CHAPTER 1

Stem cells and haemopoiesis

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Introduction

The lifelong production of blood cells occurs in haemopoietic tissue. This involves a very high level of cell turnover, demanded by the need to replace mature circulating blood cells at a rapid rate, and is necessitated by the limited lifespan of the mature cells. Granulocytes survive for only a few hours and erythrocytes for a few months, so that some 10^{13} new cells must be replaced each day to maintain steady-state blood counts. This is equivalent to an annual number of cells approximating the total body weight, but the total bone marrow of an adult human contains around 10^{12} cells, 10-fold less than daily needs. From these estimates it is clear that the blood cells required for lifelong haemopoiesis cannot be preformed in the body.

The bone marrow, which is the major site of haemopoiesis in adult humans, contains cells that represent the stages in the development of the different types of blood cells (Figure 1.1). The later stages are recognizable as belonging to the major lineages of haemopoiesis (granulocytes, erythrocytes, monocyte/macrophages, megakaryocytes, eosinophils, basophils, and T and B lymphocytes). They are the myelocytes, metamyelocytes, erythroblasts, reticulocytes, etc. Earlier stages of development become progressively less morphologically distinct in their lineage affiliation and fewer in number, whereas the least frequent cells, which cannot be discriminated morphologically, are the committed progenitor cell populations and the stem cells.

The stem cells are the most important cells in haemopoietic cell production. They are ultimately responsible for regenerating haemopoiesis following damage to the haemopoietic system by myelotoxic chemotherapy or after stem cell transplantation. This is accomplished by stem cell division, producing new stem cells to maintain the stem cell pool (stem cell renewal) and differentiating cells that are the progenitor cells of each of the blood cell lineages. Estimates of stem cell frequency in human bone marrow are about one stem cell per 20 million nucleated cells.

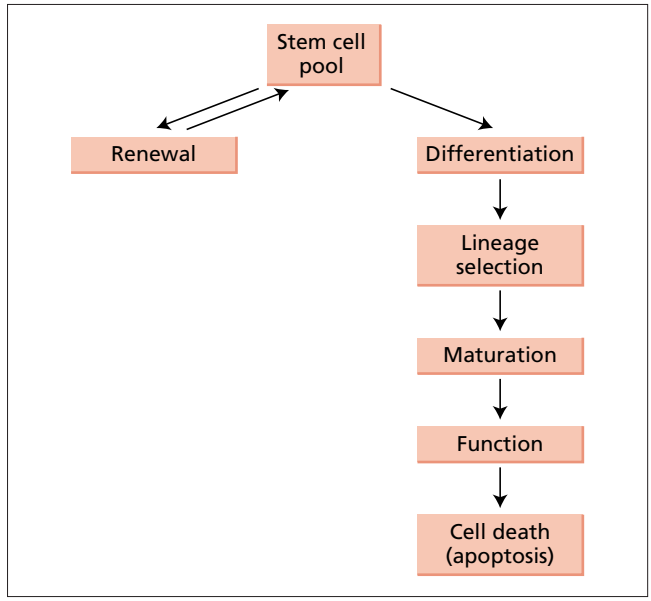

Figure 1.1 Stages in haemopoietic cell development.

They are very difficult to measure, although various assays for candidate human stem cells have been developed. These include both *in vitro* and *in vivo* assays such as long-term bone marrow culture (LT-BMC), cobblestone-area colony (CAFC) formation and the NOD/SCID mouse repopulating assay.

Haemopoiesis is regulated by soluble factors that were discovered when immobilization of bone marrow cells in a semisolid matrix containing medium 'conditioned' by the growth of a cell line in culture resulted in the growth of clonal colonies of granulocytes and macrophages. Identification of the active factors in the conditioned medium led eventually to cloning, production of recombinant protein and clinical use of cytokines in the therapy of haematological disease.

In addition to the haemopoietic system, the bone marrow contains stromal stem cells (mesenchymal stem cells), which are important for constructing the haemopoietic microenvironment. The microenvironment provides more than simply mechanical support and has been shown to be an essential component of the long-term bone marrow culture system. Moreover, damage to the microenvironment, for example by chemotherapy, has been implicated in haemopoietic insufficiency after treatment.

Studies in haemopoietic stem cell biology have now expanded to embrace the concepts of stem cell plasticity. This term refers to the ability of haemopoietic and stromal (mesenchymal) stem cells to produce cells associated with other tissues, such as liver, lung and muscle. Although this area remains highly controversial, the therapeutic applications of haemopoietic stem cell plasticity are obvious as it would provide an easily accessible source of cells that could be redirected to repair a variety of damaged tissues.

Sites of haemopoiesis

The development of the haemopoietic system is associated with the development of suitable microenvironments, which are colonized by migrating stem cells. The migration of stem cells from site to site must require mechanisms for their entry, transit and exit. These processes probably involve specific recognition and adhesive interactions between the stem cells and cells of the various microenvironments. Extracellular matrix-degrading enzymes such as the metalloproteinases have been implicated in the reversal of adhesion and exit from tissues.

There has been a long-accepted dogma that the adult haemopoietic system originates in the embryonic yolk sac. The mesenchyme of the yolk sac differentiates into endothelial cells, on the one hand, and haemopoietic stem cells on the other. At this stage, haemopoiesis consists of blood islands, consisting of primitive primordial cells (haemocytoblasts) and erythroblastoid cells surrounded by endothelial cells. The observation that endothelial and haemopoietic cell development occur in close proximity led to the hypothesis that these two cell types are derived from a common precursor, the haemangioblast, which represents the origin of the circulatory system as well as the blood cells. Following the development of the circulation, stem cells can migrate into the embryo where they sequentially seed the liver, spleen and bone marrow.

Challenging the yolk sac origin of adult haemopoiesis, embryografting experiments in birds revealed that adult haemopoiesis is derived from an intraembryonic source. This is the aorta–gonad–mesonephros (AGM) region, located in the para-aortic splanchnopleure. It is not overtly erythropoietic, unlike yolk sac haemopoiesis, but contains a spectrum of lymphoid and myeloid stem and progenitor cells. Similarly, active haemopoiesis is found in the AGM region of embryo mice. It is thought that a second wave of fetal liver colonization, originating in the AGM region, supplies stem cells for the eventual development of the adult haemopoietic system, in contrast with the primitive and temporary haemopoiesis derived from the yolk sac.

Primitive erythropoiesis persists as the major visible haemopoietic activity in the fetal blood vessels, liver and spleen but large numbers of granulocytes can be found in the connective tissue, outside the organs, for most of intrauterine life. Granulocytic cells are not produced in large numbers until haemopoiesis is established in the bone marrow. This occurs at different times in different bones and coincides with the process of ossification. Large numbers of stem cells are found in umbilical cord blood as well as in the fetal circulation, and this has led to the use of cord blood as an alternative to bone marrow as a source of cells for transplantation. At birth, haemopoietic activity is distributed throughout the human skeleton but it gradually recedes with time so that in normal adult life haemopoiesis is found mainly in the sternum and pelvis, with small amounts in other bones like the ribs, skull and vertebrae.

A small number of stem cells are present in the circulation of normal adult humans. This number increases physiologically in some circumstances, such as following exercise and during infections, and may be increased pharmacologically by administration of haemopoietic growth factors and/or cytotoxic chemotherapy. This phenomenon has been exploited to provide large numbers of circulating stem cells, which can then be collected by leucapheresis and used as a source of cells for stem cell transplantation.

The stromal microenvironment

The documentation of haemopoiesis migrating from site to site suggested that there might be specialized conditions that determine the sequence of colonization. Geiger *et al.* (1998) demonstrated the influence of the embryonic and fetal liver microenvironments by injecting adult marrow stem cells into blastocysts and finding they resumed the adult and fetal programmes of haemopoietic cell development. In contrast, when haemopoietic stem cells from fetal liver are injected into adults they develop along adult lines. Indeed, fetal liver has been used as a source of haemopoietic stem cells for clinical transplantation in some circumstances. Charbord and colleagues characterized a cell type in early-gestation fetal liver, but not in late-gestation fetal liver, with mixed endodermal and mesodermal features that supported haemopoietic cell proliferation *in vitro*. Thus, these fetal stromal cells were only present during the haemopoietic phase of liver development.

Early *in vivo* studies in adult mice demonstrated that the microenvironment in the spleen induced erythropoietic differentiation of transplanted bone marrow cells, whereas the microenvironment in the marrow induced granulopoietic differentiation, demonstrating quite clearly that different microenvironmental conditions can determine lineage expression. *In vitro*, adult bone marrow stromal cells form adherent layers that are believed to represent the haemopoietic microenvironment

and provide support for haemopoietic activity in long-term bone marrow cultures. The stromal cells consist of several cell types, namely macrophages, endothelial cells, fibroblasts and fat cells, together with their extracellular matrix, consisting of collagen, fibronectin and proteoglycan constituents.

Stem cell trafficking

The ability of stem cells to traffic around the body and to search out sites suitable for haemopoiesis is well demonstrated by (a) the 'homing' of transplanted stem cells to the bone marrow and (b) the chemotherapy and cytokine-induced 'mobilization' of stem cells into the circulation. Homing involves transendothelial migration from the bloodstream into the marrow microenvironment, whereas mobilization involves detachment from the microenvironment and transendothelial migration in the reverse direction. Together, these processes may provide a paradigm for stem cell trafficking in general (Figure 1.2). They are likely to involve multifactorial processes involving chemokines, cytokines, adhesion molecules and matrix-degrading enzymes.

In vitro experiments have shown that stromal cell-derived chemokine gradients across an endothelial barrier induce stem cells to migrate from one side to the other. Chemokines are cytokines with direct chemotactic effects on receptor-expressing target cells. However, stromal cell-derived factor 1 (SDF-1) is the only chemokine that acts on haemopoietic stem and progenitor cells. Once the stem cells have gained the extravascular spaces in the bone marrow, the stromal cells provide a plethora of potential sites for recognition by stem cells, including cell surface and extracellular matrix ligands for adhesion molecules (CAMs) on the stem cell surface. Haemopoietic stem and progenitor cells express a wide variety of cell adhesion molecules of different classes (e.g. selectins and integrins), but they are not specific for stem cells as they are also expressed by mature leucocytes.

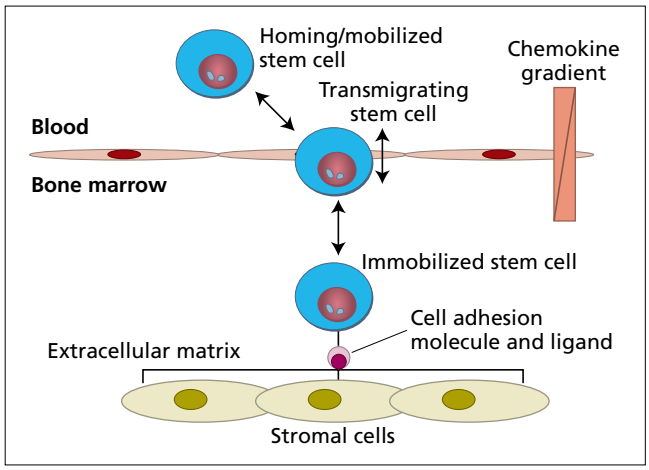

Figure 1.2 Stages in the homing and mobilization of stem cells.

Cytokines and cytokine receptors may also act in cytoadhesion as stem cell factor (SCF; c-Kit ligand) is expressed on the cell surface by stromal cells and its receptor, c-Kit, is expressed by stem cells. Similarly, the Notch ligand, Jagged, is expressed by stromal cells, whereas Notch is expressed by stem cells.

Clearly, cytokines play a role in the release of stem cells from the marrow microenvironment as cytokine administration is used to induce stem cell mobilization into the peripheral blood. Cytokines increase metalloproteinase expression, release stem cell factor from the stromal cell surface and induce stem cell migration through the endothelial barrier. Thus, stem cell trafficking may be a dynamic and continuous process, depending on the prevailing haemopoietic activity.

Organization of haemopoiesis

The haemopoietic system is a hierarchy of cells in which multipotent haemopoietic stem cells give rise to lineage-committed progenitor cells, which divide to generate the maturing and mature blood cells (Figure 1.3).

Stem cells

Assays for stem cells

Stem cells are found at a very low frequency in haemopoietic tissue and cannot be recognized in stained smears of bone marrow. In animal models, the best assay for stem cells is a repopulating assay, which tests the ability of the cells to engraft and restore haemopoiesis in a myeloablated host. For human stem cells, a variety of *in vitro* assay systems have been used, but the most widely accepted is the long-term bone marrow culture system (LT-BMC). This method reproduces the microenvironment of the bone marrow *in vitro* by growing a feeder layer of stromal cells. When haemopoietic long-term culture-initiating cells (LT-CICs) are seeded onto the stromal layer, they are induced to proliferate and produce progenitor cells that can be measured in the clonogenic assays detailed below. However, the endpoint of this assay is indirect, in that committed colony-forming cells produced by the LT-CICs are enumerated. Consequently, limiting dilution analysis is necessary to determine LT-CIC numbers. However, limiting dilution is statistically based and the procedure is very cumbersome.

A variety of clonal assays for candidate stem cells have also been used. The cobblestone area-forming cell (CAFC) assay resembles the long-term culture assay in that haemopoietic cells are seeded onto stromal layers. In this case, however, the formation of colonies containing cells of a cobblestone-like morphology is observed. The blast colony-forming cell (Bl-CFC) assay and high proliferative potential colony-forming cell assay (HPP-CFC) detect cells that share certain characteristics with stem cells but are generally considered to be less primitive than stem cells.

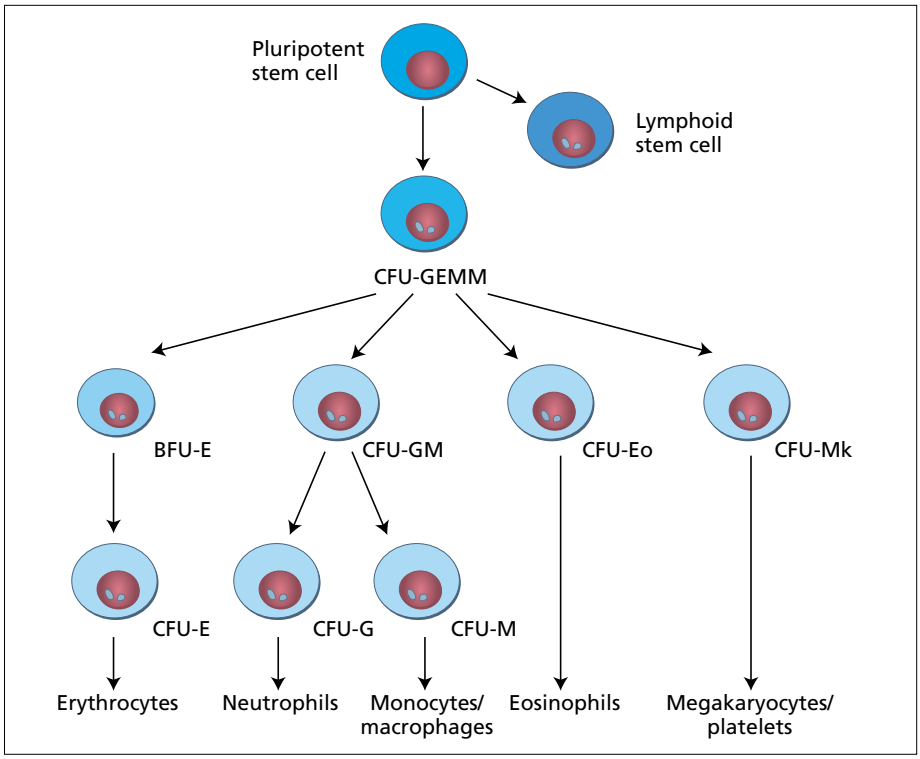

Figure 1.3 Hierarchical organization of haemopoiesis.

Stem cell properties, phenotype and purification

Morphologically, haemopoietic stem cells are undifferentiated and resemble small lymphocytes. Normally, a large fraction is quiescent, in G_0 phase of the cell cycle, which protects them from the action of cell cycle-dependent drugs such as 5′-fluorouracil, and S-phase-specific agents such as cytosine arabinoside and hydroxyurea. The quiescent state of stem cells is maintained by transforming growth factor β (TGF-β). The activity of TGF-β is mediated by p53, a tumour suppressor that regulates cell proliferation and targets the cyclin-dependent kinase inhibitor p21. The cyclin-dependent kinase inhibitors regulate the activities of cyclin–cyclin-dependent kinase (CDK) complexes. Inhibition of the cyclin–CDK complexes prevents phosphorylation of the retinoblastoma proteins that remain bound to transcription factors belonging to the E2F family. As a consequence, genes required for progression of the cell cycle are not transcribed and cells remain quiescent (Figure 1.4). Stromal cells express TGF-β, which is involved in maintaining stem cell quiescence in the bone marrow microenvironment.

The immunophenotype of haemopoietic stem cells is summarized in Table 1.1 and consists of the presence of markers that are expressed by stem cells (CD34, Thy-1) and those that are absent (CD33, CD38, HLA-DR and lineage-specific (lin) markers). CD34 is the best-known marker of human stem and progenitor cells. It is a member of the sialomucin family of glycoproteins, which are heavily glycosylated molecules with potential adhesion and signalling capabilities. CD34 has been implicated in the binding together of cells from the KG1a line and of primary human $CD34^+$ progenitor cells.

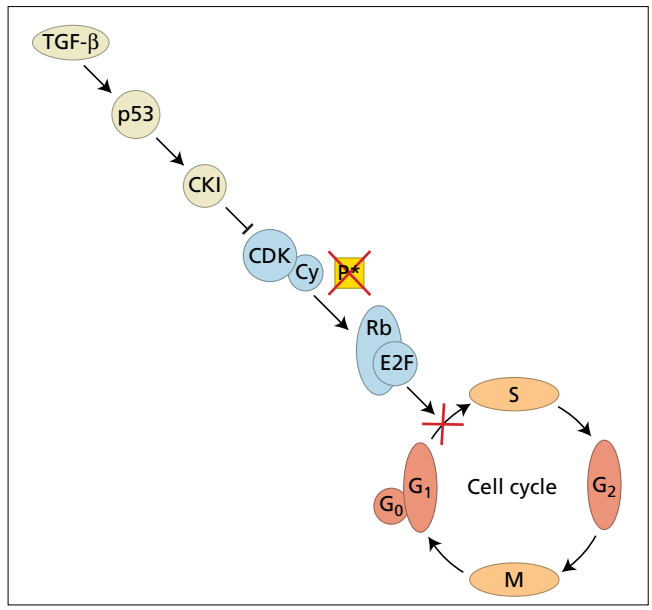

Figure 1.4 Maintenance of stem cell quiescence.

The importance, interest and rarity of stem cells have led to extensive efforts to purify them, based on stem cell characteristics that distinguish them from other cells in the haemopoietic

Table 1.1 Basic immunophenotype of haemopoietic stem cells.

Positive	*Negative*
CD34	CD33
Thy-1	CD38
AC133	Lineage markers
c-Kit	HLA-DR

system. The resistance of stem cells to cell cycle-dependent drugs, particularly to 5′-fluorouracil, has been used as one of the stages in stem cell purification. Fluorescence-activated cell sorting of cells labelled by monoclonal antibodies to phenotypic markers is a widely used strategy for stem cell purification, as is magnetic bead sorting. Despite efforts to purify stem cells to phenotypic homogeneity, the resulting populations are not functionally homogeneous.

Stem cell renewal and differentiation

Stem cells are capable of self-renewal and differentiation when they divide and are responsible for producing all the mature blood cells throughout life. This means that when steady-state stem cells divide, only 50% of the daughter cells, on average, differentiate, the remaining 50% do not differentiate, but maintain stem cell numbers. This could be accomplished by asymmetric cell division, so that each dividing stem cell forms one new stem cell and one differentiated cell (Figure 1.5a). Alternatively, balanced numbers of stem cells could divide symmetrically to form either two new stem cells or two differentiated cells (Figure 1.5b). Clearly, the asymmetric model does not allow for regeneration of the stem cell population but, by altering the proportions of renewing and differentiating stem cells, the symmetric division model can account for stem cell recovery (Figure 1.5c) because it permits an increase in the proportions of symmetrical self-renewing divisions and a reduction in the proportion of differentiating divisions. The symmetrical model of stem cell division means that self-renewal and differentiation are likely to be properties of the stem cell population at large, rather than characteristics of each individual stem cell. However, this mechanism is accompanied by extinction of the differentiating stem cells because the clones they produce will not contain any stem cells.

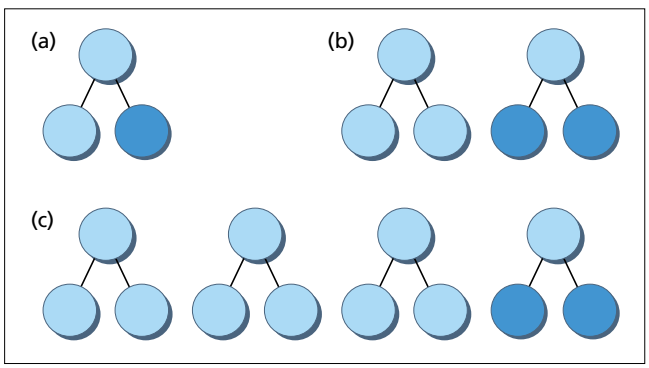

Figure 1.5 Models of stem cell self-renewal and differentiation.

Regulation of self-renewal

Control of haemopoietic stem cell proliferation kinetics is critically important for the regulation of haemopoietic cell production. Nonetheless, information about the control of stem cell renewal versus differentiation, and how this might be manipulated to improve haemopoietic cell regeneration, is still incomplete. Control mechanisms could be intrinsic or extrinsic to the stem cells, or a combination of both.

Extrinsic factors

Extrinsic control would mean that self-renewal and differentiation can be controlled by external factors, such as cell–cell interactions in the haemopoietic microenvironment or cytokines, and thereby be responsive to demands for increased haemopoietic cell production. Regulation in the bone marrow microenvironment, and in the stromal layers of long-term bone marrow cultures, may be mediated by adjacent cells or local cytokine production. SCF is produced by stromal cells and occurs as a transmembrane protein as well as a soluble protein. It binds to its receptor, c-Kit, expressed by haemopoietic stem cells and is essential for normal blood cell production. Flt3 ligand is also a transmembrane protein and is widely expressed in human tissues. It binds to Flt3 on haemopoietic cells and is important for cell survival and cytokine responsiveness. TGF-β reduces stem cell cycling and maintains stem cell multipotency.

The Notch-1–Jagged pathway may serve to integrate extracellular signals with intracellular signalling and cell cycle control. Notch-1 is a surface receptor on haemopoietic stem cells that binds to its ligand, Jagged, on stromal cells. This results in cleavage of the cytoplasmic portion of Notch-1, which can then act as a transcription factor. c-Kit, the receptor for SCF, and receptors for TGF-β and tumour necrosis factor α (TNF-α) may also act in this way.

Intrinsic factors

The expression of several transcription factors has been shown to be essential for haemopoietic cell development from the earliest stages. SCL (stem cell leukaemia haemopoietic transcription factor) and GATA-2 are required for the development of haemopoiesis in the yolk sac, whereas absence of AML-1 results in failure of fetal liver haemopoiesis, although erythropoiesis in the yolk sac is not affected. Candidate genes that are targeted by these transcription factors include c-Kit, the receptor for granulocyte colony-stimulating factor (G-CSF), globin genes and myeloperoxidase.

There is evidence from murine studies that haemopoietic progenitor cells from different inbred mouse strains vary widely in number and proliferative activity. These observations indicate

that genetically determined constitutional variation in human haemopoiesis is also likely to exist. This view is supported by the fact that parameters such as clonogenic cell frequency and numbers, proliferation ability and capacities for mobilization and expansion vary widely among individuals in the general human population. Associations have been reported between genetic markers and the frequency and activity of stem cells in mouse strains. De Haan and colleagues (2002) concluded that the expression levels of a large number of genes may be responsible for controlling stem cell behaviour. These collections of genes may be analogous to those responsible for the interindividual behaviour of human haemopoietic stem cells.

In contrast to the genetic basis for constitutional variation, certain specific genes have been demonstrated to influence haemopoietic cell kinetics. Growing evidence implicates gene products involved in cell cycle control, such as the cyclin-dependent kinase inhibitors (CKIs) p16, p21 and p27 (Figure 1.4) and the maintenance of stem cell quiescence. They have been shown to enhance proliferation and repopulating efficiency of bone marrow cells in gene knockout, knockin and gene transfer models. Loss of CKIs increases clonal expansion by haemopoietic progenitor cells and the size of the stem cell pool; the Fas and Fas ligand genes, which generally are associated with the process of cell death by apoptosis, also influence haemopoiesis as part of a mechanism suppressing progenitor cell proliferation.

Finally, lessons can be learned from studies of disease pathogenesis. Many cell cycle control genes and genes promoting cell death by apoptosis are tumour-suppressor genes that have been found to be deleted or mutated in leukaemia and other cancers. Fanconi's anaemia is an autosomal recessive bone marrow failure syndrome associated with an increased tendency for spontaneous chromosome breaks. The disease can be caused by mutations in at least seven different genes. The genes *FANCA*, *FANCC*, *FANCD1*, *FANCD2*, *FANCE* and *FANCF* have been cloned, and the corresponding proteins play important roles in DNA repair. In dyskeratosis congenita mutations have been identified in the *DKC1* gene, which encodes dyskerin. Dyskerin is a component of small nucleolar ribonuclear protein particles and the telomerase complex, indicating that the disease is due to defective telomerase.

Overall, intrinsic and extrinsic control mechanisms may be considered separately, but a picture is emerging of the integration of extracellular signalling, signal transduction, transcription factors and cell cycle control in the determination of stem cell fate.

Stem cell lineage selection

The 50% of daughter stem cells that differentiate supply cells that are destined to form all of the eight blood cell lineages. The mechanisms determining the blood cell lineages selected by the differentiating progeny of stem cells probably involve aspects of the transcriptional control of lineage-specific genes. Greaves and colleagues proposed that several lineage-specific genes are accessible to transcription factors or 'primed' in uncommitted cells. Accordingly, individual primitive cells were found to exhibit low levels of transcription of lineage-affiliated genes. Moreover, single stem cells expressed low levels of several of these genes, indicating that final lineage selection had not yet occurred. The multilineage 'priming' of stem cells is supported experimentally by the results of replating colonies composed of blast cells. This revealed that the blast cells themselves were bipotent or oligopotent progenitors for various lineages of blood cell development, and that the combinations of lineages found within individual colonies appeared to be randomly distributed, although some combinations are more common than others.

It is likely that differences in the expression levels of transcription factors determine the lineage affiliation of a differentiating cell (Figure 1.6). The transcription factors PU1 and GATA-1 have been implicated in myeloid and erythroid/megakaryocyte lineage specifications respectively. The common precursors of the myeloid, erythroid and megakaryocytic lineages coexpress PU1 and GATA-1, but GATA-1 is downregulated during myeloid cell development and PU1 during erythroid/megakaryocytic cell development. The decision of bipotent granulocyte/monocyte precursors to proceed along the granulocytic or monocyte macrophage lines of differentiation is influenced by C/EBP-α, which is required for granulocytic cell development.

Stem cell plasticity

Reports that transplanted bone marrow cells can contribute to the repair and regeneration of a spectrum of tissue types including brain, muscle, lung, liver, gut epithelium and skin have

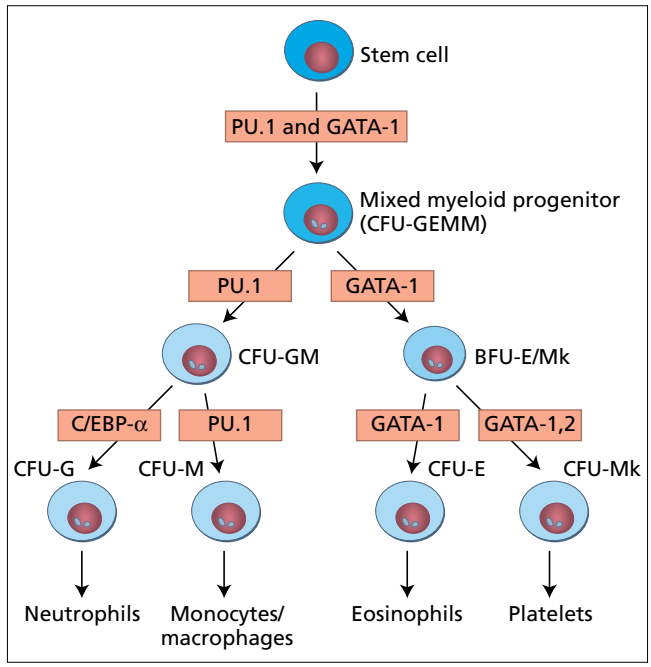

Figure 1.6 Transcription factors involved in lineage selection by haemopoietic stem and progenitor cells.

attracted considerable attention. The important implication of these observations is that haemopoietic stem cells could be used clinically for tissue replacement therapies. The multipotential nature of haemopoietic stem cells appears to be well suited to a wider role in tissue repair as they already demonstrate the capacity to make renewal, differentiation and lineage choices. Moreover, cultured blood cells can transdifferentiate from one lineage to another. Cell fusion is an alternative mechanism accounting for the contribution of bone marrow cells to tissue repair (e.g. after myocardial infarction), possibly involving the macrophage component of the marrow infusion.

A second bone marrow stem cell population with tissue-regenerating potential, the multipotent adult progenitor cells (MAPCs), representing a subpopulation of mesenchymal (stromal) stem cells, was isolated by Verfaillie and colleagues. The MAPCs develop over a period of time in culture, during which they seem to lose tissue-restricted gene expression and become able to differentiate into mesenchymal cell types (osteoblasts, chondrocytes, adipocytes and sketetal myoblasts) endothelium, neuroectoderm and hepatocytes.

Progenitor cells

The progenitor cells are the progeny of stem cells, and it is likely that some of the candidate stem cells measured in the assays mentioned above are in fact intermediate between stem and progenitor cells. As haemopoietic cell development proceeds from stem cells to progenitor cells, the probability of renewal decreases and that of differentiation increases commensurately. Thus, although the probability of self-renewal is highest within the stem cell population, it is by no means a property of stem cells alone. This has been amply demonstrated by replating progenitor cell-derived colonies grown *in vitro* and observing secondary colony formation. It is uncertain at what stage the capacity for self-renewal is lost completely. Indeed, early kinetic studies revealed that even promyelocytes divide two or three times before they differentiate into myelocytes.

Beginning in the 1960s, *in vitro* colony assays have been developed for the enumeration of clonogenic progenitor cells in haemopoietic tissue. The availability of different assays has allowed the investigation of distinct cell populations at different stages of haemopoietic cell development. The mixed lineage colony-forming cells (CFU-mix) consist of combinations of granulocytes, eosinophils, monocytes, erythrocytes and megakaryocytes and are the most primitive cells in this class. Granulocyte–macrophage colony-forming cells (CFU-GM) are bipotential and succeeded by single-lineage CFU-G and CFU-M. The erythroid lineage is represented by the burst-forming unit erythroid (BFU-E) and colony-forming unit erythroid (CFU-E), whereas separate assays exist for megakarocyte precursors (CFU-Mk).

Colony formation *in vitro* is stimulated by haemopoietic growth factors and cytokines. Some of the growth factors are named after their target cells, such as granulocyte–macrophage colony-stimulating factor (GM-CSF) and G-CSF. Others indicate which cell types they act on, such as erythropoietin (erythropoiesis), thrombopoietin (megakaryopoiesis) and SCF.

Maturing and mature cells

The maturing and mature haemopoietic cells are recognizable on stained smears of blood or bone marrow. During maturation, the cells maintain some capacity for division that can influence the blood count because each additional division would double the blood count. Eventually, however, the capacity for cell division is lost because of expulsion of the nucleus (red cells), fragmentation (platelets) or nuclear distortion (polymorphonuclear granulocytes). In contrast, mature lymphocytes have a monomorphic nucleus and retain the ability to divide. The end-products of haemopoietic cell development are cells that are highly specialized for their different functions in the body.

Cell death (apoptosis)

The final stage in the life of a blood cell is death and disposal by apoptosis. Apoptotic cell death is a mechanism for disposing of unwanted or excess cells, and it occurs widely in biological systems. It ensures the destruction of cells without releasing any lysosomal or granule contents that would cause an inflammatory reaction. Apoptosis involves a complex series of events that culminate in the activation of the caspase proteases, fragmentation of DNA and phagocytosis of apoptotic bodies by macrophages.

In haemopoiesis, apoptosis is used to dispose of mature end cells once they have fulfilled their function. In addition, it has also been proposed as a mechanism for negative regulation of cell production. Accordingly, a reduction in cell death could account for an increase in haemopoietic stem and progenitor cell numbers. However, this mechanism presupposes that substantial numbers of stem and progenitors are lost by apoptosis in steady-state haemopoiesis. Haemopoietic cytokines and growth factors act as survival factors for haemopoietic progenitor cells and prevent the death of factor-dependent cell lines *in vitro*. Also, components of the apoptotic machinery have been implicated in the feedback negative regulation of erythropoiesis and myelopoiesis, cell cycle regulation and cell differentiation. These observations suggest that the apoptotic pathways may have regulatory functions that do not culminate in cell death.

Haemopoietic growth factors and receptors

The haemopoietic growth factors and cytokines are the soluble regulators of blood cell production and are produced by several cell types in different sites in the body. They are glycoproteins

Table 1.2 Haemopoietic cytokines and their target cells.

Cytokine	*Target cell(s)*
IL-3	CFU-GEMM, HPP-CFC, CFU-GM, CFU-Eo, CFU-Baso, BFU-E, CFU-Mk
GM-CSF	HPP-CFC, CFU-GEMM, CFU-GM, CFU-Eo, CFU-Baso, CFU-Mk, BFU-E, CFU-M, CFU-G, dendritic cells
G-CSF	HPP-CFC, CFU-GEMM, CFU-GM, CFU-G
M-CSF (c-Fms ligand)	HPP-CFC, CFU-GEMM, CFU-M
Epo	CFU-E
SCF (c-Kit ligand)	HPP-CFC, CFU-GEMM, CFU-GM, CFU-Baso, BFU-E
IL-1	HPP-CFC
IL-4	CFU-GM, CFU-Baso, BFU-E, dendritic cells
IL-5	CFU-Eo
IL-6	HPP-CFC, CFU-GM, BFU-E
IL-11	CFU-Mk, CFU-GM, BFU-E
Thrombopoietin	CFU-Mk
Flt3 ligand	LT-CIC, CFU-GEMM, CFU-GM, dendritic cells
Fibroblast growth factor (FGF2 and FGF4)	CFU-GM, BFU-E, stromal cells
Leukaemia inhibitory factor (LIF)	CFU-Mk, BFU-E

References and further details will be found in Garland *et al.* (1997) and Thomson and Lotze (2003).
Baso, basophil; BFU-E, burst-forming unit erythroid; CFU, colony-forming unit; Eo, eosinophil; GEMM, granulocyte, erythrocyte, monocyte, megakaryocyte; GM, granulocyte–macrophage; HPP-CFC, high proliferative potential colony-forming cell; LT-CIC, long-term culture-initiating cell; Mk, megakaryocyte.

with little primary amino acid homology, although molecular modelling of secondary structure suggests that they possess similar structural features, such as bundles of anti-parallel α-helices joined by loops and β-sheets. Different sequences have been identified by deletional mutagenesis, which are required for secretion, biological activity and receptor binding. Some cytokines, such as SCF, exist in membrane-bound form as well as a soluble form because they lack the signal sequence responsible for release from the cell as a result of alternative splicing.

Cytokine responses and signal transduction

The responses of haemopoietic cells to cytokines include survival, proliferation, differentiation and stimulation of mature cell function. Once cytokines have bound to their receptors on the cell surface, they activate signal transduction pathways that transmit the signal to the nucleus and ultimately stimulate the transcription of regulatory genes. Haemopoietic progenitors require multiple cytokines for their optimal growth and development (Table 1.2). These growth factors act in concert to coordinate the various cellular functions that are necessary for the cell division and progressive differentiation required for the formation of mature functioning end cells. There are many examples of cytokines acting in synergy when the outcome is greater than expected from the sum of the individual cytokines acting alone. Also, cytokines are pleiotropic in their actions, and several cytokines are known to share the same functions. This level of complexity is very difficult to rationalize, but it is becoming apparent that intracellular coordination is achieved by co-localization of sequentially acting signalling proteins, or by binding interactions with adaptor complexes, cytoskeletal structures or molecular targets, for the selective activation of downstream targets. Outside the cell, haemopoietic cell responses may be modulated by controlled access of cytokines to target cell receptors, as discussed below.

Many haemopoietic growth factor and cytokine receptors belong to the haematopoietin receptor superfamily (Figure 1.7). These are type 1 transmembrane glycoproteins with modular extracellular domains. All members dimerize when they bind their ligand. G-CSF and erythropoietin (Epo) receptors homodimerize, whereas the β-chains of the interleukin (IL) 3, IL-5 and GM-CSF receptors dimerize with a common beta (β_c) chain to form a high-affinity receptor. Other members of the haematopoietin receptor superfamily (IL-6, IL-11, IL-12 and leukaemia inhibitory factor, LIF) require the presence of a transmembrane protein, gp130, to transduce a signal.

A separate group of receptors has intrinsic tyrosine kinase activity and contains receptors for SCF, hepatocyte growth factor (HGF) and Flt3 ligand (FL). It is evident that cytokine receptor systems not only act in a linear-independent manner, but also influence the activity of other cell-surface receptor systems. Biochemical studies have revealed interactions between haemopoietic cytokine receptors, including interactions of β_c with Epo and G-CSF receptors. However, it is not established

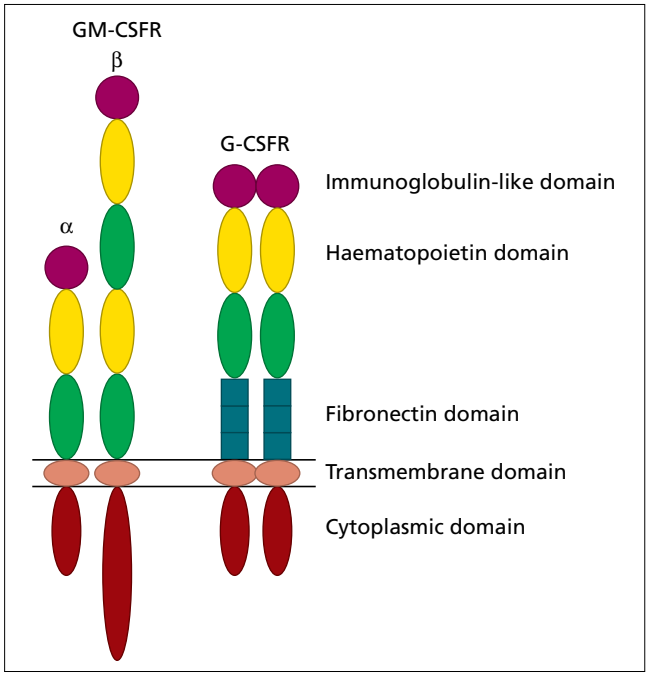

Figure 1.7 Representation of the modular structures of the haematopoietin receptor superfamily, exemplified by the receptors for GM-CSF and G-CSF (after Lewis and Gordon, 2003).

that these biochemically documented phenomena, such as receptor transmodulation, transphosphorylation and physical interaction, are biologically significant.

Cytokines regulate a variety of haemopoietic cell functions through the activation of multiple signal transduction pathways (Figure 1.8). The major pathways relevant to cell proliferation and differentiation are the Janus kinase (Jak)/signal transducers and activators of transcription (STATs), the mitogen-activated protein (MAP) kinase and the phosphatidylinositol (PI) 3-kinase pathways. Jaks function upstream of STATs, which are activated by phosphorylation, and then dimerize and migrate to the nucleus, where they bind to specific DNA motifs. Thus, the Jak–STAT pathway represents the most direct pathway for transmitting a signal from the cytokine receptor to DNA. Ras regulates the best-characterized MAPK cascade, which consists of Raf isoforms MEK1/2 and ERK1/2 and controls proliferation. PI3 kinases phosphorylate inositol lipids, which, in turn, activate downstream targets (Akt, Erk, p70s6k, vav–rac) and influence many different cellular processes. These include cell survival, cell cycle progression, proliferation and reorganization of the actin cytoskeleton. The Jak–STAT pathway is implicated in IL-3, IL-6, Epo and G-CSF signalling, the MAP kinase pathway in Epo, GM-CSF, G-CSF and IL-3 signalling, and the PI3 kinase pathway in IL-6, Epo, GM-CSF, G-CSF and M-CSF signalling.

Thus, all of the haemopoietic growth factors appear to be capable of activating all of the major signal transduction pathways simultaneously. The several pathways that have been identified and the multiple responses, pleiotropism and redundancy that are well-known features of haemopoietic cytokines raise the possibility that a particular cytokine may have different effects in different cell types and possibly utilize different signal transduction pathways for specific functions. Moreover, combinations of cytokines may cooperate to activate further signal transduction pathways that are not activated when cytokines are used individually. Such interactions among receptor-mediated signals provide a mechanism for merging the activities of different ligand–receptor systems and achieving novel cellular outcomes.

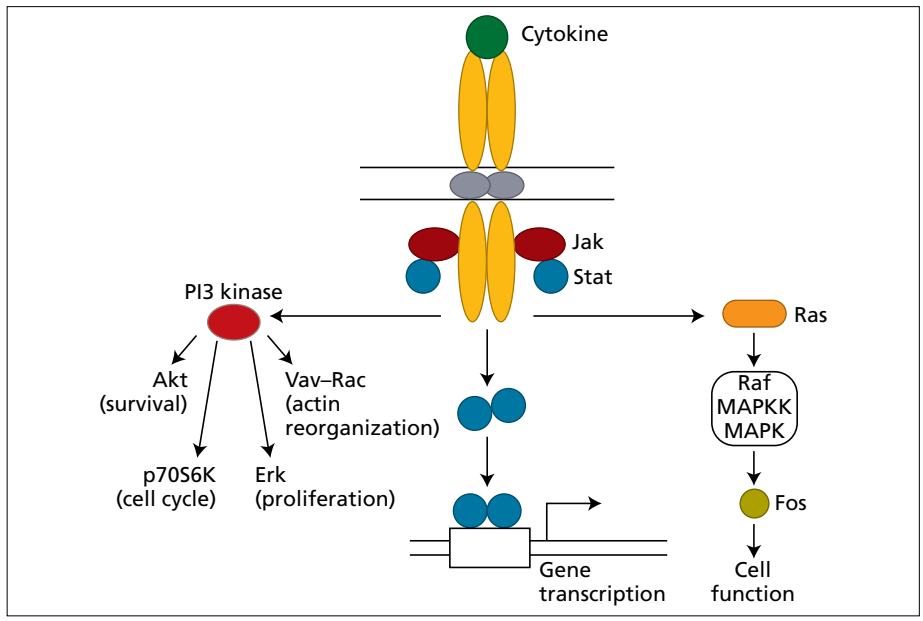

Figure 1.8 Generalized diagram of the signal transduction pathways activated by cytokines and their receptors in haemopoietic cells.

Physiology of the cytokine response

Colony formation *in vitro* is a simple model of haemopoietic regulation by cytokines, which involves the interaction of soluble proteins with specific receptors on the surface of the target cells. In culture, the cytokines are freely available at a uniform concentration and any cell which expresses the corresponding receptor will be able to respond. However, this is unlikely to be the situation *in vivo* where stem and progenitor cells are located in the haemopoietic microenvironment and there is a need to control haemopoietic cell production more precisely.

Several mechanisms have the potential to control the access of cytokines to their target cells and are likely to be physiologically important. The first mechanism is the localization of the stem and progenitor cells. The fact that these cells are found predominantly in the haemopoietic microenvironment rather than freely distributed in the bloodstream and tissues indicates that there is a mechanism to retain them there, and it has been extensively demonstrated that stem and progenitor cells express cell adhesion molecules that bind them to proteins expressed by the stromal cells of the marrow microenvironment. Cytokines can also bind to components of the microenvironment, in this case to extracellular matrix proteins produced by the microenvironmental stromal cells, and this may act to direct the cytokine to the appropriate target cell and modify the concentration or duration of exposure. Several cytokines are produced in membrane-bound or soluble forms, which can have different activities. For example, soluble SCF is active for only a short time, whereas membrane-bound SCF is more durable. In the circulation, cytokines can bind to soluble proteins, including soluble receptors, and these interactions may function as carriers for transport of the cytokine in an inactive form from its site of production to its site of action, protect the cytokine from proteolytic degradation or, in some circumstances, inactivate it.

Negative regulation of haemopoiesis

Like all homeostatic systems, haemopoiesis is regulated by a balance of positive and negative influences. They include cytokines such as the macrophage inflammatory protein, MIP-1α, and TGF-β. As well as directly inhibitory factors, a variety of other mechanisms with the potential to block cytokine action have been described. Interleukin 1α and IL-1β have an endogenous receptor agonist that blocks IL-1 binding to its receptor; several cytokines (TNF, IL-2, IL-4) are blocked by soluble receptors that compete with cell-surface receptors and some cytokines inhibit the activities of others.

The location of haemopoiesis in the marrow microenvironment, represented *in vitro* by stromal cell cultures, and the predominantly quiescent nature of the haemopoietic stem cell population indicate a negative role of the microenvironment in suppressing stem cell activity. Early studies of haemopoiesis in long-term bone marrow cultures, in which stromal cells support haemopoiesis for a prolonged period of time *in vitro*, revealed periodic oscillations in the cell cycle activity of stem cells that could be related to the presence of positive and negative cytokines implicated in maintaining homeostasis of the haemopoietic system.

Clinical applications of stem cell research

Stem cell research has provided the growth points for several clinical activities as well as for advances in experimental haematology.

Stem cell transplantation

Bone marrow and stem cell transplantation is the most obvious application of stem cell research. It originated in early studies of the haematological reconstitution of mice whose bone marrow had been ablated by ionizing radiation. It soon became apparent that haematological rescue of these animals required infusion of syngeneic marrow because transplantation of cells from a different strain resulted in a wasting condition called 'secondary disease', which is now known as graft-versus-host disease (GvHD). Both the transplantation of stem cells into mice and the recognition of the importance of histocompatibility in the murine system were important contributions to modern clinical transplantation. The identification of the stem cell immunophenotype and the development of cell separation technologies facilitated the development of graft engineering to improve the results of clinical transplantation. One application of cell separation was to deplete the graft of T lymphocytes, either by removing T cells or purifying $CD34^+$ cells. However, it soon became apparent that a graft-versus-leukaemia activity (GvL) was removed, along with the potential for GvHD, and efforts to isolate GvL from GvHD continue today.

Haemopoietic growth factors

Haemopoietic growth factors such as G-CSF, GM-CSF and erythropoietin are administered to cytopenic patients to stimulate cell production. They were originally identified by their colony-stimulating activity *in vitro*.

Stem cell mobilization

G-CSF, in particular, is widely used to mobilize stem cells (peripheral blood progenitor cells, PBPCs) into the peripheral blood from where they can be harvested by leucapheresis and used as a source of stem cells for transplantation. Initially the procedure was used for autografting but mobilization of PBPCs from allogeneic donors is practised at present.

Stem cell expansion

In cases when insufficient stem cells are available for successful engraftment, it would be advantageous to be able to increase the number of stem cells during a period of *in vitro* culture. For this, 'stem cell expansion' is conducted in the presence of combinations of cytokines, with the aim of inducing stem cell renewal and population growth. A large number of studies have investigated a variety of culture conditions and combinations of cytokines. Frequently used cytokines include Flt3 ligand, thrombopoietin, IL-3 and stem cell factor. Although large increases in numbers of colony-forming progenitor cells have been reported, there is little evidence for expansion of long-term repopulating stem cells.

Gene therapy

The self-renewal and expansion capacities of haemopoietic stem cells make them the ideal vehicle for gene therapy of genetic disorders. The transduced genes will be expressed for long periods of time in the stem cell population and in their differentiating and mature descendants (see Chapter 27).

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