Chapter 1

Understanding haemopoiesis

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This chapter aims to provide an overview of the haemopoiesis process and by the end of reading this chapter you should:

- Have a detailed knowledge of all marrow cell development and function
- Be aware of the different sites of haemopoiesis
- Be aware of the role of stem cells and how they function
- Understand growth factors and how they function

Introduction

Blood is not immediately thought of as an organ, but it is one of the largest in the body. The volume of blood in adults is approximately 4.5–5 litres and marrow cells form about 40% of this volume.

Haemopoiesis is the production of marrow cells and it is a fascinating process involving a diverse range of cytokinetic interactions, which produce cells responsible for gaseous exchange, fighting infections and haemostasis. Haemopoiesis is a complex process of proliferation, differentiation and maturation, with an intricate balance between demand and supply of marrow cells. Through ongoing research of haemopoiesis and the behaviour of stem cells, their relationship with growth factors, the microenvironment of the bone marrow and regulatory mechanisms are increasing knowledge of our haematological pathologies and informing therapeutic interventions.

Haemopoiesis is the name given to the production of marrow cells and this is further subdivided into erythropoiesis (production of red blood cells), leucopoiesis (production of white blood cells) and thrombopoiesis (production of platelets). In humans there are various haemopoietic sites, starting with the blood islands in the yolk sac, which forms the basis of marrow cell production for up to two months of gestation. Red blood cells are the first to be produced, with leucopoiesis and thrombopoiesis occurring from six weeks (Pallister 1997).

Haematology Nursing, First Edition. Marvelle Brown and Tracey J. Cutler.

During this early period the main haemoglobin being produced is Gower 1 ($\zeta 2\varepsilon 2$). The liver and spleen become the main sites for marrow cell production from about two months through to seven months' gestation and here the haemoglobin is now foetal haemoglobin, Hb F ($\alpha 2\gamma 2$). From six months' gestation the skeletal system becomes the prime source of marrow cell production and with this change in site is also a gradual change in the haemoglobin from haemoglobin F (HbF) to haemoglobin A (HbA) ($\alpha 2\beta 2$). The liver and spleen continue to produce marrow cells for at least two weeks after birth, but in much reduced quantities (Hoffbrand *et al*. 2004). The significance of the liver and spleen being sites of early marrow cell production is significant as they can revert (back) to producing marrow cells (known as 'extramedullary' haemopoiesis) when the bone marrow is unable to do so or is inefficient in production due to disease, such as haemato-oncological malignancies, sickle cell disease and thalassaemia syndromes (haemoglobinopathies).

During infancy and up to three years of age the entire skeletal system produces marrow cells. Progressively, there is replacement of marrow with yellow fatty tissue in the long bones and by the early twenties the distinct skeletal sites of the vertebrae, ribs, sternum, skull, proximal end of the humerus and femur, sacrum and pelvis become the only sites for haemopoiesis. In addition, similarly to the liver and spleen, the fatty tissue in the bone marrow can also reconvert to active haemopoiesis in times of increased demand.

The bone marrow has three types of stem cells, haemopoietic stem cells responsible for the production of marrow cells, endothelial tissues, which form the sinusoids, and, finally, mesenchymal stem cells. Mesenchymal stem cells differentiate into osteoblasts (bone tissue), chondrocytes (cartilage tissue) and myocytes (muscle tissue) (Litchtman *et al*. 2005). The existence of stems cells other than haemopoietic stem cells found in the bone marrow has generated great interest in researchers and scientists in their potential use in treating neurological and muscular diseases in the future.

All marrow cells start their development from haemopoietic stem cells, known as multipotent or pluripotent stem cells. The terms multipotent and pluripotent refer to the fact that these cells have the ability to become any type of marrow cell. These primitive progenitor cells are unrecognisable by their cell morphology but have been identified through immunological testing as being CD34+ cells. The identification of these progenitor cells as CD34+ involves the use of a monoclonal antibody (Guo *et al*. 2003; Yasui *et al*. 2003). The term CD means cluster of differentiation and relates to what are known as surface markers on a cell which are unique to that cell.

Stems cells constitute approximately 4% of the haemopoietic cells in the bone marrow and one stem cell can produce 10^6 cells after 20 divisions (Hoffbrand *et al*. 2004). The committed progenitor cells form approximately 3% and the maturing/mature cells approximately 95% of the haemopoietic stem cells (HSC) (Traynor 2006). Stem cells also have the capacity to self-renew, which is important as this means there is always a constant supply of stem cells to respond quickly to demand such as infection, haemorrhage and anaemia. Under normal circumstances, mature marrow cells are lost through normal activities and aging.

Once the HSC has been triggered all marrow cells are produced along two lines: myeloid and lymphoid. Erythrocytes, granulocytes, myelocytes and megakaryocytes are produced along the myeloid line, and lymphocytes and natural killer cells (NK) are produced along the lymphoid line. To start the differentiation process, the pluripotent stem cells can differentiate into either the precursor, colony-forming unit – granulocyte,

erythrocytes, myelocytes, megakaryocytes (CFU-GEMM), or lymphoid stem cell. Differentiation of these lines produces committed cell lines. The exception is the progenitor cell, CFU-GM, which gives rise to monocytes and neutrophils. As a cell goes through its stages of development, it progressively loses its capacity to self-renew and once matured is unable to do so at all (terminal differentiation) (see Figure 1.1).

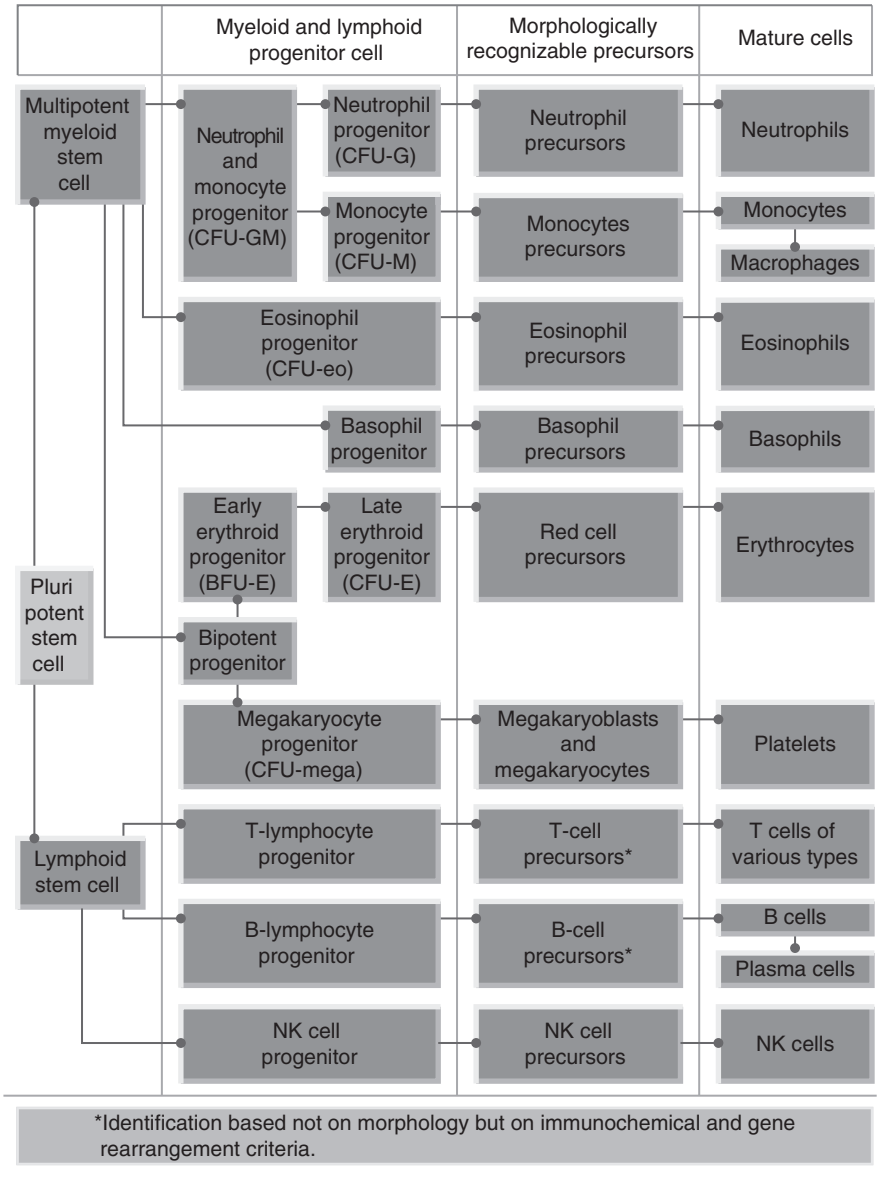

Figure 1.1 Relationships between the various types of cell involved in haemopoiesis (from Hughes-Jones *et al*. 2009). Reprinted with permission of John Wiley & Sons, Inc.

Growth factors are naturally occurring glycoproteins and determine the behaviour of the stem cell. They are produced from a number of sources: macrophages, fibroblasts, endothelial cells, monocytes, T-cells, the kidneys and the liver (Pallister 2005). Growth factors can work solely on a committed line such as erythropoietin. Others are synergistic, whilst others can influence both early progenitor cells and later committed cells. Granulocyte colony stimulating factor (G-CSF) and thrombopoietin (TPO) enhance the activities of stem cell factor (SCF), interleukin-3 (IL-3), granulocyte monocyte-colony stimulating factor (GM-CSF). In addition, G-CSF and GM-CSF enhance the function of mature cells (Hoffbrand *et al*. 2004; Metcalf and Nicola 1995). Our understanding of the activities of growth factors has aided in the pharmacological production of erythropoietin used in renal disease and recombinant granulocyte stimulating factor, (rHG-CSF), used in stem cell transplantation to reduce the period of neutropenia. Table 1.1 provides an overview of growth factors, their sources and functions.

The microenvironment of the bone marrow is known as bone marrow stroma and is a unique environment which forms the structural base, allowing stem cells to grow and develop. The stroma is made up of a structural base of cells which are macrophages, reticular connective tissue, osteoclasts, osteoblasts, adipocytes, fibroblasts and endothelial cells which form the sinusoids. The structure also enables the generation of the mesenchymal tissue providing vessels and bones to support stem cell development. The HSC form the parenchyma cells (functioning cells) of the stroma and their close proximity with the stromal layer is essential for proliferation (Kronenwett *et al*. 2000). Stromal cells produce growth factors, SCF, (kit ligand, steel factor) to ensure self-renewal of those cells. In addition, the stromal cells produce adhesive proteins such as fibronectin, forming an extra-cellular matrix. These adhesive proteins allow growth factors to become attached to receptor sites on the stem cell and once attached, set off intracellular chemical reactions to prepare the stem cell to respond to the growth factor instruction, which could be to become quiescent, proliferate, become committed, differentiate or mature. The adhesive interactions between stem cells and stromal layers are key to migration, circulation and proliferation.

It has been identified that HSCs have their own ‘space’ within the bone marrow (Traynor 2006). Erythrocyte precursors lie adjacent to venous sinus in erythroblast islands and each has its own macrophage. Megakaryocytes are found next to the venous sinus and their cytoplasmic projections extend directly into the lumen. Cytoplasmic granules, which are to become platelets, congregate at the edges of the projection, making holes in the megakaryocytes (known as platelet budding) and flow into the circulation. The precursors for monocytes and granulocytes are found deep within the cavity and when they mature are very motile and migrate to the venous sinus to enter into circulation (Pallister 1997).

Stem cells’ primary home is the bone marrow, but a small percentage enter circulation. The recognition of this has led to the development of being able to collect peripheral stem cells via apheresis for ‘harvesting’ and transplantation. Circulating progenitor cells form part of the self-renewal process and are able to re-populate stem cells in the bone marrow. Peripheral stem cells are known to be more mature than those in the bone marrow; when used for haemopoietic stem cell transplantation, they generally engraft earlier, leading to less time for the patient being neutropenic and hence reducing the potential for infections. See Chapter 2 and Chapter 20 for more details.

Table 1.1 Overview of growth factors, source and activities (adapted from Pallister 2005 and Hoffbrand *et al*. 2004).

	Growth factors	Activity	Where produced
Growth factors that influence early progenitor cells	IL-3	Stem cell production	Stromal cells T-cells
	TPO	Stimulates myeloid cell production Influences CFU-$_{MEG}$	Liver and kidneys
	GM-CSF	Stimulates production of erythrocytes, thrombocytes and phagocytic cells; neutrophils, eosinophils, monocytes	T-cells, fibroblasts, endothelial cells
	IL-7	Stimulates proliferation of all cells in the lymphoid lineage (B and T-lymphocytes and NK cells)	Fibroblasts, endothelial cells, thymus
Growth factors that act on committed cell lines	G-CSF	Stimulates production of neutrophils	Monocytes, fibroblasts
	M-CSF	Stimulates production of monocytes	Macrophages, endothelial cells
	IL-5	Influences CFU-$_{EO}$ and differentiation of activated B-cells	T-cells
	Erythropoietin	Stimulates erythrocytes production	Kidneys, limited production in the liver
	IL-7	B-cell maturation,	As above
Growth factors released from activated T-cell, macrophages	IL-1 Tumour necrosis factor (TNF)	Stimulates T-cell, macrophages, fibroblast, endothelial cells to produce GM-CSF, IL-6, G-CSF, M-CSF	IL-1 Tumour necrosis factor (TNF)

Key: SCF, stem cell factor; IL-3, interleukin-3; TPO, thrombopoietin; GM-CSF, granulocyte macrophage colony stimulating factor; IL-7, interleukin-7; G-CS, F granulocyte colony stimulating factor; M-CSF, monocyte colony stimulating factor; IL-5, interleukin-5; IL-1, interleukin-1.

Table 1.2 Down-regulators of haemopoiesis.

Transforming growth factor β (TNF-β)
Prostaglandin E
Macrophage inflammatory protein 1a (MIP-1a)
α and γ interferon
Leukaemia inhibitory factor
Tumour necrosis factor -α (TNF-α)
Lactoferrin
Inhibin

Adapted from Queensberry and Colvin (2001).

We do not as yet have the entire picture of the regulatory mechanism of HSC to understand how homeostasis is maintained within the bone marrow. Growth factors clearly up-regulate bone marrow activity but down-regulation is less well understood. The known regulatory factors are listed in Table 1.2. Ultimately, the actions of the regulators are to maintain haemopoiesis in a steady state, inhibiting mitosis or preventing apoptosis.

Apoptosis is a key factor in maintaining homeostasis of haemopoiesis. Apoptosis is programmed cell death (PCD), equated to 'cellular suicide'. It is regulated by a number of physiological processes leading to biochemical activities of intracellular proteins known as capases, taking place within the cell, resulting in a change in cell morphology. The morphological changes primarily are: changes to the cell membrane which cause loss of attachment, cell shrinkage, loss of membrane symmetry and blebbing. In addition, nuclear changes also occur, which include chromatin condensation, nuclear fragmentation and chromosomal DNA fragmentation (Charras *et al*. 2008). All these activities will ultimately lead to the death of the cell (see Figure 1.2).

Myeloid lineage bone marrow cells

Erythropoiesis

Erythropoiesis is the name for red blood cell production. Red blood cells are the most numerous of all marrow cells, as indicated in Table 1.3. Their production is triggered by hypoxia. When the blood enters the kidneys, hypoxia triggers erythropoietin (EPO) release. It is a glycoprotein synthesised by the peritubular interstitial cells of the kidneys, and chromosome 7 carries the gene for its coding (Pallister 1997).

Erythrocytes develop and mature in the bone marrow and Figure 1.3 is a schematic representation of the stages of their development. Receptor sites for EPO are found on BFU-$_E$, CFU-$_E$ and pronormoblasts. Erythropoietin shortens the pronormoblast phase, enabling faster production of red blood cells.

Development starts from the CFU-GEMM to BFU-$_E$, progressing to CFU-$_E$, which develops into the first recognisable erythroid precursor, the pronormoblast. Under Ramonsky staining the pronormoblast appears blue due to RNA ribosomes (Turgeon 2005). It has a very large nucleus to cytoplasm ratio. As it continues through stages of development, it becomes increasingly smaller in size, the haemoglobin concentration increases, giving the

cell a pink colour. The nucleus is eventually ejected at the late normoblastic phase when it then becomes known as the marrow reticulocyte. The reticulocyte stage continues for a further 1–2 days in the marrow, losing its RNA, further increasing the concentration of the haemoglobin. Reticulocytes circulate in the blood for a further 1–2 days to mature into erythrocytes. The process of maturation from pronormoblast to erythrocyte can take approximately five days and the lifespan for a normal red blood cell is 120 days (Lewis *et al*. 2001). Reticulocytosis tends to be an indication of either excessive haemolysis (as seen, for example, in sickle cell disease and thalassaemia) or excessive haemorrhage.

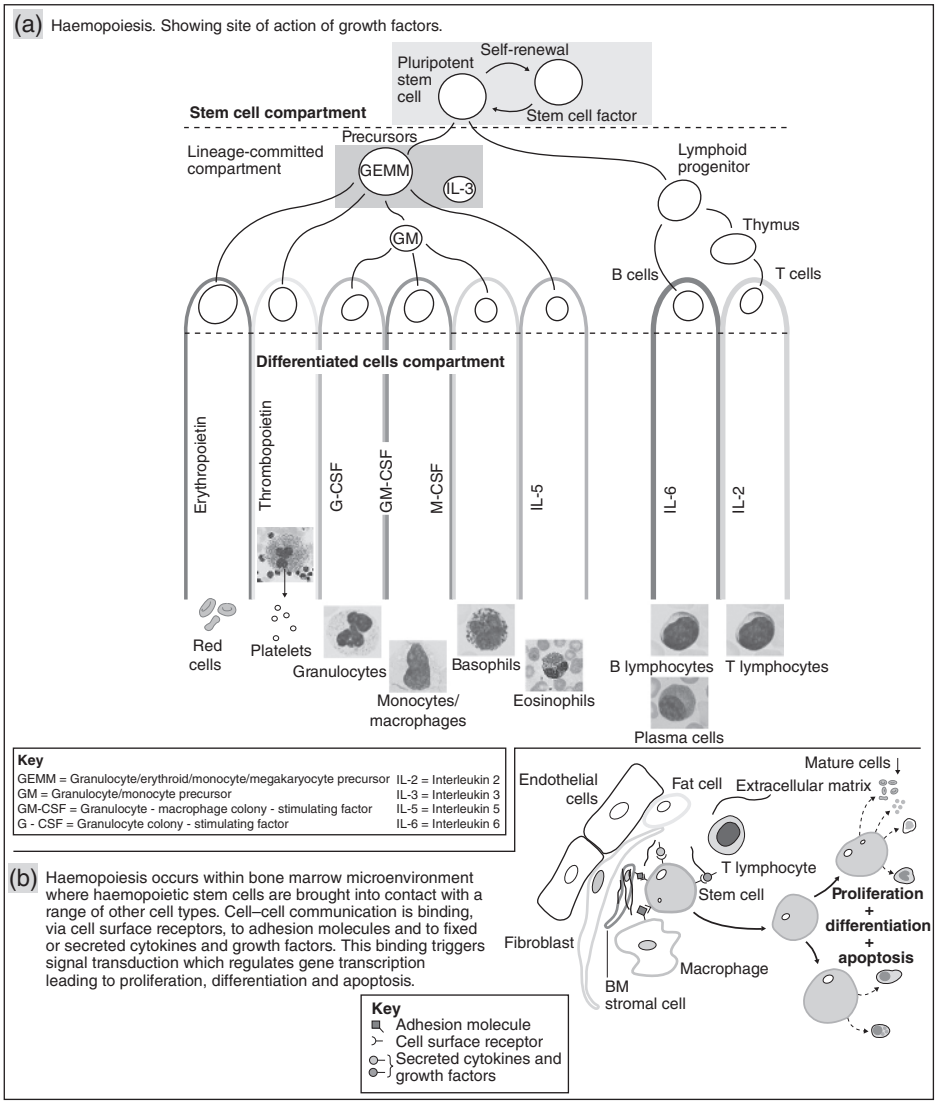

Figure 1.2 Haemopoiesis, physiology and pathology (from Mehta and Hoffbrand 2009). Reprinted with permission of John Wiley & Sons, Inc.

Table 1.3 Normal values and functions of cells.

Marrow cell	Normal count	Overview of function
Red blood cells	Women 4–5 x 10^{12}/L Men 5–5.5 x 10^{12}/L	Gaseous exchange
White cell count (WCC)	4–11/10^{9}/L	Fight infections
Platelets	150–400^{9}/L	Instigates haemostasis

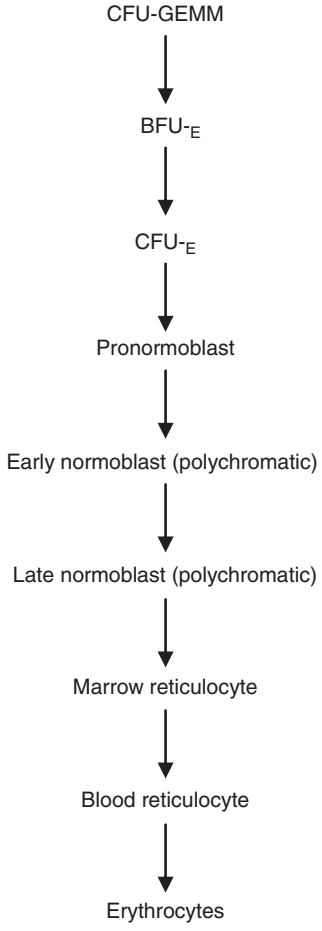

Figure 1.3 Development of erythrocytes.

Erythrocytes are anucleated, biconcave in shape, which provides a greater surface area for their function of gaseous exchange: taking oxygen (O_2) from the lungs to the tissues and carbon dioxide (CO_2) from the tissues to the lungs. The ability of the erythrocyte to

carry out its function is made possible by the haemoprotein, haemoglobin, and each erythrocyte has approximately 600 million haemoglobin molecules. The lack of nuclei and organelles also contributes to increased haemoglobin content and gas-carrying capacity. Approximately one half of the CO_2 is directly bound to haemoglobin. The rest is converted by the enzyme carbonic anhydrase, which is found in erythrocytes, into bicarbonate ions that diffuse back out into the plasma and hydrogen ions (H^+) that bind to the protein portion of the haemoglobin, thereby keeping the pH of the erythrocyte, relatively neutral.

Erythrocytes are able to make energy from breaking down glucose, known as glycolysis. This process involves enzymatic activity, which occurs along two chemical pathways known as the Embden-Meyerhof pathway (EMP) (95% of glycolysis takes place along this pathway) and pentose phosphate pathway (PPP).

Erythrocytes have structural and contractile proteins which make up the cytoskeleton. These proteins are α and β spectrin, ankyrin, actin protein 4.1 and enable erythrocytes to be deformable and flexible (Lewis *et al*. 2001). Erythrocytes have a diameter of 6–7 μm approximately and they have to pass through microvessels, which can have a diameter of 3 μm. Therefore deformability is crucial to their ability to travel to tissues. Defects of any of these proteins compromise the erythrocyte's function. The most abundant of the proteins is spectrin and a lack of it leads to a condition known as hereditary spherocytosis. The surface of erythrocytes also has antigens, some of which denote the blood group an individual has inherited (see Chapter 17).

In addition to erythropoietin, erythrocytes also require nutrients and some hormones which help to influence their development. Protein is required for the cell membrane as well as being a component of haemoglobin. Iron is an essential component of haemoglobin and vitamin B_{12} is required for nucleic synthesis. Vitamin C increases the absorption of iron and copper acts as a catalyst for iron to be taken up by haemoglobin. Vitamin B6 is required for haemoglobin synthesis and folic acid aids in maturation. Lack of the hormone thyroxin compromises EPO production and the 'sex' hormones have opposing influences on erythrocyte's development. Testosterone stimulates erythrocyte production; conversely oestrogen decreases red blood cell production. This explains why the red blood cell count is higher in men than women and why androgenic drugs are used in conditions like Fanconi's anaemia.

The reticulo-endothelial system (RES), comprising the liver, spleen, lymph nodes and bone marrow is responsible for the destruction of senescent red blood cells. Aging red blood cells are recognised by macrophages in the RES due to a number of changes which take place. The changes are caused by a reduction in the glycolytic activity, leading to reduced activities of its essential enzymes. This has the effect of making the chemical pumps (sodium pump and magnesium pump) inside the red blood cell inefficient, allowing an increased intake of water, making it unable to deform easily. The abnormal spherical shape is detected by the macrophages, particularly in the spleen, and the red blood cell is then phagocytosed.

During this process the haemoglobin molecule is removed and the globin component is broken down into amino acids to be reused for new erythrocytes. The iron is separated from the haem and is transported by apopferritin for storage as ferritin for future use in

Table 1.4 Normal values of Granulocytes and agranulocytes.

Neutrophils *	2.5–7.5 x 10^9/L
Basophils	0.01–0.1 x 10^9/L
Eosinophils	0.04–0.4 x 10^9/L
Monocytes	0.2–0.8 x 10^9/L
Lymphocytes	1.5–3.5 x 10^9/L

Adapted from (Hoffbrand *et al*. 2004).
There may be some variations on the above results depending on the population the hospital laboratory serves.
*It is well known the WCC count is lower in blacks than Caucasians, but it is not clearly understood why that occurs.

Table 1.5 Granulocytes and Agranulocytes.

Granulocytes	**Agranulocytes**		
Neutrophils Eosinophils Basophils	Monocytes Lymphocytes Natural killer cells (NK cells)	T-lymphocytes	B-lymphocytes

production of erythrocytes. When iron is required for erythrocyte production, the plasma protein transferrin transports the ferritin to the bone marrow where developing erythroblasts have receptor sites for transferrin. The haem forms biliverdin, which converts to bilirubin and is stored as bile in the gall bladder.

Leukocytosis

The entire process of leukocyte production is not fully understood, although it is known that there are specific growth factors involved in their development (see Table 1.1 on growth factors) and the production is increased during periods of infection and inflammation.

A leukocyte's prime function is to defend the body against and react to infective organisms and toxins. They are less numerous than erythrocytes and are nucleated. Leukocytes are divided into granulocytes (also known as polymorphonuclear cells because they are multi-nucleated and agranulocytes as indicated in Table 1.5). Granulocytes populate three environments, marrow, blood and tissues (Bainton 2001). Their movement is unidirectional, meaning they do not return to a previous environment, for example once they have left the marrow and entered circulation, they do not return to the marrow; similarly once in tissues, they do not return to circulation.

Granulocytes and monocytes provide innate immunity, meaning it is present from birth, is non-specific, for example attacks many pathogens and does not require sensitisation. Lymphocytes provide acquired immunity, meaning they need to be sensitised by a specific antigen, either from an infection or vaccination and produce memory cells.

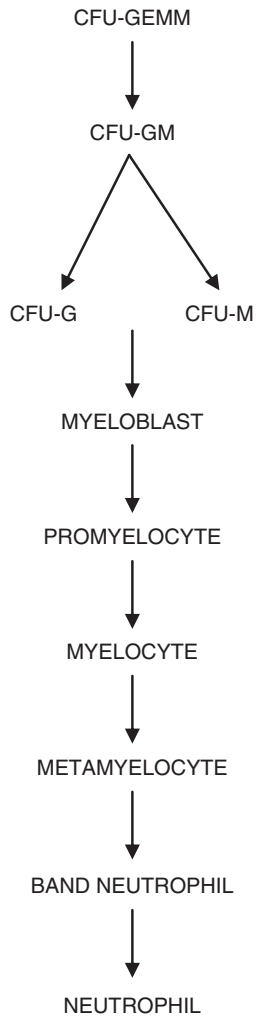

Figure 1.4 Neutrophil development.

The total white cell count (WCC) can be seen in Table 1.3, but to establish the numbers of each type of white cell a differential count is required. Table 1.4 indicates the normal differential counts.

Granulocytes

Neutrophils are the most numerous of white cells and they are produced on the myeloid line in the bone marrow, from the CFU-GM committed progenitor cell, as illustrated in Figure 1.4. Both neutrophils and monocytes share the CFU-GM which is developed from the CFU-GEMM. When the CFU-GM differentiates to CFU-G this becomes committed to producing neutrophils. The production of CFU-G progenitor cell is triggered by the growth factor, G-CSF.

The myeloblast is a large cell with a high nucleus to cytoplasm ratio, which undergoes several stages of division (as illustrated in Figure 1.4) and is the first granulocyte precursor which is recognised by cell morphology. At the promyelocyte stage primary granules are found and they contain myeloperoxidase, cathepsin G and other acid hydrolases. Secondary granules, such as lactoferrin, lysozyme and collagenase, are first seen at the myelocyte stage and in abundance in the mature neutrophil (Babior and Golde 2001).

The penultimate stage of neutrophil development is a band neutrophil. These are non-proliferating cells, and proceed to full maturation as neutrophils. Mature neutrophils are denoted by having a 3–4 lobe nucleus, held together by a thin chromatin strand and stain pink on Ramonsky staining. The whole maturity process can take up to five days, but where there is an infection, this process can be sped up to completing maturation in 48 hours. Once matured, neutrophils enter the bloodstream, making up to 60% of circulating white blood cells. Half the neutrophils will circulate and the others will loosely attach themselves to the endothelial lining of blood vessels, known as margination. This ensures there is a ready access of neutrophils to fight infections and inflammation. Neutrophils circulate for about 4–10 hours in the blood before entering tissues, where they remain for about 2–3 days, taking on the role of being non-specific against bacteria and other microbes. The daily production of neutrophils is 0.85–1.6 x 10^9/cells/kg/day (Babior and Golde 2001).

Neutrophils are microcytic, phagocytic cells; they are highly motile and act rapidly at the site of tissue injury and are the hallmark of acute inflammation. When active they have a limited lifespan of a few hours. They have the ability to respond to an invading organism in two critical ways: diapedesis and chemotaxis. Diapedesis is amoeboid movement. Neutrophils are able to elongate forming a pseudopodium, taking on a 'hand-mirror' shape to squeeze through the blood vessels and enter the interstitial tissue space. Chemotaxis is the neutrophils' ability to detect chemicals released by pathogens and from inflammatory cytokines such as interleukin-8 (IL-8), interferon-gamma (IFN-gamma), tumour necrosis factor (TNF) and opsonins, namely the complement proteins, C3a, C5a and C6. To function efficiently neutrophils rely on being presented with the antigens and they have receptor sites for IgG and complement proteins complement C3, which coats the organism (a process known as opsonisation), making it easier for the neutrophil to engulf coated organisms (Rich *et al*. 2009).

When being phagocytic, the neutrophil will engulf the organism, forming a vacuole, and the secondary granules' degranulation, releasing lysozyme, lactoferrin into the vacuole. These produce hydrogen peroxide and a highly active form of oxygen (superoxide) which ultimately leads to the death of the ingested organism.

Eosinophils

Eosinophils develop and mature in the bone marrow, being produced from the CFUeo, and go through phases of development as illustrated in Figure 1.5. The development of eosinophils is triggered in response to interleukin 3 (IL-3), interleukin-5 (IL-5) and granulocyte macrophage colony stimulating factor (GM-CSF). Mature eosinophils stain red with eosin dye using Ramonsky technique.

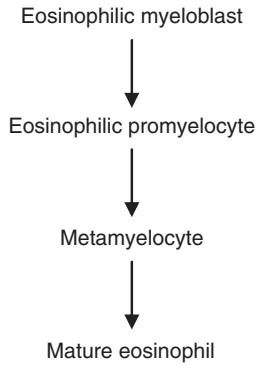

Figure 1.5 Development of eosinophils.

Following maturation they have large cytoplasmic granules and less than 1% circulate in blood, approximately 50% marginate like neutrophils and the rest enter tissues such as the skin and lower gastrointestinal tract. Eosinophils circulate in the blood for 8–10 hours and if not stimulated can survive for approximately 10–12 days. They are phagocytic, but not as aggressive as neutrophils. They are approximately the same size as neutrophils, but have large granules and respond to parasitic infections. They have a major role in controlling inflammatory responses, by neutralising histamine produced by basophils and mast cells. Along with mast cells they have a role to play in mechanisms involved in allergic reactions and asthma. Eosinophil count can vary with age, exercise and environmental stimuli such as allergens.

Basophils

Basophils are the least numerous of circulating white blood cells, forming 1% of their total and are non-phagocytic cells. They are produced from the CFU-baso and differentiation from basophilic promyelocyte → to myelocyte → to metamyelocyte and → final maturation. Their development takes place in the bone marrow and the growth factor, interleukin-5 (IL-5) initiates their development. Basophils are given their name because of their capacity to stain with base dyes and have a bluish-purple colouring on Ramonsky staining. They are the least understood of the white cells, but it is known that they are involved in hypersensitivity and have a role in instigating inflammatory response since they have granules containing substances such as heparin, histamine and platelet activating factor (Roitt 2001). Basophils also have receptor sites for antibody IgE and when bound to basophils, they aid the release of the above chemicals leading to inflammation and vascular permeability, enabling phagocytic cells to leave the circulation and enter interstitial tissue space to engulf and digest organisms.

Basophils are involved in hypersensitivity reactions and anaphylaxis. By binding to IgE this is thought to be a significant factor in the selective response of these cells to environmental stimuli such as pollen and and the clinical symptoms of an allergic reaction: watery eyes, runny nose and difficult breathing.

Platelets

The production of platelets starts with the CFU-$_{meg}$ in the bone marrow. The stage of development then proceeds from megakaryoblast to megakarocyte. The process of the development of platelets is different to that of other marrow cells as it is based on endomitotic replication (Coleman *et al*. 2005). Endocytosis is the doubling of chromosomes within a nucleus, but there is no nuclear division and the cytoplasm does not divide. Instead, the cytoplasm increases in volume. The replication of the chromosome within the nucleus can occur several times. As a consequence, with each cell cycle division of endomitosis the cell becomes larger. Once mitotic division has been completed, the nucleus within the megakaryocyte becomes lobulated and the cytoplasmic granules are formed to make the platelets. The control and production of platelets is regulated by the glycoprotein thrombopoietin, which is produced by the liver and kidneys. Platelets have receptor sites (C-MPL) for thrombopoietin, which removes it from circulation (Pallister 2005).

The megakaryocyte by its name indicates this is a very large cell, almost ten times the size of other marrow cells in the bone marrow, and each megakaryocyte can produce 2000–4000 platelets. Platelets are non-nucleated, disc-like structures which are formed from fragmentation of the megakaryocytic cytoplasm. They form holes in the megakaryocyte, known as platelet budding, to enter into circulation and are the second most numerous of all circulating marrow cells, as indicated in Table 1.3. Once in circulation, approximately one third are stored in the spleen, which acts as a reservoir and is able to be responsive to emergency demand. Platelets have a lifespan of 10–14 days in circulation.

Platelets are essential for maintaining haemostasis and critical in instigating the clotting cascade to stop haemorrhagic episodes. Platelets have glycoproteins on the surface which enable them to become adhesive following vascular injury and to allow aggregation and enable them to form a platelet plug. When activated, platelets release the contents of their granules into their canalicular system and into the surrounding blood, providing a reactive surface to enable plasma coagulation proteins to be absorbed. The platelets produce two types of granules: dense granules and α-granules. They are listed in Table 1.6.

Thromboxane A2 is a powerful vasoconstrictor and aggregator. It is produced by the platelets and plays a key role in activating other platelets. The actions of the above enable the formation of the platelet plug, which instigates action from the clotting factors.

Table 1.6 Types of granules produced by platelets.

Dense granules	α-granules
Adenosine diphosphate	Platelet factor IV
Adenosine triphosphate	Platelet derived growth factor (PDGF)
Calcium	Von Willebrand factor (vWF)
Serotonin	Heparin antagonist
	Fibrinogen
	Fibronectin
	Coagulation factors V and X111
	β-thromboglobulin

Adapted from (Harrison and Cramer 1993 and George 2000).

Through a cascading action clotting factors are activated and along with other substances form a fibrin clot.

Disorders or abnormalities of platelets are known as thrombocytopathy: an increase in platelets is thrombocytosis, a decrease is called thrombocytopenia and a decrease in function is thrombasthenia.

Lymphoid lineage marrow cells

Agranulocytes

Lymphocytes are the second most common white blood cell, with T-lymphocytes constituting approximately 60–80% of circulating lymphocytes, B-lymphocytes making approximately 10–30% and NK cells making up 2–10% (Hughes-Jones *et al*. 2009). Most lymphocytes are generally small, having a diameter of about 10–20 μm, making them larger than erythrocytes but much smaller than monocytes. In lymphocytes the nucleus is round and takes up most of the cell space.

Lymphocytes are found in large numbers in the lymph nodes, spleen, thymus, tonsils and in the Peyer's patches of the gastrointestinal tract. Unlike other blood cells which are unidirectional, some lymphocytes may leave and re-enter the circulation, surviving for about one year or more. The principal paths of re-circulating lymphocytes are through the spleen or lymph nodes (Delves and Roitt 2000). Lymphocytes enable the body to remember antigens and to distinguish self from non-self.

Lymphocytes are derived from the lymphoid multipotent stem cells in the bone marrow and provide acquired immunity. Lymphoid stem cells can divide into T or B-lymphocytes, each carrying out specific functions to defend the body against infections. Both T and B-cells travel between blood and tissues, enabling them to kill invading organisms. Differentiation between T and B-lymphocytes is based on gene rearrangements, cluster differentiation, cell membrane markers and antibody receptors (Kircher and Marquardt 2002).

T-lymphocytes

T-lymphocytes provide cellular mediated immunity and start their development in the bone marrow, continuing their maturity in the thymus gland under the influence of thymosin. T-lymphocytes develop from pro-thymocytes to mature T-lymphoblasts, becoming immunological competent. T-lymphocytes leave the thymus gland and circulate in the bloodstream to the lymph nodes and the spleen. T-lymphocytes, further subdivide into four sub-classes:

- T helper cells (CD4+)
- T suppressor cells (CD8+)
- Cytotoxic T-cell (Tc)
- Natural killer cells (NK cells)

T-lymphocytes recognise and respond to antigens that appear on cell membranes in association with other molecules, known as the major histocompatibility complex (MHC).

They are glycoproteins that present antigens in a form that is recognised by T-lymphocytes. (See Chapter 2 on Immunology and Chapter 20 Haemopoietic stem cell transplant.)

CD4+ helper cells and CD8+ suppressor cells have the responsibility of orchestrating the specific immune response. CD4+ (helper cells) help B-cells to differentiate and mature. They also recognise (MHC) molecules, class II (HLA-D, HLA-DR). CD8+ suppressor cells turn off the immune response, suppress other lymphocyte function and they recognise MHC class I antigens (HLA-A, HLA-B, HLA-C).

Cytotoxic T-cells (Tc) have a number of roles:

- Kill virally infected cells
- Destroy dysfunctional cells
- Destroy malignant cells
- Are responsible for organ rejection in transplantation

Tc cells have receptor sites (T-cell receptors) which recognise MHC class I antigen which are bound to CD8+.

Natural killer cells (NK), as their name suggest, rid the body of any harmful organism. They are cytotoxic and the granules contain proteins such as perforin and proteases (Roitt *et al*. 2001). The difference between Tc and NK cells is that Tc need to recognise antigens and MHC molecules in order to mount an immunological response to get rid of the organism (Papamichail *et al*. 2004). NK cells do not require sensitisation; they are able to lyse antibody coated cells, hence NK cells are also called antibody dependent cytotoxic cells (ADCC) but are also capable of destroying tumour and virally infected cells without presentation by an antibody. Tc and NK cells kill on contact by undergoing blast transformation releasing highly potent lymphokines such as IL-2 which destroys the organism and attract other lymphocytes, thereby increasing killing potential (Symth *et al*. 2001).

Natural killer cells can also become activated in response to interferon, a macrophage derived cytokine, and they 'hold' the virus while the adaptive immune response is generating antigen-specific cytotoxic T-cells that can clear the infection. Ultimately, T-lymphocytes are responsible for continuous surveillance of cell surfaces for the presence of foreign antigens.

B-lymphocytes

B-lymphocytes provide humoral immunity and start and complete their development in the bone marrow. They undergo several stages of development from pre-B progenitor cells to B-lymphoblast. The name B-cell was originally derived from the bursa of Fabricius, an outpouching organ in the gastrointestinal tract found in birds. CD4+ helper cells assist in the differentiation of B-cells aiding in their maturity. Once matured, B-cells migrate in the blood and then enter organs such as the lymph nodes, spleen, marrow, tonsils and appendix. The fundamental function of B-cells is to produce antibodies against antigens. Mature B-cells produce plasma cells which secrete antibodies. B-lymphocytes have many surface receptors enabling them to bind to many antigens. When mature B-cells become sensitised they not only produce plasma cells which secrete distinct antibodies (immunoglobulins) to a specific antigen, they also produce memory

cells which are the basis of immunological memory and lead to a more rapid response in the future.

Antibodies are 'Y' monomer structures and there are five classifications: IgG (gamma), IgA (alpha), IgE (Epsilon), IgM (Mu) and IgD (delta). Antibodies can live for many years and carry out a variety of functions including:

- Attacking viruses and bacteria directly
- Activating the complement system
- Inactivating toxic substances
- Helping phagocytic cells

Monocytes

Monocytes are the largest of the marrow cells when matured, almost twice the size of red blood cells, (averaging 15–18 micrometres), and they make up about 7% of the leukocytes. The cytoplasm contains large numbers of fine granules, and they are macrocytic phagocytic cells. In the bone marrow, monocytes develop from CFU-$_M$, differentiating into promonocytes and then to monocytes. The nucleus is relatively big and tends to be indented or folded rather than multilobed. Monocytes leave the bone marrow and circulate in the blood for a few hours and they migrate to tissues, continuing their development. Once in tissues they are then called tissue macrophages. Unlike erythrocytes, macrophages have the capacity for cell division and therefore have a reasonably long life.

Monocytes are highly motile and capable of ingesting infectious agents, red blood cells and other large particles. They usually enter areas of inflamed tissue later than the granulocytes and often they are found at sites of chronic infections.

Macrophages are sited in parts of the body in a strategic manner to fight infection and have particular names and form the reticulo-endothelial system (RES).

Apart from being (see table 1.7) part of the above organs, macrophages are also free wandering cells, providing non-specific immunity and are able to regulate immune responses. Through their ability to phagocytose, they prepare antigens for presentation to T-lymphocytes and enable the antigen-antibody complex which can stimulate B memory cells. They have the ability to produce chemicals known as monokines (Kircher and Marguard 2002).

Table 1.7 Sites of macrophages.

Site	Name
Skin	Langerhans cells
Spleen	Lattoral cells
Liver	Kupffer cells
Lungs	Alveolar macrophages
Brain	Microglia
Bones	Osteoclasts
Kidneys	Glomerulus mesanahgial cells
Lymphoid	Medullary sinus

Apart from their role as scavengers, macrophages play a key role in immunity by ingesting antigens, old faulty cells, microscopic particles and processing them so that they can be recognized as foreign substances by lymphocytes, thereby taking on the role of antigen presenting cells (APC). Macrophages have the ability to ingest not only other cells but also many other microscopic particles.

Conclusion

Haemopoiesis is a fascinating system, involving many complex processes to produce marrow cells. The process takes place within quite narrow margins and it is therefore not surprising that any fault within the system can in some conditions, like acute leukaemia, manifest symptoms quickly.

The continual growth in our understanding of the behaviour and action of stem cells and growth factors will impact on therapeutic modalities.

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