10. Which white cell is most closely associated with defence against parasitic infestation?
11. Which granulocyte is most closely associated with anaphylaxis?
12. Fill in the blanks in the text below:
B lymphocytes normally account for 10–30% of the circulating blood lymphocytes and play a central role in _____ (i.e. _____-mediated) immunity. B lymphocytes also play a role in processing and presenting antigen to _ cells. T lymphocytes account for 40–80% of the circulating blood lymphocytes and are responsible for _____-mediated immunity. The _____ gland is responsible for T cell education. NK cells play an important role in fighting _____ and _____ infections.

CHAPTER 2

Haemopoiesis

Learning objectives
After studying this chapter you should confidently be able to:

- **Describe the various sites of haemopoiesis throughout life**
  Haemopoiesis is conducted at a number of different anatomical sites during the process of development from embryo to adult. The earliest recognizable red cell precursors are demonstrable in 2 week old embryos. Haemopoiesis occurs in the fetal liver at 6 weeks gestation, and this organ is the primary source of fetal blood cells until about 30 weeks gestation. The fetal spleen is a secondary source of haemopoietic cells. Bone marrow rapidly becomes the sole source of blood cells by 40 weeks gestation and remains so throughout life.
- **Discuss the physiological significance of the haemopoietic stem cell compartment**
  Blood cells are produced continuously throughout life. This requires a population of precursor cells that are capable of both self-renewal and differentiation – the stem cell compartment. The common ancestral cell of all mature blood cells in humans is the totipotent stem cell. This cell can differentiate to form lymphoid or myeloid pluripotent stem cells. These stem cells retain the dual capacity for self-renewal and differentiation. Pluripotent stem cells are capable of differentiating into a number of different unipotential stem cells that are committed to a single cell line, e.g. BFU-E can only differentiate into mature red cells.
- **Outline the mechanisms of regulation of haemopoiesis**
  Haemopoiesis is a closely regulated process. The bone marrow micro-environment is important for supporting cell growth and differentiation, but the main regulatory role lies with a large family of glycoproteins known as cytokines or, more precisely, haemopoietic growth factors. The impact of growth factors on the regulation of haemopoiesis *in vivo* is highly complex and incompletely understood.
- **Outline the importance of the haemopoietic micro-environment**
  The bone marrow contains a mixture of haemopoietic and non-haemopoietic cells anchored within a structural network known as the extracellular matrix (ECM). The complete structure comprising the ECM and all of the cells of the bone marrow is known as the haemopoietic micro-environment, because all of these cells and structures interact to provide an optimal micro-environment for haemopoiesis. The non-haemopoietic cells of the haemopoietic micro-environment constitute the marrow stroma.

Under normal physiological conditions, the number of circulating blood cells is maintained within remarkably narrow limits. Because all blood cells have a limited lifespan, a dynamic equilibrium must exist between cell loss due to senescence or normal function and the synthesis and release of their replacements. The process of blood cell production is known as haemopoiesis (see *Box 2.1*). The maintenance of circulating blood cell numbers is an example of physiological homeostasis (see *Box 2.2*).

Box 2.1 Definition of haemopoiesis

Haemopoiesis is the blanket term that covers the production of all blood cells. The production of the different blood cell lineages is known as erythropoiesis (red cells), myelopoiesis (granulocytes and monocytes), granulopoiesis (granulocytes), monopoiesis (monocytes), and thrombopoiesis (platelets). The production of lymphocytes (lymphopoiesis) is also strictly part of haemopoiesis, but there are features of lymphocyte production that are markedly different from the production of the other lineages. The most significant difference is that circulating lymphocytes are not end-stage cells. When they encounter antigen they undergo a second proliferative burst that generates a clonal response.

Box 2.2 Homeostasis

The concept of homeostasis is arguably the single most profound and powerful idea that has emerged within physiology over the last 150 years. It was the French physiologist Claude Bernard, the founder of modern experimental physiology, who in 1865 first expounded the importance of the consistency of the internal environment for the continued normal functioning of the human body, with the words '*La fixité du milieu intérieur est la condition d'une vie libre et indépendante*' ('The constancy of the internal environment is the condition for a free and independent life'). This seminal observation remains the cornerstone of modern biological thought.

It was the American physiologist Walter Cannon who coined the word *homeostasis* in his book *The Wisdom of the Body* (1932). The concept of the negative feedback homeostatic loop as a mechanism for maintaining the consistency of any variable that is subject to perturbation from factors outside the feedback system itself (so-called external variables), is now recognized as being, in various forms, the mechanism whereby the vast majority of all physiological 'constants' are maintained.

Haemopoiesis is regulated by an array of protein growth factors that have been shown to stimulate the proliferation and/or differentiation of haemopoietic progenitor cells to produce the different blood cell types in precisely the proportions required to maintain normal circulating cell counts. For example, a significant haemorrhage leads to a reduction in circulating red blood cells. This triggers secretion of a red cell growth factor called erythropoietin that influences haemopoietic progenitor cells to preferentially produce red cells until the reduced red blood cell count is corrected (see also *Box 2.3*).

Box 2.3 Astonishing facts about haemopoiesis

The maintenance of a stable population requires an astonishing capacity for haemopoiesis. An average 70 kg man has a total blood volume of about 5 litres which contains a total of 25×10^{12} red cells. Since normal red cells survive for an average of 120 days, maintenance of a constant cell number requires the replacement of more than 2×10^{11} red cells every day! If the destruction and replacement of the other blood cells is taken into account, the total daily requirement for new blood cells is about 5×10^{11}. This rate of production must be maintained without pause for an average of 70 years, during which time the bone marrow will have released more than 1×10^{16} mature blood cells which, for an average 70 kg man, would weigh about 7 tonnes!

2.1 ONTOGENY OF HAEMOPOIESIS

Haemopoiesis is conducted at a number of different anatomical sites during the process of development from embryo to adult (*Figure 2.1*). Changes in the primary site of haemopoiesis

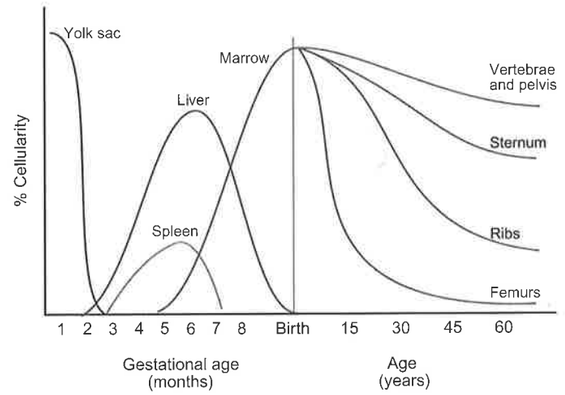

Figure 2.1
Sites of haemopoiesis throughout life.

are accompanied by simultaneous changes in the morphology of the cells produced and in the types of haemoglobin molecule synthesized within the red cell precursors.

Embryonic haemopoiesis

The earliest recognizable red cell precursors are large, nucleated cells and are demonstrable in 2 week old embryos (see *Box 2.4*). The major haemoglobin present in these cells is haemoglobin Gower I ($\zeta_2\varepsilon_2$; see *Chapter 6*). Leucopoiesis and thrombopoiesis do not commence until about 6 weeks gestation, when megakaryocytes and granulocytes can be seen in the yolk sac. In contrast to other blood cells, lymphocytes are not formed in the yolk sac, but in the lymph sacs, which begin to develop at about 7 weeks gestation. Activation of the α and γ genes occurs at about 5 weeks gestation when haemoglobin Portland ($\zeta_2\gamma_2$) and haemoglobin Gower II ($\alpha_2\varepsilon_2$) are synthesized. These three embryonic haemoglobins are undetectable by routine methods after about 10 weeks gestation. This is coincident with the end of the yolk sac phase of erythropoiesis. Haemopoietic cells share a common precursor, the haemangioblast, with endothelial cells. It is believed that it is these cells that seed the spleen, liver and bone marrow.

Box 2.4 Embryonic anatomy

The earliest recognizable blood cell precursors are demonstrable in 2 week old embryos. At this stage of development, the embryo consists of little more than two sacs – the **amniotic sac** and the **yolk sac** – separated by a wedge of tissue called the **embryonic plate**. At this stage the yolk sac is the main site of haemopoiesis. As the embryo develops, the amniotic sac expands greatly to fill the entire uterus, and the placenta is formed. The yolk sac is compressed by the expanding amniotic sac into a narrow stalk that forms the core of the umbilical cord. The embryo develops from the embryonic plate.

Fetal haemopoiesis

Haemopoietic activity is first demonstrable in the fetal liver at 6 weeks gestation. This organ is the primary source of fetal blood cells until about 30 weeks gestation. Hepatic haemopoietic activity

ceases at about 40 weeks gestation. The major haemoglobin synthesized during the hepatic phase of fetal haemopoiesis is haemoglobin F ($\alpha_2\gamma_2$).

The fetal spleen begins production of blood cells at about 10 weeks gestation and continues throughout the second trimester of pregnancy. However, even at the height of its activity, the fetal spleen is of secondary importance as a haemopoietic organ.

Bone cavities begin to form at about 20 weeks gestation and provide such an ideal environment for haemopoietic activity that the bone marrow rapidly becomes the sole source of blood cells in humans, a process complete by 40 weeks gestation. This process is associated with a gradual replacement of haemoglobin F by haemoglobin A ($\alpha_2\beta_2$).

Haemopoiesis in the developing child and adult

In the developing child, there are two demands placed on haemopoiesis: the ongoing need for replacement of senescent cells, and the pressure to increase the bone marrow volume and total blood cell pool to meet growth demands.

At birth, haemopoietically active, or red, marrow completely fills the available marrow space. This means that infants have no reserve haemopoietic capacity that can be called upon in times of increased demand. The only response open to a neonate in such circumstances is to expand the marrow volume. This is the cause of the skeletal deformities that develop in severe haemolytic states such as thalassaemia.

During early childhood, marrow volume increases in parallel with the increased marrow space made available by increased stature. The bone marrow volume in an average 3-year-old child has expanded to about 1500 ml. This is still entirely composed of active red marrow and is sufficient to meet the normal demands for blood cells of an adult. Thus, as the child grows into an adult, and the available bone marrow space expands, there is no requirement for a concurrent increase in volume of active red marrow. The expanding marrow space becomes progressively filled with inactive, or yellow, marrow. This process begins in the peripheral diaphyses of the long bones and continues until, in an adult, three-quarters of the red marrow is found in the pelvis, vertebrae, sternum and scapulae. The remainder is distributed between the skull, ribs and the epiphyseal ends of the long bones. Yellow marrow is mainly located in the middle portion of long bones. The changes in the extent of active marrow as the body matures are shown in *Figure 2.2*.

Yellow bone marrow consists mainly of fat cells (adipocytes) and serves as a fat store for the body. In times of excess haemopoietic demand that cannot be met by the existing red bone

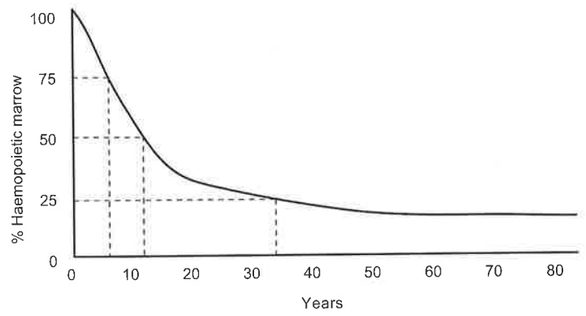

Figure 2.2
Changes in the extent of active marrow over time.

marrow, yellow bone marrow can be converted to red bone marrow to increase haemopoietic capacity. This means that adults have a reserve haemopoietic capacity of about six times normal.

In conditions where the bone marrow is unable to meet the demands of the body for blood cells, e.g. when the bone marrow space is occupied by metastatic tumours, haemopoiesis may revert to fetal sites, namely the spleen and liver. This phenomenon is known as extramedullary haemopoiesis. The most likely mechanism triggering extramedullary haemopoiesis is seeding of circulating haemopoietic stem cells in the spleen, liver or other site.

2.2 SEQUENCE OF DIFFERENTIATION OF BLOOD CELLS

Blood cells are produced in vast numbers throughout life, with no apparent sign of exhaustion of their source. This requires the existence of a population of precursor cells that are capable of both self-renewal and differentiation – the stem cell compartment. The common ancestral cell of all mature blood cells in humans is the totipotent stem cell (*Figure 2.3*). This cell can differentiate to form either a lymphoid stem cell (CFU-L) or a myeloid stem cell (CFU-GEMM). These cells are said to be pluripotent, i.e. they have the capacity to differentiate along several different cell lines but their choice is limited, as shown in *Figure 2.3* (see also *Box 2.5*). These stem cells retain the dual capacity for self-renewal and differentiation. Pluripotent stem cells are capable of differentiating into a number of different unipotent stem cells; these are committed to differentiation along a single cell line, e.g. BFU-E can only differentiate into mature red cells.

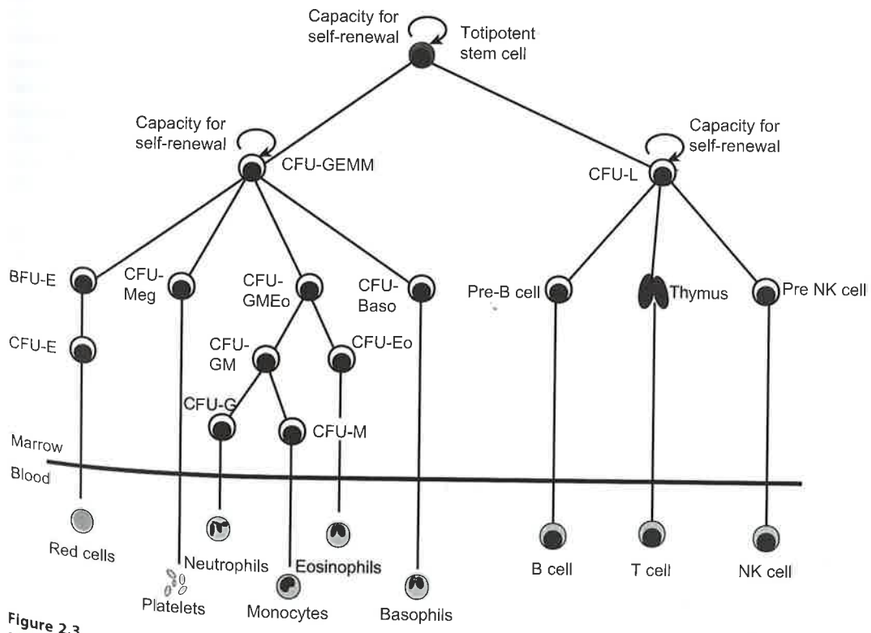

Figure 2.3
Differentiation of mature blood cells from stem cells.

Box 2.5 Pluripotent stem cells

The first unequivocal demonstration of the existence of pluripotent stem cells came in 1961 when Till and McCulloch performed experiments to determine the sensitivity of mouse bone marrow to damage by irradiation. Briefly, mice were subjected to a dose of ionizing radiation sufficient to destroy their haemopoietic capacity, and bone marrow cells from genetically identical mice were immediately transfused. After about 7 days the spleens of these mice had developed numerous macroscopic nodules which consisted of haemopoietic tissue. Subsequent experiments showed that these nodules were clonal in nature, i.e. each was derived from a single stem cell, which was given the name colony forming unit spleen or CFU-S**.** Under different experimental conditions, CFU-S could be influenced to produce granulocyte–macrophage colony forming units (CFU-GM), erythroid colony forming units (CFU-E) or megakaryocyte colony forming units (CFU-Meg) or a mixture of more than one cell line.

2.3 CONTROL OF HAEMOPOIESIS

Much of our current knowledge of the activities of haemopoietic growth factors has been derived from a mixture of animal experiments, tissue culture experiments and molecular biology. However, there is still much to learn and it is uncertain how much of the experimental data are applicable to the human *in vivo* setting.

Haemopoiesis is a closely regulated process. The bone marrow micro-environment is important for supporting cell growth and differentiation, but the main regulatory role lies with a large family of glycoproteins known as cytokines or, more precisely, haemopoietic growth factors.

Cytokines comprise a diverse family of soluble proteins and peptides that function as humoral regulators of the functions of individual cells, of the interactions between cells, and of various processes in the extracellular environment. They can act via autocrine, paracrine, juxtacrine and retrocrine mechanisms (see *Box 2.6*). In many respects, cytokines bear comparison with classical hormones, although cytokines generally impact on a wider range of cells and their secretion is not restricted to specialized glands. Haemopoietic growth factors are a subset of cytokines that exert important regulatory functions in blood cell proliferation and differentiation. Some of the most important haemopoietic growth factors are shown in *Table 2.1*.

The impact of growth factors on the regulation of haemopoiesis *in vivo* is highly complex and incompletely understood. It is clear that the naïve view: one cellular source – one cytokine – one target cell – one effect, is over simplistic. A single cell type can secrete many different cytokines in response to various stimuli. Each of the cytokines can impact on many different cell types. Each cytokine can exert many different effects on different target cells. Each of these effects can be exerted by more than one cytokine. Finally, the impact of a given cytokine can

Box 2.6 Cell signalling definitions

Autocrine – a form of cell signalling in which a cell secretes a hormone or cytokine that exerts its activity on the same type of cell.

Paracrine – a form of cell signalling in which a cell secretes a hormone or cytokine that exerts its activity on cells that are close to, but not in contact with the secreting cell.

Juxtacrine – a form of cell signalling in which a cell secretes a hormone or cytokine that exerts its activity on cells that are in contact with the secreting cell.

Retrocrine – a type of cell signalling in which soluble forms of cytokine receptors are shed by cells that interact with distant target cells expressing the relevant cytokine on their surface membranes.

differ, depending on whether it is acting alone or in concert with other cytokines and also on the sequence of cytokine activity. In short, the reality of cytokine regulation of haemopoiesis is extraordinarily complex. A simplified indication of which cytokines exert important positive effects on haemopoiesis is shown in *Figure 2.4*.

Table 2.1 Selected important haemopoietic growth factors

Growth factor family	Growth factor	Primary target	Gene locus	Source
Interleukins				
	IL-1	Stem cells, T-helper cells, B cells, NK cells, endothelial cells	IL-1α 2q13 IL-1β 2q13–21	Monocytes, activated macrophages
	IL-3	Haemopoietic progenitor cells	5q23–31	Activated T cells
	IL-4	Activated B cells, basophils	5q23–31	Activated T-helper 2 cells
	IL-5	Eosinophils, thymocytes	5q23–31	T cells
	IL-6	B cells, plasma cells, multiple non-haemopoietic cells	7p21–p1	Monocytes, fibroblasts, endothelial cells
	IL-7	B cell progenitors, NK cells, T cell progenitors	8q12–q13	Stromal cells, thymic cells
	IL-8	Neutrophils, basophils, B cells	4q12–q21	Monocytes, multiple non-haemopoietic cells
	IL-9	T-helper cells, mast cells, fetal thymocytes	5q31–32	T-helper cells
Colony stimulating factors				
	G-CSF	CFU-G, CFU-GM	17q21–q22	Monocytes, macrophages, activated neutrophils
	GM-CSF	CFU-GM, CFU-GEMM, endothelial cells	5q22–31	T cells, macrophages
	M-CSF	CFU-M, CFU-GM	5q33	Monocytes, granulocytes, fibroblasts, endothelial cells
Hormones				
	Erythro-poietin	Erythroid progenitor cells, megakaryocyte progenitor cells	7q21–22	Renal tubular, juxtatubular and interstitial cells
	Thrombo-poietin	Megakaryocyte progenitor cells	3q26–27	Liver, kidney

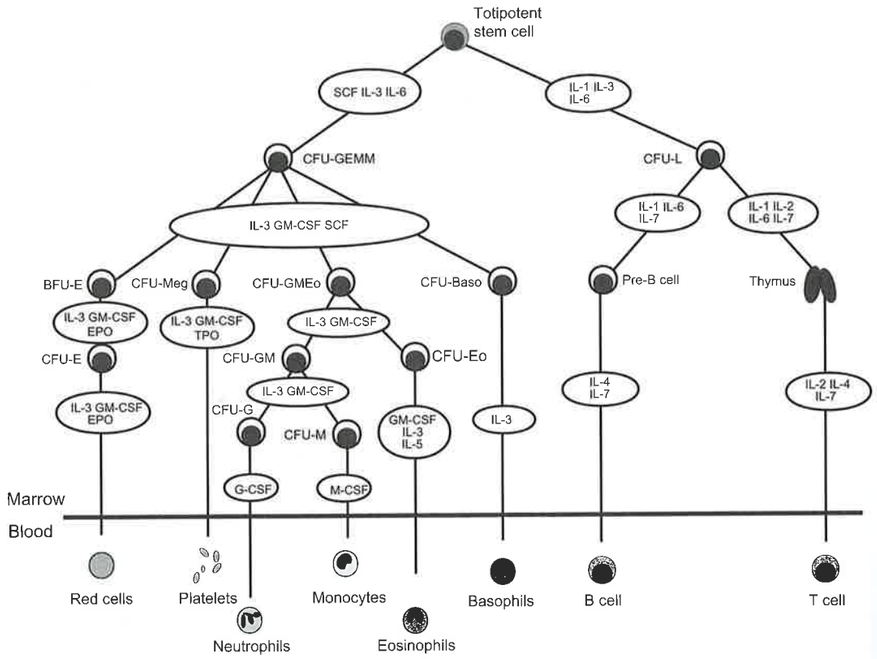

Figure 2.4
Simplified view of cytokine-mediated regulation of haemopoiesis.

One of the key features of the currently accepted model of the stem cell compartment is that a small population of self-renewing stem cells can give rise to the large number of different blood cells produced every day as shown schematically in *Figure 2.5*.

Until relatively recently the haemopoietic stem cell was regarded purely as a progenitor of blood cells but this is now known to be only part of the story. Adult haemopoietic stem cells have been shown experimentally to be capable of differentiating into a wide range of non-haemopoietic cell types, a phenomenon known as stem cell plasticity (see *Figure 2.6* and *Box 2.7*). The therapeutic potential of this observation is the focus of much scientific and clinical research. It is hoped that harvesting of haemopoietic or mesenchymal stem cells may provide a means to effect repair of damaged tissue throughout the body. If this can be achieved by differentiation of patient-derived haemopoietic stem cells, it should also avoid problems associated with immune rejection.

2.4 THE HAEMOPOIETIC MICRO-ENVIRONMENT

The bone marrow contains a mixture of haemopoietic and non-haemopoietic cells anchored within a structural network known as the ECM. Among the non-haemopoietic cells present are myofibroblasts, adipocytes, osteoblasts, osteoclasts, mesenchymal stem cells and endothelial stem cells (see *Figure 2.7*). In addition, the bone is penetrated by a

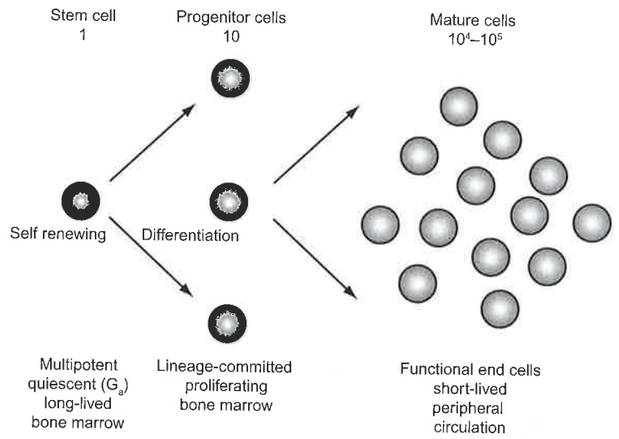

Figure 2.5
Schematic representation of stem cell expansion.

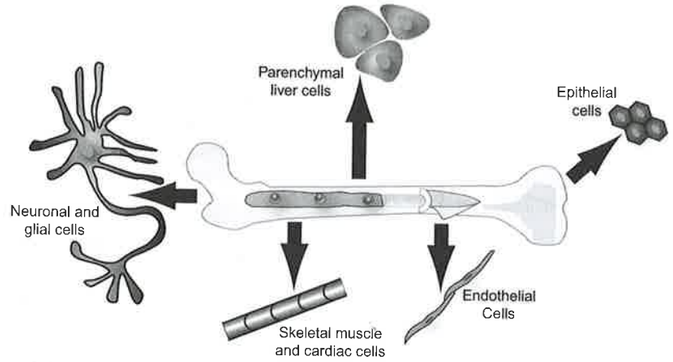

Figure 2.6
Illustration of haemopoietic stem cell plasticity.

Box 2.7 Stem cell plasticity

The first reported transplant of a human organ grown from adult haemopoietic stem cells was performed in 2008 in a cooperative effort involving researchers from Barcelona, Padua, Bristol and Milan. The patient, Claudia Castillo, had suffered a collapsed trachea due to tuberculosis, just at the point where it entered her lungs. To engineer a replacement, a section of trachea was harvested from a cadaveric donor and stripped of cells to create a skeleton of cartilage.

Haemopoietic stem cells harvested from Ms Castillo were then seeded onto the cartilage and grown on to form a new organ. The new section of trachea was successfully transplanted, without the need for immunosuppressive anti-rejection medication. This technique offers the prospect of transforming transplantation surgery.

network of blood vessels that both supply blood to the bone and form a route of egress for mature blood cells. The ECM comprises a network of fibrous proteins, glycoproteins and proteoglycans including various forms of collagen, fibronectin, laminin, haemonectin, tenascin and thrombospondin. The complete structure comprising the ECM and all of the cells of the bone marrow is known as the haemopoietic micro-environment, because all of these cells and structures interact to provide an optimal micro-environment for haemopoiesis. The non-haemopoietic cells of the haemopoietic micro-environment constitute the marrow stroma.

Several important facets of haemopoiesis are regulated by the marrow stroma, as follows.

- Many of the stromal cells (e.g. endothelial cells, fibroblasts, macrophages) are important sources of haemopoietic growth factors. The close proximity of the stromal cells to the haemopoietic progenitors facilitates paracrine growth factor activity.
- The ECM plays an important role in supporting haemopoiesis by providing a structural framework within the marrow that cells can adhere to and grow on. It may also be involved in compartmentalization of haemopoietic tissue within the marrow space.
- The marrow micro-environment plays a central role in the regulation of the release of mature blood cells to the circulation. The endothelium that lines the bone marrow sinuses is tightly packed and so normally permits only the most mature cells (e.g. red blood cells, neutrophils, platelets) to exit the marrow space. To enter the bloodstream, cells must traverse pores in the marrow vascular endothelium that are much smaller in diameter than a red blood cell. Only anucleate cells (red blood cells, platelets) or cells with a highly distensible nucleus (granulocytes) can ordinarily leave the marrow. All of the maturing blood elements have large, rigid nuclei that restrict them to the marrow.
- The adhesion molecules present on cells and the ECM also play a critical role in retaining immature cells within the marrow.
- Elements of the marrow haemopoietic micro-environment are important for the phenomenon known as stem cell 'homing'. Circulating haemopoietic stem cells are selectively attracted to and retained by the bone marrow, where they can proliferate optimally. This mechanism is important because it ensures that haemopoiesis occurs in the most hospitable environment. It is this process that is thought to govern the transfer of the sites of haemopoiesis from embryonic yolk sac to fetal liver, and from spleen to fetal bone marrow during early development. Homing is also important clinically (see *Box 2.8*).
- The ECM can be induced to selectively release haemopoietic stem cells into the circulation in response to injury or inflammation. These stress events trigger activation of neutrophils, resulting in the release of proteolytic enzymes including elastase and matrix metalloproteinases. These substances degrade and inactivate adhesion molecules, including SDF-1, VLA-4 and P/E selectins, responsible for selectively binding haemopoietic stem cells to the ECM. As a result, haemopoietic stem cells are released into the circulation. This process, known as stem cell mobilization, is exploited clinically to harvest haemopoietic stem cells for transplantation. Treatment of a donor with recombinant granulocyte colony-stimulating factor (G–CSF) markedly increases the number of haemopoietic stem cells in the peripheral blood. These can be collected by apheresis, avoiding the need for bone marrow sampling.
- The bone marrow serves as an important reservoir for mature neutrophils. In a healthy individual, over 95% of the mature neutrophils in the body are in the bone marrow, ready to be released rapidly when required, e.g. in response to bacterial infection. These neutrophils are said to be in the bone marrow's neutrophil storage pool. Bacterial endotoxin, immune complexes, and cytokines like GM–CSF and G–CSF are all capable of stimulating rapid release of the marrow storage pool of neutrophils into the peripheral blood.

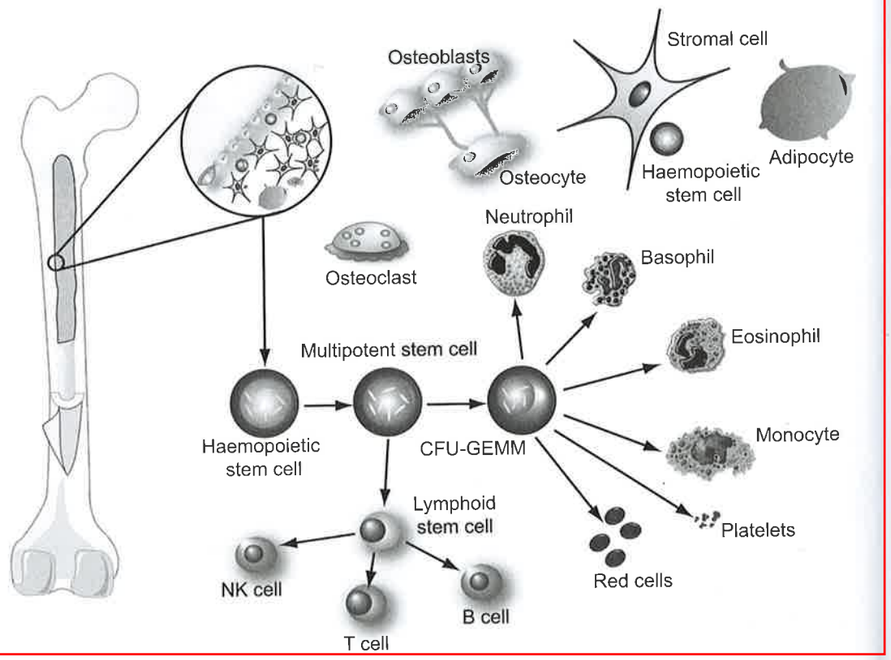

Figure 2.7
Cells of the haemopoietic micro-environment. The term stromal cell encompasses fibroblasts, endothelial cells and marrow macrophages. In some settings, the term stromal cell is used to encompass all non-haemopoietic cells found in the bone marrow.

Box 2.8 Homing and haemopoietic stem cell transplantation

The phenomenon of stem cell homing is exploited clinically in haemopoietic stem cell transplantation. In essence, patients with haematological malignancies are treated with high-dose chemotherapy to partially or completely ablate their bone marrow and they are then 'rescued' by venous infusion of allogeneic or autologous haemopoietic stem cells. The stem cells circulate in the blood only for a short time before they home to the bone marrow spaces and repopulate haemopoiesis.

2.5 ERYTHROPOIESIS

Maintenance of the circulating red cell mass within the narrow limits seen in health is achieved by a feedback mechanism, which senses body oxygen demands (tissue hypoxia) and delivery, and adjusts the rate of erythropoiesis accordingly. This feedback mechanism, mediated by the glycoprotein hormone erythropoietin is, for reasons explored in the next chapter, imperfect in pathological conditions but, when working physiologically, does so as follows.