Haematology

CLINICAL CASES UNCOVERED

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Contents

Preface

This book is a development from *Case-Based Haematology*. We have added two didactic chapters, three new cases and a number of MCQs and SAQs to help you to assess your knowledge. This book is designed to make you think. I hope it will help you, not just in haematology, but in all areas of medicine.

The basis of medical practice is still listening and looking. This book is an attempt to make you do these things well and to ask for, and interpret, appropriate investigations. Remember Denis Burkitt, who with the simple tools of observation and listening, was able to make the seminal observation which resulted in the first description of cancer caused by an infectious agent. He had no elaborate tests or DNA analysis, just his eyes and ears.

You are not expected to know the answers to all the questions, especially in the first two chapters. However, I hope you will keep this book after you graduate as a doctor and refer to it in future years.

Medicine is a very privileged profession and a very interesting one. I hope this book helps you to be a better doctor and to enjoy your medical career, wherever it may take you.

Shaun McCann

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The original stimulus to developing a teaching manual came from my old boss and now friend Professor Harry Jacob from the University of Minnesota where I had my formative training in Haematology. To him and his associates I owe a huge debt.

How to use this book

Clinical Cases Uncovered (CCU) books are carefully designed to help supplement your clinical experience and assist with refreshing your memory when revising. Each book is divided into three sections: Part 1 Basics; Part 2 Cases; and Part 3 Self-assessment.

Part 1 gives a quick reminder of the basic science, history and examination, and key diagnoses in the area. Part 2 contains many of the clinical presentations you would expect to see on the wards or crop up in exams, with questions and answers leading you through each case. New information, such as test results, is revealed as events unfold and each case concludes with a handy case summary explaining the key points. Part 3 allows you to test your learning with several question styles (MCQs, EMQs and SAQs), each with a strong clinical focus.

Whether reading individually or working as part of a group, we hope you will enjoy using your CCU book. If you have any recommendations on how we could improve the series, please do let us know by contacting us at: medstudentuk@oxon.blackwellpublishing.com.

Disclaimer

CCU patients are designed to reflect real life, with their own reports of symptoms and concerns. Please note that all names used are entirely fictitious and any similarity to patients, alive or dead, is coincidental.

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Basic science

The cellular elements in the blood are continuously destroyed and replaced. What process is required to continuously replace them and keep the number of cells within well-defined limits?

The numbers of cells produced in an individual with a lifespan of 80–90 years is astronomical (12×10^6 granulocytes are produced each day). In order to maintain haemopoiesis throughout life, a pool of resting haemopoietic stem cells (HSCs) is required. These cells, given the appropriate stimulus, can differentiate along specific pathways and produce the mature cells of the peripheral blood. Because the pool of resting stem cells must be maintained at all times, these cells must be capable of self-replication and production of progeny. Each stem cell has the capacity to differentiate into any of the cells in the blood. Hence, these stem cells are 'pluripotent' and capable of self-renewal (Fig. 1).

Where else are HSCs found besides the bone marrow?

HSCs are found in very small numbers in the peripheral blood and in large numbers in umbilical cord blood. HSCs have the appearance of a small lymphocyte.

What tragic event served as a major stimulus to stem cell research?

Much of the research that led to our understanding of haemopoiesis came from the knowledge that ionizing radiation resulted in the death of experimental animals from infection or bleeding. Thus, the development of the atom bomb served as a stimulus for many experiments. This ultimately resulted in a therapeutic manoeuvre (allogeneic stem cell transplantation), which was to test all the theories of haemopoiesis.

How does stem cell transplantation verify the existence of pluripotent stem cells?

In animals, experiments can be carried out when the HSC donor is of a different sex from the recipient. The animal is initially exposed to irradiation in order to kill all the HSCs. The animal is then 'rescued' by injecting bone marrow cells from the donor. Haemopoiesis recovers but all of the haemopoietic cells in the bone marrow and blood of the recipient are derived from the donor. These survivors were called 'radiation chimaeras' after the mythological creature, which had the head of a lion, the body of a goat and the tail of a snake (Fig. 2). Human chimaeras are created when a patient receives HSCs from a healthy donor.

What are the names of the mature blood cells in the circulation?

Erythrocytes, neutrophils, eosinophils, basophils, monocytes (Fig. 3a–f) and platelets. Erythrocytes are derived from a nucleated cell in the bone marrow called a normoblast, which undergoes a number of divisions and extrudes its nucleus before being released into the blood. Granulocytes (neutrophils, eosinophils, basophils) and monocytes are likewise derived from a nucleated cell, but lymphocytes appear to be derived from a nucleated cell, which 'differentiates' at a very early stage. Platelets are derived from giant cells called megakaryocytes.

What test is commonly used clinically to measure the frequency of HSCs in humans?

There is no commonly used test available. It is estimated that HSCs occur at the frequency of 1 in 1 million nucleated cells in the bone marrow. Most laboratories depend on a test that measures the 'committed pool' of cells, i.e.

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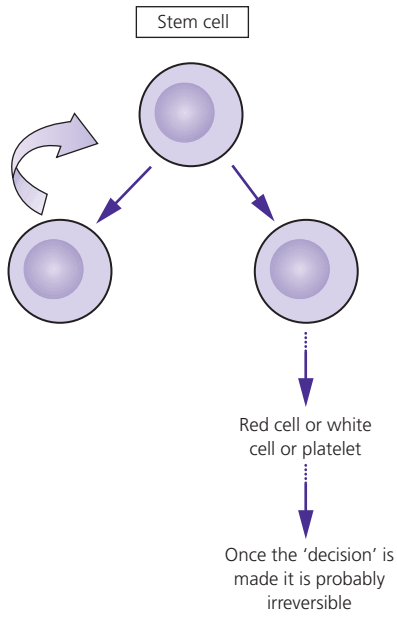

Figure 1 A schematic illustration of self-renewing stem cells.

Figure 2 Chimaera. A mythological figure with the body of a goat, head of a lion and tail of a snake. First used when mice were successfully transplanted with haemopoietic stem cells. Now used in humans after stem cell transplant to denote cells of donor or recipient origin.

primitive cells that are already committed to a particular lineage such as a red cell or a granulocyte. Mononuclear cells from the bone marrow, umbilical cord blood or peripheral blood are put into a semi-solid supporting matrix and cytokines/growth factors are added. Following *in vitro* incubation for a variable number of days, large groups of cells appear (colonies) and have the appearance of mature blood elements, e.g. red cells. Each colony represents growth from a single progenitor.

Figure 4 shows a colony of mature red cells and white cells known as a colony-forming unit – granulocyte, macrophage (CFU-GM). Figure 5 shows the different cell lineages and how they are measured. Each term, e.g. burst-forming unit – erythroid (BFU-E), refers to a colony of cells derived from a stem cell and which grows in the laboratory into a recognizable mature cell, in this case a red cell. Each colony is derived from a single stem cell.

The CD34 antigen (identified by flow cytometry) is an important clinical marker as it is a principal indicator of a pluripotent stem cell. It is expressed on haemopoietic stem cells and committed progenitor cells. The $CD34^+$ cell count is used to guide physicians when cells are being collected for stem cell transplantation.

How does a HSC differentiate into a mature blood cell?

The precise mechanism is unknown. However, it seems that cell–cell interactions (progenitors interact with mesenchymal cells in the bone marrow) and the expression of a large number of genes are important. Cytokines and growth factors may act in combination to activate signal transduction mechanisms. These intracellular factors activate the nucleus and stimulate the transcription of regulatory genes. These genes, in turn, influence proliferation, differentiation, apoptosis (programmed cell death) and development of mature cell function.

How can we recognize the potential for differentiation of HSCs?

HSCs and differentiating progenitors express antigens on their cell surface. The antigen type and frequency will change as the cell 'differentiates'. These antigens can be identified by a technique called 'flow cytometry'. The expression of different antigens allows us to identify progenitors of different lineages and to chart the development and differentiation of blood cells.

KEY POINT

The type of cell, i.e. its lineage, can be determined by the antigens on its surface. In many malignancies there is an accumulation of normal antigens in abnormal amounts.

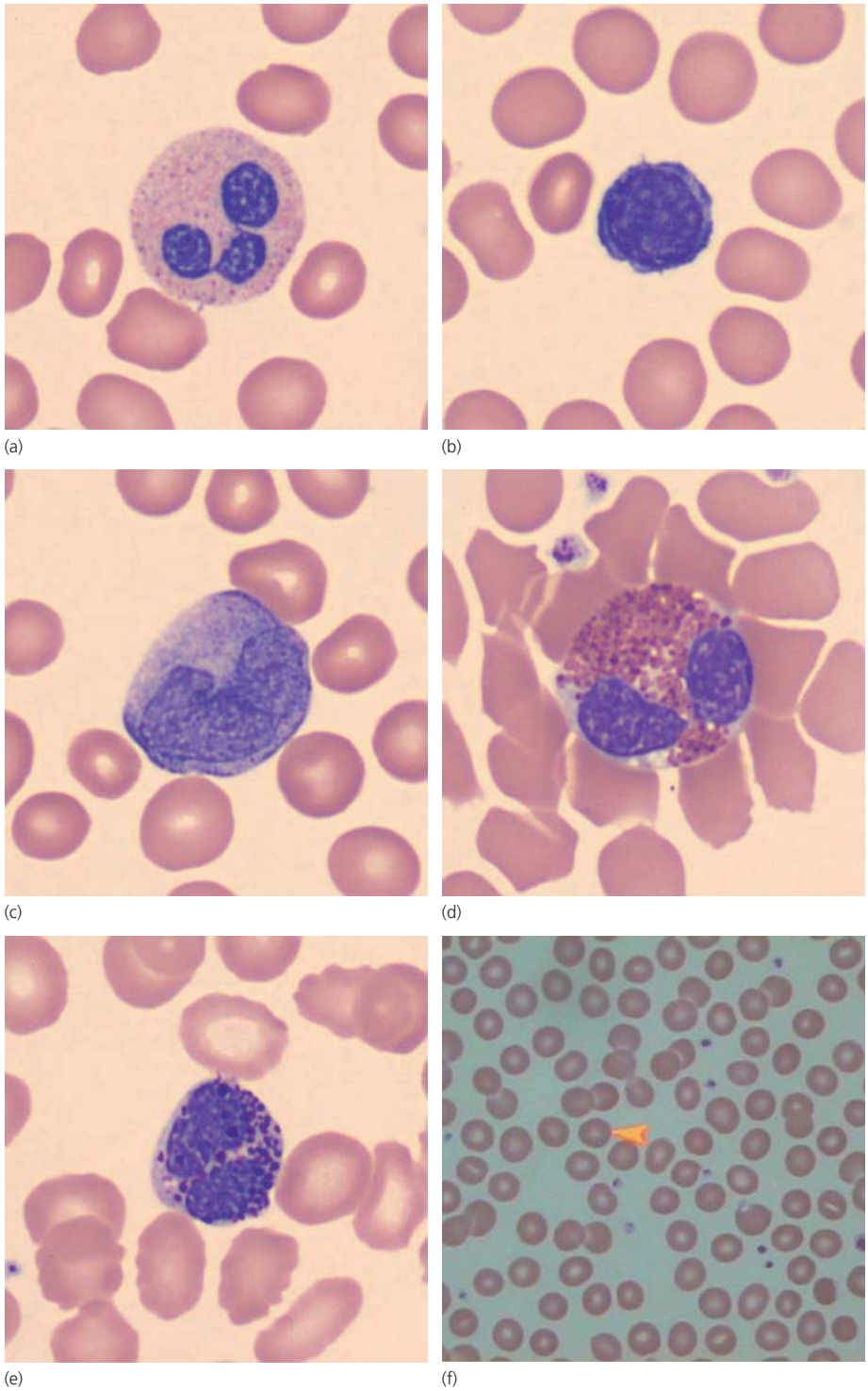

Figure 3 (a) Neutrophil, (b) lymphocyte, (c) monocyte, (d) eosinophil, (e) basophil and (f) normal erythrocyte.

Flow cytometry, or immunophenotyping, is made possible by the use of flow cytometers using the principle of hydrodynamic focusing (Fig. 6). The sample is injected, forcing the cell into the centre of the stream. As the cells intercept the light source they scatter the light and fluorochromes are excited to a higher energy state. This energy is released as a photon of light. The flow cytometer measures fluorescence per cell. After the different signals or pulses are amplified they are processed by an analogue to digital converter (ADC), which in turn allows for events to be plotted on a graphical scale (Fig. 7).

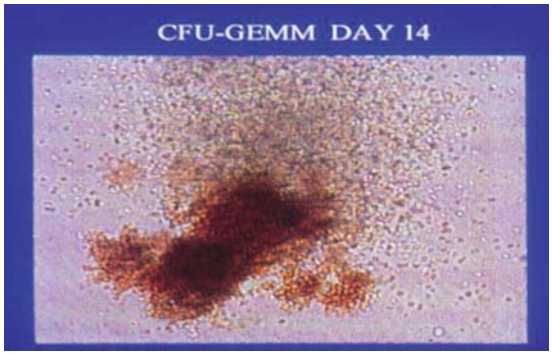

Figure 4 A colony of red cells and neutrophils grown in the laboratory and derived from a single stem cell.

Which cytokines or growth factors commonly found in the blood are available for clinical use?

Erythropoietin

Erythropoietin (EPO), a polypeptide, is the best known and is the main cytokine involved in erythrocyte differentiation, proliferation and apoptosis. It is largely produced in the kidney (Case 16). EPO production responds to hypoxia via a transcription factor complex called hypoxia inducible factor 1 (HIFI). A recombinant form is available and is used in the treatment of the anaemia of renal failure.

Granulocyte colony-stimulating factor

Granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) are glycopeptides and are secreted by granulocytes, monocytes, T lymphocytes, fibroblasts and endothelial cells. These growth factors are responsible for the production of granulocytes (G-CSF), granulocytes, eosinophils and monocytes (GM-CSF). The recombinant form of G-CSF is used in the treatment of congenital neutropenia and to 'mobilize' stem cells from the bone marrow into the peripheral circulation where they can be easily collected and used for stem cell transplantation.

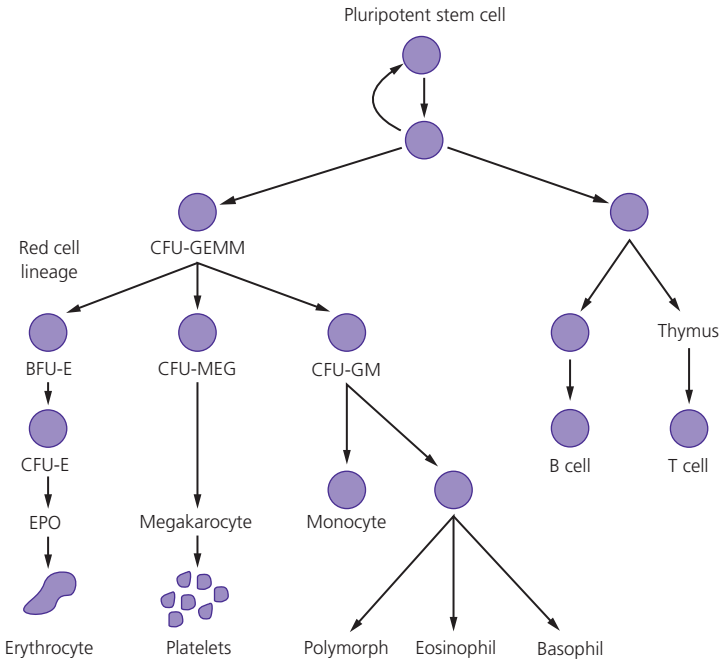

Figure 5 A schematic illustration of the different cell types, all derived from a pluripotent stem cell. Individual terms are defined in the Glossary.

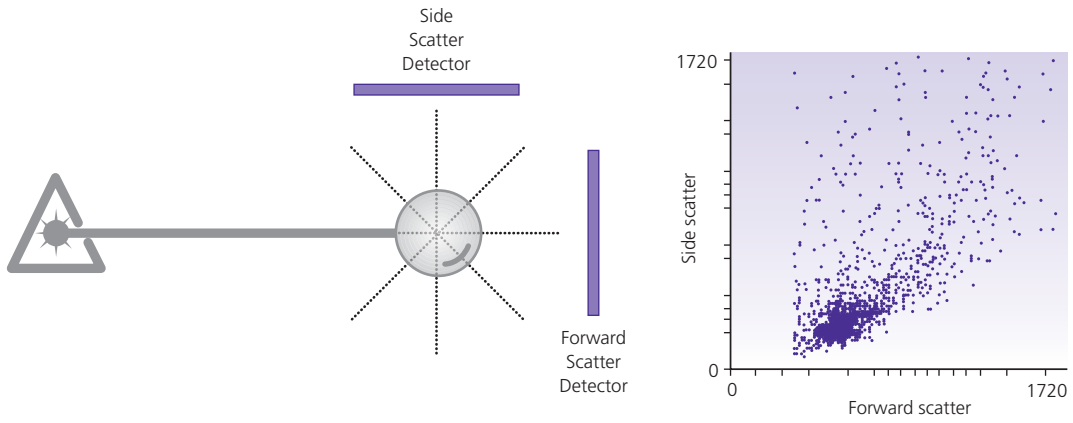

Figure 6 Flow cytometry or immunophenotyping, a method of identifying antigens on cells and thus establishing their lineage.

Flourochrom	Emission peak (nm)	Detector
FIT C	525	FL1
PE	575	FL2
PI	620	FL3

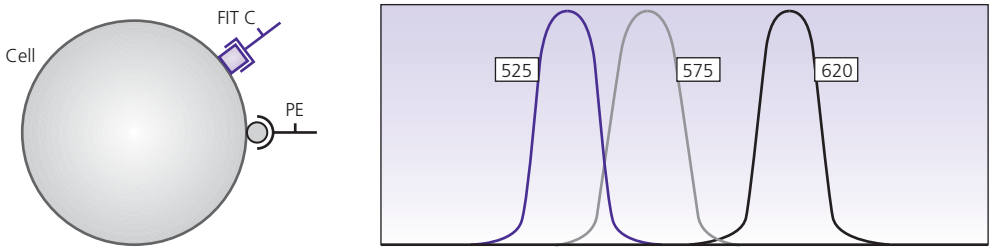

Figure 7 Flow cytometry or immunophenotyping showing the identification of antigens on cell surface using antibodies labelled with fluorochromes.

Thrombopoietin

Thrombopoietin (TPO) is a glycoprotein produced by the liver. It stimulates stem cell and platelet production. Newer formulations of recombinant TPO should soon be available to treat thrombocytopenia.

Stem cell factor

Stem cell factor is a glycoprotein produced by stromal cells and binds to the receptor c-Kit. It is essential for stem cell differentiation and proliferation.

How does apoptosis (programmed cell death) affect haemopoietic cells?

Apoptosis (programmed cell death) is a complex event, which terminates with the activation of caspases, DNA fragmentation and phagocytosis.

What is meant by the term stem cell plasticity?

Under experimental circumstances human pluripotent haemopoietic stem cells may be made to differentiate into non-haemopoietic tissues of mesenchymal origin such as muscle or cartilage. Whether this phenomenon can be reproduced in patients is unclear to date.

What major events occur during red cell (erythrocyte) development?

Fetal erythropoiesis develops in the yolk sac perhaps from a common progenitor with the endothelial cell known as the haemangioblast. The liver then predominates as the site of haemopoiesis followed by a period when the cells circulate and finally in the last 3 months of fetal life the bone marrow predominates as the major

site of haemopoiesis. Haemoglobin (Hb) synthesis in fetal life differs from childhood and adult Hb (Case 8). It seems that undifferentiated stem cells contain most of the genes associated with the different cell lineages but over time many genes become silent and others are transcribed at a higher level. The first recognizable red cell precursor is a nucleated 'normoblast'. Following cell division these cells become smaller, develop haemoglobinized cytoplasm and eventually extrude their nucleus. A mature erythrocyte is a non-nucleated biconcave disc.

As red cells precursors mature, gene expression is upregulated for blood group antigens, membrane proteins, glycolytic and haem synthetic enzymes. The final two steps are the synthesis of globin chains and haem. About 2% of circulating red cells are non-nucleated but not biconcave discs. They are larger than mature red cells, contain ribosomes and synthesize small amounts of Hb. They are called reticulocytes and mature into erythrocytes after about 48 hours.

KEY POINTS

Red cell lifespan is 120 days. Erythrocyte production rate is approximately 10^{10} per hour. EPO is the most important growth factor. EPO, following any hypoxic stimulus, binds to trans-membrane receptors and triggers a signalling system that results in red cell proliferation and inhibition of apoptosis. A critical factor is the activation of the JAK-2 kinase. Mutations in JAK-2 are now fundamental to our understanding of diseases in which proliferation of red cells, white cells and platelets predominate (Case 16).

What is the main function of the immune system and what cells predominate?

The immune system has a fundamental function in regulating defence mechanisms against foreign attacks, primarily infective agents. In addition, it also has an important role in the development of cancers (malignancy). The effectors of the immune system are represented by lymphocytes. We identify and recognize different types of lymphocytes (known as subsets) which have various intrinsic functional properties.

Lymphocytes with different functions originate from pluripotent stem cells. Humoral responses are mediated by B lymphocytes through their capacity to produce and secrete specific antibodies.

Cell-mediated immunity is regulated by T lymphocytes. During the process of physiologic development, common lymphocyte progenitors, which are derived from pluripotent stem cells, differentiate in lymphoid organs, including fetal liver and bone marrow in the case of B lymphocytes and the thymus for T lymphocytes. There is a third population of lymphocytes named natural killer (NK) cells which mediate responses against virus-infected and tumour cells.

What was the major discovery that allowed us to unravel the functional subsets of lymphocytes in the human immune system?

The turning point was the development of the monoclonal antibody (MoAb) technology in 1975 by Köhler and Milstein (who later won the Nobel Prize for Medicine) which rapidly led to the production of MoAbs directed against antigens expressed by different lymphocyte subsets (B, T, NK). This collection of antigens is known as the 'immunophenotypic' profile.

MoAbs were grouped according to the antigen identified. Thus, all the MoAbs directed against a well-defined antigen are identified according to a cluster of differentiation (CD). A table showing the MoAbs frequently used to identify different lymphocyte subsets is shown in the Appendix.

What laboratory technique is used to identify lymphocyte subsets?

Flow cytometry (Fig. 8).

What is the major function of B lymphocytes?

B lymphocytes are important in 'humoral immunity'. They produce antibodies also called immunoglobulins (Igs). These are glycoproteins that bind to specific antigens, neutrophils, lymphocytes and basophils. The antigen is initially processed by antigen presenting cells (APCs, monocytes and macrophages) and T cells are activated. In turn, the B lymphocyte proliferates and differentiates into a plasma cell, which produces specific antibody which will destroy or opsonize the antigen.

What is meant by the term opsonization?

Opsonization is when antibodies make a pathogen ready for digestion.

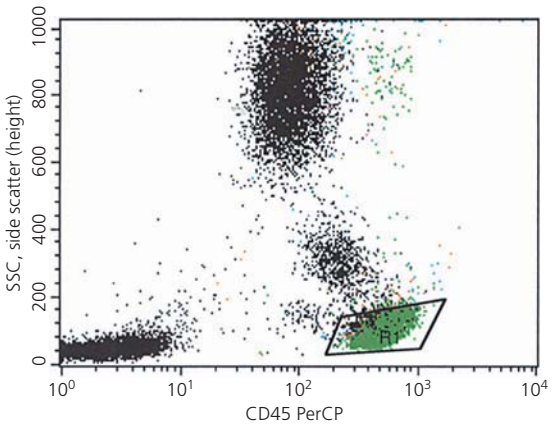

Figure 8 A dot plot of white cells labelled with an antibody to CD45 (green), an antigen present on all white cells.

Table 1 B-cell surface antigens at different stages of differentiation.

Immature B cells	CD10 (CALLA)
B cells beginning heavy chain rearrangement	CD19, TdT
B cells beginning light chain rearrangement	CD20, surface Ig

CALLA, common acute lymphoblastic leukaemia antigen; Ig, immunoglobulin; TdT, terminal deoxynucleotidyl transferase.

How do B cells recognize antigens and produce specific antibodies?

Each antibody (Ig) molecule has two heavy and two light chains. During early development in the bone marrow the genes for heavy and light chains are rearranged in that order. Once rearrangement is complete, the cell will express the antibody molecule on its surface. Mature B lymphocytes express IgM and IgD on their cell surface, which is important for cell survival. The cell surface antigen profile is shown in Table 1. Maturing B lymphocytes leave the bone marrow and migrate to lymph nodes where they congregate in an area called the germinal centre. Having recognized an antigen by binding to it, the B lymphocyte then undergoes a process called somatic hypermutation, i.e. mutations (rearrangements) occur in the variable region genes. The cells will divide and proliferate into plasma cells or B memory cells and produce specific antibody, as shown in Table 1.

How do T cells exert their function?

T cells are important in 'cell mediated immunity'. T-cell maturation occurs in the thymus. There are four T-cell receptor (TCRs) genes: α, β, γ and δ. Like in the B lymphocyte, the TCR genes undergo rearrangement so that a mature T cell will only express α/β or γ/δ (5%) receptors on its surface. The TCR recognizes a major histocompatibility complex (MHC) molecule and the T cell leaves the thymus. T memory cells provide the immune system with a memory so that these cells will rapidly proliferate if subsequently exposed to the same antigen.

What major events take place during white blood cell development?

Granulocytes are white blood cells (WBCs), neutrophils, eosinophils and basophils found in the circulation. Granulocytes are derived from haemopoietic stem cells in the bone marrow under the influence of cytokines (G-CSF is the most important). Neutrophils have a lifespan of around 7 hours in the circulation and leave the circulation as part of the inflammatory reaction. There is a large reserve of granulocyte precursors in the bone marrow. Normal individuals can increase the production of WBCs by 10–20 times. Approximately 50% of the granulocytes and monocytes are 'marginating', i.e. adherent to the sidewall of blood vessels but are 'available' if required. The predominant granulocyte is the neutrophil and its function is phagocytosis of microorganisms.

KEY POINT

Following bacterial infection, neutrophils are attracted by chemotactic factors. Neutrophils ingest antibody and complement-coated bacteria to form a phagosome. The neutrophil degranulates and various enzymes are released. H_2O_2 is produced and interacts with O_2^- in the presence of iron to generate singlet oxygen and hydroxyl radicals, both of which are toxic to bacteria.

What other types of granulocytes are present in the circulation?

Eosinophils and basophils are also derived from haemopoietic stem cells in the bone marrow. Eosinophils are important in the response to parasitic infection, allergy and drug reactions. Basophils are also implicated in allergy and drug reactions.

What other WBCs besides granulocytes are important and what is their function?

Monocytes are also derived from a haemopoietic stem cell in the bone marrow under the influence of the cytokine GM-CSF and are very closely related to granulocytes. They have a similar phagocytic and killing function (enhanced by GM-CSF), and are found in the spleen and liver as well as the circulation.

What other cells are found in the blood besides erythrocytes and white cells?

Platelets. These are very small non-nucleated structures derived from the 'shedding' of the cytoplasm of giant cells in the bone marrow called megakaryocytes. Megakaryocytes are polyploid (increased DNA content) and have multilobed nuclei. Unlike other bone marrow cells, megakaryocytes become larger as they mature. Platelets have a lifespan of about 7 days.

What is the function of platelets?

The principal function of platelets is to enhance the generation of thrombin (blood clotting). They do this by acting as a catalytic surface. The important structural elements of this catalytic surface include: (i) plasma membranes rich in glycoproteins and phospholipids; and (ii) secretory granules. The plasma membrane is a highly reactive surface on which haemostatic (procoagulation and anticoagulation) reactions can take place.

The plasma membrane is predominantly in deep invaginations and the main glycoproteins are GP IIb–IIIa (the most plentiful). GP IIb–IIIa acts as a receptor for fibrinogen. GP Ib–IX–V is the major von Willebrand receptor and binding is the initial step that localizes platelets to the site of vascular injury.

The phospholipids in the platelet membrane provide the surface to mediate Ca^{2+} dependent binding of vitamin K dependent coagulation factors through their γ-carboxyglutamic acid residues.

Platelet granules (α granules, dense granules and lysosomes) contribute to platelet adhesion and aggregation, and to blood clotting. For a diagram and explanation of blood clotting see Case 18.

KEY POINT

Platelet adhesion and aggregation are sufficient to stop bleeding from small vessels. Coagulation factor activation and fibrin formation, together with platelet aggregation and adhesion, are required to stop bleeding from larger wounds.

Approach to the patient

Many patients are seen initially in a primary care setting and may have vague symptoms. In the hospital the majority of patients are referred for investigation and expert opinion because of abnormal blood test results. Others are referred to the haematologist because abnormal blood test results occur during the diagnosis or management of another apparently unrelated condition.

As always, the most important diagnostic event is the history-taking.

What should be your initial interaction with the patient?

In an ambulatory patient it is important to observe the patient's general demeanour and ease of movement. It is important to greet the patient by asking their name while offering to shake hands at the same time. (In some cultures it is impolite for a man to shake hands with a woman.) You should introduce yourself by your full name and title.

Why is it important to observe the patient and what can you learn from a handshake?

You can observe if the gait is steady and if the patient is in pain when they walk. An unsteady gait might suggest a peripheral neuropathy or subacute degeneration of the spinal cord seen in vitamin B_{12} deficiency (Case 3). Pain may be bony and reflect malignant disease or a fracture such as in multiple myeloma. A dorsal kyphosis should also arouse the same suspicion (Case 13). A handshake provides information about the patient's state of mind. Excessive sweating may be the result of nervousness or hyperthyroidism. Rheumatoid disease and other forms of arthritis may be evident. As you shake hands you can carry out a rapid general inspection noting the presence of jaundice, seen in haemolysis or cyanosis, or plethora in erythrocytosis.

Why should you ask the patient their name?

Although you might know the patient's name it is important to ask. First, simply to make sure the patient you are interviewing is who you think he/she is. Secondly, to test the patient's memory and ability to understand and speak. Providing the patient with their name and asking for confirmation is not adequate as he/she might affirm without understanding the question. Chronic alcoholism, multiple small strokes, Alzheimer's disease or vitamin B_{12} deficiency can impair memory or understanding.

Why should you introduce yourself by name and title?

It is important for the patient to know your name as it provides a sense of security and gives the patient confidence. Your title is important as it also lets the patient know precisely with whom they are dealing. Never call a patient by their first name unless they ask you to as it conveys disrespect or over-familiarity. You can be friendly and put the patient at their ease without using first names.

Frequently a patient will ask you if they may bring a spouse, relative or friend into the interview. What should be your response?

Gently but firmly you should deny the request. A second person can often inhibit the patient from revealing certain details about their complaint to avoid embarrassment. Likewise you could be inhibited from asking certain questions. Tell the patient that it would be perfectly acceptable to bring in their friend/relative at the end of the interview when you can explain the probable

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