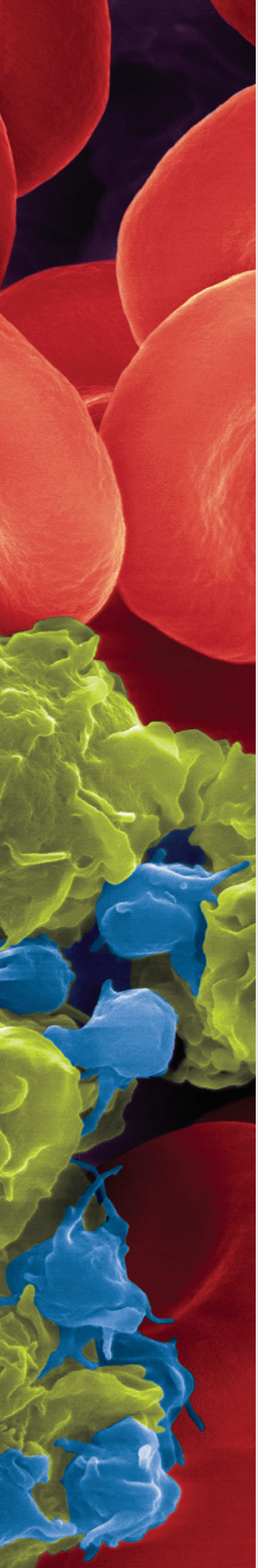
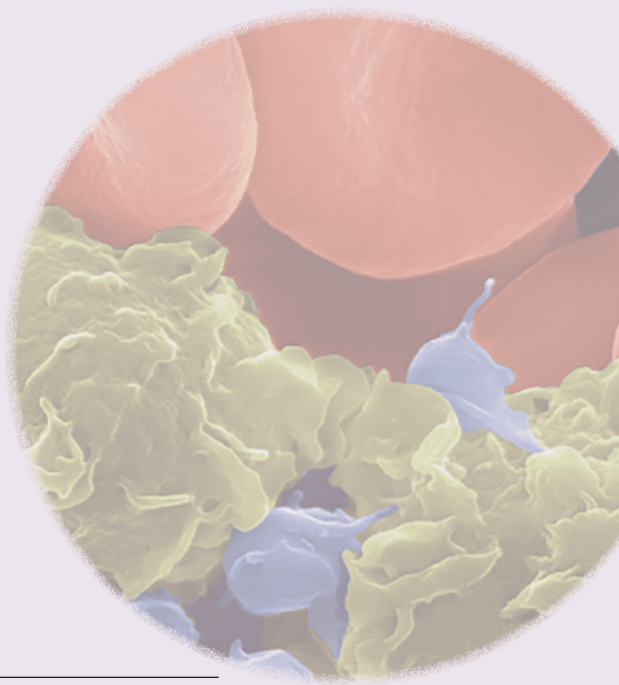


CHAPTER 1

Haemopoiesis

Key topics



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

Companion website: www.wileyessential.com/haematology

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

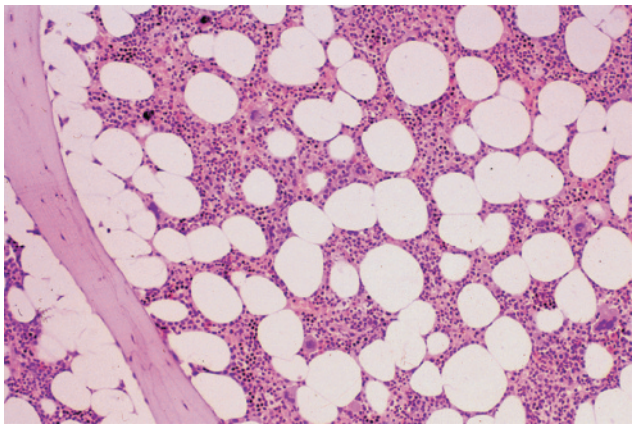


Figure 1.1 Normal bone marrow trephine biopsy (posterior iliac crest). Haematoxylin and eosin stain; approximately 50% of the intertrabecular tissue is haemopoietic tissue and 50% is fat.

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

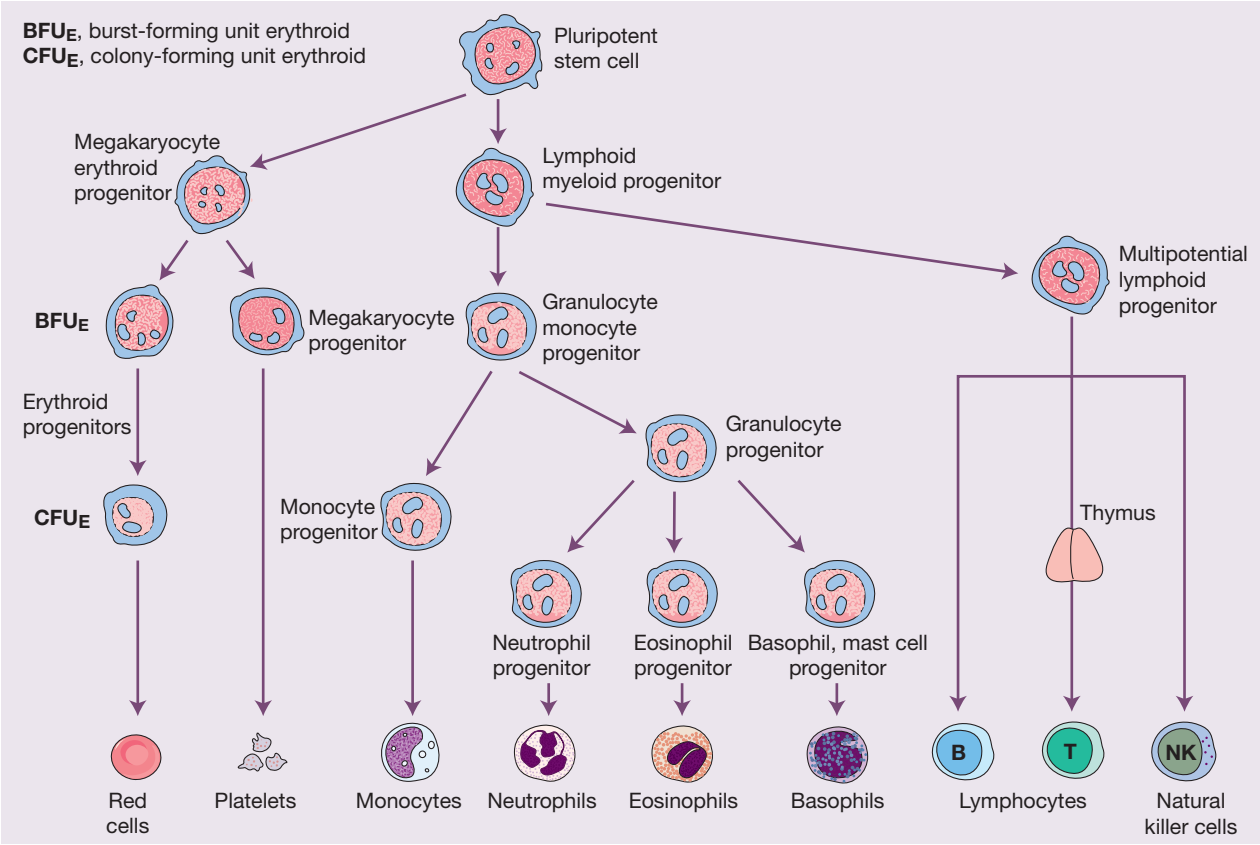


Figure 1.2 Diagrammatic representation of the bone marrow pluripotent stem cells (haemopoietic stem cells, HSC) and the cell lines that arise from them. A megakaryocytic/erythroid progenitor (MkEP) and a mixed lymphoid/myeloid progenitor are formed from the pluripotent stem cells. Each gives rise to 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).

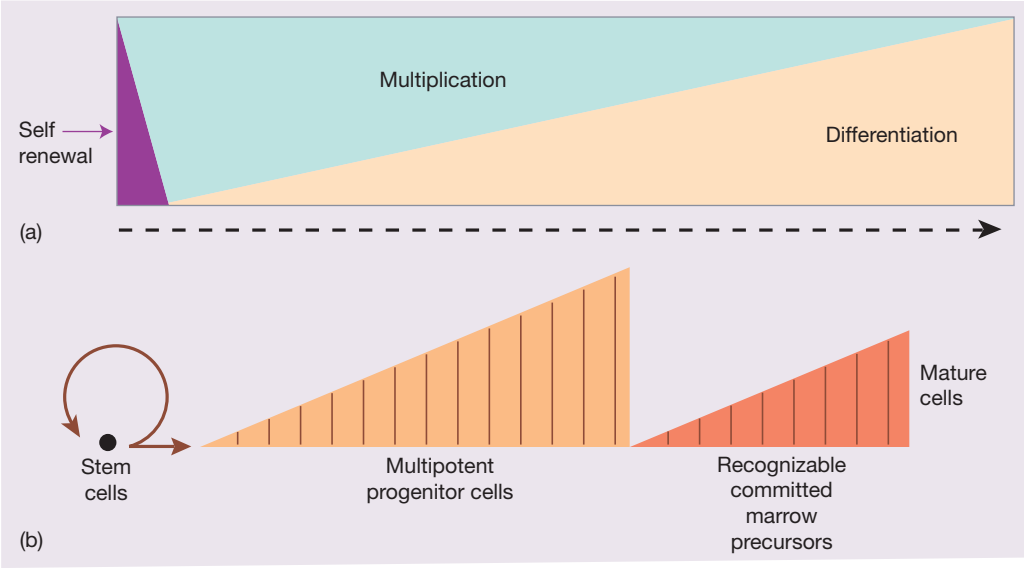


Figure 1.3 **(a)** Bone marrow cells are increasingly differentiated and lose the capacity for self-renewal as they mature. **(b)** A single stem cell gives rise, after multiple cell divisions (shown by vertical lines), to >10^6 mature cells.

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.

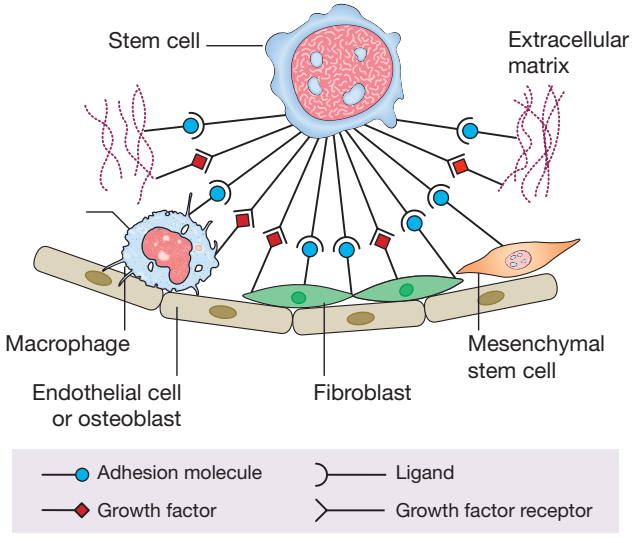


Figure 1.4 Haemopoiesis occurs in a suitable microenvironment ('niche') provided by a stromal matrix on which stem cells grow and divide. The niche may be vascular (lined by endothelium) or endosteal (lined by osteoblasts). There are specific recognition and adhesion sites; extracellular glycoproteins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

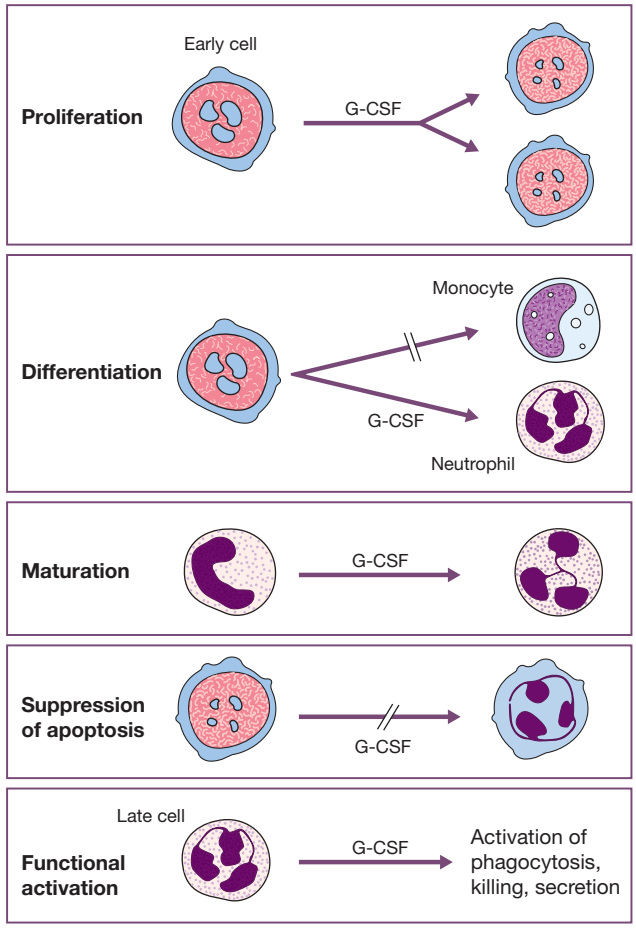


Figure 1.5 Growth factors may stimulate the proliferation of early bone marrow cells, direct differentiation to one or other cell type, stimulate cell maturation, suppress apoptosis or affect the function of mature non-dividing cells, as illustrated here for granulocyte colony-stimulating factor (G-CSF)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

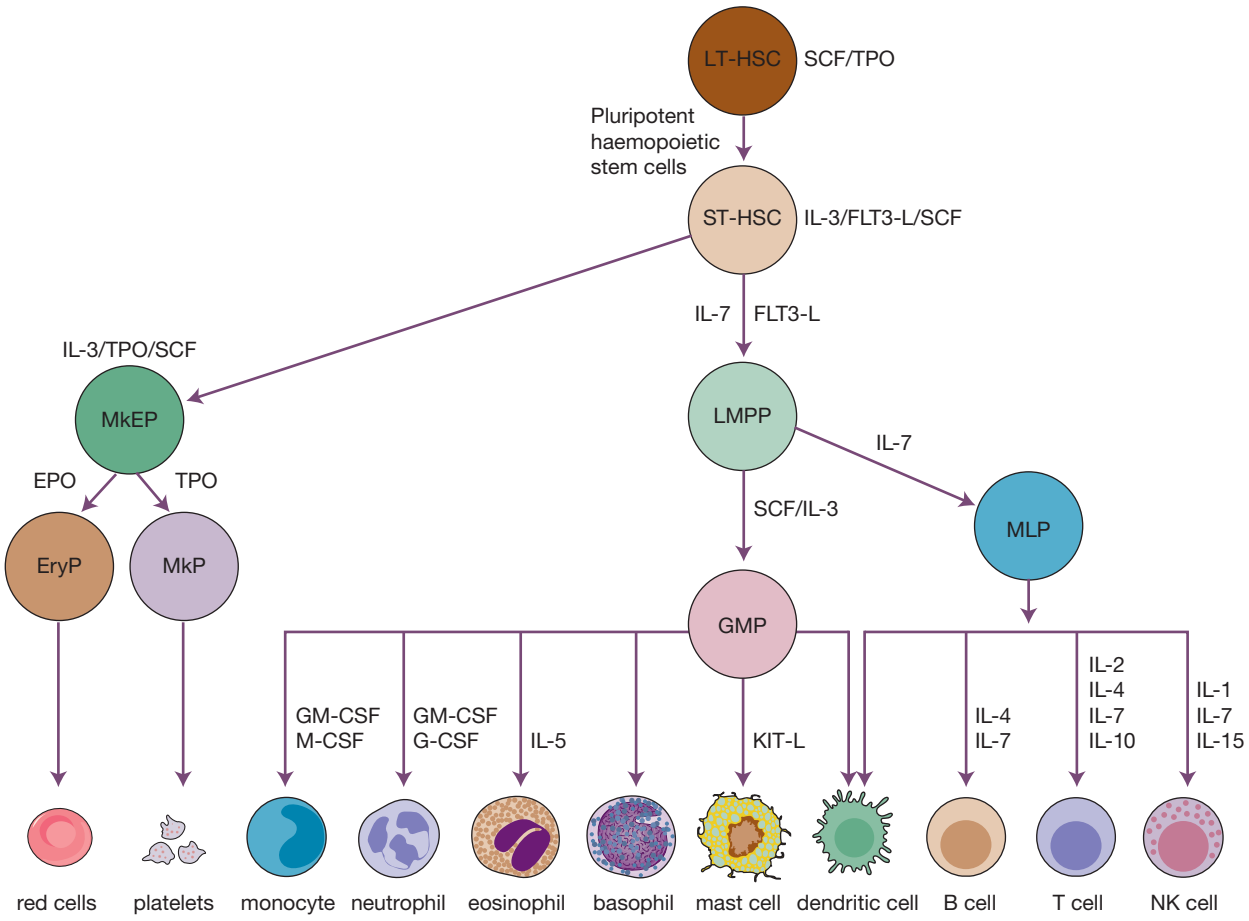


Figure 1.6 The role of growth factors in normal haemopoiesis. Multiple growth factors act on the earlier marrow stem and progenitor cells. EPO, erythropoietin; EryP, erythroid progenitor; FLT3-L, FLT3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating 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.

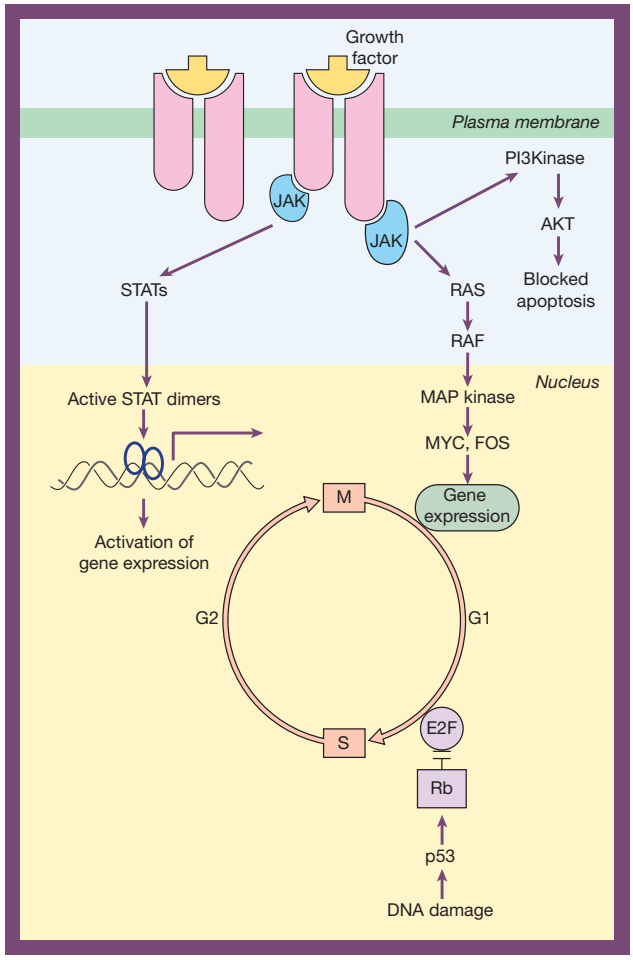


Figure 1.7 Control of haemopoiesis by growth factors. The factors act on cells expressing the corresponding receptors. Binding of a growth factor to its receptor activates the JAK/STAT, MAPK and phosphatidyl-inositol 3-kinase (PI3K) pathways (see Fig. 15.2), which leads to transcriptional activation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

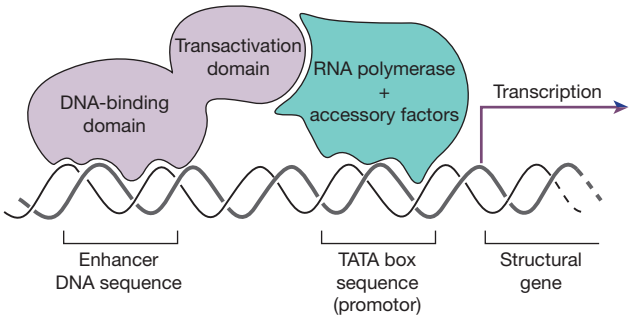


Figure 1.8 Model for control of gene expression by a transcription factor. The DNA-binding domain of a transcription factor binds a specific enhancer sequence adjacent to a structural gene. The transactivation domain then binds a molecule of RNA polymerase, thus augmenting its binding to the TATA box.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

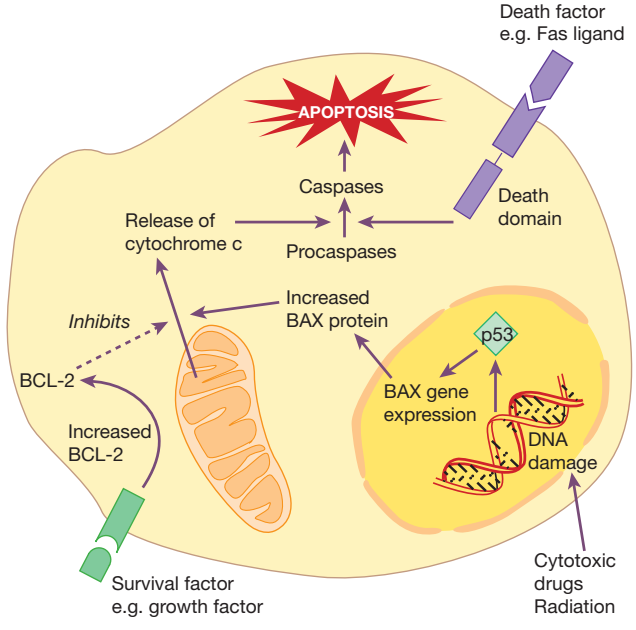


Figure 1.9 Representation of apoptosis. Apoptosis is initiated via two main stimuli: (i) signalling through cell membrane receptors such as FAS or tumour necrosis factor (TNF) receptor; or (ii) release of cytochrome c from mitochondria. Membrane receptors signal apoptosis through an intracellular death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

Now visit **www.wileyessential.com/haematology** to test yourself on this chapter.