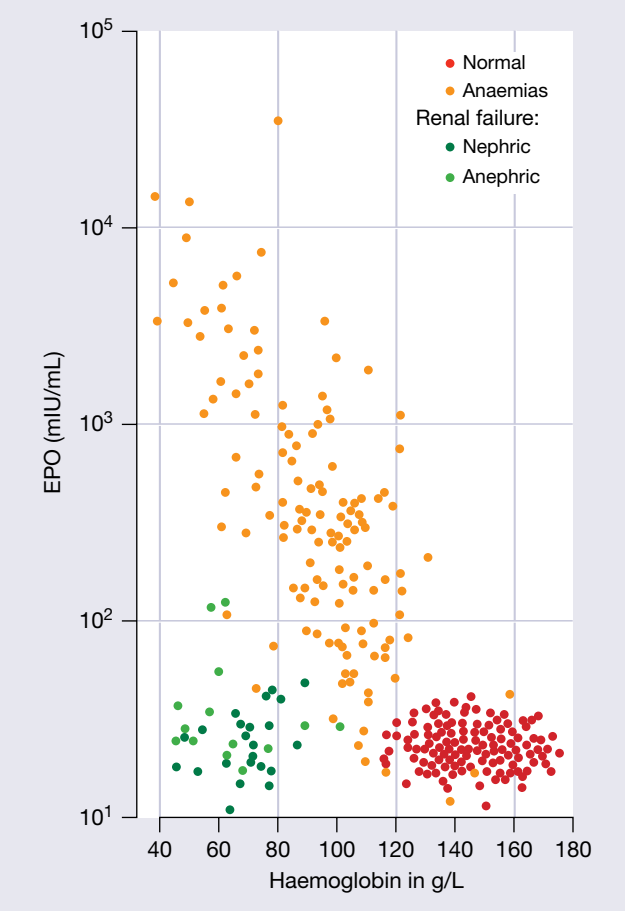


Figure 2.6 The relation between erythropoietin (EPO) in plasma and haemoglobin concentration. Anaemias exclude conditions shown to be associated with impaired production of EPO. Source: Modified from M. Pippard *et al.* (1992) *Br. J. Haematol*. 82: 445. Reproduced with permission of John Wiley & Sons.

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

The main function of red cells is to carry O_2 to the tissues and to return carbon dioxide (CO_2) from the tissues to the lungs. In order to achieve this gaseous exchange they contain the specialized protein haemoglobin. 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 fetus and adult is discussed in more detail in Chapter 7.

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

Haem synthesis occurs largely in the 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 (ALA) synthase (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

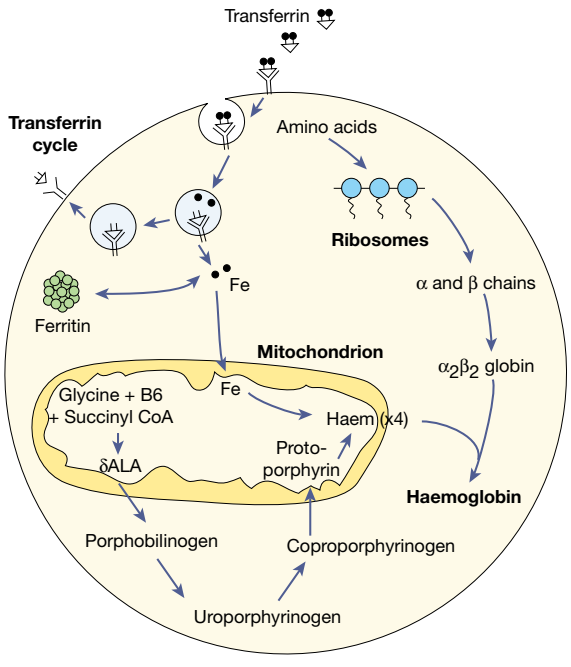


Figure 2.7 Haemoglobin synthesis in the developing red cell. The mitochondria are the main sites of protoporphyrin synthesis, iron (Fe) is supplied from circulating transferrin and globin chains are synthesized on ribosomes. δ-ALA, δ-aminolaevulinic acid; CoA, coenzyme A.

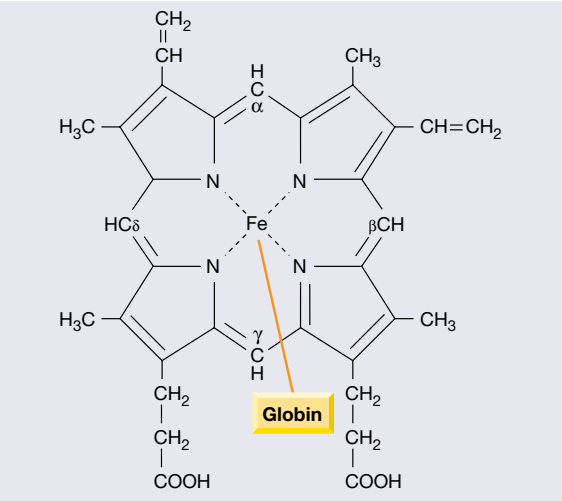


Figure 2.8 The structure of haem.

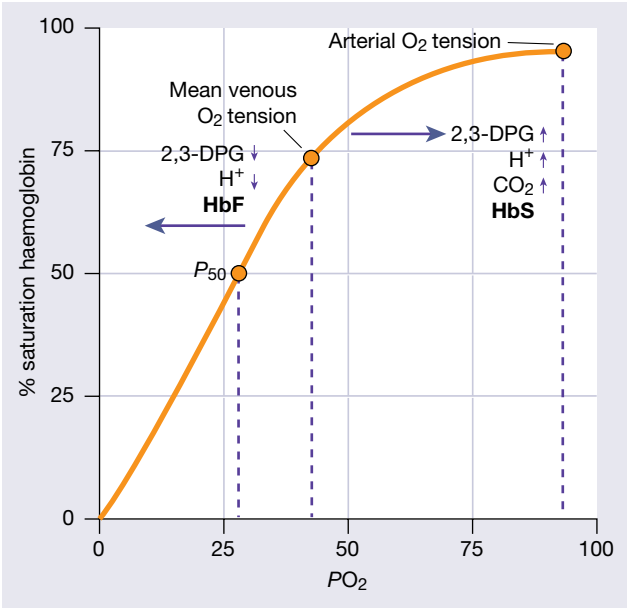


Figure 2.10 The haemoglobin oxygen (O_2) dissociation curve. 2,3-DPG, 2,3-diphosphoglycerate.

(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).

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} (i.e. 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 (i.e. the P_{50} falls), while with decreased affinity for O_2, the curve shifts to the right (i.e. 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 CO_2, and the presence of sickle haemoglobin (Hb S), shift the curve to the right (oxygen is given up more easily), whereas fetal 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.

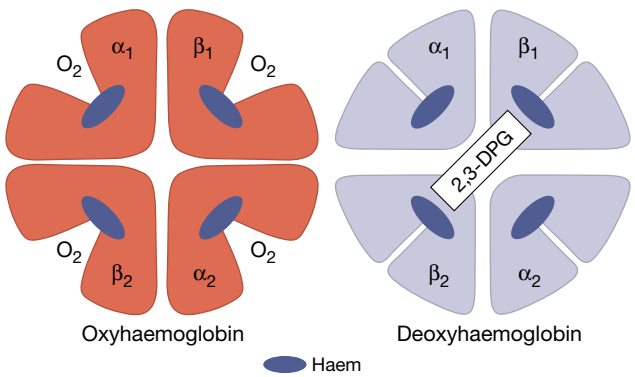


Figure 2.9 The oxygenated and deoxygenated haemoglobin molecule. α, β, globin chains of normal adult haemoglobin (Hb A); 2,3-DPG, 2,3-diphosphoglycerate.

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 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 be able to pass repeatedly through the microcirculation, whose minimum diameter is 3.5 µm; to maintain haemoglobin in a reduced (ferrous) state; and to 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 lifespan 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 as nicotinamide adenine dinucleotide (NADH) by this pathway and as reduced nicotinamide adenine dinucleotide phosphate (NADPH) by the hexose monophosphate shunt (see 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 also 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, reduced haemoglobin containing ferrous ions. The Luebering–Rapoport shunt, or side arm, of this pathway (Fig. 2.11) generates 2,3-DPG, important in the regulation of haemoglobin's oxygen affinity (Fig. 2.9).

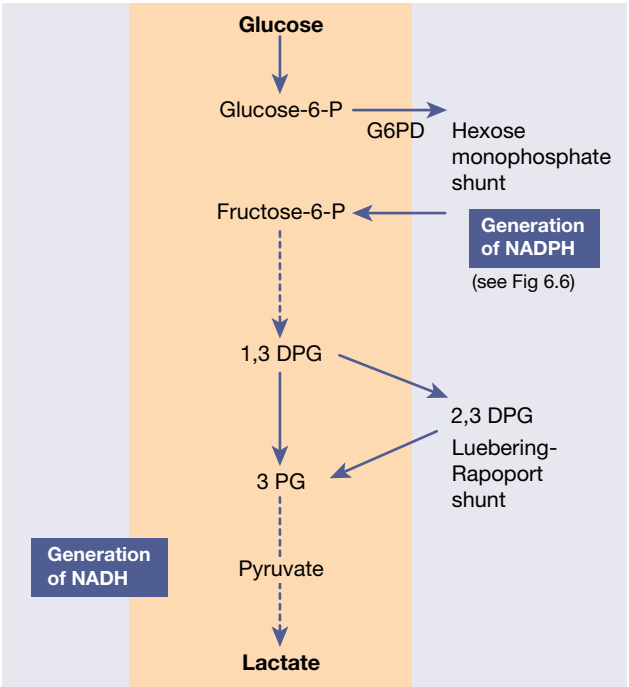


Figure 2.11 The Embden–Meyerhof glycolytic pathway. The Luebering–Rapoport shunt regulates the concentration of 2,3-diphosphoglycerate (2,3-DPG) in the red cell. ATP, adenosine triphosphate; NAD, NADH, nicotinamide adenine dinucleotide; PG, phosphoglycerate.

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 ribulose-5-phosphate (see 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 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 (see p. 70).

Red cell membrane

The red cell membrane comprises a lipid bilayer, integral membrane 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 peripheral or integral, penetrating the lipid bilayer. 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).

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 on the internal side of the red cell membrane and are 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 are attached to protein band 4.1. At the head end, the β spectrin chains attach to ankyrin, which connects to band 3, the transmembrane protein that acts as an anion channel ('vertical connections'; 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; see 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 (see Fig. 2.16).