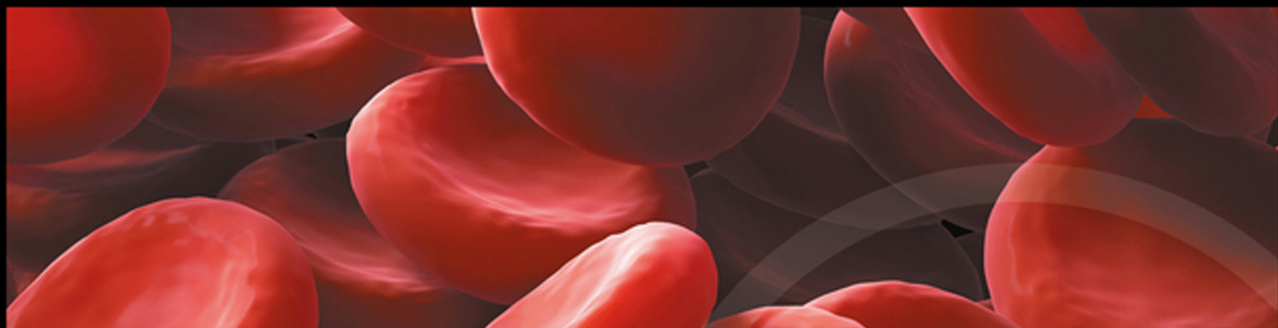# HAEMATOLOGY

Lecture Notes

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10th Edition

WILEY Blackwell

Haematology
Lecture Notes

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Preface

Modern haematology in the UK remains at the interface between clinical and laboratory practice, and is one of the disciplines in which an increasing appreciation of the importance of the molecular basis of disease has translated directly into patient care. We have reflected this in the 10th edition of *Haematology Lecture Notes*, with a new chapter outlining the molecular and cellular techniques that are central to haematology. Our online companion website also features self-assessment questions which allow the reader to apply his or her knowledge to clinical cases.

As always, we are grateful to our colleagues for their help and advice in the production of this book. We are especially grateful to Professor Kevin Gatter of the Nuffield Division of Clinical Laboratory Sciences for kindly allowing us to use his excellent histological images, and to Drs Angela Hamblin, Robert Danby, Jaimal Kothari and Adam Mead, consultant haematologists, for their advice and suggestions. We are also grateful to those readers who have provided feedback on previous editions.

We hope that students and junior doctors continue to find *Haematology Lecture Notes* a useful introduction to this rapidly changing specialty.

About the companion website

This book is accompanied by a companion website:

www.wiley.com/go/hatton/haematology/10e

The website includes:

- Interactive multiple choice questions for each chapter
- Chapter overviews
- Further reading

An introduction to haematopoiesis

Learning objectives

✔ To understand the process of formation of blood cells
✔ To understand the concept of a stem cell
✔ To appreciate the processes of lineage specification of blood cells
✔ To recognize the different types of mature blood cell
✔ To understand the normal role of each mature cell type in the blood

Where is blood formed?

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. 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 extra-embryonic 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 haemato-poiesis in healthy individuals. During the first few years of life, nearly all the marrow cavities contain red haematopoietic marrow, but this recedes such 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.

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 haematopoi-etic tissue may also appear in the adult liver and spleen and other tissues (known as extramedullary haematopoiesis).

Haematopoietic stem cells

The process of haematopoiesis involves both the spec-ification of individual blood cell lineages and cellular proliferation to maintain adequate circulating num-bers of cells throughout life. 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 1.1). Thus, the immediate progeny of HSCs are the multipotent progenitor
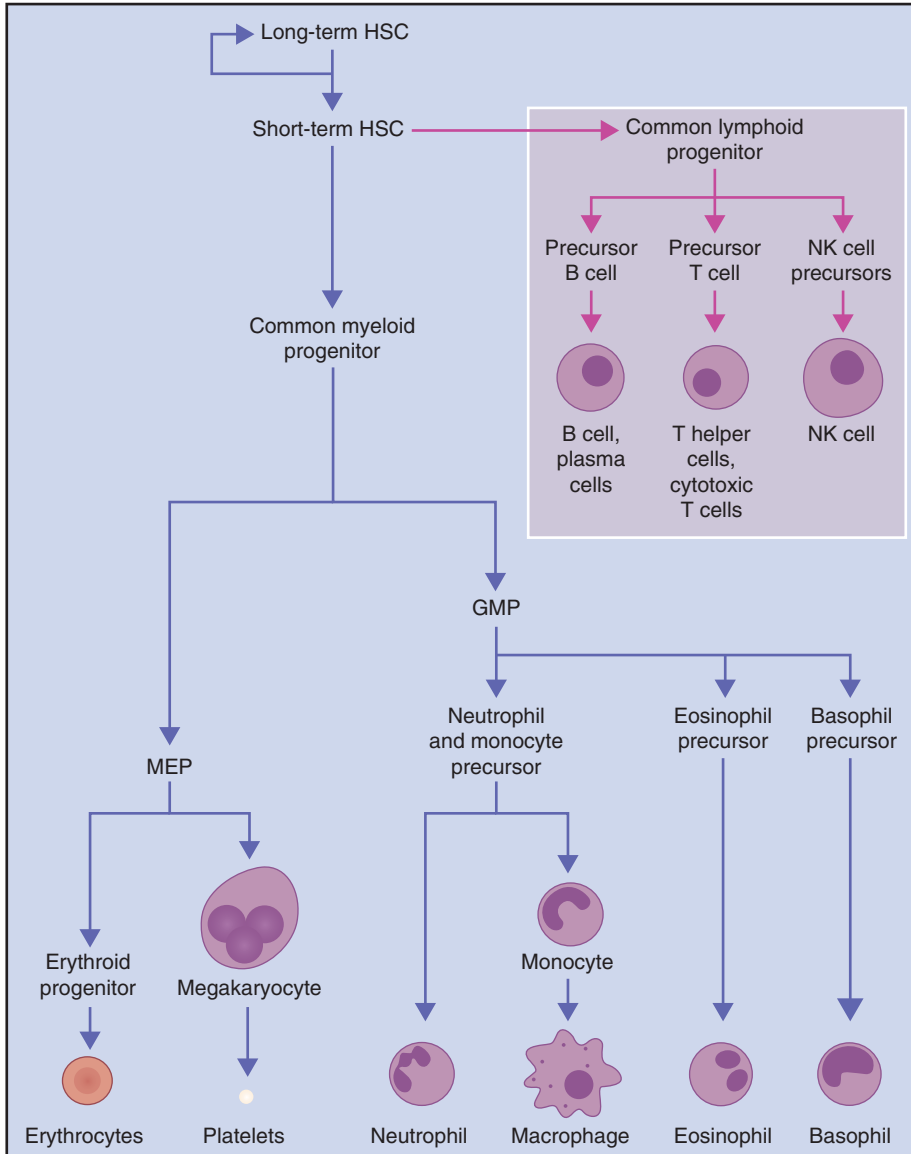
Figure 1.1 A schematic representation of the process of haematopoiesis. Multipotent stem cells give rise to lymphoid (pink) and myeloid (blue) lineages. The myeloid lineage further divides into granulocytic, erythroid and megakaryocytic (platelet) lineages. As cells progress through this process of differentiation, they accrue more functional specialization and lose their multipotency. GMP, granulocyte macrophage progenitor; HSC, haematopoietic stem cell; MEP, megakaryocyte/erythroid progenitor; NK, natural killer.

cells, which have limited self-renewal 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 1.1, 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.

HSCs 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 5) 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 stem cell transplantation.

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.

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, core binding factor 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 11).

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

Morphologically, myeloblasts are the earliest recognizable granulocytic cells. They are large cells, with open nuclear chromatin (Figure 1.2a). The successive stages through which a myeloblast matures into circulating neutrophil granulocytes are termed promyelocytes (Figure 1.2b), myelocytes (Figure 1.2c), metamyelocytes and band forms. Cell division occurs in myeloblasts, promyelocytes and myelocytes, but not normally in metamyelocytes or band cells.

The maturation process of the neutrophil lineage is characterized by a reduction in the size of the cell, along with the development of granules containing agents essential for their microbicidal function. The nucleus also gradually begins to adopt its characteristic segmented shape (Figure 1.3).

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
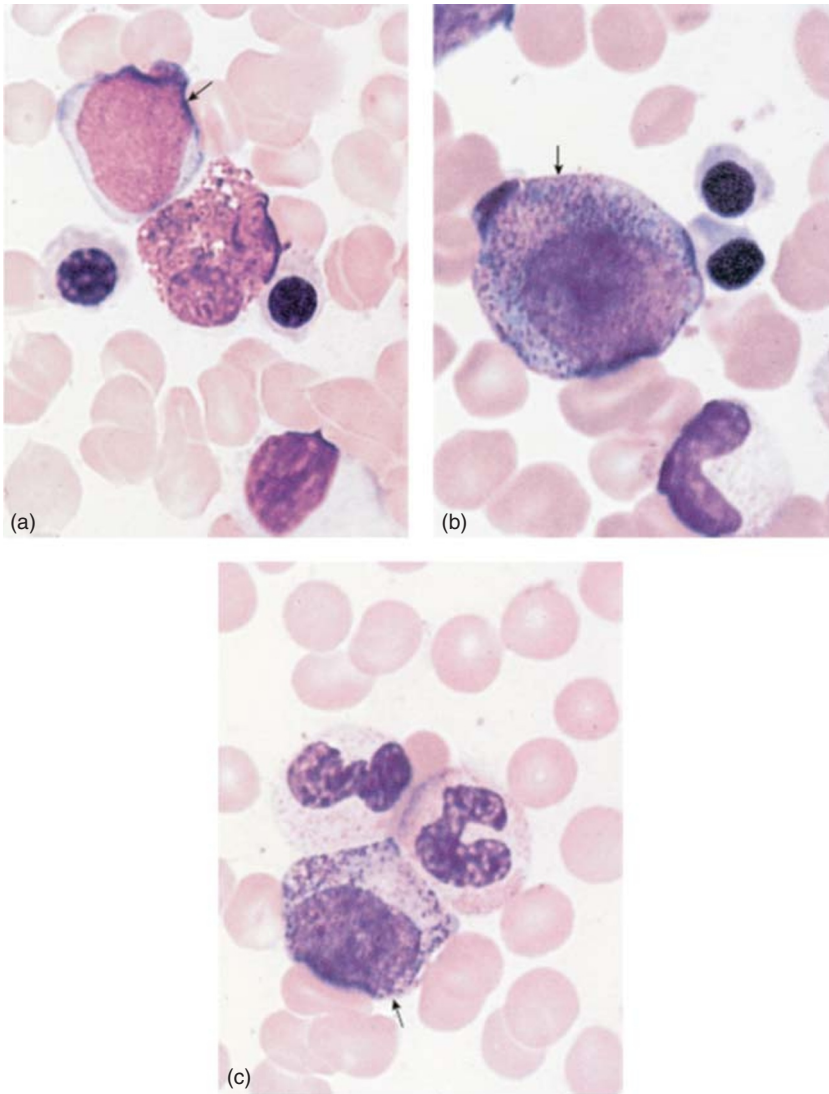
Figure 1.2 Neutrophil precursors from normal bone marrow. (a) Myeloblast (arrowed); the other nucleated cells near the myeloblast are an eosinophil granulocyte (centre) and two polychromatic erythroblasts. (b) Promyelocyte (arrowed); the other nucleated cells are two polychromatic erythroblasts and a neutrophil metamyelocyte. (c) Neutrophil myelocyte (arrowed); there are two neutrophil band cells adjacent to the myelocyte.

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 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
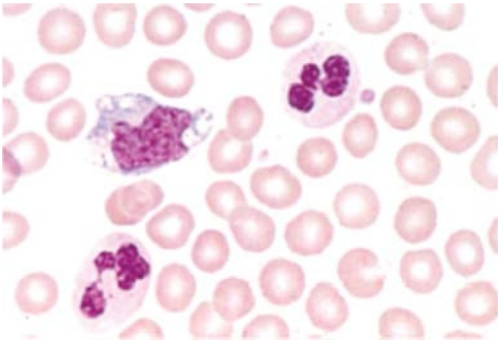
Figure 1.3 Monocyte and two neutrophil granulocytes – the monocyte has a pale, greyish-blue vacuolated cytoplasm.

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 on H&E staining, 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 specific reactive conditions.

Monocytopoiesis and monocyte function

The cell classes belonging to the monocyte–macrophage lineage are, in increasing order of maturity, monoblasts, promonocytes, marrow monocytes, blood monocytes and tissue macrophages. Their synthesis is controlled in part by the activity of GM-CSF. 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 a contribution 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 involves superoxide dependent and O_2-independent mechanisms.

Megakaryocytes and platelet function

Megakaryocytes are the cells that give rise to platelets. 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 mononucleate cells that are markedly polyploid. A mature megakaryocyte is illustrated in Figure 1.4. 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
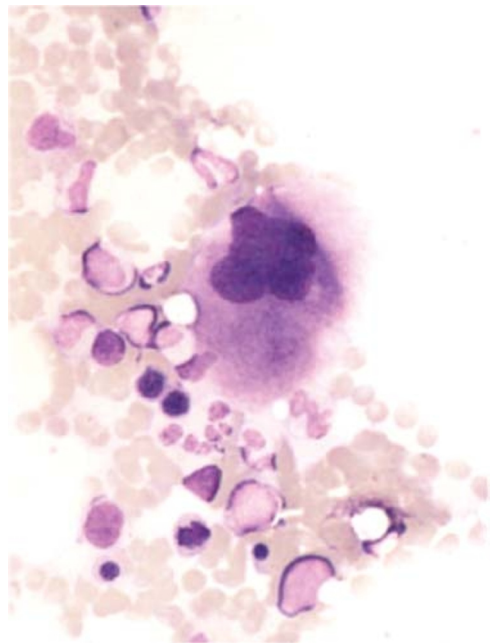
Figure 1.4 Mature megakaryocyte (centre). This is a very large cell with a single lobulated nucleus. Compare the size of the megakaryocyte with that of the other nucleated marrow cells in this figure.
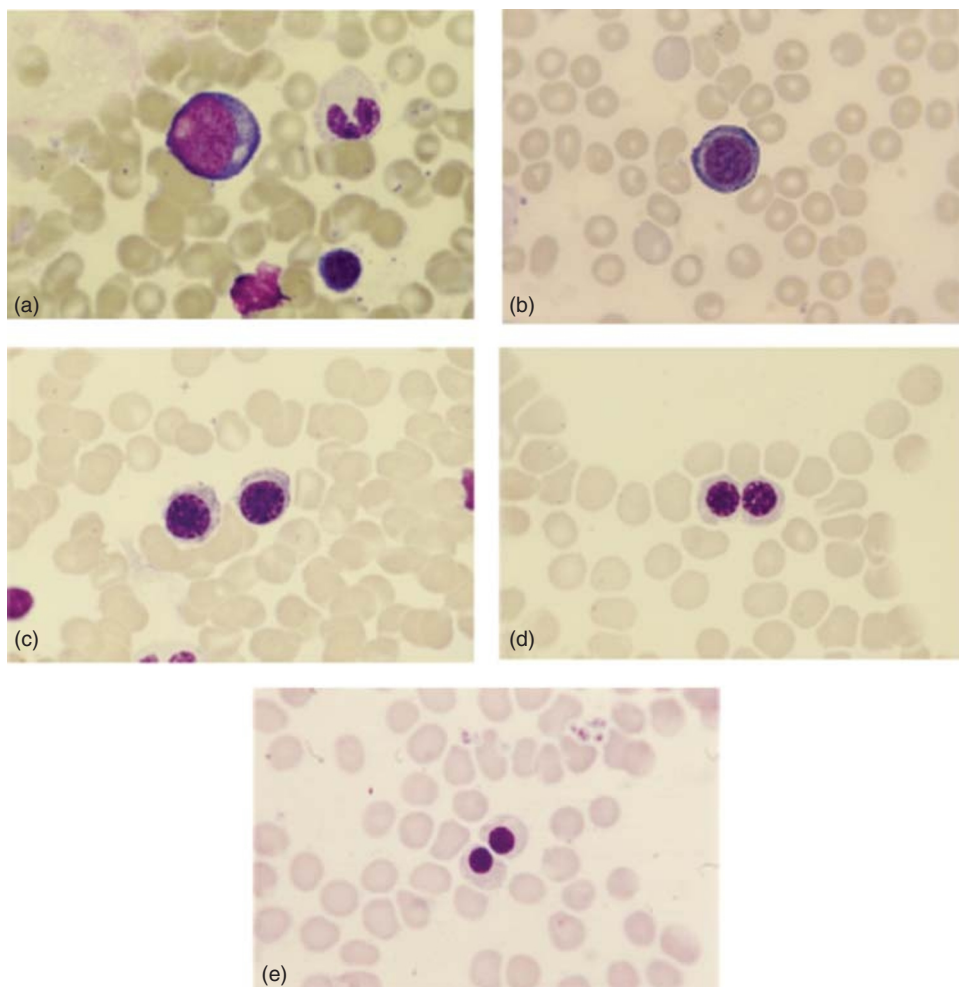
Figure 1.5 (a) Proerythroblast, (b) basophilic normoblast, (c) two early polychromatic normoblasts, (d) two late polychromatic normoblasts and (e) two more mature late polychromatic normoblasts. The condensed chromatin in the basophilic normoblast is slightly coarser than in the proerythroblast. The nuclei of the late polychromatic normoblasts contain large masses of condensed chromatin.

platelets themselves; thus, when platelet numbers are high, TPO is sequestered on the platelet membranes, 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 14).

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.
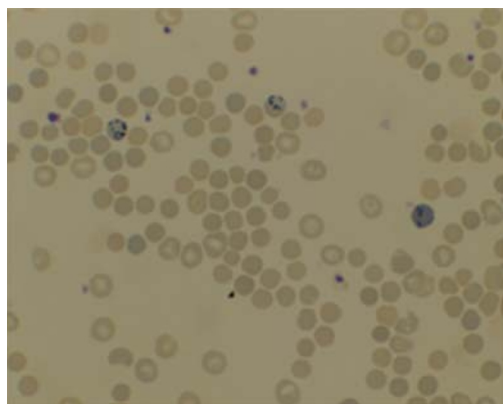
Figure 1.6 Reticulocytes in peripheral blood stained supravitally with brilliant cresyl blue. Note the reticulum of precipitated ribosomes.

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 1.5. 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 1.5). 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 1.6) 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 4.

Lymphopoiesis

The structure and function of lymphoid tissue are the focus of Chapter 6. Lymphoid cells 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.

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) 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.

T cells, by contrast, are formed in the thymus, where lymphocyte progenitors from the fetal liver migrate in early gestation. 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 T-cell receptor (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. During the process of maturation, T cells acquire both CD4 and CD8 cell surface markers (double positive thymocytes) and undergo a process of positive selection to ensure the survival only of those 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 down-regulate their CD8 expression and become CD4 T cells. A further phase of negative selection