network of blood vessels that both supply blood to the bone and form a route of egress for mature blood cells. The ECM comprises a network of fibrous proteins, glycoproteins and proteoglycans including various forms of collagen, fibronectin, laminin, haemonectin, tenascin and thrombospondin. The complete structure comprising the ECM and all of the cells of the bone marrow is known as the haemopoietic micro-environment, because all of these cells and structures interact to provide an optimal micro-environment for haemopoiesis. The non-haemopoietic cells of the haemopoietic micro-environment constitute the marrow stroma.

Several important facets of haemopoiesis are regulated by the marrow stroma, as follows.

- Many of the stromal cells (e.g. endothelial cells, fibroblasts, macrophages) are important sources of haemopoietic growth factors. The close proximity of the stromal cells to the haemopoietic progenitors facilitates paracrine growth factor activity.
- The ECM plays an important role in supporting haemopoiesis by providing a structural framework within the marrow that cells can adhere to and grow on. It may also be involved in compartmentalization of haemopoietic tissue within the marrow space.
- The marrow micro-environment plays a central role in the regulation of the release of mature blood cells to the circulation. The endothelium that lines the bone marrow sinuses is tightly packed and so normally permits only the most mature cells (e.g. red blood cells, neutrophils, platelets) to exit the marrow space. To enter the bloodstream, cells must traverse pores in the marrow vascular endothelium that are much smaller in diameter than a red blood cell. Only anucleate cells (red blood cells, platelets) or cells with a highly distensible nucleus (granulocytes) can ordinarily leave the marrow. All of the maturing blood elements have large, rigid nuclei that restrict them to the marrow.
- The adhesion molecules present on cells and the ECM also play a critical role in retaining immature cells within the marrow.
- Elements of the marrow haemopoietic micro-environment are important for the phenomenon known as stem cell 'homing'. Circulating haemopoietic stem cells are selectively attracted to and retained by the bone marrow, where they can proliferate optimally. This mechanism is important because it ensures that haemopoiesis occurs in the most hospitable environment. It is this process that is thought to govern the transfer of the sites of haemopoiesis from embryonic yolk sac to fetal liver, and from spleen to fetal bone marrow during early development. Homing is also important clinically (see *Box 2.8*).
- The ECM can be induced to selectively release haemopoietic stem cells into the circulation in response to injury or inflammation. These stress events trigger activation of neutrophils, resulting in the release of proteolytic enzymes including elastase and matrix metalloproteinases. These substances degrade and inactivate adhesion molecules, including SDF-1, VLA-4 and P/E selectins, responsible for selectively binding haemopoietic stem cells to the ECM. As a result, haemopoietic stem cells are released into the circulation. This process, known as stem cell mobilization, is exploited clinically to harvest haemopoietic stem cells for transplantation. Treatment of a donor with recombinant granulocyte colony-stimulating factor (G–CSF) markedly increases the number of haemopoietic stem cells in the peripheral blood. These can be collected by apheresis, avoiding the need for bone marrow sampling.
- The bone marrow serves as an important reservoir for mature neutrophils. In a healthy individual, over 95% of the mature neutrophils in the body are in the bone marrow, ready to be released rapidly when required, e.g. in response to bacterial infection. These neutrophils are said to be in the bone marrow's neutrophil storage pool. Bacterial endotoxin, immune complexes, and cytokines like GM–CSF and G–CSF are all capable of stimulating rapid release of the marrow storage pool of neutrophils into the peripheral blood.

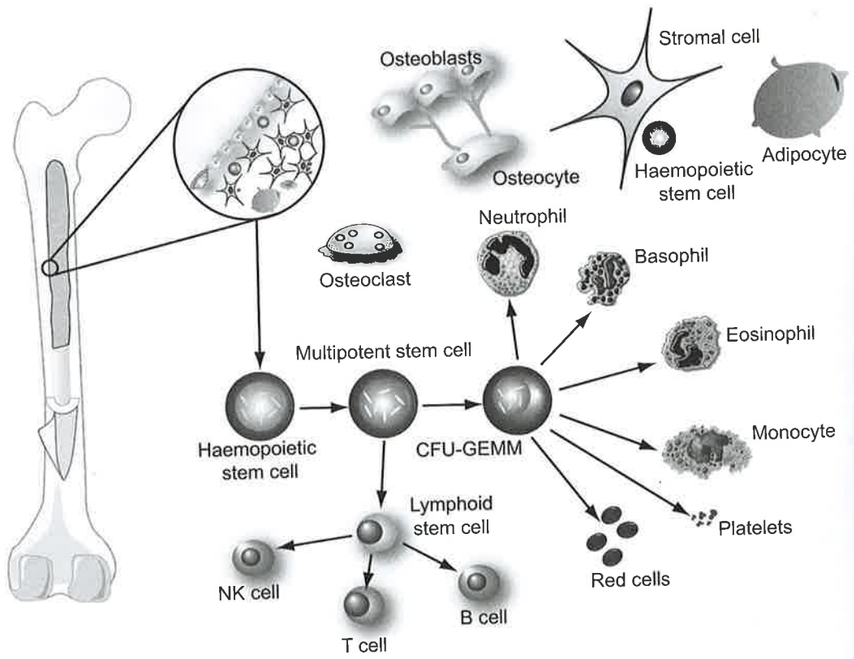

Figure 2.7
Cells of the haemopoietic micro-environment. The term stromal cell encompasses fibroblasts, endothelial cells and marrow macrophages. In some settings, the term stromal cell is used to encompass all non-haemopoietic cells found in the bone marrow.

Box 2.8 Homing and haemopoietic stem cell transplantation

The phenomenon of stem cell homing is exploited clinically in haemopoietic stem cell transplantation. In essence, patients with haematological malignancies are treated with high-dose chemotherapy to partially or completely ablate their bone marrow and they are then 'rescued' by venous infusion of allogeneic or autologous haemopoietic stem cells. The stem cells circulate in the blood only for a short time before they home to the bone marrow spaces and repopulate haemopoiesis.

2.5 ERYTHROPOIESIS

Maintenance of the circulating red cell mass within the narrow limits seen in health is achieved by a feedback mechanism, which senses body oxygen demands (tissue hypoxia) and delivery, and adjusts the rate of erythropoiesis accordingly. This feedback mechanism, mediated by the glycoprotein hormone erythropoietin is, for reasons explored in the next chapter, imperfect in pathological conditions but, when working physiologically, does so as follows.

- A fall in the circulating red cell mass leads to decreased haemoglobin, which in turn leads to reduced delivery of oxygen to the tissues, and hypoxia develops.
- Tissue hypoxia is sensed by an enzyme-linked mechanism in the kidney (see *Box 2.9*) and increased synthesis of erythropoietin (EPO) by the peritubular endothelial cells of the kidney is stimulated. There are other minor sites that can be called into play, but this is the main one by far.
- EPO binds to specific receptors on BFU-E and CFU-E in the bone marrow, resulting in a shortening of cell-cycle time, an increased rate of maturation and an increased rate of release of red cells from the bone marrow.
- The resulting increased red cell mass, and hence [Hb], improves oxygen delivery to the tissues, the hypoxia is corrected and EPO synthesis is decreased.

The EPO gene is located at 7q21–q22. The feedback regulation of erythropoiesis is considered in more detail in *Chapter 3*. *Figure 2.8* shows a lineage tree for normal erythropoiesis and the progenitor cells that are influenced by EPO.

Box 2.9 Erythropoietin and hypoxia

The question of how the kidney senses tissue hypoxia and triggers transcription of erythropoietin has recently been elucidated. The first step came in 1992 when Semenza and Wang identified a novel transcription factor that bound to the EPO gene and induced transcription. They called this new protein hypoxia inducible factor-1 (HIF-1). It was subsequently shown that, in the presence of oxygen, hydroxylation of specific proline residues in the α chain of HIF-1 triggers binding of von Hippel–Lindau tumour suppressor protein (VHL). The resulting complex is rapidly degraded by the proteasome. In the absence of oxygen, prolyl hydroxylation and proteasomal degradation are slowed, resulting in stabilization and accumulation of HIF-α. The HIF-α subunit translocates to the nucleus where it dimerizes with HIF-β, binds to the hypoxia response elements of HIF-target genes (such as EPO), and activates their transcription. We now know that HIF proteins are closely involved in many different physiological responses to hypoxia, including glycolysis, inflammation and neutrophil apoptosis.

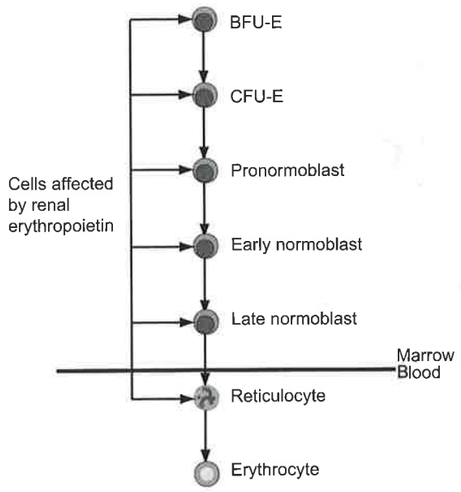

Figure 2.8
The impact of erythropoietin on erythropoiesis.

2.6 GRANULOPOIESIS AND MONOPOIESIS

Granulocyte and monocyte production is regulated by the combined actions of haemopoietic growth factors. IL-3 and GM–CSF act together on CFU-GEMM to stimulate production of CFU-GMEo. The action of G–CSF on these cells stimulates neutrophil production, M–CSF stimulates monocyte production and IL-5 stimulates eosinophil production. Basophil production is stimulated by the action of IL-3 on CFU-GEMM. A lineage tree for normal granulopoiesis and monopoiesis is shown in *Figure 2.9*.

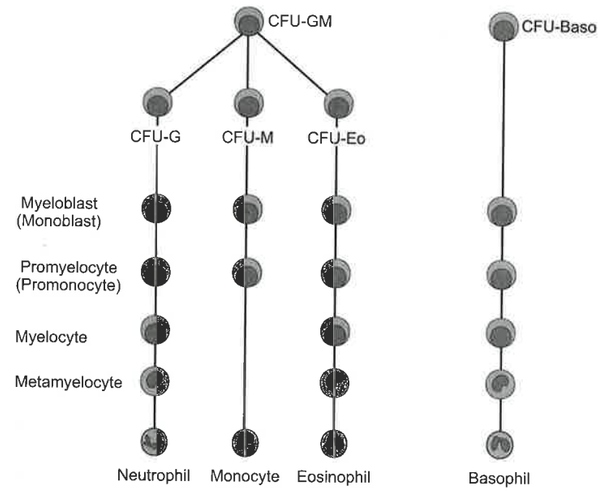

Figure 2.9
Lineage tree for granulopoiesis and monopoiesis.

2.7 THROMBOPOIESIS

Megakaryoblasts are formed from CFU-Meg by a unique process called **endomitotic replication**. In this process, DNA replication and expansion of cytoplasmic volume occur, but not cellular division. Thus, with each complete cycle of endomitosis, the cell becomes progressively larger and increasingly polyploid. Morphologically recognizable megakaryocytes may have up to 64*n* DNA content (i.e. 32 times the normal diploid (2*n*) content).

Once endomitotic replication has ceased, the megakaryocyte nucleus becomes lobulated and the cytoplasm matures, with the formation of ribbon-like structures that project through the endothelium into the venous sinus. It is from the ends of these projections that the platelets are shed into the circulation. This process is estimated to take from 2 to 3 days. Each megakaryocyte is capable of producing between 2000 and 7000 platelets.

Megakaryocyte proliferation and differentiation is stimulated by a glycoprotein hormone called thrombopoietin (TPO). This hormone is synthesized in the liver by parenchymal cells and sinusoidal epithelial cells, and in the kidney by proximal convoluted tubule cells. TPO is also synthesized by striated muscle and bone marrow stromal cells. The TPO gene is located at 3q26.3–27. Abnormalities of 3q are frequently found in a range of haematological malignancies.

A lineage tree for normal thrombopoiesis is shown in *Figure 2.10*.

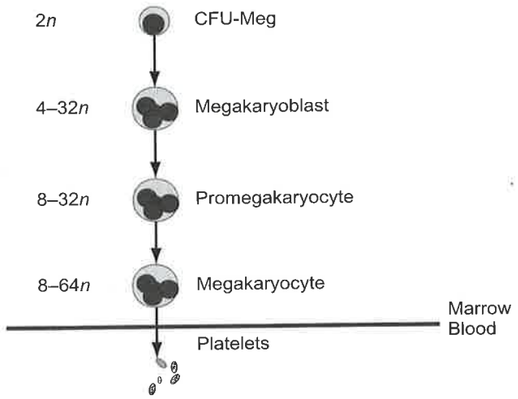

Figure 2.10
Lineage tree for thrombopoiesis.

2.8 LYMPHOPOIESIS

In contrast to the other forms of haemopoiesis, lymphopoiesis is not a one-way process of differentiation and maturation into end-stage cells. Two distinct phases of lymphopoiesis are distinguishable:

- antigen-independent differentiation, in which the lymphoid stem cell differentiates to form mature antigen-committed lymphocytes. This process occurs in the primary lymphoid organs: T lymphocyte differentiation occurs in the thymus gland, while B lymphocyte differentiation takes place in the fetal liver and adult bone marrow. As the name suggests, antigen-independent differentiation is the process that populates the body with B and T cells that are capable of responding to antigen, but is not driven by antigen exposure. The T and B cells produced by this process are known as naïve or virgin cells because they have not yet encountered antigen.
- antigen-dependent differentiation occurs when a naïve T or B cell encounters and recognizes a foreign antigen. Binding of an antigen triggers the T or B cell to undergo a process called 'blast transformation' that includes a burst of proliferation to form a clone of cells specifically able to target the offending antigen. This process is an important component of the immune response to a foreign antigen. Antigen-dependent differentiation occurs in secondary lymphoid tissue such as the spleen, lymph nodes and mucosa-associated lymphoid tissue (MALT).

B lymphopoiesis

Antigen-independent B cell differentiation

During antigen-independent B cell differentiation, there are a series of important changes in the developing cells that prepare them for their role in immune surveillance. A central feature of the human immune system is that, for every conceivable foreign antigen, a B cell already exists that carries a specific surface receptor that can recognize it. This requires that there are hundreds of millions of different B cell receptor molecules available and, because each B cell only carries one type of receptor, that there are millions of distinct B cells circulating around the body.

As shown in *Figure 2.11*, the different stages of B cell differentiation include:

- precursor B cells that have undergone immunoglobulin gene rearrangement, but are not yet expressing immunoglobulin in their cytoplasm and carry no surface immunoglobulin receptor;
- immature B cells in which immunoglobulin heavy chains are expressed in the cytoplasm of the cell but not on the cell surface;
- naïve B cells, which represent the final stage of antigen-independent B cell differentiation. These cells carry two forms of immunoglobulin surface receptor: IgM and IgD, and are capable of recognizing and responding to, but have not yet been exposed to, foreign antigen.

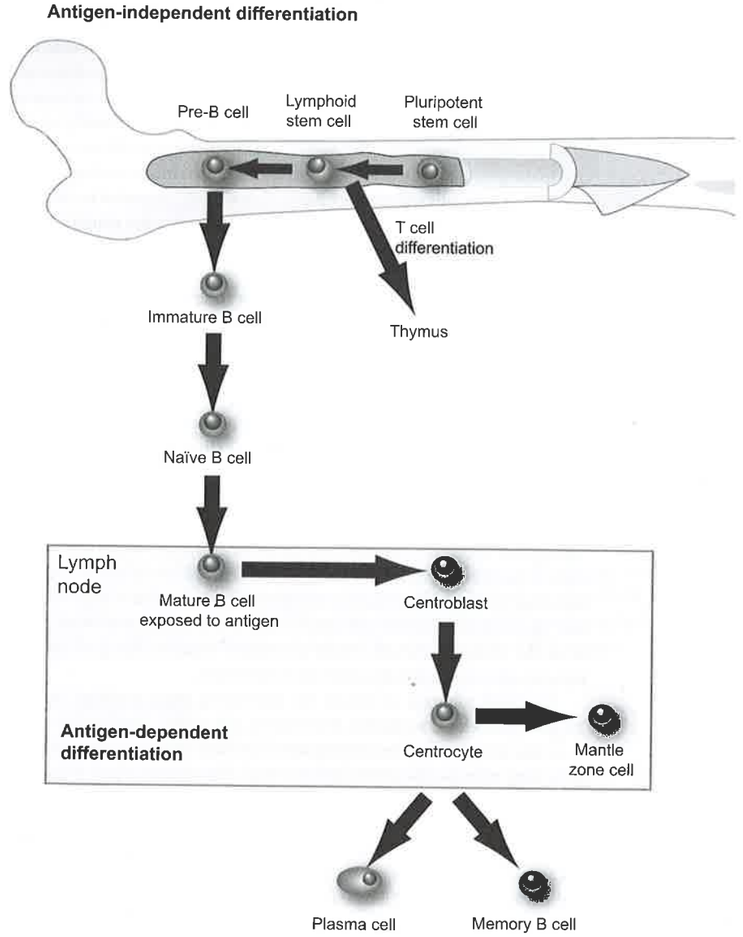

Figure 2.11
The stages of B cell differentiation.

Naïve B cells circulate in the bloodstream and are also found in relatively small numbers in primary lymphoid follicles and follicle mantle zones.

Antigen-dependent B cell differentiation

As the name suggests, antigen-dependent B cell differentiation represents a second wave of differentiation following encounter of a naïve B cell with foreign antigen, and is an important component of the immune response.

Following engagement of a B cell receptor with antigen, the cell proliferates to form a clone of lymphocytes that mature into plasma cells and which mount a specific antibody response to the inducing antigen. This stage of antigen-dependent B cell differentiation, sometimes known as the immunoblastic reaction, occurs in the paracortical region of lymph nodes, and leads to IgM-producing plasma cells accumulating in the medullary cords.

Within a few days of the immunoblastic reaction a second reaction, known as the germinal centre reaction, occurs. During this reaction, proliferating B cells differentiate into centroblasts and undergo a process called somatic hypermutation of the immunoglobulin genes, that increases the antigen affinity of the antibody that they will eventually secrete.

Centroblasts mature into centrocytes, which no longer proliferate. These cells interact with T cells in the germinal centres of lymph nodes and differentiate into high-affinity immunoglobulin-secreting plasma cells and memory B cells. Some B cells generated by the germinal centre reaction migrate outwards from the lymphoid follicle to populate the marginal and mantle zones of the lymphoid follicle. These post-germinal centre B cells are capable of rapid immune responses if re-challenged with their inducing antigen.

Some of the pre-plasma cells formed by the germinal centre reaction migrate from the lymph node to the bone marrow, where they mature into antibody-secreting plasma cells. A proportion of the pre-plasma cell population become resident in the lymph node and mature to form nodal plasma cells.

All of the B cell differentiation processes described for lymph nodes also occur in mucosa-associated lymphoid tissue (MALT), such as Waldeyer's ring, Peyer's patches, and mesenteric nodes. B cells and plasma cells formed in MALT circulate but return preferentially to their site of origin rather than to lymph nodes.

B cell markers

Throughout B cell development, the proteins expressed within the cell cytoplasm and on its surface membrane evolve and change. This fact can be exploited in the laboratory to identify each stage of B cell differentiation, by identifying the pattern of these 'cell markers' carried by the cell. These markers include cell surface receptors, enzymes, and other proteins in the cytoplasm or on the surface of the cell, whose presence or location change during differentiation and the presence of immunoglobulin gene rearrangements.

The most common technique for identifying these markers, immunophenotyping, uses 'tagged' monoclonal antibodies that bind to and enable visualization of the markers present in or on the cell. Immunophenotyping can be performed on cell suspensions or on biopsy material from bone marrow, blood or lymphoid tissue. The ways in which some of the most useful B cell markers change during differentiation is shown in *Figure 2.12*.

The earliest change that marks a precursor cell as belonging to the B cell lineage is the rearrangement of the μ immunoglobulin heavy chain genes located on chromosome 14q32. This is followed by rearrangements of the κ immunoglobulin light chain genes on chromosome 2p12, and the λ immunoglobulin light chain genes on chromosome 22q11. The defining feature of the pre-B cell is the appearance of μ immunoglobulin heavy chains in the cytoplasm of the cell. As the pre-B cell continues to differentiate, intact IgM and IgD immunoglobulin are expressed on the cell surface, which act as the B cell antigen receptor.

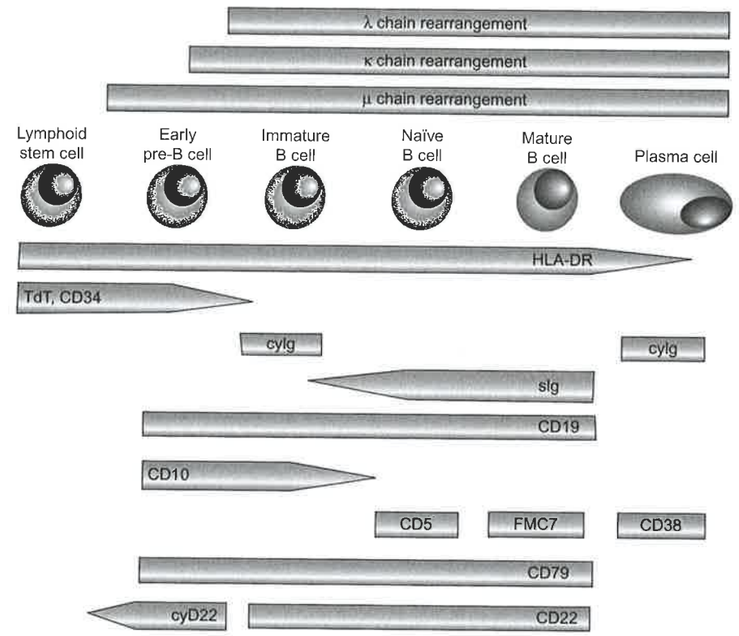

Figure 2.12
Changes in B cell markers during B cell differentiation (adapted from Rezuke *et al.* 1997).

A variety of cellular antigens are expressed in a predictable way as B cells differentiate. These are designated by CD (cluster of differentiation) numbers, e.g. CD10 as shown in *Figure 2.12*. The earliest antigens expressed in B cells are the nuclear enzyme terminal deoxynucleotidyltransferase (TdT), and the cell surface antigen HLA-Dr. Neither of these antigens is specific to B cells; they can also be found in other cell types. However, their presence is a useful indicator of the stage of differentiation. As the cell matures, antigens that are specific to B cells such as CD19, CD20, and CD10 are expressed. The terminally differentiated plasma cell can be recognized by the absence of the majority of B cell-associated antigens and the presence of the CD38 antigen.

T cell differentiation

Antigen-independent T cell differentiation

All T cells are derived from haemopoietic stem cells in the bone marrow. During fetal development, T lymphoid stem cells (CFU-L) populate the subcapsular region of the thymus gland where, under the influence of epithelial nurse cells they proliferate and differentiate. The progeny of these lymphoid stem cells progress through the cortical and medullary regions of the thymus and, from there, enter the circulation as mature T cells. During their journey, the T cells acquire surface receptors that are responsible for the specificity of their function.

In the process of differentiation into fully mature T cells, cortical T lymphoblasts progress through four distinct phases. The most primitive cortical T lymphoblasts express the intranuclear enzyme TdT and the surface marker CD7. The earliest events in the process of differentiation are

the rearrangement of the T cell receptor genes in a manner analogous to immunoglobulin gene rearrangement in B cells, and the expression of the adhesive molecule CD2. These markers of differentiation are associated with large cortical thymocytes. The next stage of differentiation involves the acquisition and simultaneous expression of the accessory molecules CD4 and CD8. CD4 is responsible for recognition of MHC class II molecules while CD8 recognizes MHC class I molecules. These accessory molecules play an important role in the 'selection' of T cells within the thymus gland.

T cell selection is an important protective mechanism that helps to ensure that circulating T cells do not react against self-antigens. The vast majority of T cells produced in the thymus die in the process of maturation and are never released into the circulation. It is thought that all immature T cells are predestined to undergo apoptosis in the thymus unless they are specifically selected for 'rescue'. There are two stages in this selection process.

1. Positive selection occurs in the thymic cortex where the CD3+ CD4+ CD8+ thymocytes are exposed to MHC class I and II molecules on the surfaces of cortical epithelial cells. Any T cell that fails to recognize self-MHC molecules will undergo apoptosis. This step ensures that circulating T cells can only recognize foreign antigens expressed in association with self-MHC class I or II molecules on the surface of antigen-presenting cells.
2.. Negative selection occurs in the thymic medulla and involves T cells at the next stage of differentiation when they express either CD4 or CD8, never both. Here, they come into contact with antigen-presenting cells that carry processed host antigens in association with self-MHC molecules. Any T cell that recognizes and binds strongly to self-antigen undergoes apoptosis. This step ensures that circulating T cells do not recognize self-antigens expressed in association with self-MHC class I or II molecules on the surface of antigen-presenting cells.

Thymocytes that survive both positive and negative selection exit the thymus as naïve T cells and are characterized by expression of CD62L and absence of the activation markers CD25, CD44 and CD69. These cells circulate in the blood but are preferentially attracted to lymph nodes and other secondary lymphoid tissues, a process known as 'homing'.

Antigen-dependent T cell differentiation

Foreign antigen is recognized and processed by antigen-presenting cells such as dendritic cells, which then migrate to lymph nodes and other secondary lymphoid tissues where they present processed antigen to naïve T cells. If a naïve T cell binds strongly to a presented antigen, it undergoes a burst of proliferation to produce an expanded clone of cells capable of binding to the inducing antigen. These activated T cells undergo further differentiation and migrate to sites of inflammation where they help to direct various facets of the immune response.

NK cell differentiation

NK cells are derived from haemopoietic stem cells, but the stages involved in their differentiation are not well understood. They function to directly kill tumour cells and virus-infected cells. Precisely how NK cells recognize target cells is unclear, but recognition of an 'altered-self' state has been proposed.

NK cells do not respond to antigen in the same way as T and B cells. Instead, they carry a more primitive surface receptor that recognizes a component of all antibodies, which enables them to recognize and kill cells that have bound antibody. They also express receptors that recognize cells with low concentrations of MHC class I molecules. NK cells are activated by, and undergo proliferation in response to cytokines released during the immune response such as interferon.

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SELF-ASSESSMENT QUESTIONS

1. Where are the earliest recognizable blood cell precursors formed?
2. Name the three different embryonic haemoglobins and show their globin structure.
3. What is the functional difference between red and yellow marrow in adults? Where are these found?
4. Place the following in order of increasing maturity: promyelocyte; myelocyte; myeloblast; CFU-GEMM.
5. Why is chronic renal failure associated with anaemia?
6. Complete the following: post-natal antigen-independent differentiation in B cells occurs in the _____ _____, while for T cells it occurs primarily in the _____ _____.