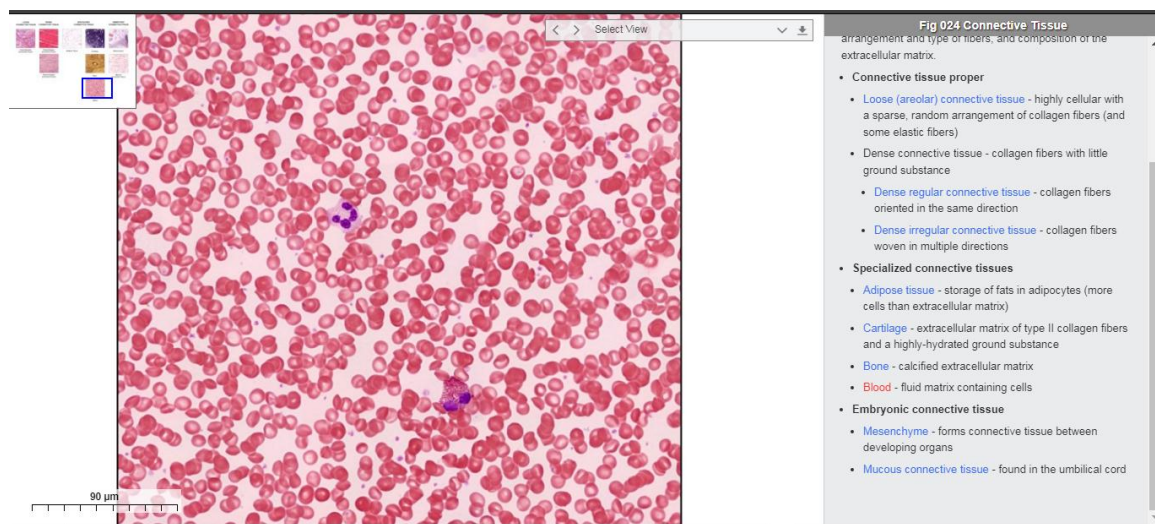


BLOOD

Blood is sometimes considered to be a fluid connective tissue because of the mesenchymal origin of its cells and a low ratio of cells to liquid intercellular substance, the blood plasma. In human adults about 5 l of blood contribute 7-8 % to the body weight of the individual. The contribution of red blood cells (erythrocytes) to the total volume of the blood (haematocrit) is about 43%.

Erythrocytes are the dominant (99%) but not the only type of cells in the blood. We also find leucocytes and, in addition, blood platelets. Cells in the blood are also being referred to as the formed elements of the blood. Erythrocytes and blood platelets perform their functions exclusively in the blood stream. In contrast, leucocytes reside only temporarily in the blood and leave the blood stream through the walls of capillaries and venules and enter either connective or lymphoid tissues.



Erythrocytes

Erythrocytes do not contain a nucleus. They do contain haemoglobin, which fills almost the entire cytoplasm. Erythrocytes are unable to move actively, but they are remarkably elastic and can withstand deformation. They are typically biconcave disks although their shape is influenced by osmotic forces. The average diameter of the disk is ~7 μm. Since erythrocytes can be found in the vast majority of histological sections - in small numbers even in perfused tissues - they will often allow us to estimate the size of other structures or cells. Mature erythrocytes do not contain organelles, and their cytoplasm looks fairly homogenous - even in the EM.

At high magnification some granularity may be visible in EM images. The granular appearance is caused by haemoglobin molecules. Early foetal erythrocytes (up to the 4th month of gestation) are larger than "adult" erythrocytes, and they are nucleated. The latter feature they share with erythrocytes of other animal classes (e.g. birds).

Functions

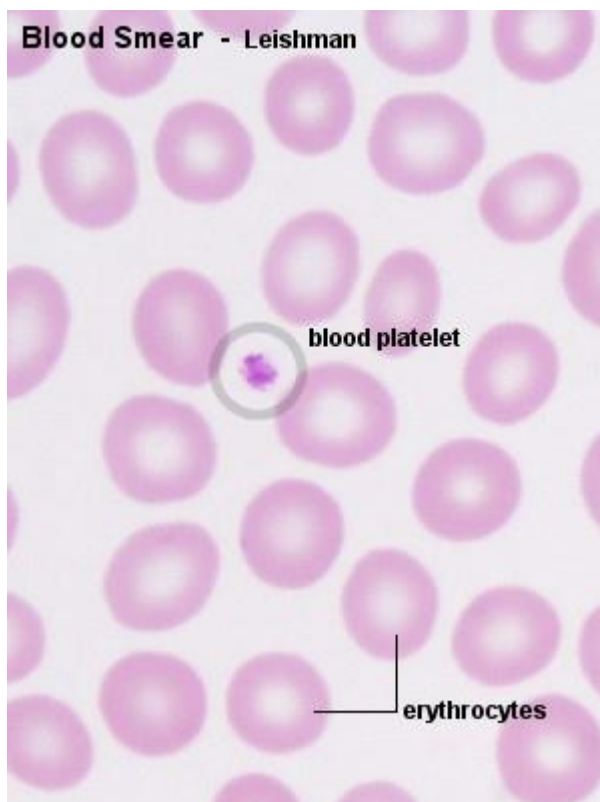
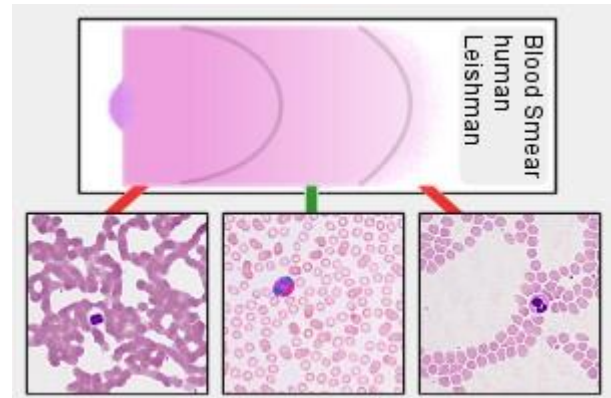
Erythrocytes function in the transport of oxygen. Haemoglobin, the oxygen binding protein in erythrocytes, contributes about 30% of the weight of an erythrocyte. The lifespan of an erythrocyte in the bloodstream is 100-120 days. About 5×10^{11} erythrocytes are formed/destroyed each day.

Suitable Slides

blood smear - Leishman, Wright's, Giemsa or May-Grünwald-Giemsa stains

? Where to look for cells in a blood smear
The density of cells varies across the smear. Cells will be "heaped and piled" close to the point where the drop of blood was placed on the slide. White blood cells appear shrunken, and some types are difficult to distinguish from each other.

There are fewer cells close to the tip of the smear. In this region, white blood cells are sometimes damaged and erythrocytes may be deformed. The best viewing area is between these two regions. Where it is located exactly and how wide it is will depend on the smear, but the middle of the smear is a good starting point.



Blood Smear, human - Leishman stain
How does the shape of the erythrocyte facilitate its function? How would you expect an erythrocyte to look like if it is in an extracellular fluid of very low or very high osmotic pressure?
Identify and draw a few erythrocytes.

It is a good idea to do one large composite drawing for all types of blood cells.

Leucocytes

Leucocytes can be further subdivided into granular leucocytes, i.e. neutrophils, basophils and eosinophils, and non-granular leucocytes, i.e. monocytes and lymphocytes.



In healthy individuals the relative numbers of circulating leucocyte types are quite stable. A differential leucocyte count would typically produce the following cell frequencies (numbers in parentheses are the range of normal frequencies reported in different texts):

- ~ 60% neutrophils (50% - 70%)
- ~ 3% eosinophils (>0% - 5%)
- ~ 0.5% basophils (>0% - 2%)
- ~ 5% monocytes (1% - 9%)
- ~ 30% lymphocytes (20% - 40%)

Changes in their relative numbers indicate that something abnormal is happening in the organism. For example, a larger than usual number of neutrophils (neutrophilia) would indicate an acute or chronic infection. The number of basophils and eosinophils may increase (eosinophilia or basophilia) as a consequence of allergic disorders.

Granular Leucocytes

Granular leucocytes are all approximately the same size - about 12-15 μm in diameter. Their nuclei form lobes, and nucleoli cannot be seen. The number of nuclear lobes varies according to cell type. All granulocytes are motile.

The term granulocytes refers to the presence of granules in the cytoplasm of these cells. The granules correspond to secretory vesicles and lysosomes. Specific granules are the granules which are only found in one particular type of granulocytes.

Neutrophil granulocytes (or neutrophils)

have a very characteristic nucleus. It is divided into 3-5 lobes which are connected by thin strands of chromatin. The number of lobes increases with cell age. Up to 7 lobes can be found in very old neutrophils (hypersegmented cells).

Neutrophils (like all other granulocytes, monocytes and lymphocytes) contain all the organelles that make up a typical cell. In addition to the usual complement of organelles, they also contain two types of granules. Primary granules (or A granules) contain lysosomal enzymes and are likely to be primary lysosomes, although they are larger (0.4 μm) than the "ordinary" primary lysosome. Secondary granules (or B granules), the specific granules of the neutrophils, contain enzymes with strong bactericidal actions. The specific granules of neutrophils stain only weakly if they are at all visible - they are "neutral", hence the term neutrophil.

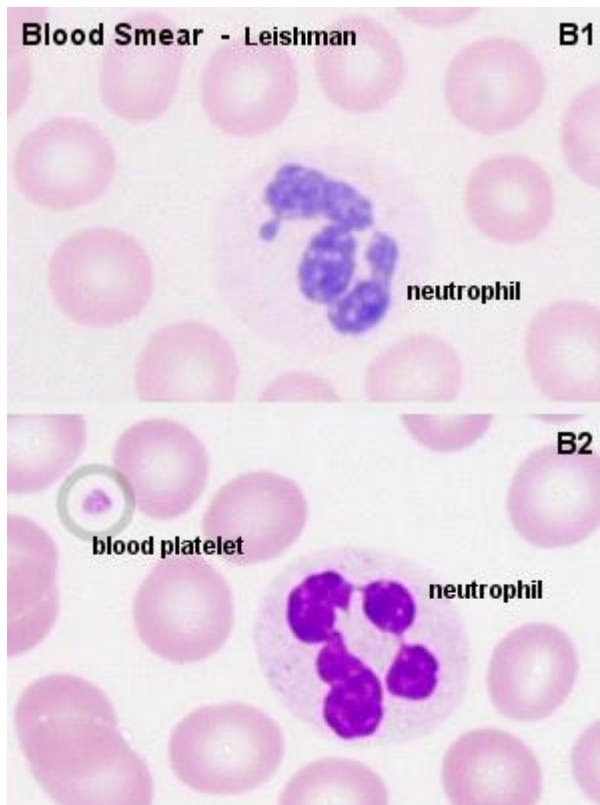
Functions

Neutrophils play a central role in inflammatory processes. Large numbers invade sites of infection and begin to phagocytose tissue debris and foreign bodies, e.g. bacteria. They are the first wave of cells invading sites of infection, and their phagocytotic activity is stimulated if invading microorganisms are "tagged" with antibodies (or opsonised). Neutrophils cannot replenish their store of granules. The cells die once their supply of granules has been exhausted. Dead neutrophils and tissue debris are the major components of pus. Their lifespan is only about one week.

Lost neutrophils are quickly replenished from a reserve population in the bone marrow. Because they are younger, their nuclei have fewer lobes than the "average" neutrophil. A high proportion of neutrophils with few nuclear lobes indicates a recent surge in their release from the bone marrow.

Suitable Slides

see lab section on [erythrocytes](#)



Blood Smear, human - Leishman stain
Neutrophil granulocytes are easy to find. They are the most frequent type of white blood cells, and the complex shape of their nucleus identifies them unequivocally. In darkly stained smears it is possible to see some faintly purple, very small granules in the cytoplasm. These granules represent the primary granules of neutrophils. Have a close look at the nucleus of a number of neutrophils, and make a [qualified guess](#) at the gender of the individual, which donated blood for the slides. Identify and draw one or two neutrophil granulocytes.

How the neutrophils and other leucocytes exactly will look like depends somewhat on how the stain turned out. In some batches (B2) nuclei are dark and crisp, and the cytoplasm is well demarcated. In other batches (B1) nuclei and cytoplasm are lighter and their boundaries are less well defined. While the morphology appears clearer in the darker stained smears, it will usually be more difficult to identify eosinophils and basophils ([see below](#)).

Eosinophil granulocytes (or eosinophils)

Their nucleus usually has only two lobes. Almost all of the cytoplasm appears filled with the specific granules of the eosinophils. As the term "eosinophil" indicates, these granules are not neutral but stain red or pink when eosin or a similar dye is used in the staining process. Aside from the usual complement of organelles eosinophils contain some large rounded vesicles (up to 1 μm) in their cytoplasm. These granules correspond to the eosinophilic grains that we see in the light microscope. The specific granules contain, in addition to enzymes that otherwise are found in lysosomes, an electron-dense, proteinaceous crystal. This crystal is composed of major basic protein (MBP)

Functions

The presence of antibody-antigen complexes stimulates the immune system. Eosinophils phagocytose these complexes and this may prevent the immune

system from "overreacting". Their granules also contain the enzymes histaminase and arylsufatase. These enzymes break down histamine and leukotrienes, which again may dampen the effects of their release by basophils or mast cells. MBP, which can also function as a cytotoxin, and its release by eosinophils may be involved in the response of the body against parasitic infections, which are accompanied by an increase in the number of eosinophils.

Suitable Slides

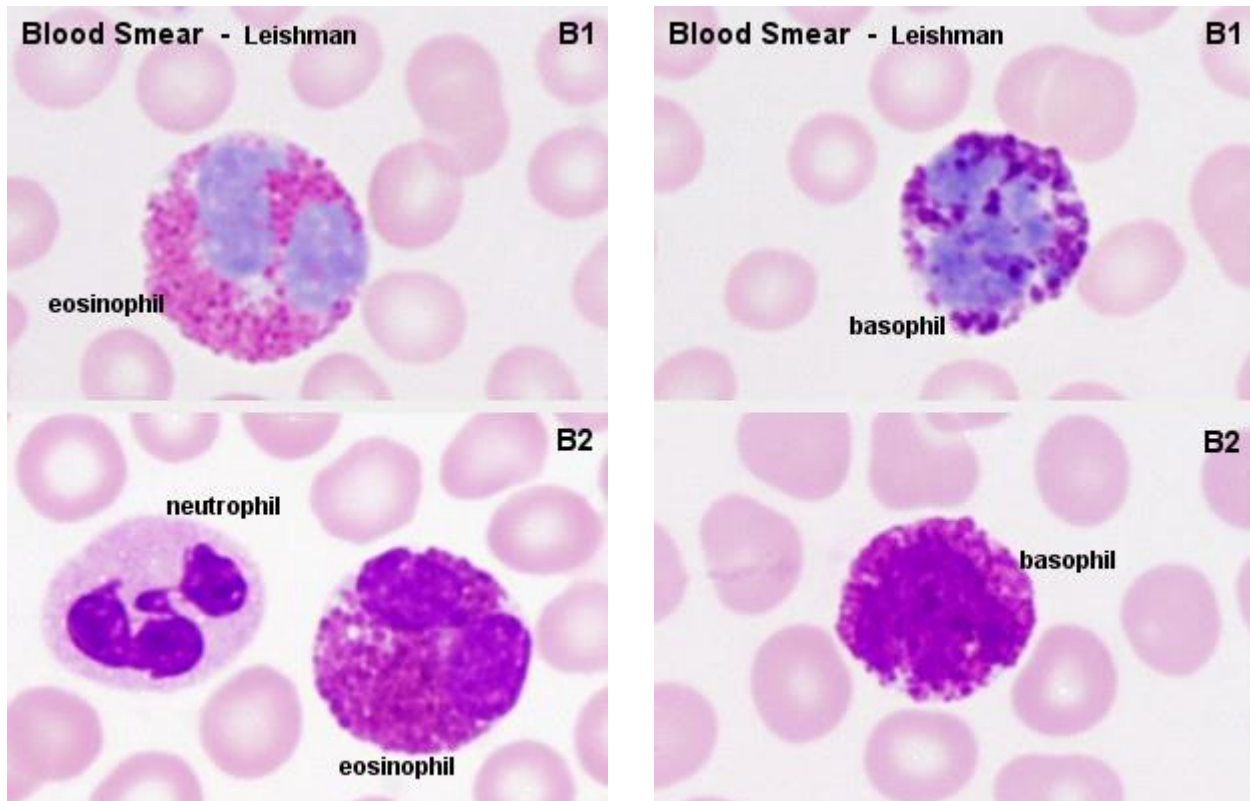
see lab section on [erythrocytes](#)

Blood Smear, human - Leishman stain

Eosinophils and basophils may initially be difficult to distinguish - in particular in darker smears. If you see them side by side in your drawing the difference between them should become apparent. Chances are 6:1 that the you find an eosinophil before you find a basophil. The two lobes of the nucleus of eosinophils are usually well-defined and of about equal size. The nucleus is embedded in a cytoplasm crowded with granules, which seem to form a solid mass in the cell. The 2-3 nuclear lobes of basophils are not as well defined as those of eosinophils, granules are not as numerous as in eosinophils and pretty much all of them can be identified "as individuals" rather than the dense mass they form in eosinophils.

Note that eosinophils and basophils are much easier to distinguish in B1. In B2, the difference in the staining of their of granules is not as pronounced, and the nuclei do not stand out as clear as in B1.

Identify, draw and label an eosinophil and a basophil.



Basophil granulocytes (or basophils)

Basophilic granulocytes have a 2 or 3 lobed nucleus. The lobes are usually not as well defined as in neutrophilic granulocytes and the nucleus may appear S-shaped. The specific granules of basophils are stained deeply bluish or reddish-violet. Their colour corresponds closely to the colour of the nucleus which sometimes is difficult to see amongst or behind the granules. The granules are not as numerous as those in eosinophils. The specific granules of basophils (about 0.5 μm) appear quite dark in EM pictures. They contain heparin, histamine, lysosomal enzymes and leukotrienes (the latter correspond to the slow-reacting substance of anaphylaxis or SRS-A).

Functions

Heparin and histamine are vasoactive substances. They dilate the blood vessels, make vessel walls more permeable and prevent blood coagulation. As a consequence, they facilitate the access of other leucocytes and of plasma-borne substances of importance for the immune response to the tissue - e.g. access of neutrophils and antibodies to a site of infection. The release of the contents of the granules of basophils is receptor-mediated. Basophils do not produce antibodies, but their plasma membrane contains receptors which bind

antibodies (IgE) produced by plasma cells (activated B-lymphocytes; [see below](#)). If these antibodies come into contact with their antigens, they induce the release of the contents of the basophil granules.

Non-granular leucocytes

Monocytes

These cells can be slightly larger than granulocytes (about 12-18 μm in diameter). Their cytoplasm stains usually somewhat stronger than that of granulocytes, but it does not contain any structures which would be visible in the light microscope using most traditional stains (a few very fine bluish grains may be visible in some monocytes). The "textbook" monocyte has a C-shaped nucleus. Monocytes contain granules (visible in the EM) which in appearance and content correspond to the primary granules of neutrophils, i.e. the granules correspond to lysosomes.

Functions

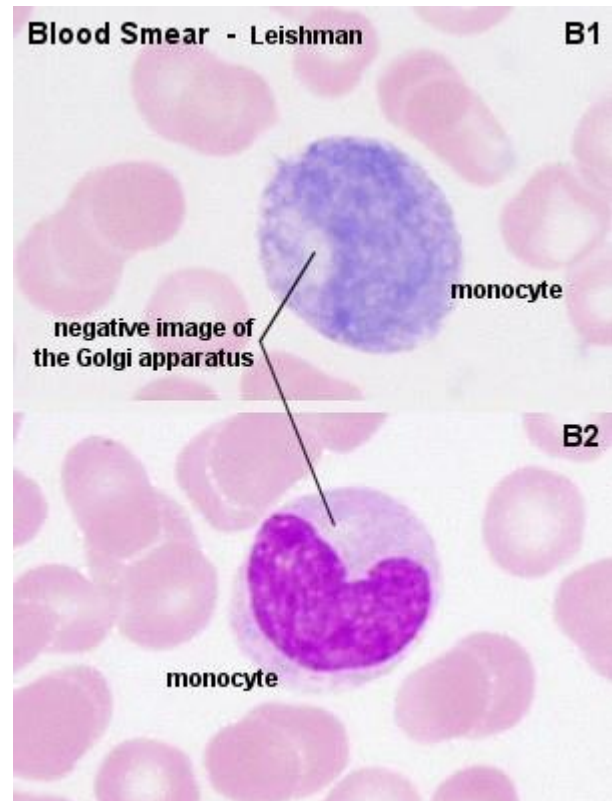
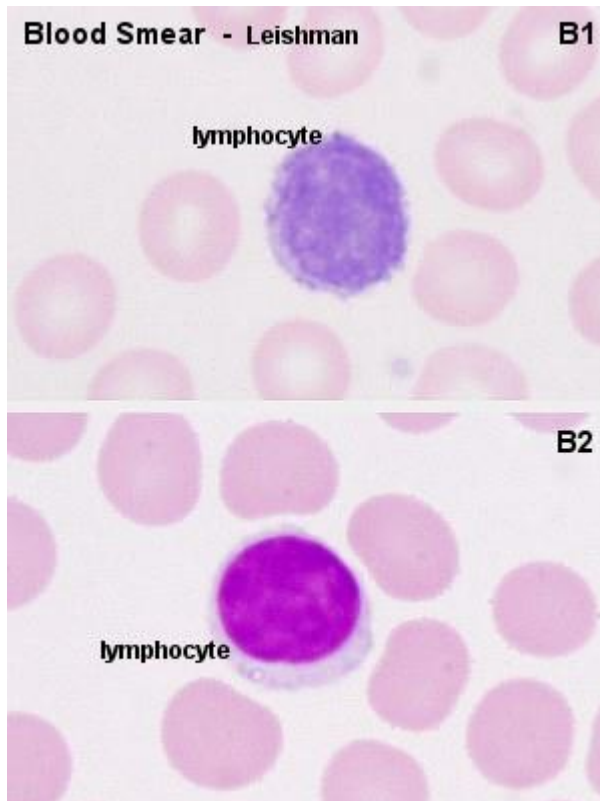
Once monocytes enter the connective tissue they differentiate into macrophages. At sites of infection macrophages are the dominant cell type after the death of the invading neutrophils. They phagocytose microorganisms, tissue debris and the dead neutrophils. Monocytes also give rise to osteoclasts, which are able to dissolve bone. They are of importance in bone remodelling.

Suitable Slides

[see lab section on erythrocytes](#)

Blood Smear, human - Leishman stain

Monocytes and lymphocytes definitely look much prettier in darker stained smears (B2) than in lighter ones (B1) - mainly because of a clearer distinction between cytoplasm and nucleus. The cell is very likely to be a lymphocyte if the nucleus is round and surrounded by a narrow rim of cytoplasm. The C-shaped nucleus of a textbook monocyte may not be easy to find. The nuclei will vary from a peanut- to a "fat" S-shape in smears. Whatever the shape of the nucleus, it is usually not lobed, and it is bound, at least on its concave side, by a wide rim of non-granular cytoplasm. Note also the light area of monocyte cytoplasm which is often visible close to the concave surface of the nucleus. The Golgi apparatus is located in the area. The Golgi apparatus does not stain as well as the remainder of the cytoplasm and leaves a light "impression" - the phenomenon is also called a "negative image".
Identify and draw lymphocytes and a monocyte.



Lymphocytes

These cells are very variable in size. The smallest may be smaller than erythrocytes (down to $\sim 5 \mu\text{m}$ in diameter) while the largest may reach the size of large granulocytes (up to $15 \mu\text{m}$ in diameter). How much cytoplasm is discernible depends very much on the size of the lymphocyte. In small ones, which are the majority of lymphocytes in the blood, the nucleus may appear to fill the entire cell. Large lymphocytes have a wider rim of cytoplasm which surrounds the nucleus. Both the nucleus and the cytoplasm stain blue (and darker than most other cell types in the blood). The typical lymphocyte only contains the usual complement of cellular organelles. The appearance of lymphocytes may change drastically when they are activated (see below).

Functions

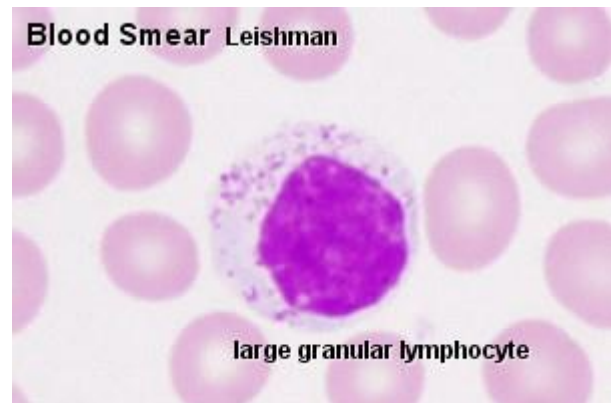
Most lymphocytes in the blood stream belong to either the group of B-lymphocytes ($\sim 5\%$) or the group of T-lymphocytes ($\sim 90\%$). Unless they become activated, the two groups can not easily be distinguished using routine light or electron microscopy.

Upon exposure to antigens by antigen-presenting cells (e.g. macrophages) and T-helper cells (one special group of T-lymphocytes) B-lymphocytes

differentiate into antibody producing plasma cells. The amount of cytoplasm increases and RER fills a large portion of it.

T-lymphocytes represent the "cellular arm" of the immune response (cytotoxic T cells) and may attack foreign cells, cancer cells or cells infected by a virus.

T-lymphocytes and B-lymphocytes form the vast majority of lymphocytes in the blood stream, but they do not add up to 100%, and they usually are small lymphocytes. The much less frequent medium-sized or large lymphocytes may represent e.g.



- natural killer (Nk-) cells which belong to the group of large granular lymphocytes, or
- [haemopoietic stem cells](#) of which a few will be circulating in the blood stream.

Blood Platelets

Blood platelets (or thrombocytes) do not contain a nucleus. Unlike erythrocytes, which also lack a nucleus, the blood platelets of mammals have never been nucleated cells. Instead, blood platelets are fragments of the cytoplasm of very large thrombocyte precursor cells, megakaryocytes. Like other cells involved in the formation in blood cells, megakaryocytes are found in the bone marrow.

Platelets are about 3 μm long but appear somewhat smaller in the microscope. This is because their cytoplasm is divided into two zones: an outer hyalomere, which hardly stains, and an inner granulomere, which contains bluish staining granules. These granules are usually not individually visible with the highest magnification on your microscope, and the granulomere appears more or less homogeneously blue.

In addition to different types of vesicles (i.e. the granules), mitochondria, ribosomes, lysosomes and a little ER are present in the thrombocyte granulomere. Different types of vesicles contain either serotonin (electron-dense delta granules; few) or compounds important for blood coagulation (alpha granules - they also contain platelet-derived growth factor (PDGF) which may play a role in the repair of damaged tissue). The hyalomere contains cytoskeletal fibres, which include actin and myosin.

Functions

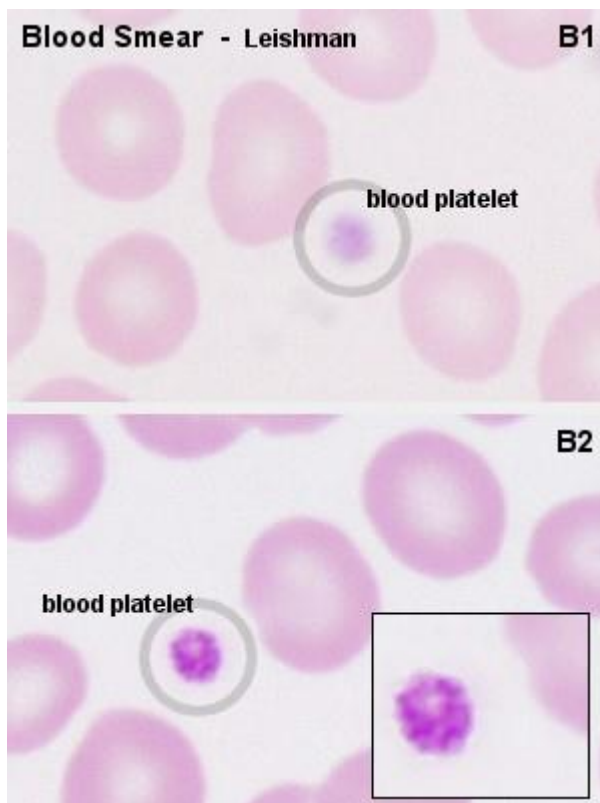
Platelets assist in haemostasis, the arrest of bleeding. Serotonin is a potent vasoconstrictor. The release of serotonin from thrombocytes, which adhere to the walls of a damaged vessels, is sufficient to close even small arteries. Platelets, which come into contact with collagenous fibres in the walls of the vessel (which are not usually exposed to the blood stream), swell, become "sticky" and activate other platelets to undergo the same transformation. This cascade of events results in the formation of a platelet plug (or platelet thrombus). Finally, activating substances are released from the damaged vessel walls and from the platelets. These substances mediate the conversion of the plasma protein prothrombin into thrombin. Thrombin catalyzes the conversion of fibrinogen into fibrin, which polymerizes into fibrils and forms a fibrous net in the arising blood clot. Platelets captured in the fibrin net contract leading to [clot retraction](#), which further assists in haemostasis.

Blood coagulation is a fairly complex process, which involves a large number of other proteins and messenger substances. Deficiencies in any one of them, either inherited or acquired, will lead to an impairment of haemostasis.

Suitable Slides

blood platelets (thrombocytes) : [see lab section on erythrocytes](#)

megakaryoblasts and megakaryocytes: [see lab section on haemopoiesis](#)



Blood Smear, human - Leishman stain
In lightly stained smears (B1), blood platelets will appear like light blue, fairly ill-defined specks between the other blood cells. In darker smears (B2), you will be able to see that the blue specks are formed by an accumulation of small bluish grains, the granules of the blood platelets.

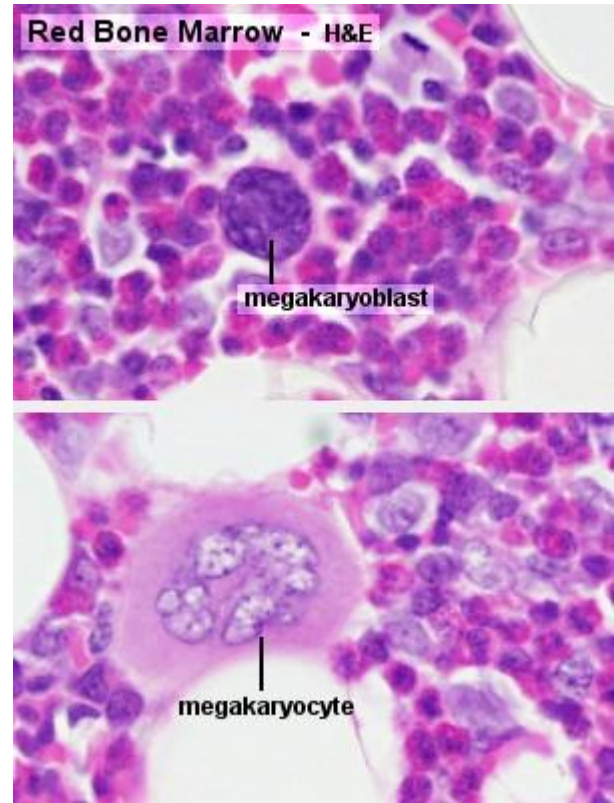
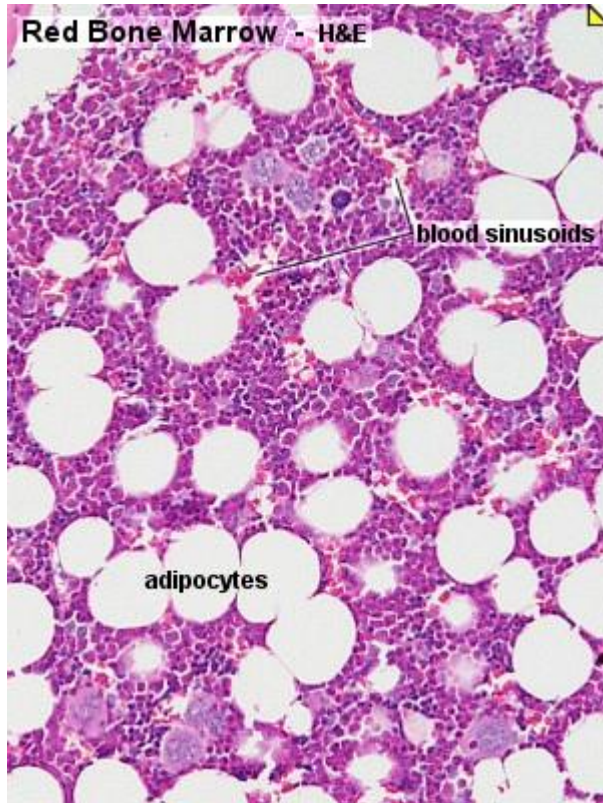
Identify and include a platelet in one of your other drawings.

Red Bone Marrow, rabbit - H&E
The marrow cavity of this bone is filled with red bone marrow. H&E is not the method of choice for looking at haemopoietic cells, but a few of the numerous named types or broader groups can actually be recognized.

Precursors of platelets are the haemopoietic cells easiest to find in red bone marrow. The very dark and large

megakaryoblast and the even larger but light megakaryocytes are clearly visible even at low magnifications.

Identify and draw a megakaryocyte and megakaryoblast.



Haemopoiesis

During foetal development, the formation of blood cells (haemopoiesis) commences in wall of the yolk sac. After the second month of foetal development, the [liver](#), and, slightly later, the spleen, become the dominant sites of haemopoiesis. From the 6th month, and dominating from the 7th month onwards, the formation of blood cells occurs in bone marrow, which is the major site of haemopoiesis in normal adult humans.

Yellow bone marrow, which harbours mainly adipocytes, dominates in the hollow of the diaphysis of adult long bones. Haemopoiesis occurs in red bone marrow, which is typically found between the trabeculae of spongy bone. Both age and demands on haemopoiesis may effect the relative amounts of red and yellow bone marrow.

Haemopoietic cells surround the vascular sinusoids and are supported by reticular connective tissue. In addition to the endothelial cells of the sinusoids and the reticulocytes of the connective tissue, macrophages are frequent in red bone marrow.

Haemopoietic Cells

The basis of haemopoiesis is a small population of self-replicating stem cells, which ultimately can generate all types of blood cells. Their progeny may develop into either lymphocytic stem cells or pluripotent haemal stem cells (colony-forming unit - stem cell - CFU-S). The latter type gives rise to stem cells which can form the major groups of blood cells other than lymphocytes. Depending on their progeny it is possible to differentiate

- burst-forming unit of the erythroid line (BFU-E),
- colony-forming unit - granulocytes and macrophages (CFU-G/M), and
- colony-forming unit - megakaryocytes (CFU-Mk).

Erythrocytes

The first identifiable stage of erythropoiesis is the proerythroblast - a large, slightly basophilic cell, which contains a large, lightly stained nucleus. Proerythroblasts proliferate to generate a sequence of cells which show a gradual decrease in size and condensation of their chromatin. They are named after changes in the staining characteristic of their cytoplasm (basophilic erythroblast, polychromatophilic and orthochromic normoblasts). The nucleus is finally extruded from the normoblast. The cell enters circulation as a reticulocyte, which still contains some organelles. Reticulocytes remain for a few days in either the bone marrow or the spleen to mature to erythrocytes.

Granulocytes

Myeloblast appear light-microscopically similar to proerythroblast. They proliferate to generate promyelocytes. Promyelocytes begin to accumulate non-specific granules, but they are still able to divide. The maturation of their progeny, the myelocytes, is characterised by the accumulation of specific granules and changes in nuclear morphology. Metamyelocytes have a C-shaped nucleus.

Thrombocytes

are, as mentioned above, fragments of the cytoplasm of megakaryocytes.

Megakaryocytes are very large cells (up to 160 μm), which contain very large, highly lobulated, polyploid nuclei. Megakaryocytes are in turn the product of the differentiation of basophilic megakaryoblasts.

The nomenclature employed for haemopoietic cells (but not the number of stages recognized) is somewhat variable across texts. Note also that these cell types refer to stages of development along a morphologically more or less continuous spectrum.

Precursors of blood cells which are usually only found in the bone marrow can be found in peripheral blood in a variety of pathological conditions.

If a Rh-negative mother has been immunised by erythrocytes of a Rh-positive foetus, a condition called Erythroblastosis fetalis may develop during subsequent pregnancies. It would show itself in the foetus or newborn by the presence of erythrocyte precursors in peripheral blood - although other, more severe symptoms should be obvious. Chronic myeloid leukemia is another condition - in this case showing itself by the presence of all types of granulocyte precursors in peripheral blood.

Suitable Slides

sections of red bone marrow - H&E or a bone marrow smear - Leishman, Wright's, Giemsa or May-Grünwald-Giemsa stains

Red Bone Marrow, rabbit - H&E

Most of the haemopoietic cells visible will be of the erythroblastic line. The only cell type of this line which is easy to distinguish in H&E stained sections are normoblasts. A very condensed nucleus is seen in late (orthochromic) normoblast. Granulocyte and erythrocyte precursors will mostly intermingle, but may be distinguished by nuclear morphology and/or size. A bent nucleus is found in metamyelocytes - this shape is very pronounced in the last, immature form of neutrophils, which are also called stab or band cells. If the cell (1) is large, with a distinct "clearing" in the otherwise pink cytoplasm and (2) has an ovoid or slightly indented nucleus, it is likely to be a myelocyte. Cells with large light nuclei and almost unstained cytoplasm are either reticulocytes or macrophages.

Macrophages are frequently associated with normoblasts, and together these cells form erythroblastic islands. The name for macrophages in these islands, nurse cells, may tell you a bit about their function in addition to the scavenging of the expelled nuclei.

Identify normoblasts, myelocytes and metamyelocytes and include them in your drawing of the megakaryocyte/blast.

If you still have some time and are desperate to get frustrated, try to hunt up a nice basophilic erythroblast - a basophilic cell with homogeneously staining nucleus that is somewhat smaller than the nuclei of granulocyte-precursors.

