Chapter 1

Normal Haemoglobin, Blood Cells and Haemopoiesis

Learning objectives

- To have a basic understanding of the structure and functions of the haemoglobin molecule.
- To know about the general features of globin genes — their location, structure, transcription and translation.
- To be able to identify the various types of normal blood cells in photographs.
- To know the functions, concentration and lifespan of various types of blood cell.
- To understand the concept of a stem cell.
- To understand how blood cells are produced from pluripotent haemopoietic stem cells and how haemopoiesis is regulated.
- To be able to identify erythroblasts, neutrophil precursors and megakaryocytes in photographs.
- To be aware of differences in the sites of haemopoiesis during human development and the reappearance of extramedullary haemopoiesis in some haematological disorders.

Normal haemoglobin and its synthesis

Structure and function

Normal human haemoglobins (Hbs) are tetramers consisting of two pairs of unlike globin chains; each of the four chains is associated with one haem group located within a hydrophobic crevice. In adult Hbs, α-chains are associated mainly with β-chains (HbA; $\alpha_2\beta_2$) and to a much lesser extent with δ-chains (HbA_2; $\alpha_2\delta_2$). In fetal Hb, α-chains are associated with γ-chains (HbF; $\alpha_2\gamma_2$). In the embryonic Hbs, ζ-chains are associated with ε-chains (Hb Gower 1; $\zeta_2\varepsilon_2$) or γ-chains (Hb Portland; $\zeta_2\gamma_2$) and α-chains are associated with ε-chains (Hb Gower 2; $\alpha_2\varepsilon_2$). There are two types of γ-chains in HbF which differ only in the amino acid at position 136, which may be glycine ($^{G}\gamma$-chains) or alanine ($^{A}\gamma$-chains).

When the percentage O_2 saturation of Hb at various O_2 tensions (in mmHg) is determined in the laboratory and the two values are plotted against each other, a sigmoid O_2 dissociation (O_2 affinity) curve is obtained (Fig. 1.1). This is because the binding of one O_2 molecule to the haem group on one globin chain of the tetrameric Hb molecule promotes the binding of the next O_2 molecule to the haem group on another globin chain. This haem–haem interaction results from a small shape change in the molecule that occurs when O_2 combines with haem. In the deoxygenated state, the two β-chains are separated slightly, such that one molecule of 2,3-diphosphoglycerate (2,3-DPG) can enter the Hb molecule and bind to the β-chains; in the oxygenated state, the 2,3-DPG is ejected. The partial pressure of O_2 at which normal Hb is half saturated with O_2 (P_{50}) is 26 mmHg

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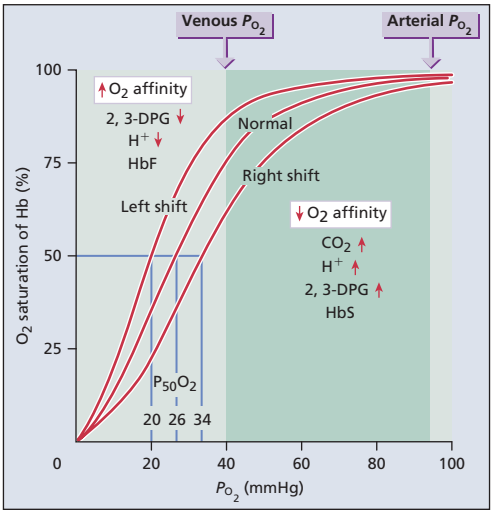

Figure 1.1 Oxygen dissociation curves of human blood.

(at pH 7.4, 37ºC). *In vivo*, exchange of O_2 normally occurs between a P_{O_2} of 95 mmHg (95% saturation) in arterial blood and a P_{O_2} of 40 mmHg (70% saturation) in venous blood.

The O_2 affinity of Hb is decreased (the O_2 dissociation curve is shifted to the right) by an increase in the amount of CO_2 in the blood (Bohr effect). The CO_2 generates hydrogen ions by reacting with water, and the reduced O_2 affinity results from the combination of hydrogen ions with deoxyhaemoglobin; these hydrogen ions are released when Hb is oxygenated. Thus the Bohr effect facilitates the release of O_2 in tissues and the unloading of CO_2 in the lungs. A second mechanism by which CO_2 generated in the tissues decreases the O_2 affinity of Hb (i.e. facilitates the release of O_2) is by reacting with the amino groups of the α-globin chains to form carbamates. When Hb combines with O_2 in the alveoli, this CO_2 is released.

The O_2 affinity of Hb is increased by a decrease in 2,3-DPG levels as occurs in stored blood, and decreased by an increase in 2,3-DPG levels as occurs in hypoxia. The O_2 affinity of HbF is higher than that of HbA because γ-chains bind to 2,3-DPG more weakly than β-chains. The high affinity of HbF facilitates O_2 transport from the mother to the fetus. O_2 affinity is decreased in sickle cell anaemia and also by an increase in body temperature (e.g. during fever).

Synthesis

The genes for the ε-, $^{G}\gamma$-, $^{A}\gamma$-, δ- and β-chains are found in this order (5′ to 3′) in a linked cluster on chromosome 11. The α gene is duplicated so that there are two α genes close to the ζ gene on chromosome 16 in the linkage order ζ, α_2, α_1 (5′ to 3′). Interestingly, in both chromosomes 11 and 16, the genes are arranged in the order in which they are switched on during intrauterine life.

There are a number of conserved sequences in the upstream flanking regions of the globin genes. Such sequences are involved in the regulation of globin gene expression and are known as promoters. In addition, there are other local regulatory elements termed enhancers situated at various distances either 5′ or 3′ to the gene. The promoters are involved in the attachment and correct positioning of the transcription initiation complex, which includes RNA polymerase (the enzyme involved in mRNA synthesis). The globin gene promoters and enhancers are recognized by non-specific (ubiquitous) transcription factors, erythroid tissue-specific transcription factors such as GATA-1, GATA-2 and erythroid Krüppel-like factors (EKLF), and developmental stage-specific transcription factors. Expression of genes in the entire β-globin gene cluster is influenced by a remote regulatory region known as the β-locus control region (β-LCR), which is situated upstream of the ε gene. Expression of genes in the entire α-globin gene cluster is controlled by a regulatory element known as HS-40 located upstream of the ζ gene.

Each globin gene contains two non-coding regions, also known as intervening sequences (IVSs) or introns (i.e. regions that are not represented in the mature mRNA) and three coding regions or exons (Fig. 1.2). The initial mRNA transcript (mRNA precursor) is large and complementary to all regions (coding and non-coding) of the globin gene, but the regions complementary to the base sequences of the introns are soon removed by excision and ligation (spliced) and are absent in the mature mRNA. Within the nucleus,

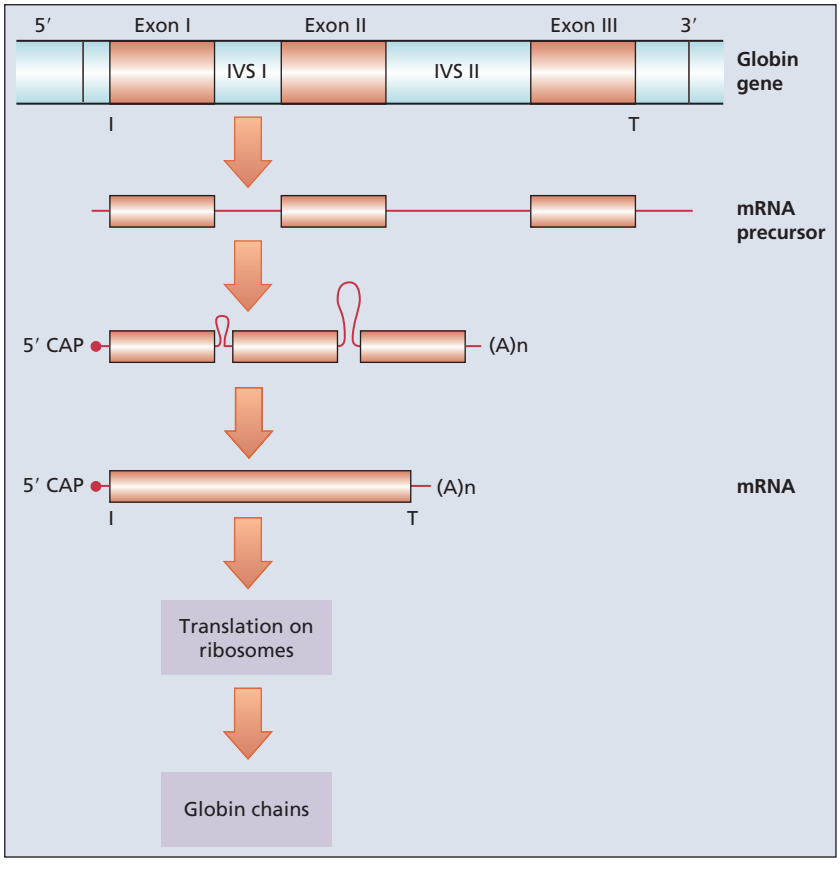

Figure 1.2 Expression of globin genes. *Notes*: IVS — intervening sequence; I — initiator codon; T — termination codon; (A)n — polyadenylation site; 5′ CAP — structure containing 7-methyl guanosine.

the mRNA is modified at the 5′ end by the formation of a CAP structure and is stabilized by polyadenylation at the 3′ end. The mature mRNA enters the cytoplasm and attaches to ribosomes on which globin chain synthesis (translation) occurs.

Blood cells

Morphology

On Romanowsky-stained blood smears, normal erythrocytes appear as red, anucleate cells with circular outlines and have diameters between 6.7 and 7.7 μm (mean 7.2 μm). Blood-cell morphology should be assessed in a region of the blood smear in which only occasional red cells overlap. In such

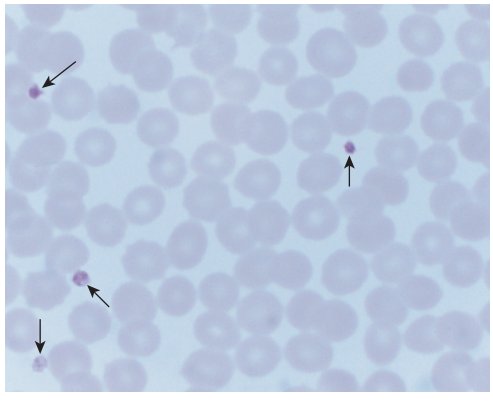

Figure 1.3 Smear of normal peripheral venous blood. The red cells are round and do not vary greatly in size. They are well filled with haemoglobin; the central area of pallor is small. A few platelets (arrowed) are also present.

Table 1.1 Morphology of normal white cells in Romanowsky-stained smears of peripheral blood.

		Cytoplasm			
Cell type	**Cell size (μm)**	**Colour**	**Ratio of cytoplasmic volume to nuclear volume**	**Granules**	**Nucleus**
Neutrophil granulocytes	9–15	Slightly pink	High	Numerous, very fine, faint purple	Usually two to five segments
Eosinophil granulocytes	12–17	Pale blue	High	Many, large and rounded, reddish-orange	Usually two segments
Basophil granulocytes	10–14		High	Several, large and rounded, dark purplish-black	Usually two segments, granules overlie nucleus
Monocytes	15–30	Pale greyish-blue, cytoplasmic vacuoles may be seen	Moderately high or high	Variable number, fine, purplish-red	Various shapes (rounded, C- or U-shaped, lobulated), skein-like or lacy chromatin
Lymphocytes	7–12 (small lymphocytes)	Pale blue	Low or very low	Few, fine purplish-red	Rounded with large clumps of condensed chromatin
	12–16 (large lymphocytes)	Pale blue	Higher	Several, coarser, purplish-red	Less condensation of chromatin

a region, each red cell (which is biconcave in shape) has a central area of pallor whose diameter is about a third of the red cell diameter (Fig. 1.3).

In addition to red cells, blood smears contain platelets and various types of white cell. On average, the ratio between red cells, platelets and leucocytes is 700:40:1. The platelets are small anucleate cells, about 2–3 μm in diameter (Fig. 1.3). They stain light blue and contain a number of small azurophilic granules, which are often concentrated at the centre. The important features of the morphology of normal leucocytes are summarized in Table 1.1. Neutrophil, eosinophil and basophil granulocytes (Fig. 1.4) are also described as polymorphonuclear leucocytes or polymorphs: the two or more nuclear masses in each cell are joined in series by fine strands of nuclear chromatin. Normally, the proportion of neutrophil polymorphs with five or more nuclear segments is 3% or less. A monocyte is shown in Fig. 1.4(c). Small lymphocytes (Fig. 1.5) account for about 90% of lymphocytes in the blood; large lymphocytes for the remaining 10%. Many of the large lymphocytes contain several prominent purplish-red granules in their cytoplasm (Fig. 1.6).

Number and lifespan

The reference ranges for concentrations of various types of blood cell in adults are given in Table 1.2, together with data on their lifespan in the blood. Ranges for the Hb and packed cell volume (PCV) in healthy individuals are given on p. 16. Normal red cells circulate for 110–120 days and at the end of their lifespan are phagocytosed in the bone marrow, spleen, and liver by macrophages

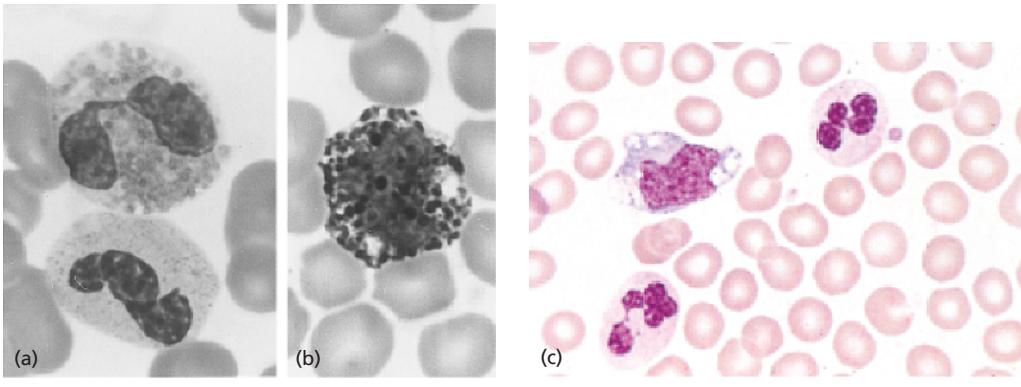

Figure 1.4 Smear of normal peripheral venous blood: (a) neutrophil granulocyte and eosinophil granulocyte; (b) basophil granulocyte; and (c) monocyte and two neutrophil granulocytes — the monocyte has a pale, greyish-blue vacuolated cytoplasm. Figure (c) is at a lower magnification than (a) and (b).

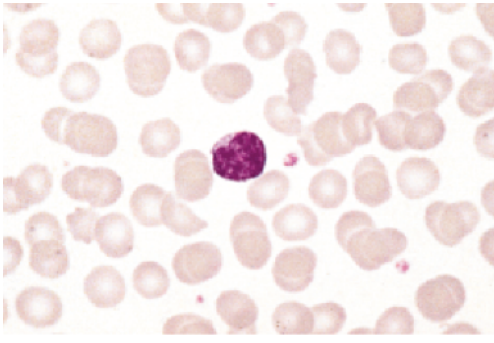

Figure 1.5 A small lymphocyte in a normal blood smear.

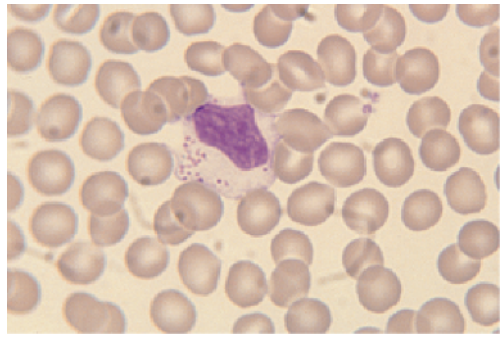

Figure 1.6 Large lymphocyte with several azurophilic cytoplasmic granules. Large granular lymphocytes (LGLs) include cytotoxic T cells and natural killer (NK) cells (p. 7). *Source*: Courtesy of Dr. Barbara Bain.

Table 1.2 Ninety-five percent reference limits for the concentrations of various types of circulating blood cell in adults and their lifespan in the blood.

Cell type	Reference range (95% reference limits)	Lifespan in blood
Red cells	Males $4.4–5.8 \times 10^{12}$/L Females $4.1–5.2 \times 10^{12}$/L	110–120 days
White cells (leucocytes)	$4.0–11.0 \times 10^{9}$/L*	
Neutrophil granulocytes	$1.5–7.5 \times 10^{9}$/L*	$t_{1/2}$ approx. 7 h
Eosinophil granulocytes	$0.02–0.60 \times 10^{9}$/L	$t_{1/2}$ approx. 6 h
Basophil granulocytes	$0.01–0.15 \times 10^{9}$/L	
Monocytes	$0.2–0.8 \times 10^{9}$/L	$t_{1/2}$ approx. 70 h
Lymphocytes	$1.2–3.5 \times 10^{9}$/L	
Platelets	$160–450 \times 10^{9}$/L	9–12 days

Note: *Applies to Caucasians.

Table 1.3 The main functions of blood cells.

Type of cell	Main functions
Red blood cells (erythrocytes)	Transport O_2 from lungs to tissues; transport CO_2 from tissues to lungs (see p. 1)
Neutrophil granulocytes	Chemotaxis, phagocytosis, killing of phagocytosed bacteria
Eosinophil granulocytes	All neutrophil functions listed above, effector cells for antibody-dependent damage to metazoal parasites, regulate immediate-type hypersensitivity reactions (inactivate histamine and leukotrienes released by basophils and mast cells)
Basophil granulocytes	Mediate immediate-type hypersensitivity (IgE-coated basophils react with specific antigen and release histamine and leukotrienes), modulate inflammatory responses by releasing heparin and proteases
Monocytes (and macrophages*)	Chemotaxis, phagocytosis, killing of some microorganisms, antigen presentation, release of IL-1 and TNF which stimulate bone marrow stromal cells to produce GM-CSF, G-CSF, M-CSF, and IL-6 (p. 15)
Platelets	Adhere to subendothelial connective tissue, participate in blood clotting (see p. 147)
Lymphocytes	Involved in immune responses and production of haemopoietic growth factors (p. 7)

Note: *Macrophages are tissue cells derived from monocytes.

(a component of the reticuloendothelial system). The neutrophil granulocytes in the blood are distributed between a marginated granulocyte pool (consisting of cells that are loosely attached to the endothelial lining of small venules) and a circulating granulocyte pool. There is a continuous exchange of cells between these two pools and in healthy subjects, the circulating granulocyte pool accounts for between 16% and 99% (average, 44%) of all blood granulocytes. When cell counts are determined on samples of peripheral venous blood, only the circulating granulocytes are being studied. In healthy Caucasian adults, the reference range for the absolute neutrophil count is $1.5–7.5 \times 10^9$/L. The lower limit for the reference range is lower in healthy blacks, being about 1.0×10^9/L. Neutrophil granulocytes leave the circulation exponentially, with an average $t_{1/2}$ of about 7 h, and probably survive in tissues and secretions for about another 30 h.

Lymphocytes continuously recirculate between the blood and lymphatic system. They leave the blood between the endothelial cells of the postcapillary venules of lymph nodes, migrate through the lymph node into efferent lymphatics and re-enter the blood via the thoracic duct. The majority of human lymphocytes are long-lived, with average lifespans of 4–5 years and maximum lifespans of greater than 20 years. The short-lived lymphocytes survive for about 3 days.

Functions

The main functions of blood cells are summarized in Table 1.3. More details of platelet function are given on p. 147.

Neutrophils and monocytes

Monocytes are the precursors of tissue macrophages. Phagocytosis of microorganisms and cells coated with antibody (with their exposed Fc fragments) and complement (especially C3b) occurs via binding to Fc and C3b receptors on the surface of neutrophils, monocytes and macrophages. Bacteria and fungi that are not antibody coated are phagocytosed after binding to mannose receptors on the phagocyte surface. The killing of phagocytosed microorganisms involves superoxide (O_2^-)-dependent and O_2-independent mechanisms.

The superoxide-dependent microbicidal agents include H_2O_2, hypochlorous acid, chloramines and hydroxyl radicals ($OH^{\bullet}$). Superoxide (O_2^-) is produced by neutrophils and some γ-interferon-stimulated macrophages during the respiratory burst which follows their activation and lasts from a few seconds to 15 min. The generation of O_2^- results from the reduction of O_2 by NADPH, which is catalysed by NADPH oxidase, one component of which is membrane-bound flavocytochrome b_{558}. The O_2^- undergoes dismutation to O_2 and H_2O_2. It also generates hydroxyl radicals by reaction with H_2O_2. Myeloperoxidase, found in the primary granules of neutrophils, catalyses the formation of the strong microbicidal agent hypochlorous acid from Cl^- and H_2O_2. The hypochlorous acid so formed reacts with amines to form chloramines.

Non-O_2-dependent microbicidal mechanisms include a reduction of pH within phagocytic vacuoles (phagosomes) and the release into phagosomes of (i) lysozyme (found within azurophilic and specific granules), which causes swelling and rupture of bacteria; (ii) defensins (peptides) and bacterial permeability increasing protein (both found in primary granules) that damage and make leaky the membranes of microorganisms; and (iii) the iron-binding protein lactoferrin, which may prevent ingested bacteria from taking up iron.

Lymphocytes

Between 65% and 80% of peripheral blood lymphocytes are T cells, 10–30% are B cells and 2–10% are non-T and non-B cells (null cells). Both B and T cells are formed with specific antigen-recognizing molecules on their cell surface which determine that each cell recognizes a specific antigenic determinant. The antigen-recognizing molecules for B and T cells are, respectively, immunoglobulin and the T-cell receptor (TCR) molecule. The cells are triggered into proliferation when they react with the specific antigen in the presence of appropriate accessory cells; their progeny develop into effector cells or memory cells.

The effector T-lymphocytes include helper cells (CD4+ cells), which promote the function of B cells and are required for the maturation of other types of T cell, and suppressor–cytotoxic cells (CD8+ cells), which inhibit the function of other lymphocytes and are cytotoxic towards foreign and virus-infected cells. The ratio of helper to suppressor cells is 1.5–2.5:1. Thus, the functions of the T cells include:

1. Mediation of cellular immunity against viruses, fungi and low-grade intracellular pathogens such as mycobacteria.
2. Participation in delayed hypersensitivity reactions, tumour rejection and graft rejection.
3. Interaction with B cells in producing antibodies against certain antigens.
4. Suppression of B-cell function.

Activated T cells also produce interleukin-5 (IL-5, eosinophil colony-stimulating factor) which is involved in the regulation of eosinophil granulocytopoiesis and IL-3, one of the multilineage haemopoietic growth factors. (This explains the excessive eosinophil production in some T-cell lymphomas.) They also produce other haemopoietic growth factors (IL-6, granulocyte–macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF)) (p. 15).

The percentages of B cells that express IgM, IgD, IgG and IgA molecules on their surface are, respectively, 40%, 30%, 30% and 10%. Many B cells have both IgM and IgD on their surface but others usually have only IgG or IgA. A single B cell expresses immunoglobulins of only one light chain type, and there are twice as many cells with κ light chains as there are with λ light chains. B cells that are activated by reaction with a specific antigen develop into antibody-secreting plasma cells or into B memory cells. Most of the antibodies formed during a primary antibody response consist of IgM, and almost all of the antibodies formed during a secondary antibody response (which results from the activation of B memory cells) consist of IgG.

The null cells are now known as natural killer (NK) cells. These cells lyse antibody-coated target cells and are therefore also called antibody-dependent cytotoxic cells (ADCC). The NK cells also kill tumour cells and virus-infected cells in the absence of antibody.

Further information on B cells, T cells and NK cells is provided in Chapter 7.

Haemopoiesis in the adult

In normal adults, haemopoiesis (production of blood cells) only occurs in the marrow contained within certain bones (p. 15).

General considerations and early events

Haemopoietic systems of adults are examples of steady-state cell renewal systems in which the rate of loss of mature cells (red cells, granulocytes, monocytes, lymphocytes and platelets) from the blood is balanced fairly precisely by the rate of release of newly formed cells into the blood. Mature cells are lost either because of ageing or during the performance of normal functions.

The formation of blood cells involves two processes:

1 Progressive development of structural and functional characteristics specific for a given cell type (cytodifferentiation or maturation).
2 Cell proliferation.

A schematic representation of haemopoiesis is shown in Fig. 1.7. The stem cells and progenitor cells are involved early in haemopoiesis; they cannot be recognized morphologically in marrow smears but can be studied by functional tests. In man, these cells (colony-forming units or CFU) have been identified and characterized on the basis of their ability to produce small colonies of one or more cell types when grown in semi-solid media containing appropriate haemopoietic growth factors. The most primitive haemopoietic cell is the pluripotent haemopoietic stem cell. This gives rise to two types of committed stem cell, namely multipotent myeloid stem cells and lymphoid stem cells. The essential characteristics of stem cells are:

1 An extensive capacity to maintain their own number by cell proliferation (self-renewal).
2 The capacity to mature into other cell types.

The lymphoid stem cells give rise to lymphocyte progenitor cells that eventually mature into all types of T and B lymphocytes and NK cells. The multipotent myeloid stem cells differentiate into various types of myeloid progenitor cell which eventually generate erythrocytes, neutrophils, eosinophils, basophils, monocytes, platelets, mast cells and osteoclasts. Unlike the stem cells, the lymphoid and myeloid progenitor cells have only a limited capacity for self-renewal.

The more immature myeloid progenitor cells are committed to two or three differentiation pathways. With increasing maturity, their differentiation potential becomes progressively limited, eventually to one pathway only. The unipotent progenitor cells committed to the production of erythrocytes, neutrophil granulocytes, eosinophil granulocytes, basophil granulocytes, monocytes/macrophages and megakaryocytes are, respectively, called CFU-E, CFU-G, CFU-eo, CFU-baso, CFU-M and CFU-mega. They mature into the earliest morphologically recognizable cells of the corresponding cell lineage (pronormoblasts, myeloblasts, monoblasts and megakaryoblasts).

Pluripotent haemopoietic stem cells account for about one per 10,000–100,000 nucleated marrow cells. They are also found in very small numbers in circulating blood so that stem cells used for marrow transplantation can be derived not only from bone marrow but also from peripheral blood. Haemopoietic progenitor cells are also found both in marrow and in blood. Despite their presence in blood, myeloid stem cells and progenitor cells normally develop into morphologically recognizable haemopoietic cells only within the microenvironment of the bone marrow.

In addition to haemopoietic stem cells, the marrow contains a more primitive cell type known as mesenchymal stem cells that gives rise to the pluripotent haemopoietic stem cells but may also differentiate under appropriate conditions *in vitro* and/or *in vivo* into a variety of non-haemopoietic cell types such as chondrocytes, osteoblasts, adipocytes, cardiac myocytes, hepatocytes and even neuronal cells.

Morphologically recognizable haemopoietic cells derived from myeloid stem cells

In every myeloid cell lineage other than that involved in platelet production, the early precursors

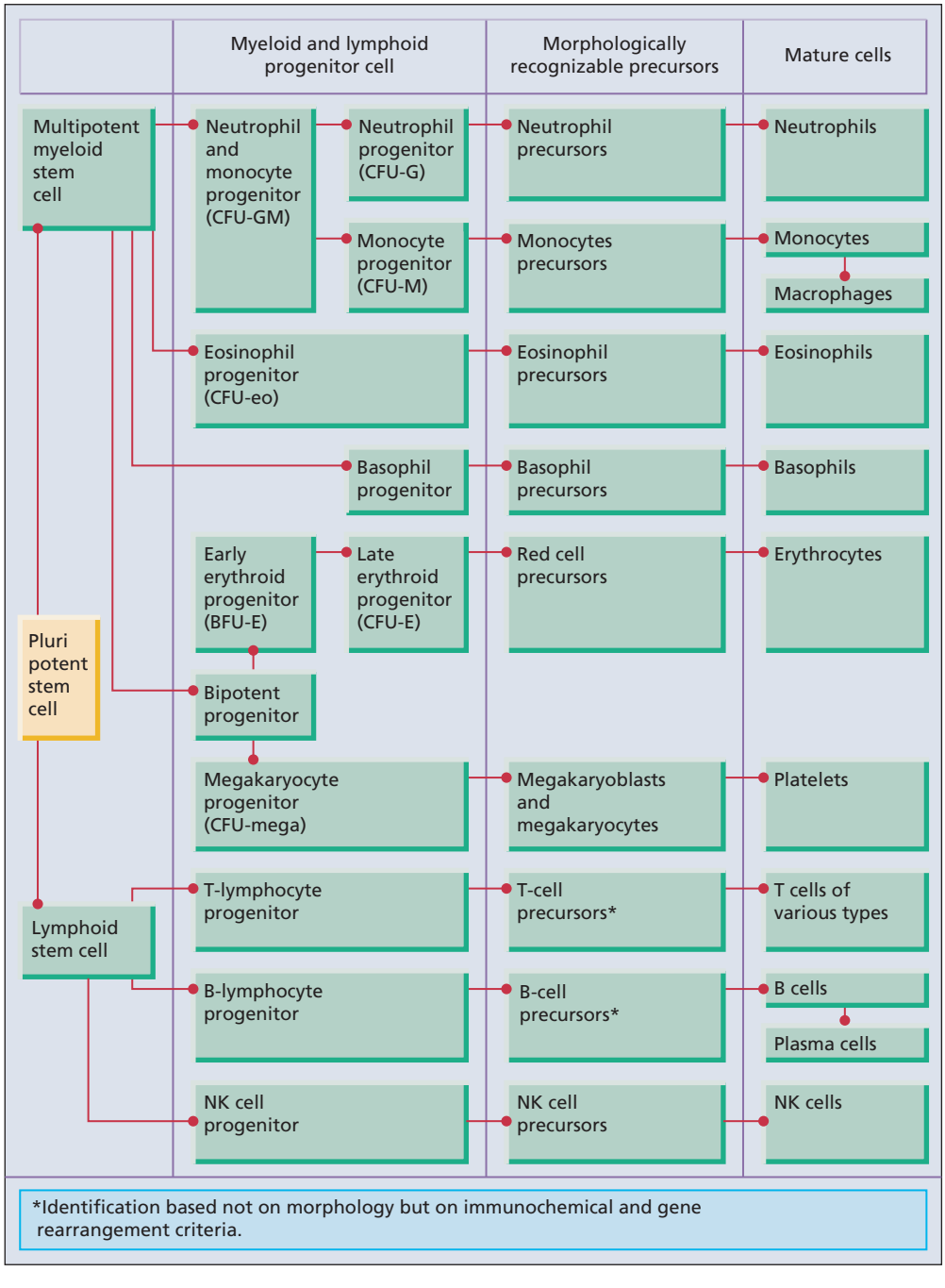

Figure 1.7 Relationships between the various types of cell involved in haemopoiesis.

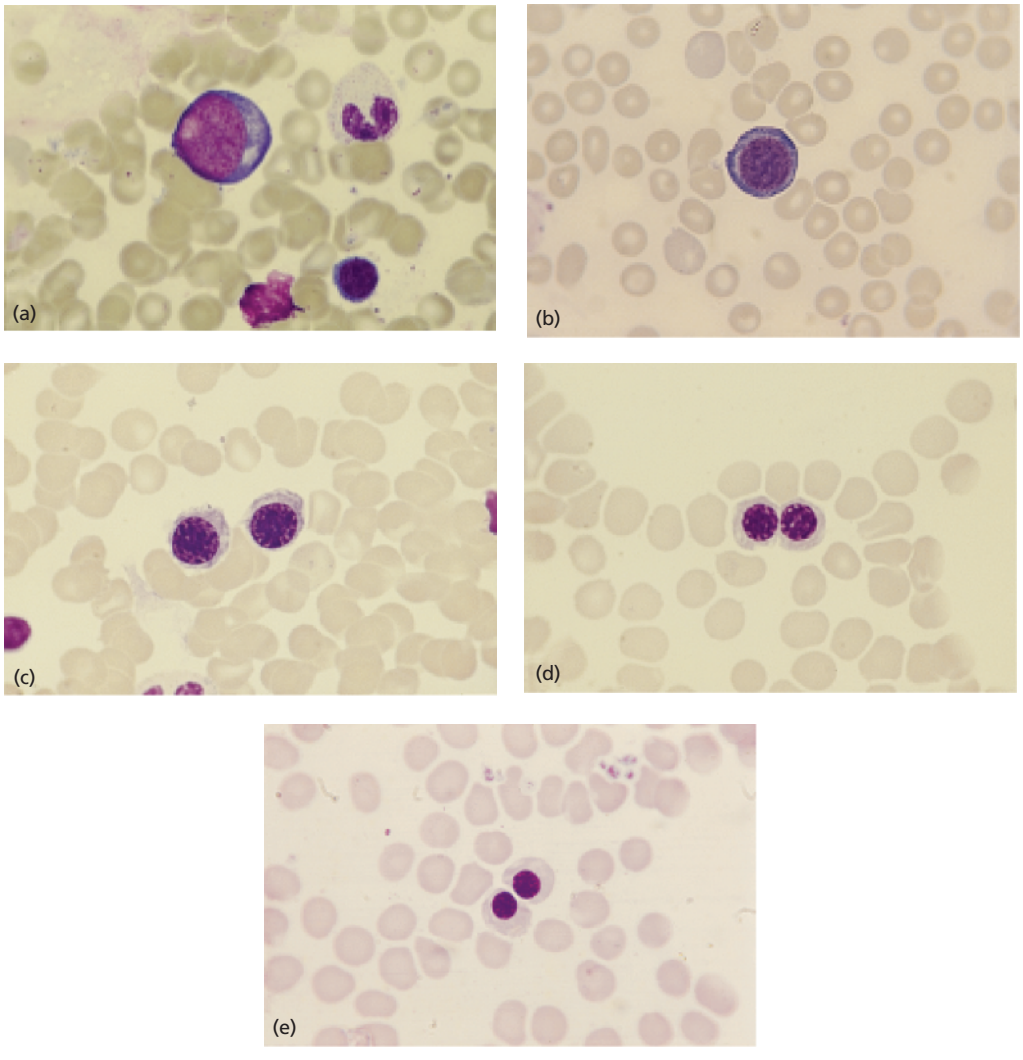

Figure 1.8 (a) Pronormoblast, (b) basophilic normoblast, (c) two early polychromatic normoblasts, (d) two late polychromatic normoblasts and (e) two more mature late polychromatic normoblasts. The granules of condensed chromatin in the basophilic normoblast are slightly coarser than in the pronormoblast. The nuclei of the late polychromatic normoblasts contain large masses of condensed chromatin.

that can be identified on morphological and cytochemical criteria are capable of both dividing and maturing. The late precursors do not divide but continue to mature. The proliferative activity during haemopoiesis serves as an amplifying mechanism and ensures that a large number of mature blood cells are derived from a single cell that becomes committed to any particular lineage.

Erythropoiesis

The pronormoblast is a large cell with a small quantity of agranular intensely basophilic cytoplasm (due to the presence of numerous ribosomes) and a large nucleus containing finely dispersed nuclear chromatin and nucleoli (Fig. 1.8(a)). The successive stages through which a pronormoblast develops into erythrocytes are

termed basophilic normoblasts (Fig. 1.8(b)); early and late polychromatic normoblasts (Fig. 1.8(c)–(e)); marrow reticulocytes and blood reticulocytes. Pronormoblasts, basophilic normoblasts and early polychromatic normoblasts undergo cell division and the late polychromatic normoblasts do not. Nucleated cell classes of increasing maturity show: (i) a progressive reduction in cell and nuclear size; (ii) a progressive increase in the quantity of condensed nuclear chromatin; (iii) a progressive increase in the ratio of cytoplasmic volume to nuclear volume; and (iv) a progressive increase in Hb (which stains pink) and a progressive decrease in ribosomal RNA (which stains blue), resulting in polychromasia (grey–pink colour). The late polychromatic normoblast extrudes its nucleus and becomes a marrow reticulocyte. The marrow reticulocytes enter the blood stream and circulate for 1–2 days before becoming mature red cells.

In Romanowsky-stained marrow and blood smears, reticulocytes appear as rounded, faintly polychromatic cells whose diameters are slightly larger than those of mature red cells. When living polychromatic red cells are incubated with brilliant cresyl blue (supravital staining), the ribosomes form a basophilic precipitate of granules or filaments, or both; in the most immature of these cells the precipitated RNA appears as a basophilic reticulum (hence the term 'reticulocyte') (Fig. 1.9). Mature red cells lack ribosomes.

On the basis of their morphological features, nucleated red cell precursors (erythroblasts) found in normal marrow are called normoblasts and normal erythropoiesis is described as being normoblastic in type. The characteristic feature of normoblastic erythropoiesis is the presence of moderate quantities of condensed nuclear chromatin in early polychromatic erythroblasts. Even in healthy individuals, a few erythroblasts fail to develop normally and such cells are recognized and phagocytosed by bone marrow macrophages. This loss of potential erythrocytes due to the intramedullary destruction of red cell precursors is described as ineffective erythropoiesis. The extent of ineffective erythropoiesis in normal marrow is slight.

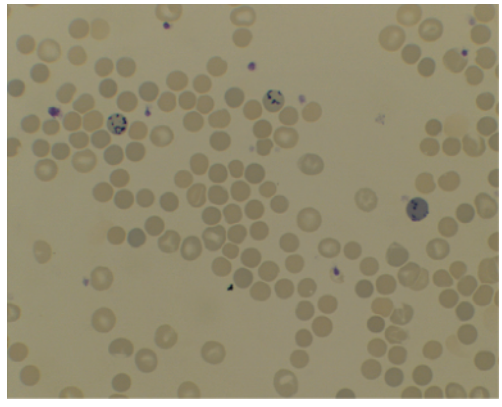

Figure 1.9 Reticulocytes in peripheral blood stained supravitally with brilliant cresyl blue. Note the reticulum of precipitated ribosomes.

Neutrophil granulocytopoiesis

The myeloblasts superficially resemble pronormoblasts except that their cytoplasm is less basophilic (Fig. 1.10(a)). The successive cytological classes through which a myeloblast matures into circulating neutrophil granulocytes are termed promyelocytes (Fig. 1.10(b)), neutrophil myelocytes (Fig. 1.10(c)), neutrophil metamyelocytes and neutrophil band cells (stab cells). During this maturation the following changes occur:

1. A progressive reduction of cytoplasmic basophilia and a progressive increase in the quantity of condensed chromatin after the promyelocyte stage.
2. The formation of coarse, purplish-red (azurophilic) cytoplasmic granules (primary granules) at the promyelocyte stage, which remain visible at the myelocyte stage but not later.
3. The formation of fine neutrophilic granules (specific granules) at the myelocyte and metamyelocyte stages.
4. Indentation of the nucleus which is moderate at the metamyelocyte stage (C-shaped nucleus) and more marked at the band cell stage (U-shaped, curved or coiled band-like nucleus).
5. A progressive segmentation of the U-shaped or band-like nucleus of the band cell leading to the formation of granulocytes with two to five nuclear lobes.

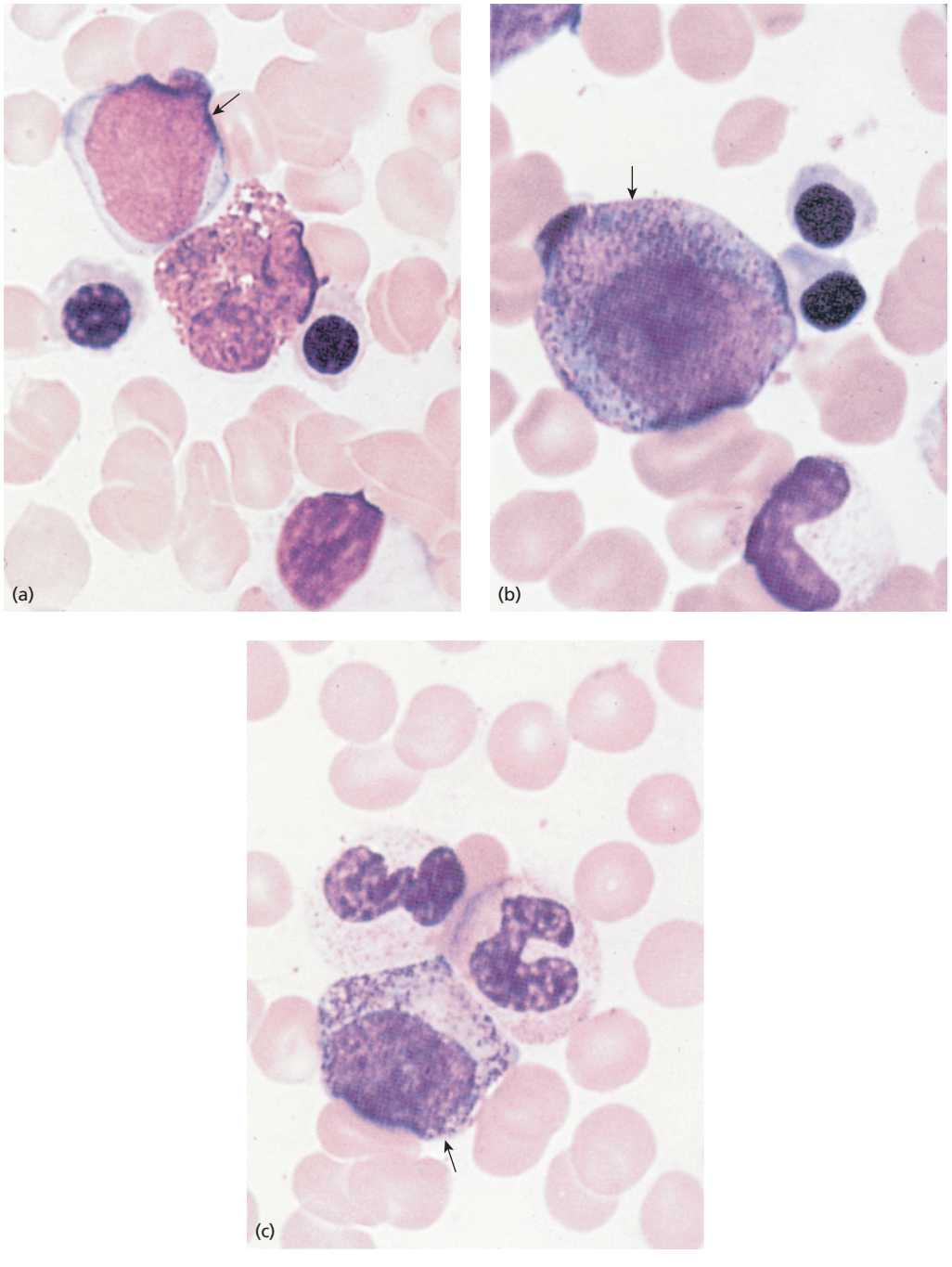

Figure 1.10 Neutrophil precursors from normal bone marrow. (a) Myeloblast (arrowed); the other nucleated cells near the myeloblast are an eosinophil granulocyte (centre) and two polychromatic erythroblasts. (b) Promyelocyte (arrowed); the other nucleated cells are two polychromatic erythroblasts and a neutrophil metamyelocyte. (c) Neutrophil myelocyte (arrowed); there are two neutrophil band cells adjacent to the myelocyte.

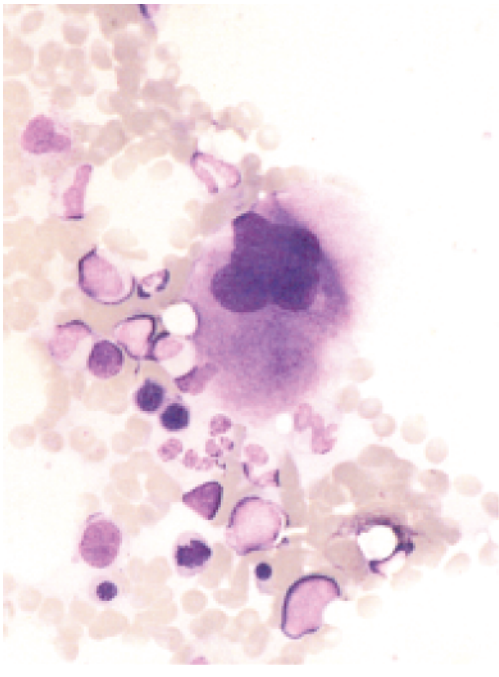

Figure 1.11 Mature megakaryocyte (centre). This is a very large cell with a single lobulated nucleus. Compare the size of the megakaryocyte with that of the other nucleated marrow cells in this figure.

Cell division occurs in myeloblasts, promyelocytes and myelocytes but not normally in metamyelocytes and band cells.

Megakaryocytopoiesis

During megakaryocytopoiesis, there is replication of DNA without nuclear or cell division which leads to the generation of very large uninucleate cells with DNA contents between 8c and 64c (other haemopoietic cells have DNA contents between 2c and 4c; 1c is the DNA content of a germ cell). There is a rough correlation between the DNA content of a megakaryocyte nucleus and both its size and extent of lobulation. A mature megakaryocyte is illustrated in Fig. 1.11. Large numbers of platelets are formed from the cytoplasm of each mature megakaryocyte; these are rapidly discharged directly into the marrow sinusoids. The residual 'bare' megakaryocyte nucleus is phagocytosed by macrophages.

Monocytopoiesis

The cell classes belonging to the monocyte–macrophage lineage (the mononuclear phagocyte system) are, in increasing order of maturity: monoblasts, promonocytes, marrow monocytes, blood monocytes and tissue macrophages.

Lymphocytopoiesis

The lymphoid stem cell in the bone marrow generates B-cell progenitors within that tissue. The B-cell progenitors undergo maturation into B cells in the microenvironment of the marrow and then travel via the blood into the B-cell zones of peripheral lymphoid tissue (follicles and medulla of lymph nodes and splenic follicles).

Either the lymphoid stem cells or primitive T-cell progenitors derived from them migrate from the marrow, via the blood, into the thymus where maturation into T cells takes place; those T cells that recognize self are deleted. The T cells later migrate to the T-cell zones of peripheral lymphoid organs (paracortical areas and medulla of lymph nodes and periarteriolar lymphoid sheaths of the spleen).

The terms used to describe cells at various stages of B-lymphocyte differentiation in the bone marrow and T-lymphocyte differentiation in the thymus are as follows:

Pre-pre-B cell → pre-B cell → immature B cell → mature B cell

Pre-T cell (thymic lymphoblast) → early thymocyte (large cortical thymocyte) → intermediate thymocyte (small cortical thymocyte) → late thymocyte (medullary thymocyte) → mature T cell

All these stages have the morphological features of either lymphoblasts or lymphocytes. The identification of different lymphocyte precursors is therefore based not on morphology but on various properties like reactivity with certain monoclonal antibodies, immunoglobulin gene rearrangement status, presence of immunoglobulin

Table 1.4 The sequence of events during B-cell differentiation.

Characteristic	Pre-pre-B cell	Pre-B cell	Immature B cell	Mature B cell	Plasma cell
Heavy-chain genes rearranged	+	+	+	+	+
Light-chain genes rearranged	−/+	+	+	+	+
Terminal deoxynucleotidyl-transferase	+	+/−	−	−	−
Cytoplasmic μ-chains expressed	−	+	−	−	−
Surface IgM (but not IgD) expressed	−	−	+	−	−
Surface IgM and IgD expressed	−	−	−	+	−
Cytoplasmic Ig expressed	−	−	−	−	+
cALLA (CD10)	+	+	−	−	−
CD19 and CD20	+	+	+	+	+

Note: cALLA — common acute lymphoblastic leukaemia antigen.

Table 1.5 The sequence of events during T-cell differentiation.

Characteristic	Pre-T cell	Early thymocyte	Intermediate thymocyte	Late thymocyte	Mature T cell
CD7	+	+	+	+	+
Terminal deoxynucleotidyl-transferase	−/+	+	+	−	−
TCR γ genes rearranged/deleted	−	+	+	+	+
TCR β genes rearranged	−	−	+	+	+
TCR α genes rearranged	−	−	−/+	+	+
CD2	−	+	+	+	+
CD3	−	+	+	+	+
CD4 and CD8	−	−	−/+	−	−
CD4 or CD8	−	−	−	+	+

Note: TCR — T-cell receptor.

on the surface membrane, presence of μ-chains or immunoglobulin within the cytoplasm, terminal deoxynucleotidyl-transferase (TdT) activity and TCR gene rearrangement status (Tables 1.4 and 1.5) (also see Chapter 7).

Regulation of haemopoiesis

The regulation of haemopoietic cells, including haemopoietic stem cells and progenitor cells, depends on intimate contact with one or

more types of bone marrow stromal cell (macrophages, endothelial cells, fibroblasts, adipocytes and osteoblasts) and with stromal-cell-derived extracellular matrix components. The stromal cells (and also some T-lymphocytes) influence the proliferation and maturation of the haemopoietic stem cells and progenitor cells by producing: (i) a number of membrane-bound, matrix-bound and soluble haemopoietic growth factors; and (ii) inhibitory cytokines (e.g. transforming growth factor-β, tumour necrosis factor (TNF), and interferons). Haemopoietic growth factors are also produced by the liver (thrombopoietin (TPO) and <10% of the erythropoietin) and kidneys (most of the eythropoietin). One of the important growth factors acting on stem cells is called stem-cell factor (SCF, kit ligand or Steel factor). Growth factors influencing early progenitor cells include SCF, IL-3, TPO and GM-CSF. Those acting on the lineage-committed bipotent or unipotent progenitor cells include G-CSF, M-CSF, IL-5 (influencing CFU-eo), TPO (influencing CFU-mega) and erythropoietin. Haemopoietic growth factors react with specific receptors on the cell membrane of target cells and mediate their effects on survival, proliferation and differentiation via second messengers. In their absence, the target cell undergoes programmed cell death (apoptosis). All the haemopoietic growth factors are glycoproteins and some (erythropoietin, GM-CSF, G-CSF) have been genetically engineered and are available as therapeutic agents. Growth factors such as G-CSF and GM-CSF not only influence haemopoiesis but also enhance the function of the mature cells.

The details of the steady-state regulation of blood cells other than red cells are still not entirely clear. The rate of erythropoiesis is primarily regulated by the hormone erythropoietin, which is secreted mainly by the kidneys, probably by peritubular cells. The production of erythropoietin is stimulated when the supply of oxygen to renal tissue falls (e.g. when the red cell count falls). Erythropoietin increases red cell production mainly by stimulating the rate of conversion of CFU-E to pronormoblasts. It also shortens the total time taken for a pronormoblast to mature into marrow reticulocytes and for the latter to be released into the circulation.

Intrauterine haemopoiesis and postnatal changes

The production of blood cells begins in the yolk sac of the 14- to 19-day human embryo. The fetal liver becomes the main site of haemopoiesis in the second trimester of pregnancy and the fetal bone marrow in the third trimester. The majority of the haemopoietic cells in the yolk sac and fetal liver are erythroblasts. The embryonic Hbs, Gower 1 ($\zeta_2\varepsilon_2$), Gower 2 ($\alpha_2\varepsilon_2$) and Portland 1 ($\zeta_2\gamma_2$), are synthesized by erythroblasts in the yolk sac, fetal Hb (HbF, $\alpha_2\gamma_2$) by erythroblasts in the fetal liver, and both HbF and HbA ($\alpha_2\beta_2$) by those in the fetal bone marrow. The main site of granulocytopoietic activity in intrauterine life is the fetal bone marrow.

After birth, the marrow is the sole site of haemopoiesis in healthy individuals. During the first 4 years of life, nearly all the marrow cavities contain red haemopoietic marrow with very few fat cells. Thereafter, increasing numbers of fat cells appear in certain marrow cavities. By the age of 25 years, the only sites of active haemopoiesis are the skull bones, ribs, sternum, scapulae, clavicles, vertebrae, pelvis, the upper half of the sacrum, and the proximal ends of the shafts of the femur and humerus. All the remaining marrow cavities contain yellow fatty marrow. Even at sites of active haemopoiesis, about half the volume of the marrow normally consists of fat cells.

In a number of diseases (e.g. chronic haemolytic anaemias, megaloblastic anaemias and some leukaemias) there may be: (i) a partial or complete replacement of fat cells by haemopoietic cells in marrow cavities normally supporting haemopoiesis; (ii) the extension of haemopoietic marrow into marrow cavities normally containing non-haemopoietic fatty marrow (e.g. in long bones); and (iii) the appearance of foci of haemopoietic tissue in the liver and spleen (extramedullary haemopoiesis).