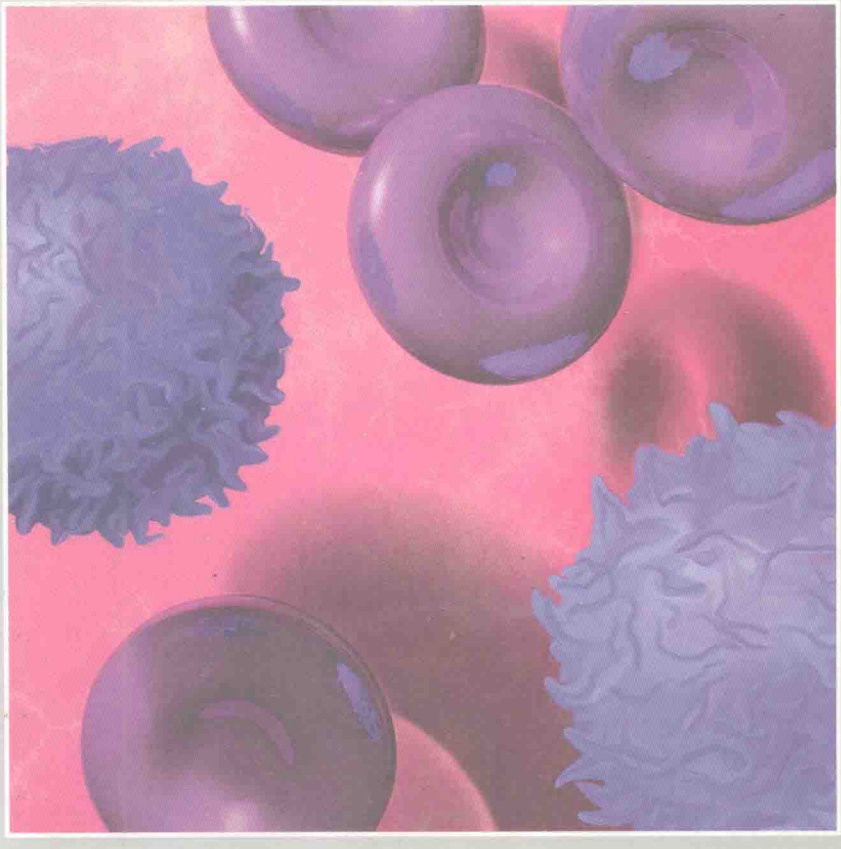
Essential Haematology

A. V. HOFFBRAND
J. E. PETTIT

THIRD EDITION



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Essential Haematology

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Third Edition

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Preface to Third Edition

In the 8 years since the publication of the previous edition of *Essential Haematology* there have been great advances in molecular biology. These have had a major impact on the understanding of normal physiological processes and the basis of disease within the field of haematology. It has proved a difficult task, therefore, in writing this third edition to maintain the size and style of the previous editions. The authors appreciate that the scope of this book is now beyond that strictly required by an undergraduate course, but we feel that the extra information is necessary for an understanding of this broad and scientifically advanced subject. The sections on haemopoiesis, genetic defects of haemoglobin, antenatal diagnosis, leucocytes, leukaemias, haemostasis and blood transfusion, in particular, have been expanded to incorporate this new knowledge. Advances in therapy (such as the use of haemopoietic growth factors), broadening indications for allogeneic and autologous bone marrow transplantation, and the use of thrombolytic agents have also been incorporated. We have attempted to reflect the relative frequency of diseases more equitably by increasing the space allotted to such topics as the blood in systemic diseases, AIDS, myelodysplasia and thrombosis, as compared with rarer entities.

New figures and tables appear in all chapters, and most illustrations are now in colour. The authors and publishers are grateful to Gower Medical Publishing for use of the following figures from *Sandoz Atlas of Clinical Haematology* (Hoffbrand & Pettit, 1988): 2.14a–b, 2.15, 3.4a–c, 4.6, 4.7, 4.8, 5.4b, 6.4, 6.11, 6.21, 7.2, 8.7, 8.8f, 9.6, 9.7, 9.8, 10.4a, 10.7, 11.10, 12.10a, 13.9a–c, 13.14, 14.6d, 14.7a–b, 15.3a, 18.3, 18.4, 20.6, 20.7a, 20.10a–b, 20.12c and 21.1; and to Mosby-Wolfe for Fig. 16.6 redrawn from *Color Atlas of Clinical Hematology* (Hoffbrand & Pettit, 1994).

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AVH, JEP

Note to the corrected reprint
For this new printing of the third edition we have indicated by a red vertical line in the margin those sections of the text which we consider less 'essential' for undergraduate medical students approaching their final examination.

Preface to 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 Jony Russell of Blackwell Scientific Publications for his invaluable help and patience.

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Contents

Chapter 1
Blood Cell Formation (Haemopoiesis)

This first chapter mainly concerns general aspects of blood cell formation (haemopoiesis) and the early stages of formation of red cells (erythropoiesis), granulocytes and monocytes (myelopoiesis) and platelets (thrombopoiesis).

Site

In the first few weeks of gestation the yolk sac is the main site of haemopoiesis. From 6 weeks until 6–7 months of foetal life the liver and spleen are the main organs involved and they continue to produce blood cells until about 2 weeks after birth (Table 1.1, Fig. 6.1b). The bone marrow is the most important site from 6–7 months of foetal life and, 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 and 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, 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 haemopoietic areas, approximately 50% of the marrow consists of fat (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. Morever, the liver and spleen can resume their foetal haemopoietic role (so-called 'extramedullary haemopoiesis').

Haemopoietic stem and progenitor cells

It is now thought that a common (pluripotential) stem cell gives rise after a number of cell divisions and differentiation steps to a series of progenitor cells for three main marrow cell lines: (a) erythroid, (b) granulocytic and monocytic, and (c) megakaryocytic, as well as to a common lymphoid stem cell (Fig. 1.2). Although the appearance of the pluripotential stem cells is probably similar to that of small- or intermediate-sized lymphocytes, their presence can be shown in mice, at least, by culture

• Foetus	0–2 months—yolk sac 2–7 months—liver, spleen 5–9 months—bone marrow
• Infants	Bone marrow (practically all bones)
• Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

Table 1.1 Sites of haemopoiesis.

techniques. The existence of the separate progenitor cells, which also resemble lymphocytes, for the three cell lines has also been demonstrated by *in vitro* culture techniques. The earliest detectable myeloid precursor gives rise to granulocytes, erythrocytes, monocytes and megakaryocytes and is termed CFU_{GEMM} (CFU = colony-forming unit in agar culture medium). More mature and specialized progenitors are then formed (Fig. 1.2).

The stem cell also has the capability of self-renewal (Fig. 1.3) so that, although the marrow is a major site of new cell production, its overall cellularity remains constant in a normal healthy steady state. The precursor cells are, however, capable of responding to haemopoietic growth factors with increased production of one or other cell line when the need arises. There is considerable amplification in the system: one stem cell, for example is normally capable of producing about 10^6 mature blood cells after 20 cell divisions (Fig. 1.3).

The bone marrow is also the primary site of origin of lymphocytes in humans (Chapter 9) and there is evidence for a common precursor cell of both the myeloid and lymphoid systems.

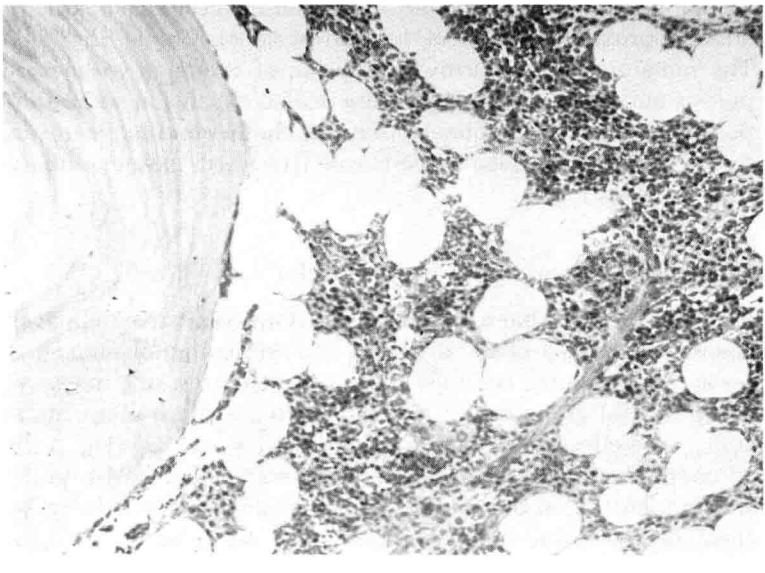

Fig. 1.1 A normal bone marrow trephine biopsy (posterior iliac crest). Haematoxylin and eosin stain; approximately 50% of the intertrabecular tissue is haemopoietic tissue and 50% is fat.

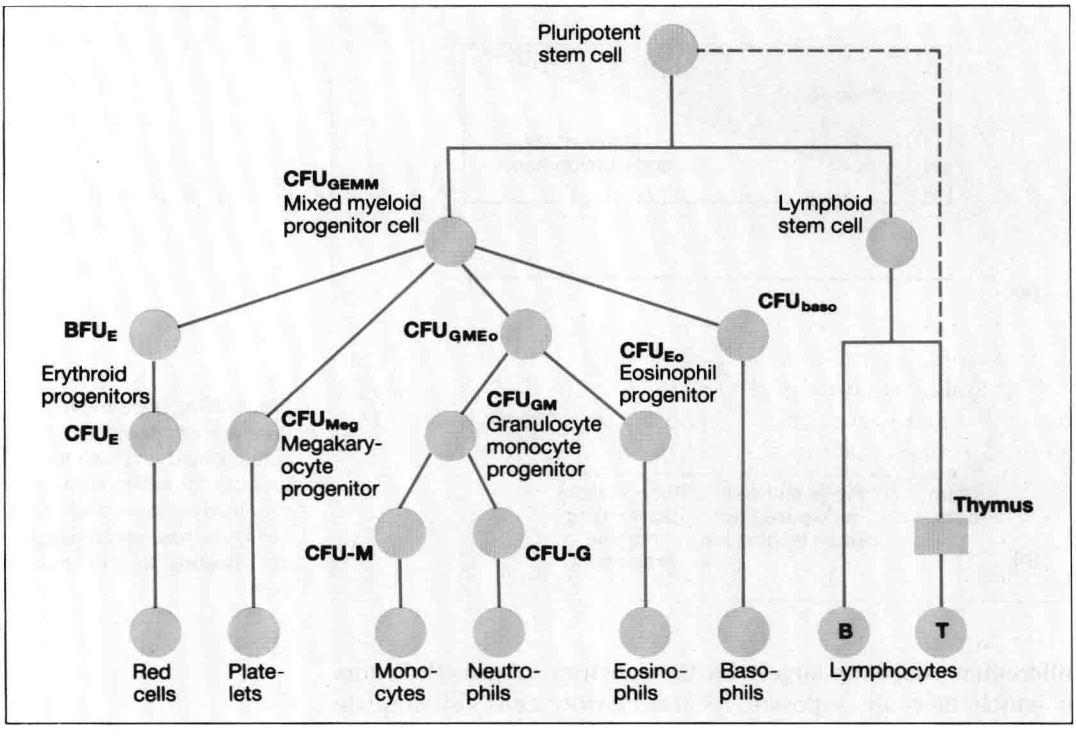

Fig. 1.2 Diagrammatic representation of the bone marrow pluripotent stem cell and the cell lines that arise from it. Various progenitor cells can now be identified by culture in semi-solid medium by the type of colony they form. BFU_E, burst-forming unit, erythroid; CFU, colony-forming unit; E, erythroid; Eo, eosinophil; GEMM, mixed granulocyte, erythroid, monocyte, megakaryocyte; GM, granulocyte, monocyte; Meg, megakaryocyte.

Haemopoietic stem cells also give rise to osteoclasts which are part of the monocyte–phagocyte system. The development of the mature cells—red cells, granulocytes, monocytes, megakaryocytes and lymphocytes—is further considered in other sections of this book.

Bone marrow stroma

The bone marrow forms a suitable environment for stem cell growth and development. It is composed of stromal cells and a microvascular network (Fig. 1.4). If haemopoietic stem cells are infused intravenously into a suitably prepared recipient, they circulate and seed the marrow successfully but do not thrive at other sites. This is the basis of bone marrow transplantation performed for a number of serious bone marrow and other diseases. The direction in which stem and progenitor cells

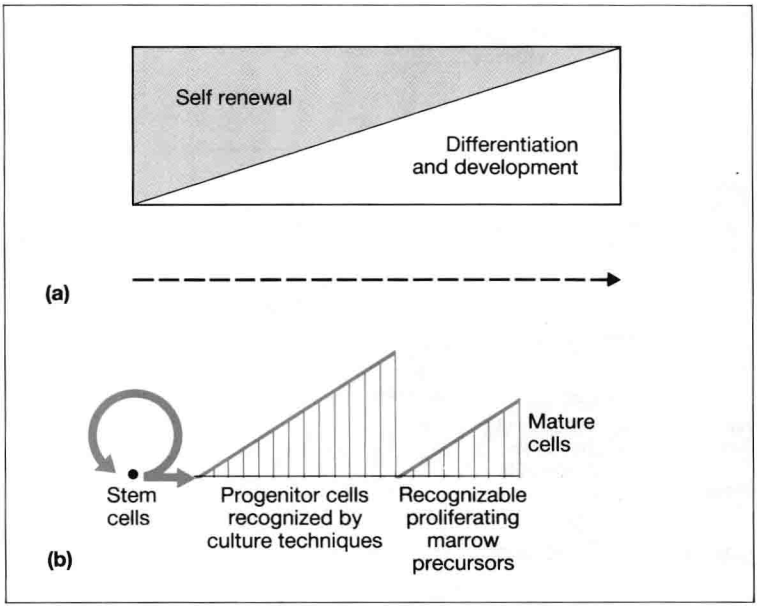

Fig. 1.3 (a) Bone marrow cells are increasingly differentiated and lose the capacity for self-renewal as they mature. (b) A single stem cell gives rise, after multiple cell divisions, to $>10^6$ mature cells.

differentiate depends largely on the spectrum of growth factors to which they are exposed. As the marrow cells differentiate they lose cell adhesion molecules (CAMs), changes allowing the cells to leave the marrow and enter the circulation. Stem cells also circulate and in the early foetus, the yolk sac, and in later foetal life the liver and spleen, as well as the bone marrow, provide the correct environment for stem cell survival and proliferation.

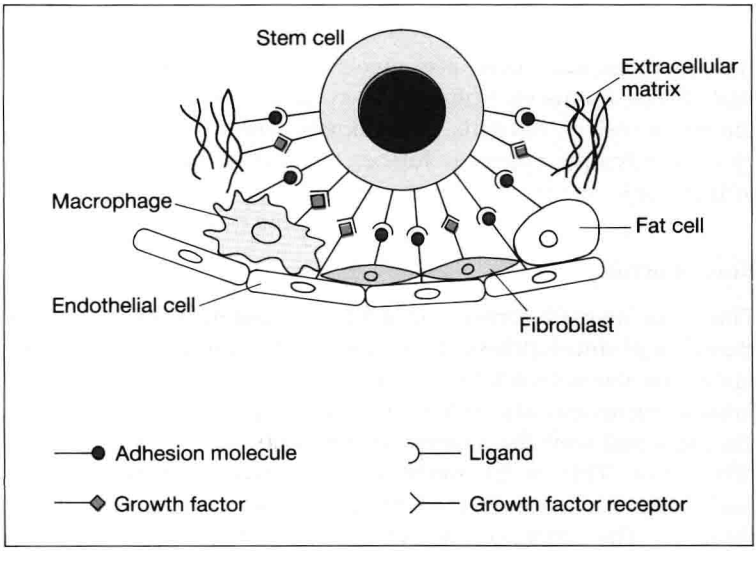

Fig. 1.4 Haemopoiesis occurs in a suitable microenvironment provided by a stromal matrix on which stem cells grow and divide. There are probably specific recognition and adhesion sites; extracellular glycoproteins and other compounds are involved in the binding (Table 1.2).

Table 1.2 The composition of the bone marrow microenvironment.

Extracellular matrix	Stromal cells
Fibronectin (binds erythroid precursors)	Macrophages
Haemonectin (binds granulocyte precursors)	Fibroblasts
Laminin	Endothelial cells
Collagen	Fat cells
Proteoglycans (acid mucopolysaccharides), e.g. chondroitin, heparan	Reticulum ('blanket') cells

Haemopoietic growth factors

The haemopoietic growth factors are glycoprotein hormones that regulate the proliferation and differentiation of haemopoietic progenitor cells and the function of mature blood cells (Table 1.3). They may act locally at the site where they are produced or circulate in plasma. They share a number of common properties (Table 1.4). T lymphocytes, monocytes (and macrophages), endothelial cells and fibroblasts (stromal cells) are the major cell sources except for erythropoietin, 90% of which is synthesized in the kidney. The biological effects of the growth factors are mediated through specific receptors on target cells. Some growth factors are found normally in plasma but others cannot be detected except when there is an inflammatory or other stimulus. Antigens or endotoxins activate T lymphocytes or macrophages to release IL-1 (interleukin-1) and tumour necrosis factor (TNF) which then stimulate other cells including endothelial cells, fibroblasts and other T cells and macrophages to produce GM-CSF (CSF = colony-stimulating factor), G-CSF, M-CSF, IL-6 and other growth factors in an interacting network (Fig. 1.5). T lymphocytes appear to be the sole source of IL-3 and IL-5. 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. Within the marrow the growth factors, particularly those acting at the earliest stages of haemopoiesis, may act locally by cell to cell contact or by binding to the extracellular matrix to form niches to which stem and progenitor cells adhere.

IL-1 has a wide variety of biological activities mainly related to inflammation. Stem cell factor acts locally on the pluripotential stem cells and on early myeloid and lymphoid progenitors (Fig. 1.6). IL-3 and GM-CSF are multi-potential growth factors with overlapping activates, IL-3 being more active on the earliest marrow progenitors. IL-3 activity also leads to increased platelet as well as granulocyte and monocyte production. IL-1 and IL-6 enhance the effects of SCF, IL-3 and GM-CSF on survival

Table 1.3 Haemopoietic growth factors.

Act on stromal cells
IL-1, TNF } stimulate production of GM-CSF, G-CSF, M-CSF, IL-6
Act on pluripotential cells
SCF (stem cell factor, kit ligand, Steel factor)
Act on early multipotential cells
IL-3
IL-6
GM-CSF
Act on late cells committed to one (or two) lineages
G-CSF
M-CSF
IL-5 (Eo-CSF)
Erythropoietin
Thrombopoietin

Notes:
1 TGFβ (transforming growth factor-β) acts as a growth-inhibiting factor for a wide variety of haemopoietic and non-haemopoietic cells. Both TNF and IL-4 also have inhibitory effects for late myeloid progenitors.
2 IL-3 and GM-CSF have synergistic effects with the more restricted growth factors on later cells.
3 A number of other factors (IL-8, IL-9, IL-10, IL-11) are described but less well characterized.
4 Growth factors acting on lymphoid progenitors are described in Chapter 9.
CSF, colony-stimulating factor; Eo, eosinophil; G, granulocytes; IL, interleukin; M, monocyte; TNF, tumour necrosis factor.

Table 1.4 General characteristics of myeloid and lymphoid growth factors.

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

and differentiation of the early haemopoietic cells. Together these factors maintain a pool of haemopoietic stem and progenitor cells on which later acting factors may act to stimulate increased production of one or other cell lineage in response to the body's needs, e.g. in infection (Fig. 1.5), haemorrhage or hypoxia.

Erythropoietin, G-CSF, M-CSF, IL-5 (an eosinophilic growth factor) and thrombopoietin act on later cells which are more

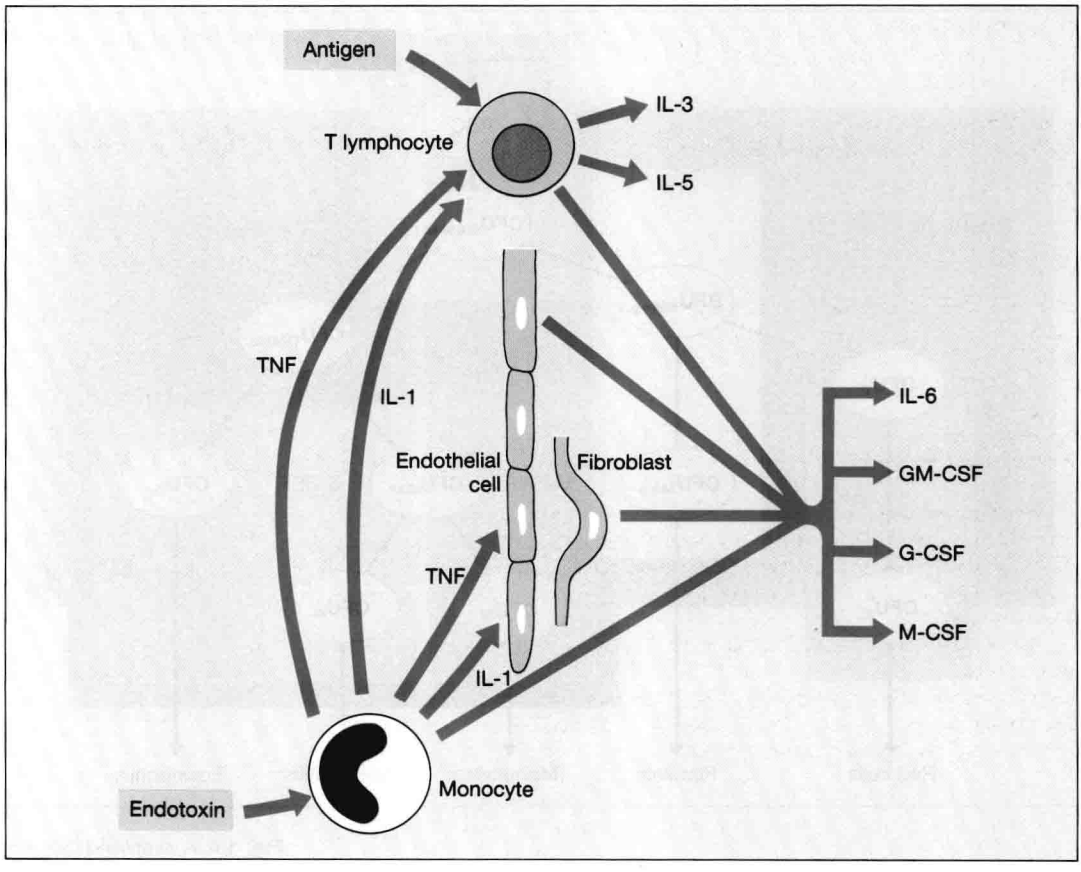

Fig. 1.5 Regulation of haemopoiesis; pathways of stimulation of leucopoiesis by endotoxin, for example from infection. It is likely that endothelial and fibroblast cells release basal quantities of GM-CSF and G-CSF in the normal resting state and that this is enhanced substantially by the monokines TNF (tumour necrosis factor) and IL-1 (interleukin-1), released in response to infection. IL-1 and TNF also stimulate T cells and antigen may stimulate T cells directly. CSF, colony-stimulating factor; G, granulocyte; M, monocyte.

committed to one cell lineage (Fig. 1.6). IL-6 also has a particular role in megakaryocyte formation. The growth factors also affect the survival and function of mature cells, e.g. GM-CSF potentiates viability and microbial killing and the production of cytokines by mature neutrophils, monocytes and eosinophils (Fig. 1.7).

A common action of the growth factors is to inhibit apoptosis (programmed cell death) of target cells. Apoptosis is a gene-directed process requiring ongoing protein synthesis in which Ca^{2+} ions activate endonucleases, the dying cells being removed by phagocytosis.

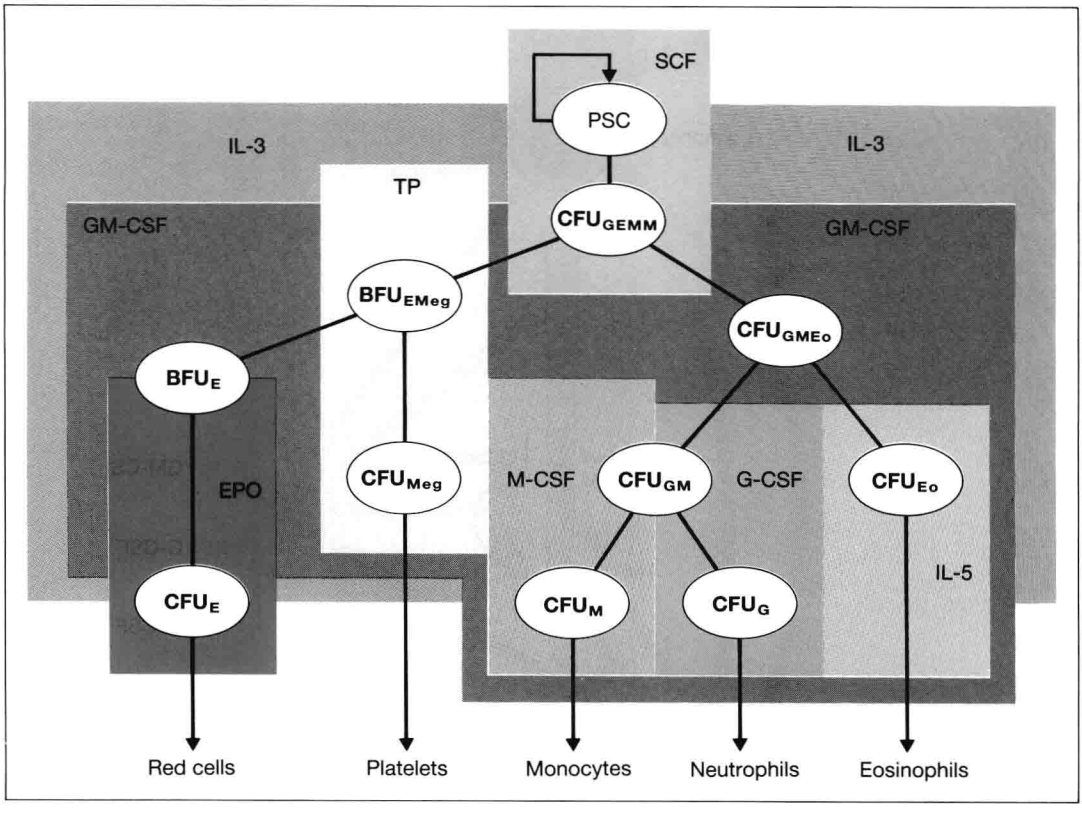

Fig. 1.6 A simplified diagram of the role of growth factors in normal haemopoiesis. Multiple growth factors act on the earlier marrow stem and progenitor cells. EPO, erythropoietin; TP, thrombopoietin; PSC, pluripotential stem cell; SCF, stem cell factor. For other abbreviations see Fig. 1.2 and Table 1.3.

Signal transduction

Several different mechanisms have been identified by which a growth factor signal for the cell to proliferate or differentiate is transduced through the target cell membrane to the cell nucleus (Fig. 1.8 and 1.9). The first event, however, is the binding of the growth factor to its receptor on the cell membrane.

Growth factor receptors

Each of the growth factors binds with high affinity to its corresponding receptor on the target cell. Most of the receptors belong to a structurally related set of membrance glycoproteins, the haematopoietin receptor family (Table 1.5). Binding of the growth factor to these receptors causes alteration of the intracellular domains of the receptor protein. Dimerization of two identical, or the two different, receptor molecules in response to growth factor binding appears to be necessary for signal transduction