1

An introduction to haematopoiesis

LEARNING OBJECTIVES

After reading this chapter, you should be able to:

- ✔ describe the site and process of formation of blood cells
- ✔ understand the concept of a stem cell, and the idea of lineage specification of blood cells
- ✔ recognize the different types and functions of mature blood cells
- ✔ describe the basic structure of lymph nodes
- ✔ define B cells and T cells through their respective receptor gene rearrangements
- ✔ understand that malignant diseases of haematological cells derive from their recognizable normal counterparts.

An introduction to the blood and its diseases

An appreciation of blood and its normal components is essential for the care of patients in nearly every clinical specialty. Simple tests of haematological function inform our understanding of the responses to infection and inflammation, nutrition and gastrointestinal dysfunction, as well as renal and liver disease, and can sometimes provide the earliest indicators of malignant disease in tissues which are otherwise clinically hard to assess. This alone makes an understanding of basic haematology essential for every doctor.

Beyond this, haematology also encompasses some of the most common acquired and inherited disorders responsible for morbidity and mortality around the world, and some of the most aggressive and yet treatable malignancies. It has also provided transformational insights into the molecular basis of disease that have informed therapeutics in many specialties.

This opening chapter provides an overview of the formation and function of the cellular components of blood, which will be needed for each of the subsequent sections of this text.

The formation of blood

As the developing embryo grows, it starts to require a means of delivering oxygen to tissues for respiration. The circulation and blood develop at the same time, from around 3 weeks' gestation, and there are close links between the cellular origins of the first blood cells and the vasculature.

Haematology Lecture Notes, Eleventh Edition. Deborah Hay, Andrew King and Michael Desborough.

Companion website: www.wiley.com/go/Hay/Haematology/11e

Haematopoietic stem cells originate in the para-aortic mesoderm of the embryo. Primitive red blood cells, platelet precursors and macrophages are initially formed in the vasculature of the extraembryonic yolk sac, before the principal site of haematopoiesis shifts to the fetal liver at around 5–8 weeks' gestation. The liver remains the main source of blood in the fetus until shortly before birth, although the bone marrow starts to develop haematopoietic activity from as early as 10 weeks' gestation.

After birth, the marrow is the sole site of haematopoiesis in healthy individuals. During the first few years of life, nearly all the marrow cavities contain red haematopoietic marrow, but this recedes so that by adulthood, haematopoiesis is limited to marrow in the vertebrae, pelvis, sternum and the proximal ends of the femora and humeri, with minor contributions from the skull bones, ribs and scapulae (Figure 1.1). It is the posterior superior iliac spine of the pelvis – a bony landmark readily palpable in most individuals - which is the usual site of bone marrow biopsy for the clinical assessment of the process of haematopoiesis.

Although the sites of haematopoiesis in the adult are therefore relatively limited, other sites retain their capacity to produce blood cells if needed. In conditions in which there is an increased haematopoietic drive (such as chronic haemolytic anaemias and chronic myeloproliferative disorders), haematopoietic tissue will expand and may extend into marrow cavities that do not normally support haematopoiesis in the adult. Foci of haematopoietic tissue may also appear in the adult liver and spleen and other tissues (known as extramedullary haematopoiesis).

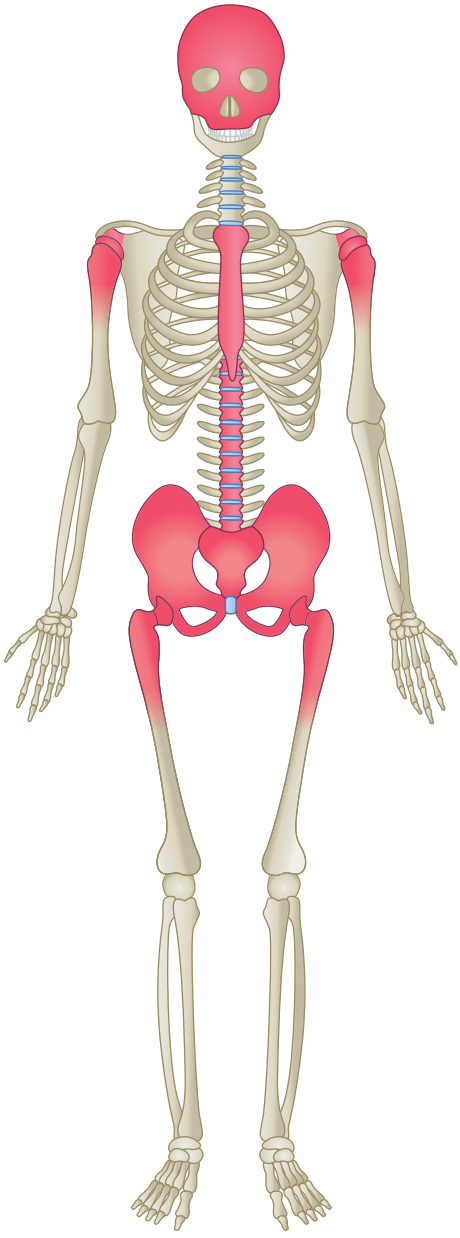

Figure 1.1 The red shading indicates the position of red (blood-forming) marrow in the adult. The posterior superior iliac spine of the pelvis is the usual site of bone marrow biopsy for clinical assessment of the process of haematopoiesis.

Haematopoietic stem cells

The process of haematopoiesis needs to generate adequate numbers of specialised blood cells throughout life from a small pool of precursor cells. This is accomplished using the unique properties of haematopoietic stem cells.

Long-term haematopoietic stem cells (HSCs) in the bone marrow are capable of both self-renewal and differentiation into the progenitors of individual blood cell lineages. The progenitor cells of individual lineages then undergo many rounds of division and further differentiation in order to yield populations of mature blood cells. This process can be represented as a hierarchy of cells, with HSCs giving rise to populations of precursor cells, which in turn give rise to cells increasingly committed to producing a single type of mature blood cell (Figure 1.2). Thus, the immediate progeny of HSCs are the multipotent progenitor cells, which have limited self-renewal

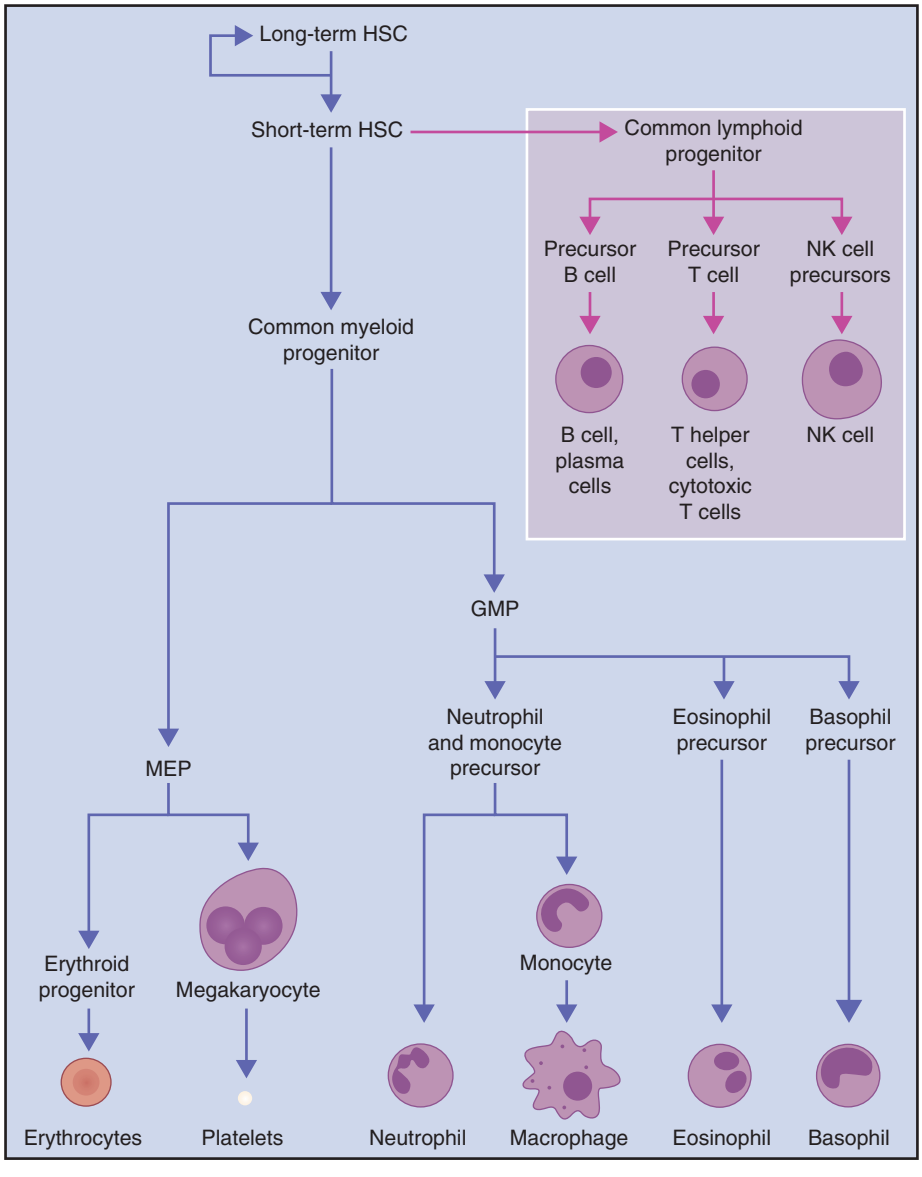

Figure 1.2 A schematic representation of the process of haematopoiesis. Multipotent stem cells give rise to lymphoid (pink) and myeloid (blue) lineages. The myeloid lineage further divides into granulocytic, erythroid and megakaryocytic (platelet) lineages. As cells progress through this process of differentiation, they accrue more functional specialization and lose their multipotency. GMP, granulocyte macrophage progenitor; HSC, haematopoietic stem cell; MEP, megakaryocyte/erythroid progenitor; NK, natural killer.

capacity but retain the ability to differentiate into all blood cell lineages. Although there is still debate about exactly how lineage-restricted subsequent precursors are, the concept of sequential and irreversible differentiation is widely accepted. In Figure 1.2, the HSC is seen giving rise to two major lineages: the lymphoid lineage, in which a common lymphoid progenitor gives rise to B cells, T cells and natural killer (NK) cells; and a myeloid lineage, with a common myeloid progenitor giving rise to red cells, granulocytes and platelets. The division of haematopoiesis into myeloid and lymphoid compartments is fundamental to an understanding of haematological disease.

The process of haematopoiesis outlined above has several advantages. First, it permits the massive expansion of cell numbers needed to maintain an adequate population of mature blood cells. It also means that the production of each type of mature blood cell can be controlled individually, tailoring production to specific physiological requirements. Finally, it requires relatively little proliferative activity on the part of the long-term HSCs themselves, thereby minimizing the risk of developing mutations in these crucial cells during DNA replication and cell division.

Haematopoietic stem cells were first detected and defined functionally through experiments in which a subset of cells from the bone marrow was shown to produce blood cells of all lineages when transplanted into lethally irradiated mice, which have no haematopoietic potential of their own. Subsequent work has used cell surface markers and flow cytometric techniques (see Chapter 7) to define this population: positivity for the cell surface marker CD34 combined with negativity for CD38 describes a population of multipotential cells that is capable of regenerating all cell lineages from the bone marrow. The cell surface marker CD34 is also used to isolate cells with multipotency and self-renewal capacity for autologous and allogeneic stem cell transplantation (see Chapter 12).

Differentiating blood cells

Precisely how the ultimate lineage choice of differentiating progenitor cells is determined remains a subject of research. It has been argued that factors intrinsic to the HSC itself, such as stochastic fluctuations in transcription factor levels, may direct lineage specification. However, it is also known that proper regulation of HSCs and progenitor cells requires their interaction with extrinsic factors, such as non-haematopoietic cells in the bone marrow niche (e.g. endothelial cells and osteoblastic progenitors). HSCs and progenitor cells are not randomly distributed in the marrow, but exist in ordered proximity relative to mesenchymal cells, endothelial cells and the vasculature. Signalling from these non-haematopoietic cells, plus physicochemical cues such as hypoxia and blood flow, are therefore likely to influence the transcriptional activity and fate of HSCs. It is clear, however, that the process of lineage specification is accompanied by a gradual reduction in the multipotency of the cell.

Myelopoiesis

Signalling through myeloid growth factors such as granulocyte-macrophage colony stimulating factor (GM-CSF) is essential for the survival and proliferation of myeloid cells. The specification of the myeloid lineage is also known to require the interaction of a series of specific transcription factors, including C/EBPα, RUNX1-core binding factor complex and c-Myb. As well as being essential for the normal formation of myeloid cells, it is becoming clear that an appreciation of these factors and others like them is critical for an understanding of myeloid diseases such as acute myeloid leukaemia (see Chapter 8).

The separation of the erythroid and megakaryocytic components of myelopoiesis requires the action of transcription factors GATA1, NF-E2 and SCL, and signalling through the growth factors thrombopoietin and erythropoietin.

Granulocytes and their function

Granulocytes, cells of the innate immune system characterized by the presence of specific cytoplasmic granules containing microbicidal and immunologically active molecules, are the most abundant white cells in the blood. The maturation of the normal bone marrow is outlined in Figure 1.3, with the least mature granulocytic cell, the myeloblast, passing through a series of successive stages termed promyelocytes (Figure 1.3b), myelocytes (Figure 1.3c), metamyelocytes and band forms. Cell division occurs in myeloblasts, promyelocytes and myelocytes, but not normally in metamyelocytes or band cells.

As well as a reduction in the size of the cell and the development of granules containing agents essential for their microbicidal function, the maturing granulocyte also gradually begins to adopt its characteristic nuclear segmented shape (Figure 1.4).

Mature neutrophils have the ability to migrate to areas of inflammation (chemotaxis), where they become marginated in the vessel lumen and pass into the tissues through interaction with selectins, integrins and other cell adhesion molecules. Once primed by cytokines such as tumour necrosis factor α (TNFα) and γ-interferon (IFNγ), neutrophils are able to phagocytose opsonized microbes, and destroy them by deploying their toxic intracellular contents. This release of reactive oxygen species (the 'respiratory burst') provides a substrate for the enzyme myeloperoxidase (MPO), which then generates

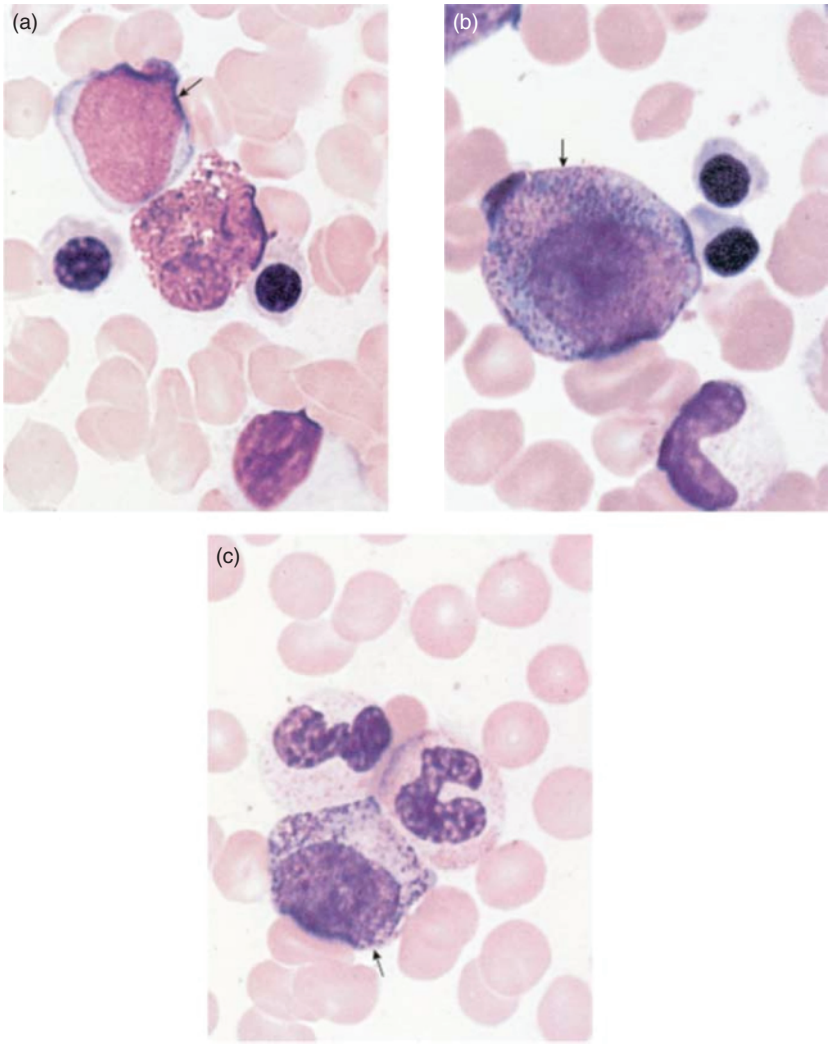

Figure 1.3 Neutrophil precursors from normal bone marrow. (a) Myeloblast (arrowed); the other nucleated cells near the myeloblast are an eosinophil (centre) and two polychromatic erythroblasts. (b) Promyelocyte (arrowed); the other nucleated cells are two polychromatic erythroblasts and a metamyelocyte. (c) Myelocyte (arrowed); there are two neutrophil band cells adjacent to the myelocyte. May-Grünwald-Giemsa (MGG) staining is used throughout for blood films.

hypochlorous acid with direct cytotoxic effects. The granules of neutrophils also contain an array of antimicrobial agents, including defensins, chymotrypsin and gelatinases.

Eosinophils, a subset of granulocytes with bright pink granules on haematoxylin and eosin-stained (H&E) blood films, have a similar ability to phagocytose and destroy micro-organisms, but are classically associated with the immune response to parasitic infection. They are often found in high numbers in patients with allergy and atopy. Interleukin 5 (IL-5) signalling appears to be critical for their differentiation from granulocyte precursors.

Basophils are the least common of the granulocytes. They contain very prominent cytoplasmic granules which have stores of histamine and heparin as well as proteolytic enzymes. They are involved in a variety of immune and inflammatory responses, but it is unusual to see a marked elevation or depression in their numbers in reactive conditions. Figure 1.5 shows examples of each of the normal mature granulocytes.

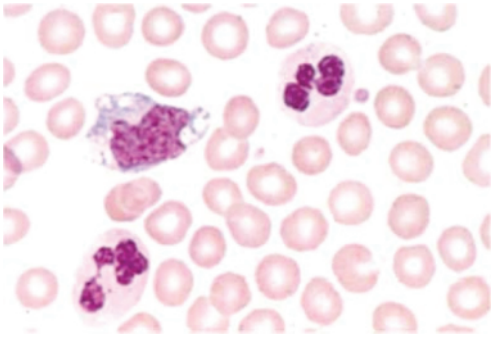

Figure 1.4 Monocyte and two neutrophil granulocytes – the monocyte has a pale, greyish-blue vacuolated cytoplasm.

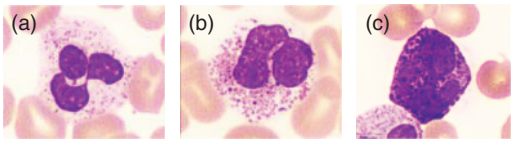

Figure 1.5 Mature granulocytes. (a) Neutrophil: note the polylobed nucleus and fine cytoplasmic granules. (b) Eosinophil, with a typical bilobed nucleus and pinkish orange (eosinophilic) granules. (c) Basophil, with granules so darkly staining as to obscure the nuclear contours

Monocytopoiesis and monocyte function

Functionally, monocytes have a variety of immune roles. As the precursors of tissue macrophages and dendritic cells, their roles include phagocytosis, antibody presentation to other immune cells and contributing to the cytokine milieu. Phagocytosis of micro-organisms and cells coated with antibody (with their exposed Fc fragments) and complement occurs via binding to Fc and C3b receptors on the surface of monocytes and macrophages. Bacteria and fungi that are not antibody-coated are phagocytosed after binding to mannose receptors on the phagocyte surface. As with neutrophils, the killing of phagocytosed micro-organisms by monocytes/macrophages may involve superoxide dependent and O_2-independent mechanisms.

The cell classes belonging to the monocyte–macrophage lineage are, in increasing order of maturity, monoblasts, promonocytes and monocytes (with the latter only typically seen in the blood), and tissue macrophages. Their synthesis is controlled in part by the activity of GM-CSF.

Megakaryocytes and platelet function

Megakaryocytes are the cells that give rise to platelets, the cellular fragments needed for primary haemostasis. During megakaryocyte formation, driven by the action of the growth factor thrombopoietin (TPO), there is replication of DNA without cell division. This leads to the generation of very large cells that are markedly polyploid, with a lobulated nucleus. A mature megakaryocyte is illustrated in Figure 1.6. Large numbers of platelets are formed from the cytoplasm of each mature megakaryocyte; these are rapidly discharged directly into the marrow sinusoids. The residual 'bare' megakaryocyte nucleus is then phagocytosed by macrophages.

TPO is the key regulator of normal platelet production. This protein, which is produced by the liver, binds to TPO receptors on the megakaryocyte membrane. Downstream signalling through mechanisms including the JAK/STAT pathway allows an increase in megakaryocyte ploidy, and also cytoplasmic maturation such that increased numbers of platelets are released. TPO is also able to bind to the surface of platelets themselves; thus, when platelet numbers are high, TPO is sequestered on the platelet membranes,

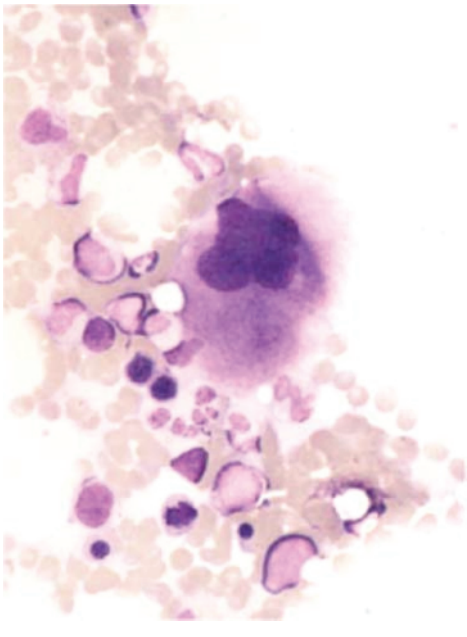

Figure 1.6 Mature megakaryocyte (centre). This is a very large cell with a single lobulated nucleus. Compare the size of the megakaryocyte with that of the other nucleated marrow cells in this figure.

leaving less available to act on the megakaryocytes to promote further platelet production. In this way, a negative feedback loop is created, maintaining platelet numbers within stable limits.

The fundamental role of platelets is in primary haemostasis, through their interactions with von Willebrand factor and the exposed collagen of damaged endothelial surfaces (see Chapter 13).

Erythropoiesis and red cell function

The specification of the erythroid lineage requires a balanced interaction between transcription factors GATA1 and other haematopoietic transcription factors, including PU.1 and FOG1. Once committed to an erythroid fate, the expansion of erythroid precursors takes place, driven largely by signalling through the erythropoietin receptor.

The hormone erythropoietin (epo) is expressed principally in the cortical interstitial cells of the kidney, where its transcription is modulated in response to hypoxaemia. The transcription factor hypoxia inducible factor (HIF-1) is induced in cells exposed to hypoxaemic conditions and enhances expression of the erythropoietin gene. Increased levels of erythropoietin are therefore available to interact with the epo receptor on red cell progenitor membranes, activating an erythroid-specific signal transduction cascade and leading to enhanced proliferation and terminal differentiation of erythroid cells.

Morphologically, the differentiation and maturation of erythroid cells are shown in Figure 1.7. Proerythroblasts are early erythroid progenitors in the bone marrow recognizable by their large size, their dark blue cytoplasm, their dispersed nuclear chromatin and nucleoli. As the cells mature, they become smaller with less basophilic cytoplasm (Figure 1.7).

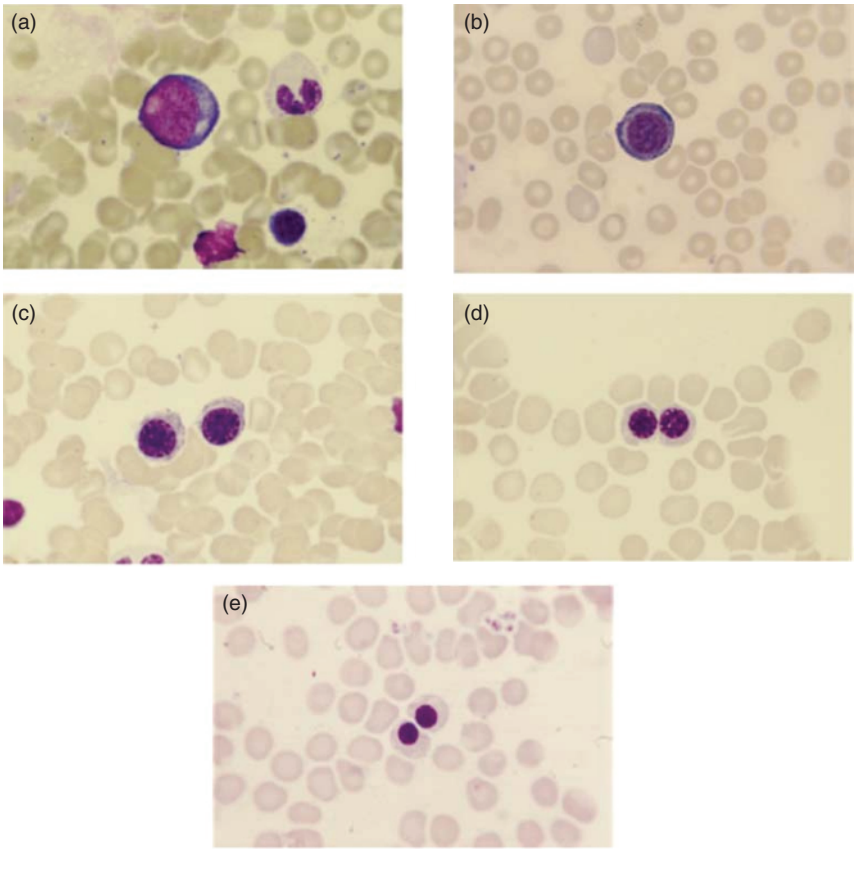

Figure 1.7 (a) Proerythroblast, (b) basophilic normoblast, (c) two early polychromatic normoblasts, (d) two late polychromatic normoblasts and (e) two very mature late polychromatic normoblasts. The condensed chromatin in the basophilic normoblast is slightly coarser than in the proerythroblast. The nuclei of the late polychromatic normoblasts contain large masses of condensed chromatin.

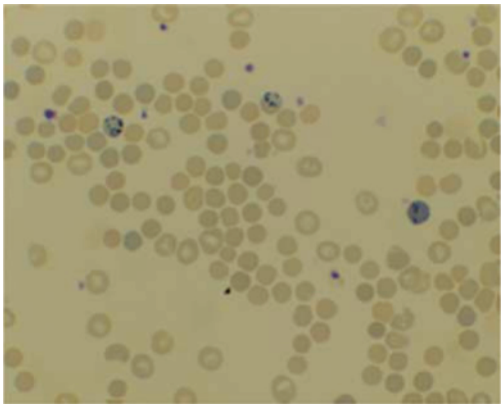

Figure 1.8 Reticulocytes in peripheral blood stained supravitally with brilliant cresyl blue. Note the reticulum of precipitated ribosomes.

Cell division continues until the cells reach the late polychromatic normoblast stage, when cells extrude their nucleus. At this point, the cell is termed a reticulocyte (Figure 1.8) and is released from the marrow into the peripheral blood. Reticulocytes are characterized by their slightly larger size and bluish staining (due to higher RNA content) contrasted with mature red cells. After 1–2 days in circulation, reticulocytes lose their remaining ribosomes and become mature red cells.

The red cell function is to carry oxygen, bound to the haem moiety of haemoglobin, from the lungs to the peripheral tissues. The details of haemoglobin structure and function (and diseases resulting from perturbation of these) are discussed further in Chapter 3.

Lymphopoiesis

B cells are needed for antibody production, and T cells for cell-mediated immunity. They are thought to arise from multilymphoid progenitor cells in the fetal marrow. Although incompletely characterized, these progenitors are known to feature CD45 and CD7 cell surface markers. The transcription factor Ikaros has been shown to be critical for lymphopoiesis in mouse models; Pax5 is among several transcription factors needed for B-cell development, while GATA3 and Notch signalling are essential for T-cell maturation.

B-cell development

The development of B lymphocytes commences in the fetal liver and fetal marrow. Here, progenitor B cells develop into pre-B cells (defined by the presence of the cytoplasmic μ chain of the B-cell receptor, see below) and then into mature B cells. During this time, the genes for the immunoglobulin light and heavy chains are rearranged, allowing the production of immunoglobulins with a wide array of antigenic specificities. Subsequent B-cell maturation requires antigen exposure in the lymph nodes and other secondary lymphoid tissues, with the mature B cell having the capacity to recognize non-self-antigens and produce large quantities of specific immunoglobulin.

Immunoglobulin structure and gene rearrangement

The defining feature of B-cell development is the production of immunoglobulin with a wide range of antibody specificities. Immunoglobulins are made up of two identical heavy chains and two identical light chains (either κ or λ) (Figure 1.9). The five major classes of immunoglobulin – IgA, IgG, IgM, IgD and IgE – are defined by their heavy chains (α, γ, μ, δ and ε) and the two heavy chains and two light chains are held together in a Y-shaped structure. The amino terminal portions of the heavy and light chains are known as 'variable' regions (V_H or V_L), because differences in their amino acid sequence create unique antigen-binding sites, each of which can recognize a different epitope. In contrast, the carboxy terminal regions are the 'constant' regions (CH or CL), because their structure is similar for all immunoglobulins of the same class. The enzyme papain cleaves the immunoglobulin molecule into an Fc fragment consisting of the carboxy terminal regions of the heavy chain and two Fab fragments containing the antigen-binding site. A number of cell types (e.g. splenic macrophages) possess Fc receptors that can bind the Fc portion of immunoglobulin molecules.

Human plasma contains all types of immunoglobulin, but IgG is present at the highest concentration. IgA is the second most common type of immunoglobulin and is found at mucosal surfaces in the gut and respiratory system. IgM represents <10% of the immunoglobulin found in plasma and is the antibody most commonly produced during a primary immune response. IgM has a very large molecular weight

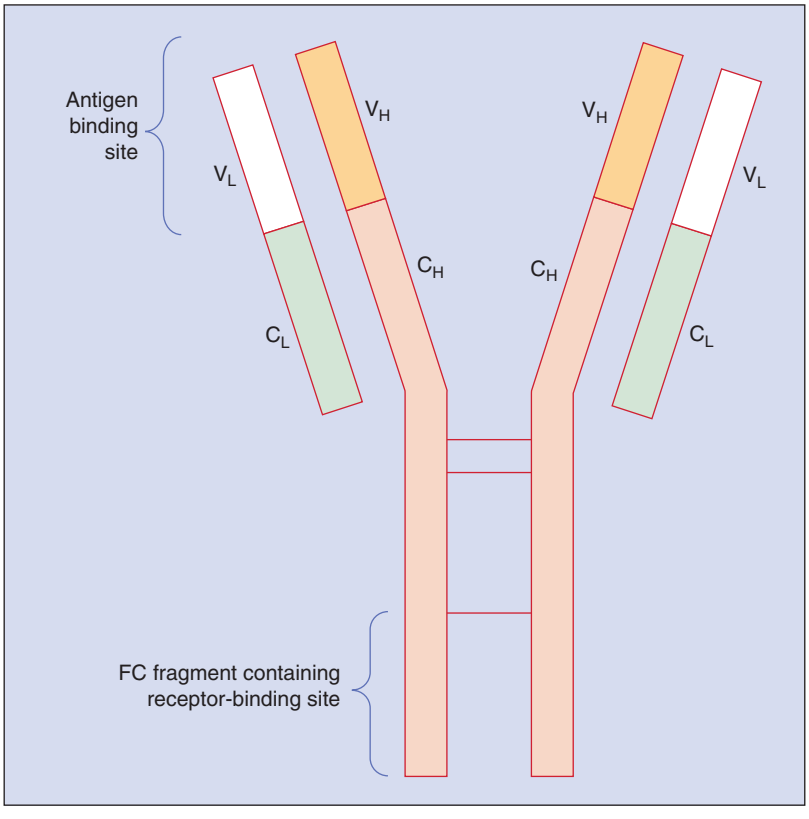

Figure 1.9 Schematic model of an IgG molecule.

(approximately 900 000 kDa) and is pentameric in structure – relevant in the clinical presentation of Waldenström macroglobulinaemia (see Chapter 9).

Antibody diversity

Immunoglobulin gene rearrangement is the mechanism by which a single variable gene (V_H or V_L) in either a heavy or light chain gene complex is juxtaposed to genes encoding the constant region (C_H or C_L), generating differences from one immunoglobulin molecule to another in antigen-binding specificities (Figure 1.10). Diversity is increased by the introduction of randomly generated sequences, as well as by mutations within the variable regions. In consequence, a B-cell clone can synthesize immunoglobulin molecules with unique antigen-binding sites. It may be added that at different stages of differentiation, a single B cell can synthesize heavy chains with different constant regions but with the same variable regions and thus antigen specificity. Thus, a B cell can start by expressing IgM but following antigen binding can produce IgG, IgA or IgE.

B-cell selection and maturation

B cells arising from progenitor cells in the bone marrow make their way to the germinal centre of lymphoid tissue, where they encounter antigen already bound to dendritic cells. If the surface immunoglobulin (sIg) on the B cells binds this antigen, the cross-linking provides a survival signal. Otherwise, the B cells undergo apoptosis, a process that ensures selection of 'useful' B cells, which become memory B cells and plasma cells.

Lymph node structure

Lymph nodes contain densely packed T and B lymphocytes, organized in a manner that allows the

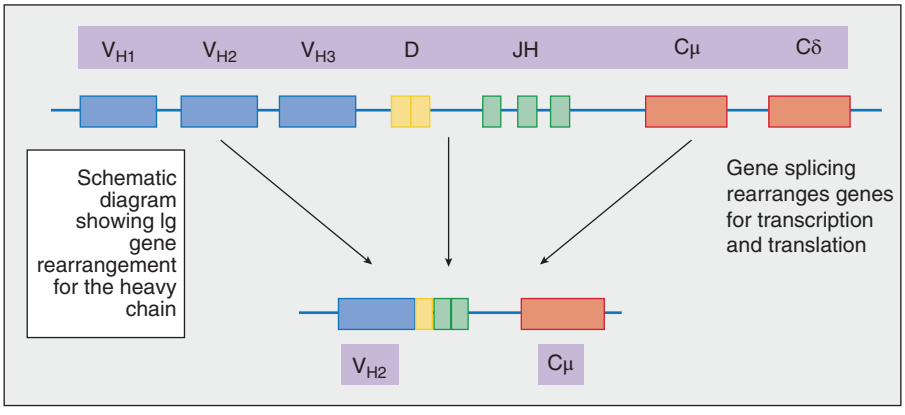

Figure 1.10 A diagram illustrating gene rearrangement enabling antigen-binding diversity. During differentiation, a single B cell can synthesize heavy chains with different constant regions coupled to the same variable region. Note that it is the genetic locus itself that undergoes rearrangement, not merely the transcripts it generates. The T-cell receptor (TCR) undergoes gene rearrangement in a very similar way in T cells. C, constant region gene; D, diversity gene; JH, joining sequence; VH, variable gene.

presentation of antigen to produce an effective immune response. In addition to having a blood supply, lymph nodes receive 'afferent' lymphatic vessels, which drain antigen-rich lymph from the tissues.

Within the unstimulated lymph node, resting B cells are organized into structures called primary follicles (Figure 1.11). When exposed to antigen (e.g. from micro-organisms), these structures enlarge and germinal centres develop, comprising proliferating B cells lying within a meshwork of follicular dendritic cells. Surrounding the follicles are sheets of lymphocytes, which become increasingly rich in T cells towards the lymph node medulla. The medulla contains several types of lymphocytes, including plasma cells.

Other lymphoid tissue

Lymphoid tissue is found in many sites other than lymph nodes. These sites include tissues directly exposed to external pathogens (e.g. respiratory and gastrointestinal tracts) and also central sites such as the bone marrow and spleen. The spleen is the largest single lymphoid mass in the body, and its white pulp component bears relation to a lymph node, though with no afferent lymphatic; instead, the lymphoid cells in the white pulp of the spleen respond to antigen borne directly in the blood, making it critical in the early immune response to bacteraemia. The red pulp of the spleen is the site of removal of senescent red cell and platelets from the circulation.

Plasma cells are found in many lymphoid tissues, particularly at sites where they can secrete immunoglobulin into the circulation or mucous secretions (e.g. into the respiratory or gastrointestinal tracts). The spleen is the principal source of antibodies in the circulation.

T-cell development

T cells originate from lymphoid precursors in the marrow and fetal liver. These earliest immature T cells express neither CD4 nor CD8 and undergo rearrangement of the T-cell receptor genes to permit cell surface expression of the TCR. As with the surface immunoglobulin or B-cell receptor, the process of rearrangement yields a vast collection of potential TCRs, with the ability to recognize a wide range of different antigens. The TCR is formed from two polypeptide chains, usually α and β or less commonly γ and δ. Interaction of the TCR and a binding ligand results in T-cell activation and proliferation.

During the process of maturation, T cells migrate from the bone marrow to the thymus, acquire both CD4 and CD8 cell surface markers (termed 'double-positive' thymocytes) and undergo a process of positive selection to ensure the survival only of those

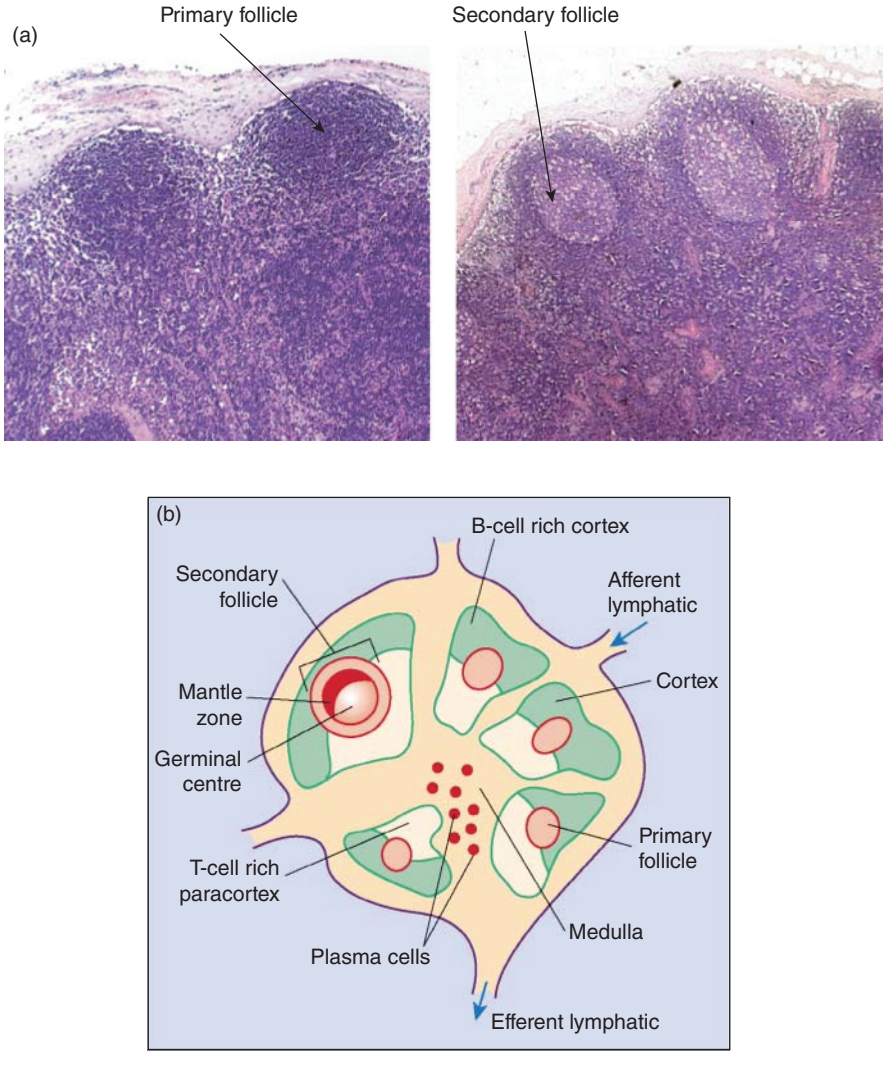

Figure 1.11 (a) Histological section through a lymph node and (b) illustration of a stylized lymph node.

that are able to interact adequately with major histocompatibility complex (MHC) molecules on antigen-presenting cells. T cells that interact with MHC class I become CD8 positive only, while those that interact with MHC class II downregulate their CD8 expression and become CD4 T cells. Any T cells that fail to recognize MHC will die by apoptosis, and the same fate awaits cells that bind with very high affinity. As a consequence, the T cells that emerge from the thymus all carry TCRs with an intermediate affinity for 'self' MHC molecules. This ensures that they will subsequently continuously bind to (and disengage from) self-MHC molecules. However, if alteration of the MHC molecule by the presence of a peptide (e.g. from a virus) increases the affinity of TCR binding, the cell presenting the peptide with the MHC molecule will become the target for specific recognition and killing.

CD4+ lymphocytes are known as T 'helper' (Th) cells, and form the majority of the circulating T-cell population. Their roles include the production of cytokines to promote an inflammatory response in

the presence of the appropriate antigen. Such cytokines include IFNγ (from the Th1 class of CD4+ cells) and interleukins 4, 5 and 13 (from the Th2 subset of CD4+ cells). The effects of cytokine production include activation of the monocyte/macrophage system, the promotion of granulocyte maturation and the induction of antibody synthesis by B cells.

CD8+ lymphocytes are T-suppressor/cytotoxic cells, comprising approximately one-quarter of the T cells in the peripheral blood. Their function is to destroy any cells expressing a peptide to which their TCR can bind (e.g. virally infected cells).

Natural killer cells

A small minority of mature lymphocytes are distinct from both B- and T-cell lineages. These are the NK cells, which have a role in the innate immune system, through cell-mediated cytotoxicity. NK cells differ from T cells in that they do not express TCRs, and therefore CD3, but they are nevertheless capable of mediating cell lysis. This is achieved through surface receptors that suppress NK activity when they engage self MHC molecules on a cell. However, the absence of MHC molecules on a cell removes this inhibition and the NK cells then initiate cytotoxic destruction of the target cell.

Natural killer cells express FcγRIIIA (CD16) – a receptor that recognizes antibody on the surface of a cell – and thereby induce antibody-dependent cell-mediated cytotoxicity (ADCC). Furthermore, NK cell activation leads to the production of cytokines, including IFNγ, macrophage and granulocyte-macrophage colony-stimulating factor (M-CSF, GM-CSF), IL-3 and TNFα. These cytokines effect neutrophil recruitment and function, in addition to helping to activate the monocyte–macrophage system. NK cells can lyse virus-infected cells and may be able to lyse tumour cells.

Identifying lymphoid cells in the blood and marrow

All the stages of both B- and T-cell development described above have the morphological features of either lymphoblasts or lymphocytes. The identification of different lymphocyte precursors is therefore based not on morphology but on properties including reactivity with certain monoclonal antibodies and the presence of immunoglobulin or TCR on the cell surface membrane (Tables 1.1 and 1.2). In the peripheral blood, lymphocytes may be small and compact or may appear large with azurophilic cytoplasmic granules (Figure 1.12). Such large granular lymphocytes include cytotoxic T cells and NK cells.

Table 1.1 The sequence of events during B-cell differentiation.

Characteristic	Pre-pre-B cell	Pre-B cell	Immature B cell	Mature B cell	Plasma cell
Heavy-chain genes rearranged	+	+	+	+	+
Light-chain genes rearranged	–/+	+	+	+	+
Terminal deoxynucleotidyl-transferase	+	+/–	–	–	–
Cytoplasmic μ-chains expressed	–	+	–	–	–
Surface IgM (but not IgD) expressed	–	–	+	–	–
Surface IgM and IgD expressed	–	–	–	+	–
Cytoplasmic Ig expressed	–	–	–	–	+
CD10	+	+	–	–	–
CD19	+	+	+	+	+

Ig, immunoglobulin.

Table 1.2 The sequence of events during T-cell differentiation.

Characteristic	Pre- T cell	Early thymocyte	Intermediate thymocyte	Late thymocyte	Mature T cell
CD7	+	+	+	+	+
Terminal deoxynucleotidyl-transferase	–/+	+	+	–	–
TCR γ genes rearranged/deleted	–	+	+	+	+
TCR β genes rearranged	–	–	+	+	+
TCR α genes rearranged	–	–	–/+	+	+
CD2	–	+	+	+	+
CD3	–	+	+	+	+
CD4 and CD8	–	–	–/+	–	–
CD4 or CD8	–	–	–	+	+

TCR, T-cell receptor.

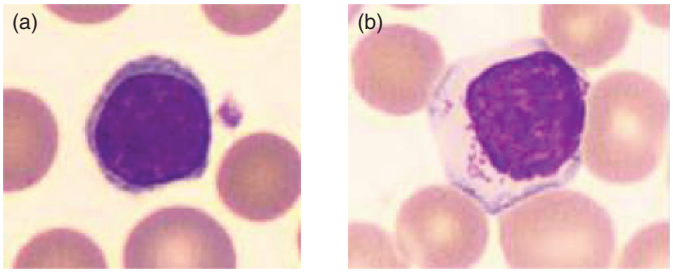

Figure 1.12 (a) A normal small lymphocyte. (b) A large lymphocyte with several azurophilic cytoplasmic granules. Large granular lymphocytes include cytotoxic T cells and natural killer (NK) cells.

Normal numbers of blood cells

The descriptions above give some sense of the way in which normal numbers of blood cells are maintained in the circulation. For red cells, this is achieved by linking the sensing of hypoxia to red cell production, while for platelets there is a balance between TPO binding to mature platelets and their bone marrow precursor, the megakaryocyte. For granulocytes, cytokine signalling related to inflammation and infection induces release from the marrow, and for lymphocytes antigenic stimulation of a pre-existing pool of lymphoid cells is key. For a healthy individual in the steady state, the numbers of each type of cell therefore exist within a predictable range. This is termed the normal or reference range, and is discussed in more detail in Chapter 2.

The non-cellular components of blood

Although the great bulk of this chapter (and the rest of the text) focuses on the function and dysfunction of blood cells, the non-cellular component also

forms a key part of haematological study. Blood plasma is the liquid portion remaining following removal of blood cells by centrifugation. Principally composed of water, it also contains all the clotting factors essential for normal haemostasis (see Chapter 13); the immunoglobins synthesized and secreted by plasma cells (see Chapter 10); albumin, key for transport of some small molecules through the blood and needed to maintain oncotic pressure; and all the electrolytes, hormones and transport proteins required for the normal function of blood. Many biochemical tests are therefore performed on blood plasma (or serum, its equivalent once clotting factors have been removed).

Summary

Table 1.3 summarizes the role of each mature cell type in the peripheral blood. It is the abnormal production, function or destruction of these cells that constitutes the study of clinical haematology, which forms the basis for the rest of this book.

Table 1.3 The main functions of blood cells.

Red blood cells (erythrocytes)	Transport O_2 from lungs to tissues (see Chapters 2 and 4)
Neutrophil granulocytes	Chemotaxis, phagocytosis, killing of phagocytosed bacteria
Eosinophil granulocytes	All neutrophil functions listed above, effector cells for antibody-dependent damage to metazoal parasites, regulate immediate-type hypersensitivity reactions (inactivate histamine and leukotrienes released by basophils and mast cells)
Basophil granulocytes	Mediate immediate-type hypersensitivity (IgE-coated basophils react with specific antigen and release histamine and leukotrienes), modulate inflammatory responses by releasing heparin and proteases
Monocytes and macrophages	Chemotaxis, phagocytosis, killing of some micro-organisms, antigen presentation, release of IL-1 and TNF that stimulate bone marrow stromal cells to produce GM-CSF, G-CSF, M-CSF and IL-6
Platelets	Adhere to subendothelial connective tissue, participate in primary haemostasis
Lymphocytes	Critical for immune responses and cytokine production

G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; Ig, immunoglobulin; IL, interleukin; M-CSF, macrophage colony-stimulating factor; TNF, tumour necrosis factor.