PRPP	5′ phosphoribosyl-1-pyrophosphate
PS	phosphatidylserine
PSGL	P-selectin glycoprotein ligand
PT	prothrombin time
PTLD	post-transplantation lymphoproliferative disorders
PUS-1	pseudo-uridine synthase-1
PV	polycythaemia vera
R-5-P	ribose-5-phosphate
RA	refractory anaemia
RAEB	refractory anaemia with excess blasts
RAEB-t	refractory anaemia with excess blasts in transformation
RALS	right angle light scatter
RARS	refractory anaemia with ring sideroblasts
RBC	red blood cell
RCMD	refractory cytopenia with multilineage dysplasia
RCMD–RS	refractory cytopenia with multilineage dysplasia and ring sideroblasts
RCUD	refractory cytopenia with unilineage dysplasia
RE	reticulo-endothelial
REAL	Revised European–American Classification of Lymphoid Neoplasms
RN	refractory neutropenia
ROTI	related organ or tissue impairment
RT	refractory thrombocytopenia
Ru-5-P	ribulose-5-phosphate
SAHA	suberoylanilide hydroxamic acid
SCCS	surface-connected canalicular system
SDS	sodium dodecyl sulphate
sIg	surface immunoglobulin
SK	streptokinase
SLE	systemic lupus erythematosus
SLL	small lymphocytic lymphoma
SPF	S-phase promoting factor
T-ALL	T-lymphoblastic leukaemia / lymphoma
t-AML	therapy-related AML
TAR	thrombocytopenia with absent radii
TAT	thrombin–antithrombin
TBI	total body irradiation
TC	transcobalamin
TdT	terminal deoxynucleotidyltransferase
TEC	transient erythroblastopenia of childhood
TF	tissue factor
TFPI	tissue factor pathway inhibitor
TfR	transferring receptor
TIA	transient ischaemic attack
TIBC	total iron binding capacity
TNF	tumour necrosis factor
tPA	tissue plasminogen activator
T-PLL	T-cell prolymphocytic leukaemia
TPMT	thiopurine methyl transferase
TPO	thrombopoietin
TTP	thrombotic thrombocytopenic purpura
u-PA	urokinase-type plasminogen activator
UPG III	uroporphyrinogen III
VEGF	vascular endothelial growth factor
VSD	ventricular septal defect
vWD	von Willebrand's disease
vWF	von Willebrand Factor
WAS	Wiskott–Aldrich syndrome
WHO	World Health Organization
WM	Waldenström macroglobulinaemia
WPSS	WHO prognostic scoring system

CHAPTER 1

Content of the blood

Learning objectives

After studying this chapter you should confidently be able to:

- **Describe the cellular and fluid components of normal blood**

The blood is one of the largest organs of the body, with a volume of about five litres. Normal peripheral blood is composed of three types of cell; red cells (erythrocytes), white cells (leucocytes) and platelets (thrombocytes), suspended in a pale yellow fluid called plasma. There are five different types of white cell normally present. Red cells are responsible for gaseous transport, thrombocytes for the arrest of bleeding and white cells for specific and non-specific immune defence against foreign material or organisms.

The blood is one of the largest organs of the body, with a volume of about five litres and a weight of 5.5 kg in an average 70 kg man. Blood circulates throughout the body, supporting the function of all other body tissues. Because blood and bone marrow are sampled easily, the haemopoietic system is probably the most intensively studied organ of the body. This has led to an explosion of knowledge about blood and blood-forming tissue in both health and disease (see also *Box 1.1*).

Box 1.1 Beliefs about the blood

From the time of Hippocrates (460–370 BC), curiosity about the blood and its functions combined with ignorance and mysticism to produce a plethora of mistaken beliefs, myths and legends. The aura of mystery that surrounds the blood has still not completely been dispelled. Examples of the vestiges of primitive belief abound in the work of poets, playwrights and authors. Obvious examples include tales of vampires who must regularly imbibe the blood of virgins to sustain life, and the constant reference to blood to symbolize murder and the pangs of conscience of the anguished Lord and Lady Macbeth following the murder of Duncan.

Our everyday language is enriched by expressions such as 'hot-blooded', which denotes passion or 'cool as a cucumber'. Both are rooted in medieval beliefs about the power and properties of the blood.

Histologically, blood is classed as a connective tissue. However, blood differs from most other connective tissues in having a fluid rather than a gelatinous matrix or 'ground substance'. The fluid component of blood is called plasma. The cells of the blood are suspended in the plasma.

Visualization of the cellular components of blood is most commonly performed by smearing a drop of blood on a glass slide to form a thin film a single cell thick. The smear is then air dried, fixed in alcohol and stained with a type of dye mixture known as a Romanowsky-type stain. It is the stained appearance of blood cells that is the basis of their identification and characterization under a light microscope. This simple technique still forms an important component of haematological diagnosis (see *Box 1.2*).

Box 1.2 Staining characteristics

Specific dye mixtures have been named after their originators, for example Wright, Giemsa, or May–Grünwald (which is not, strictly, a true Romanowsky stain, but is very similar). Romanowsky stains include a basic dye (positively charged) such as methylene blue (or a derivative – see below) and an anionic dye (negatively charged) such as eosin. Methylene blue binds to negatively charged sites in the cells, such as nucleic acid in nuclei and ribosomes, giving them a blue to purple colour. Eosin binds to positively charged sites, for example most cytoplasmic proteins, giving them a pink to red colour. In a strict Romanowsky stain (as against a Romanowsky-*type* stain) methylene blue is oxidized to produce a range of oxidation products, a process called '**polychroming**'. The proportion of the various oxidation products depends on the method of oxidation. The polychromed methylene blue is then mixed with eosin, and the resulting dyes are known as azures. May–Grünwald is not a 'true' Romanowsky stain since it is made from the azures rather than polychromed methylene blue.

1.1 CONTENT OF THE BLOOD

Normal peripheral blood is composed of three types of cell; red cells (erythrocytes), white cells (leucocytes) and platelets (thrombocytes), suspended in a pale yellow fluid called plasma. The cells occupy about 40–50% of the total volume.

Plasma

Plasma occupies about 50–60% of the total blood volume. It is a pale yellow aqueous solution of electrolytes, proteins and small organic molecules such as glucose. The major extracellular cation is Na^+, which has a plasma concentration of about 140 mmol l^{-1}. Other important plasma cations include K^+, Ca^{2+}, Fe^{3+} and Mg^{2+} but these are all found at much lower concentration. The relative concentrations of Na^+ and K^+ in the plasma contrast with their intracellular concentrations where K^+ is present at a higher concentration.

The major plasma anions are Cl^- and HCO_3^-, although SO_4^{2-} and HPO_4^{2-} are also present at lower concentration. Plasma is always electrically neutral, i.e. the concentrations of anions and cations are always such that the total numbers of negative and positive charges exactly balance.

A large number of different plasma proteins exist. The major protein component is albumen, normally comprising around 60% of the total. It is albumen that is primarily responsible for maintaining the osmotic balance between the fluid components of blood and tissue. Like most plasma proteins, albumen is synthesized in the liver. The remainder fall broadly into four distinct families:

- **haemostatic proteins**, such as the coagulation factors, fibrinolytic factors and their inhibitors.
- **immunoglobulins**, which are antibody molecules synthesized by plasma cells. There are five different classes of immunoglobulin (IgA, IgD, IgE, IgG and IgM) which are structurally related but perform distinct biological functions.
- **innate immune system proteins**, which consist of a variety of plasma proteins that are important in the induction of inflammation and immunity against microbial infection, acute phase proteins, complement, interferons and others, all of which are involved with non-specific immunity.
- **transport proteins**, which ferry nutrients, waste products and other substances around the body. These are α and β globulins, and include transferrin (iron), transcobalamin (vitamin B_{12}), and caeruloplasmin (copper), low density lipoproteins that solubilize and transport dietary or stored lipids (which all transport 'nutrients'), hormone-binding proteins that transport steroid hormones and, for example, haptoglobin which transports any haemoglobin that is released into the circulation, thus becoming a 'waste product'.

Myriad other proteins can be found in the plasma, including cytokines, hormones and growth factors, but these are not usually thought of as plasma proteins, as they are simply using the blood as a transport medium.

Red cells

Mature red cells, or erythrocytes, are the most numerous of the blood cells: about 5×10^{12} are normally present in each litre of blood. They constitute about 45% of blood by volume, a measure known as the haematocrit (see *Box 1.3*). Red cells survive in the circulation for about 120 days before being sequestered in the spleen and consumed by the phagocytic cells of the reticuloendothelial system. The normal mature circulating red cell has no nucleus.

The normal red cell is a biconcave discoid shape with a diameter of about 8.4 μm and a volume of about 88 fl (*Figure 1.1*; see also *Box 1.4*). This characteristic shape imparts flexibility to the cell, allowing it to traverse the smallest blood capillaries which have a diameter of only 3 μm, and also facilitates gaseous exchange across the cell membrane by maximizing the surface area:volume ratio of the cell, thereby bringing more haemoglobin molecules closer to the cell surface. The primary function of red cells is to transport oxygen from the lungs to the tissues, but they also play an important role in the reverse transportation of carbon dioxide.

The oxygen-carrying pigment, haemoglobin, is present in high concentration in mature red cells and is responsible for the characteristic red colour of the blood. Haemoglobin consists of two parts, an iron-containing porphyrin, haem, and a protein, globin. As globin is slightly acidic at physiological pH, haemoglobin stains pink with Romanowsky dyes (see *Chapter 6*). There are around $5–6 \times 10^6$ molecules of haemoglobin in each red cell, constituting an approximately 33% solution.

Normal circulating red cells contain little other than haemoglobin. They have no nucleus or ribosomes, which means they are incapable of protein synthesis. They also lack mitochondria, which means that they are limited to the anaerobic glycolytic pathway to provide the entire requirement of the red cell for energy and reducing potential (see *Chapter 8*).

Box 1.3 Factitious blood count results

Before the days of automation, three red cell parameters were measured, namely red blood cell count (RBC), haemoglobin concentration (Hb) and packed cell volume (PCV) or haematocrit. PCV was measured by centrifugation under strictly controlled conditions. From these three measured parameters three further parameters were derived, namely mean cell haemoglobin (MCH), which was derived by dividing Hb by RBC, mean cell (or corpuscular) haemoglobin concentration (MCHC), which was derived by dividing Hb by PCV, and mean cell volume (MCV), which was derived by dividing PCV by RBC. MCV, MCH and MCHC are thus, strictly, all derived values and as such cannot be measured directly.

The advent of automation brought huge problems regarding the measurement of PCV. Attempted solutions included conductiometric cell volume (CCV), and continuously spinning flow through centrifugation methods. Neither system proved reliable and accurate.

Paralleling these developments was the development of the Coulter principle, whereby cells were counted as they interrupted the passage of an electric current. Not only did this system allow for the number of cells to be counted, but the size of each interference peak was related to the volume of the cell causing the peak. It rapidly became the standard to measure the size of these peaks and to report this as a measured MCV. However, this method does not measure cell size in plasma, but in an isotonic diluent, so this value is not a true MCV. In some cases, for example in hyperglycaemia, dilution in isotonic saline can cause artefactual swelling of the cells and lead to misreporting of MCV. It is important to be aware of the causes of factitious results like these, many of which are specific to each cell counting system, to be able to spot them and so avoid misreporting and potential misdiagnosis.

have been demonstrated on the red cell membrane, including those for insulin, parathyroid hormone, vitamin E, the complement components C3b and C4b, opiates and oestradiol. In most cases, any function of these receptors in the context of red cell function remains obscure.

Red cell membrane peripheral proteins. The red cell membrane peripheral proteins interact to form a cytoskeleton, which acts as a tough supporting framework for the lipid bilayer. Four proteins play a key role in the structure of the red cell cytoskeleton: spectrin, ankyrin, band 4.1 and actin, although many others play ancillary roles in this complex structure.

Platelets

The second most numerous type of cell in the blood is the platelet or thrombocyte: about 150–440 x 10^9 platelets are present in each litre of blood (see *Chapter 18*). Normal peripheral blood platelets are discoid, anucleate cells, or, more correctly, cell fragments, with a granular cytoplasm. They are derived from cells in the marrow called megakaryocytes, and are produced by a process of budding off from megakaryocyte cytoplasm. They have a volume of about 7 fl, a diameter of about 3 μm and are about 1 μm thick. Platelets survive in the circulation for 10–12 days.

The structure of the platelet plasma membrane is broadly similar to that of the red cell, but is significantly more complex in its detailed structure and function. The external leaflet of the membrane is exceptionally rich in receptors that help to drive the multiple functional processes of platelets. Important platelet receptors include GPIb, a primary receptor for von Willebrand factor (vWF), which serves to mediate the initial adhesion of platelets to sub-endothelium; GPIIb/IIIa which acts as a receptor for fibrinogen, fibronectin, and vWF, thereby mediating platelet aggregation. The platelet membrane also includes receptors for ADP, thrombin, adrenaline (epinephrine) and serotonin, which play a role in platelet aggregation.

Another important feature of the platelet membrane is that the distribution of membrane lipids is asymmetric. The membrane is rich in phospholipids, but phosphatidylserine (PS) is present mainly in the inner cytoplasmic leaflet. This is important because PS is required for formation of the prothrombinase complex during clot formation (see *Chapter 18*). Platelet activation is accompanied by exposure of PS at the platelet surface, which forms a substrate for prothrombinase formation. Because PS normally is 'hidden' from the exterior, resting platelets do not activate coagulation.

The outer surface of the platelet membrane is coated in a layer of glycolipids, mucopolysaccharides and adsorbed proteins called the glycocalyx. This layer confers a negative charge to the platelet surface, which helps to prevent platelet–platelet and platelet–endothelium adhesion in the resting state. The glycocalyx is rich in adhesion molecules and protein receptors, and plays an important role in platelet function. The membrane and glycocalyx make up the peripheral zone of the platelet.

The platelet membrane has multiple contortions that penetrate deeply into the cytoplasm but retain their connection with the cell surface. These structures are collectively known as the surface-connected canalicular system (SCCS) and act as a route for trafficking of molecules between the plasma and platelet interior, including granule release during activation. The SCCS also provides extra intact membrane that allows the platelet to spread and change shape during cell adhesion.

Platelets also contain a dense tubular system (DTS) that, unlike the SCCS, is a closed-channel system. The DTS consists of smooth endoplasmic reticulum and regulates intracellular calcium transport and acts as a site of prostaglandin synthesis. It is the release of calcium from the DTS that triggers platelet contraction and activation of platelets.

The platelet cytoskeleton largely consists of actin, whose assembly and disassembly between

globular and filamentous forms provides the motor for platelet filopod formation, spreading and shape change during the early steps of activation and, later, for clot retraction. Beneath the cytoskeleton lies a rim of microtubules that help to maintain platelet shape. The cytoskeleton and microtubule layer constitute the sol-gel zone of the platelet.

Beneath the sol-gel zone lies the organelle zone, which is responsible for the metabolic activities of the platelet. Platelets contain three morphologically distinct types of storage granules: α granules, dense granules and lysosomes. α granules are more common, with each platelet containing up to 200. Dense bodies are less common (2–10 per platelet). These granules act as storage sites for substances required during platelet activation. The contents of both α and dense granules are released during the energy-dependent platelet release reaction. Platelets are rich in mitochondria that provide the energy needed by the platelets.

Adequate numbers of functionally normal platelets are essential for optimal haemostasis. Platelets do not normally adhere to endothelial cells, but they do specifically adhere to underlying tissue matrix should this become exposed by any gaps in the endothelial lining of injured blood vessels. Such binding triggers platelets to release their granule contents, leading to the induction of vasoconstriction, the promotion of aggregation with other platelets to form a plug, and the promotion of fibrin clot formation, all of which contribute to the arrest of haemorrhage. Platelet-derived substances also induce the localized promotion of cell proliferation which facilitates wound healing, and increase vascular permeability which promotes the egress of antibodies and leucocytes to deal with any potentially damaging micro-organisms that may have penetrated the wound. There is also, however, a 'down side', because platelets are also involved in the early stages of the development of atherosclerosis, which can lead to arterial disease and thrombosis.

White cells

The least numerous of the blood cells is the white cell or leucocyte: about 5×10^9 white cells are present in each litre of blood. They consist of a variety of cells specialized for specific and non-specific immune defence against foreign material or organisms. There are five different types of white cell normally present in the peripheral blood: neutrophils, eosinophils, basophils, monocytes and lymphocytes (see also *Box 1.6*).

The neutrophils, eosinophils and basophils are characterized by the presence of cytoplasmic granules and so are known collectively as **granulocytes**. Their names are based on their Romanowsky staining characteristics, which in turn are based on the nature of their specific granule contents. These cells comprise a rapid response force: when triggered by the presence of foreign material, they mount a generalized defensive reaction, involving degradative enzymes, toxins and signalling substances contained within their storage granules. The different granulocytes store different types of secretory substances that contribute to their differing functions. Collectively, the granulocytes make up 50–75% of the total circulating leucocytes.

Box 1.6 Reference values

Reference values ($\times 10^9$/l):

WBC	4.0–11.0	
Neutrophils	2.1–7.2	(55–65%)
Lymphocytes	1.5–4.0	(20–40%)
Monocytes	0.2–0.8	(4–10%)
Eosinophils	0–0.45	(1–3%)
Basophils	0–0.2	(0–1%)

The lymphocytes and monocytes are known as mononuclear leucocytes because they hav a non-segmented, round or indented nucleus. These cells are sometimes, incorrectly, know as agranulocytes. This term implies that these cells have no granules but, although they lac the prominent secondary granules that characterize the granulocytes, they can contain fi azurophilic granules.

The monocytes and lymphocytes mount a slower, but more powerful, defensive reactio than the granulocytes. Lymphocytes are responsible for antigen-specific immune response Monocytes are non-specific phagocytic cells that are the circulating equivalent of tiss macrophages. The mononuclear cells collectively represent 20–50% of circulating leucocytes.

Neutrophils

The neutrophil is the most numerous white cell in adults: about 60% of circulating white ce are neutrophils (6 x 10^9 per litre of whole blood). The neutrophil is typically around 12–15 μm diameter. Its nucleus is divided into a varying number of lobes, joined by thin chromatin strands. Because of this, the neutrophil is sometimes called a polymorphonuclear leucocyte, or 'poly' for short; however, this term is potentially misleading since the other two granulocytes also have polymorphic nuclei, albeit usually with fewer lobes.

Neutrophil cytoplasm contains numerous fine granules, which stain pale pink with Romanowsky dyes. The appearance of these cytoplasmic granules distinguishes the neutrophil from its granulocytic cousins. The granules are of two types, the more abundant, pink-staining, specific or secondary granules, and the larger, reddish-purple staining, azurophilic or primary granules. The neutrophil-specific granules contain a battery of enzymes and other inflammatory substances, including lysozyme which degrades bacterial cell walls, and collagenase which degrades collagen fibres and opens up the tissue matrix. The azurophilic granules also contain digestive enzymes, including lysozyme, glycosidases, proteases, nucleases, and myeloperoxidase. Neutrophils have also been shown to contain tertiary granules and secretory vesicles that contain various membrane proteins that can be expressed during activation and contribute to the anti-microbial role of the cell.

Neutrophils spend about 8–10 hours in the circulation before they exit to the tissues, passing between endothelial cells by a process known as diapedesis. Once in the tissues, neutrophils are responsible for non-specific defence against bacterial and fungal infection. Having left the vessels, neutrophils can survive in the tissues for several days; indeed there are several hundred times more neutrophils in the tissues than there are in the blood at any one time. Neutrophils that do not encounter microbes are thought to undergo apoptosis within 24–48 hours. This mechanism of cell death is important because apoptotic neutrophils are phagocytosed intact by tissue macrophages. This avoids cell lysis, which would release the inflammatory substances within the cell into the surrounding tissues, triggering an inappropriate inflammatory response.

During their time in the peripheral circulation, a proportion of neutrophils, around half under resting conditions, are 'marginated' along the walls of the vascular endothelium or are sequestered in the spleen or lungs (see *Box 1.7*). Marginated neutrophils are perfectly placed both to receive, as early as possible, chemotactic signals radiating from a site of tissue infection, and to respond to such signals by entering the tissues to deal with their source.

Marginated neutrophils are not static; they roll along the vascular endothelial surface. Margination occurs partly due to the hydrodynamic forces of the blood flow and partly to loose interactions between adhesion molecules known as selectins on the neutrophil and endothelial cell surface. Selectins are glycoprotein molecules bearing a free NH_2 terminal lectin domain, and are present on both neutrophils (L-selectins) and endothelial cells (P and E selectins). L-selectins are also present on the surface of monocytes and lymphocytes.

strong, a considerable localized build-up of neutrophils and monocytes, live, dead and decaying, can build up in a protein-rich liquid, which we call 'pus', sometimes creating the painful swollen area known as an abscess. This powerful toxic mess can be a threat to surrounding tissue, and considerable necrosis can occur before the final clearance is mediated by monocytes and macrophages (see also *Box 1.8*).

Box 1.8 Colour of pus

Pus is characteristically a whitish-yellow colour, but may acquire a distinctly green tinge. This is caused by the presence of neutrophil myeloperoxidase, a digestive enzyme found in neutrophil secondary granules. Other colour changes to pus include a reddish tint if blood is present or a bluish tint in the presence of pyocyanin, a pigment present in the pathogen *Pseudomonas aeruginosa*.

Eosinophils

About 1% of the circulating white cells are eosinophils. This name is derived from the staining characteristics of the large cytoplasmic granules, which stain strongly with the acidic dye eosin. Typically, the eosinophil nucleus is bi-lobed. Eosinophils are responsible for limiting inflammatory responses, particularly helping to dampen any allergic response, and have an important role in defence against parasitic infestation. Tissue eosinophils are also capable of responding, albeit inefficiently, to bacterial and fungal infection in a similar manner to neutrophils. Eosinophils circulate in the bloodstream for about 4–5 hours before they exit to the tissues where they can remain for up to 14 days. Relatively large numbers may be found under epithelial surfaces, such as skin, gut, and the respiratory and urinary tracts, ready to take action against parasites that might enter via these routes.

Attraction of eosinophils to reactive sites is under the influence of various chemokines, including CCL11 (eotaxin-1), CCL24 (eotaxin-2), CCL5 (RANTES), and certain leukotrienes including leukotriene B4 (LTB4). Type 2 cytokines, including IL-5, GM–CSF, and IL-3, released from a specific subset of helper T cells (T_H2) at the reactive site, are responsible for eosinophil activation. Eosinophils are only weakly phagocytic, but they are capable of ingesting and killing micro-organisms in a similar way to neutrophils. They deal with macroparasites that are far too large to ingest by spilling their toxic granule contents directly onto their targets. Eosinophils also ingest antigen–antibody complexes, which are then destroyed by degradative enzymes, and this is an important aspect of their anti-inflammatory, anti-allergic capability.

The strong eosinophilia (acidophilia) of the granules is due to the presence of crystals of MBP (major basic protein), which is rich in the positively charged amino acid arginine. MBP is particularly toxic to many parasites, especially macroparasites such as helminths (worms) and amoebae. The granules also contain a variety of other substances, including enzymes (lysozyme, peroxidase, proteases, lysophospholipase) and chemotactic factors which attract more eosinophils to a reactive site. Eosinophil cationic protein (ECP), another granular protein released into the environment on activation, has the ability to punch holes in the membrane of target organisms, thus permitting the entry of destructive enzymes. Eosinophils also secrete enzymes that inactivate various inflammatory mediators, for instance arylsulfatase and histaminase, which destroy leukotriene and histamine respectively. This is the second important aspect of their anti-inflammatory, anti-allergic capability.

Finally, on activation eosinophils release cell signalling compounds of various kinds, including eicosanoids (leukotrienes including LTC_4, LTD_4 and LTE_4, and prostaglandins including PGE_2), growth factors including TGFβ, VEGF and PDGF, and cytokines including IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-13 and TNFα.

Basophils

Basophils are the least numerous circulating white cell; less than 1% of circulating white cells are basophils. The large cytoplasmic granules are characterized by their avidity for the basic dye methylene blue due to their content of the anionic polysaccharide, heparin. The granules also contain histamine and other inflammatory mediators (e.g. chemotactic factors, proteases, and cytokines). Basophils are involved in anaphylactic, hypersensitivity and inflammatory reactions. They release their granule contents when surface receptors bind IgG or IgE antibodies, the complement components C3a and C5a, basic polypeptides such as bradykinin and neurotensin, or histamine-releasing factors such as RANTES or IL-8.

Chemotactic factors that are released attract eosinophils and more basophils to the site. In this way a very quick defensive reaction (immediate hypersensitivity reaction) is mounted against foreign antigens. In some cases this is experienced as an allergic response, such as hay fever or asthma. Some of the substances released serve to limit the extent of tissue damage and to begin repair processes. For example, heparin acts as an anticoagulant, while other factors regulate vascular permeability, blood flow, tissue oedema and fibroblast differentiation. Mast cells are probably more closely involved in the post-injury phase than basophils.

Basophils can be thought of as the circulating equivalent of fixed tissue mast cells, although mast cells arise from a distinct precursor and are not derived from emigrant basophils. Mast cells have the disadvantage of being fixed in tissue sites, whereas basophils can move responsively and rapidly to inflammatory sites, and can reach high concentration at such sites, where they mount immediate hypersensitivity responses.

Lymphocytes

Lymphocytes are the second most common white cell in the peripheral blood: in adults about 33% of circulating white cells are lymphocytes. They are responsible for specific immunity to recognized molecules, known as antigens, through the responsive production of antibodies specific to the antigens. Morphologically, there are two populations of lymphocytes. Small lymphocytes are consistently sized at 10–12 μm in diameter, and thus form a useful size scale when examining peripheral blood films. They have much less cytoplasm than monocytes. Small lymphocyte cytoplasm often appears as a thin perinuclear rim, rich in ribosomes. In contrast to the monocyte, the lymphocyte nucleus is round, intensely basophilic and almost fills the cell. A few lymphocytes; the so-called 'large lymphocytes', have more abundant cytoplasm, and can mimic monocytes; however, they may be differentiated both by their cytoplasmic colour and by their nuclear shape. Lymphocytes have a variable lifespan of between a few days and many years. The biology of lymphocytes is hugely complex and beyond the scope of this text – readers should consult a specific immunology text for more detail.

There are three different main functional types of lymphocytes, each with several subtypes, which play distinct roles in specific immunity; the functional subtypes do not correlate with morphological types.

B lymphocytes normally account for 10–30% of the circulating blood lymphocytes and play a central role in humoral (i.e. antibody-mediated) immunity (see also *Box 1.9*). B lymphocytes carry antigen receptors on their cell surface and when they encounter and recognize foreign

Box 1.9 Why B lymphocytes?

B lymphocytes are so called because, in chickens, they are 'educated' in a specialized organ known as the Bursa of Fabricius. Glick and associates defined the importance of this organ in 1961, when 'bursectomized' chickens were shown to fail to synthesize antibody and to succumb rapidly in response to *Salmonella* infection. In humans, B lymphocyte education occurs predominantly in the bone marrow.

antigen, they migrate to lymph nodes where they undergo a proliferative burst that results in the generation of a clone of identical B cells, which subsequently mature into clonal plasma cells that synthesize and secrete antibody specific to the inducing antigen. A minority of the stimulated B lymphocytes mature to form long-lived memory cells that are capable of mounting a rapid antibody response on a second encounter with antigen. B lymphocytes also play a role in processing and presenting antigen to T cells.

T lymphocytes account for 40–80% of the circulating blood lymphocytes and are responsible for cell-mediated immunity. The thymus gland is responsible for T cell education (see *Box 1.10*). Several T lymphocyte subsets have been identified, each with a distinct immune function. T lymphocytes carry surface antigen receptors that are analogous to those on B cells. Encounter with a specific antigen triggers an immune response. Important T lymphocyte subsets include:

- T helper (T_H) lymphocytes, which secrete cytokines that regulate the immune response.
- T cytotoxic (T_C) lymphocytes, which directly kill tumour cells and virus-infected cells and play a central role in transplant rejection.
- memory T lymphocytes, which are long-lived and can trigger a rapid immune response on a second encounter with specific antigen.
- regulatory or suppressor T (T_S) lymphocytes, which are responsible for maintenance of immune tolerance.

Natural killer cells (NK cells) are a type of large granular cytotoxic lymphocyte that play an important role in fighting cancer and viral infections. NK cells have cytoplasmic granules that contain cytotoxins such as perforin and granzyme. They kill target cells by engagement and direct release of these proteins, triggering target cell apoptosis.

Box 1.10 Why T lymphocytes?

The pivotal role of the thymus gland in cellular immunity was first demonstrated in 1961 when it was shown that thymectomized mice had a greatly impaired capacity to fight infection. T lymphocytes are named to show that they are educated in the thymus gland.

Monocytes

About 5% of circulating white cells are monocytes; the vast majority are the precursors of fixed tissue macrophages, the remainder being immature dendritic cells, the two types of cells being morphologically indistinguishable. Blood monocytes circulate for about 10 hours before they exit to the tissues where they mature into and join the pre-existing population of actively phagocytic tissue macrophages, which are responsible for the removal and processing of aged red cells and other debris. They may undergo limited proliferation in the tissues, and can exist there for periods of months or even years. There is a degree of specialization of tissue macrophages that is determined largely by the influence of the various interleukins and other cell signalling compounds that individually and collectively effect the maturation in response to specific need. Dependent on location, tissue macrophages and dendritic cells are known by different names in different locations (e.g. Küpffer cells in liver, Langerhans cells in skin, microglial cells in brain, osteoclasts in bone, etc.). Together, the monocytes, the fixed tissue macrophages and their precursors constitute the reticulo-endothelial (RE) system.

The blood monocyte is a large cell (16–22 μm in diameter) with a kidney-shaped or distinctly cleft nucleus and a scattering of delicate azurophilic granules in the cytoplasm, giving it an overall grey-blue appearance. They can resemble large lymphocytes; however, their cytoplasm is typically more grey-blue in colour, their nucleus is less intensely stained, shows a more uniform

granularity and often appears 'folded over' to give a kidney or more complex shape. They are a heterogeneous population.

Tissue macrophages play several important roles, primarily in the non-specific immune system. Although less numerous than the neutrophils, they are actively phagocytic and have a much wider spectrum of digestive enzymes within their lysosomes, enabling them to be active against a much wider range of targets, including soluble target materials, which they remove in a process known as pinocytosis. On their surface they carry a range of receptors, which trigger the ingestion of bound ligands, an example being the Fc receptor, which promotes the ingestion of opsonized targets. They respond to a variety of chemotactic signals, which attract them to areas of inflammation, and they release a variety of growth factors and cytokines, which regula responses of other cell types. On activation they, like neutrophils, undergo a respiratory bur and thus produce both reactive oxygen and reactive nitrogen species, which, together with t large array of enzymes, enable them to be effective against a wide variety of targets.

Tissue macrophages also play an important role in specific immunity, since they are involv in the processing and presentation of antigen to T lymphocytes. However, it is in this role th dendritic cells predominate. They display fragments of digested material on their surface, combination with specific receptors for lymphocytes, in a process called 'antigen presentation

SUGGESTED FURTHER READING

Bain, B.J. (2006) *Blood Cells: a Practical Guide*. Blackwell Publishing, Oxford.
ASH Image Bank – http://ashimagebank.hematologylibrary.org/
Bloodline Image Atlas – www.bloodline.net/external/image-atlas.html

SELF-ASSESSMENT QUESTIONS

1. Which of the following statements are true?
   a. The plasma Na^+ concentration is lower than the K^+ concentration.
   b. The red cell Na^+ concentration is lower than the K^+ concentration.
   c. Plasma is always electrically neutral.
   d. The major plasma protein component is immunoglobulin.
2. Approximately how many red cells are present in each litre of blood in an adult male?
   a. $4.4–5.9 \times 10^9$.
   b. $4.4–5.9 \times 10^{12}$.
   c. $3.8–5.2 \times 10^{12}$.
   d. $3.8–5.2 \times 10^9$.
3. Aged red cells are sequestered by which organ?
4. Is red cell spectrin an integral or a peripheral protein?
5. Name the second most numerous blood cell in a healthy person.
6. Fill in the blanks in the text below:
   The outer surface of the platelet membrane is coated in a layer of glycolipids, mucopolysaccharides and adsorbed proteins called the _____. This layer confers a _____ charge to the platelet surface, which helps to prevent platelet–platelet and platelet–endothelium adhesion in the resting state. The membrane and glycocalyx make up the _____ zone of the platelet.
7. Platelets contain two channel systems, the SCCS and the DTS. Which of these is a closed-channel system?
8. Name the three morphologically distinct types of storage granules found in platelets.
9. Name the most numerous white cell in a healthy person.