THE UNIVERSITY OF QUEENSLAND

Faculty of Medicine
School of Biomedical Sciences (SBMS)
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MEDI7111 Clinical Science 1

Histology of Blood Cells

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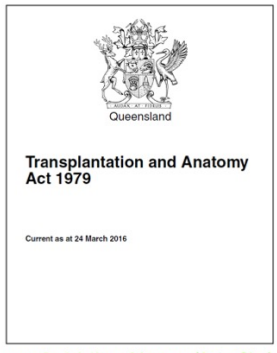

www.legislation.qld.gov.au/Acts_SLs/Superseded/SUPERS_T/Transpl_AnatA79.htm

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The objectives of this online resource are

1. To become familiar with the cells of blood and identify these in a blood smear.

VIRTUAL SLIDES (WEEK 2) – http://sci-histology.bacs.uq.edu.au
Username: uqmd1
Password: student
**** This is a shared password. Do not change this password***

VIRTUAL SLIDES (Lesson 83) – http://sci-histology.bacs.uq.edu.au

Slide 12 - long bone (TS), kitten
Slide 13 - knee joint, kitten
Slide 15 - Bone marrow smear, Human, Grunwald Giemsa
Slide BT29 - blood smear, dog
Slide 46 - longitudinal section of femur, cat
Slide 79 - blood smear, human

Slide 47 - Lymph node, tuberculosis, H&E
Slide 88 - Acute Appendicitis, Human H&E
Slide 270 - Heart, recent infarction, Human, H&E
Slide 280 - Rheumatoid arthritis synovium, Human, H&E
Slide 298 - Pilonidal sinus, Human, H&E

A. INTRODUCTION

Blood is specialised connective tissue, consisting of cells and plasma (extracellular matrix). As we will see examples of blood cells in many of the upcoming histology resources, we examine the specific cell types here.

There are three functional classes of blood cells:

1. **Erythrocytes:** Red blood cells transport blood gases and function only within the vascular system.
2. **Leukocytes:** White blood cells are major constituents in the defence and immune systems of the body. They function primarily in tissues outside the circulation, and those found within the vascular system are usually travelling between places of activity, and not currently active.
    A. Polymorphonuclear Leukocytes/Granulocytes (Neutrophils, Eosinophils, Basophils), contain granules in their cytoplasm, nuclei are often multi-lobed or different shapes.
    B. Mononuclear Leukocytes/Agranulocytes (Lymphocytes, Monocytes), no granules are visible in their cytoplasm
3. **Thrombocytes:** Cell fragments rather than whole cells. The platelets function in haemostasis or control of bleeding. They plug holes in blood vessels and contribute to the activation of the blood-clotting cascade.

The characteristic colours of the neutrophils, eosinophils and basophils are due to the staining of a blood smear or film with Romanovsky stains. There are several versions of these, *e.g.* May-Grunwald, Giemsa, Wright and Leishman; all have similar staining reactions. Basic tissue components attract and stain orange with eosin and acidic

tissue components stain blue with methylene blue. Other colours are produced by interactions between the dye molecules and tissue components (metachromasia).

The detailed chemistry of these stains is beyond this class, however, if you are interested, you may find more information in this article:

Horobin RW (2011) How Romanowsky stains work and why they remain valuable Biotech Histochem 86: 36-51

There are four staining reactions:

Basophilia (deep blue): acid structures attract a blue stain, *eg.* DNA in nuclei
Eosinophilia (orange-pink): basic structures attract eosin *e.g.* haemoglobin in RBCs
Azurophilia (purple): structures that attract an azure stain, e.g. lysosomes found in white blood cells
Neutrophilia (light pink to lilac): attract a dye previously thought to be neutral in pH (hence neutrophilia) *e.g.* the granules in the cytoplasm of the neutrophil.

Where to look in a smear:

As is shown in the online slide guide that accompanies these notes, the process of producing a blood or bone marrow smear does not result in all parts of the smear being equally good for examination.

Over most of the smear, the blood cells will be too dense to see anything properly. At the feathered end of the smear, the cells often have distorted morphology, and there is a region - the region of best morphology, where the red blood cells are numerous, but spread out sufficiently that they are not overlapping. The white blood cells settle onto the slide and spread out a little bit, meaning that their nuclei are more easily visible, and they aren't overlapped by the red blood cells either. Always look in this region of best morphology, which is found just in from the feathered edge of the smear.

Bone marrow smears are very complex - in addition to mature cells, they contain many immature cells. The less mature white blood cells are, the more similar the lineages look, so bone marrow smears contain many large cells with large nuclei and bluish cytoplasm which cannot be pinned down to a particular cell type.

However, examine the bone marrow sections in the Virtual Microscope (these are not smears, but sections of bone containing marrow) to see what these cells look like. Because these sections are typically stained with H&E, the nuclear and granular distinctions produced by the Romanovsky stains are absent - another reason that the immature cells will all tend to look alike.

The Histology Guide website contains an excellent Romanovsky-stained bone marrow smear, if you wish to learn more about immature blood cells.
http://histologyguide.com/slideview/MH-034ahr-bone-marrow-smear/08-slide-1.html?x=0&y=0&z=-1&page=1

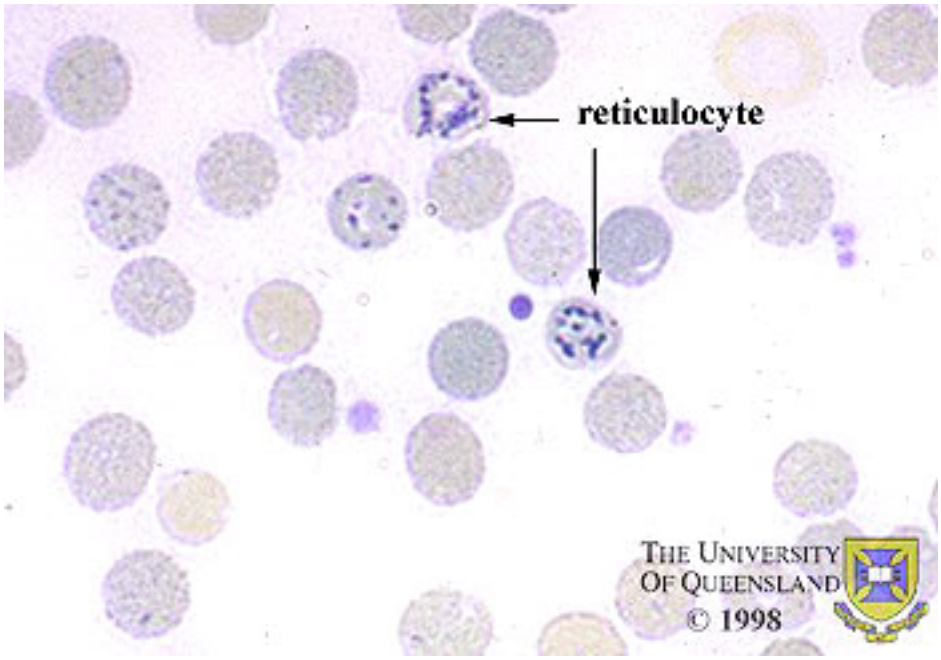
Also note that there are increasing numbers of adipocytes in bone marrow with age. You should now be able to identify the adipocytes.

MATURE BLOOD CELLS

Red blood cells (Erythrocytes)

Red blood cells (RBCs) are non-nucleate biconcave discs, ~7-8μm in diameter. This gives the characteristic pale central area in the RBCs seen in blood smears. The size of RBCs in a normal blood smear is fairly uniform. This is described as normocytic. Variations in the size of RBCs are described as microcytic (small RBCs), macrocytic (large RBCs) or anisocytic (RBCs of irregular sizes).

Reticulocytes are immature RBCs. RBCs are released from bone marrow when they are almost mature, then finish maturation (which takes about a day) in the blood stream. They usually number about 0.2-2% of red cell count (more in infants). A special reticulocyte stain is used to obtain a reticulocyte count. The stain demonstrates the last remnants of ribosomal RNA in the cell. Increase in the number of reticulocytes may be due to increased erythropoiesis, e.g. after blood loss, as the bone marrow hurriedly releases immature RBCs to make up for the loss.



Chan, 2004

RBCs have a life span of about 120 days. Old red blood cells are removed from the circulation by macrophages in the spleen, bone marrow and liver.

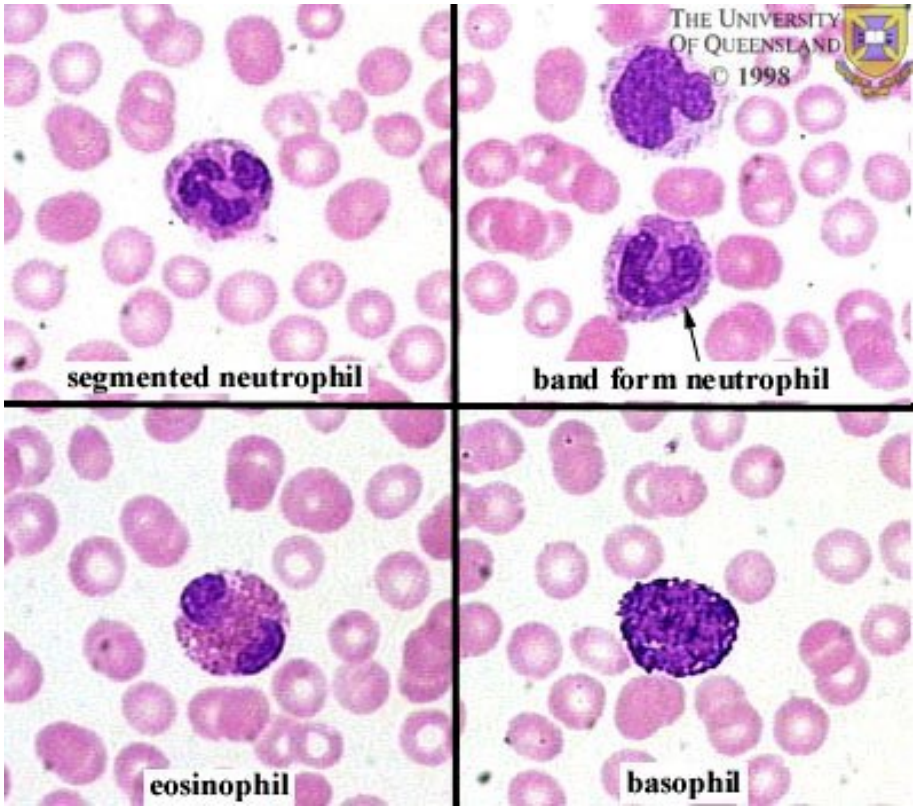
Platelets

Platelets (thrombocytes) are non-nucleate, basophilic fragments of cytoplasm, ~2-3μm. They are usually seen in clumps in a blood smear. Platelets have a life span of about 7-10 days. They are primarily removed by the spleen.

White blood cells

Granulocytes

Granulocytes are characterised by the presence of specific granules in the cytoplasm.



Chan, 2004

Neutrophils are also known as polymorpho-nuclear leukocytes (polymorphs/polys): cytoplasm with fine granules, multi-lobed nucleus, ~12-14μm. The majority of neutrophils have a 3-5 lobed nucleus. These are known as segmented forms ("segs"). Neutrophils with fewer lobes are less mature. 'Band form' neutrophils (nucleus with 2 lobes) are present (3-5%) in normal blood smears. Increase in the number of these immature forms (shift to the left) indicates an increased demand and formation of these white cells, e.g. blood loss or bacterial infection. If a shift to the right is present (more mature and less immature forms), that indicates suppression of production in the bone marrow.

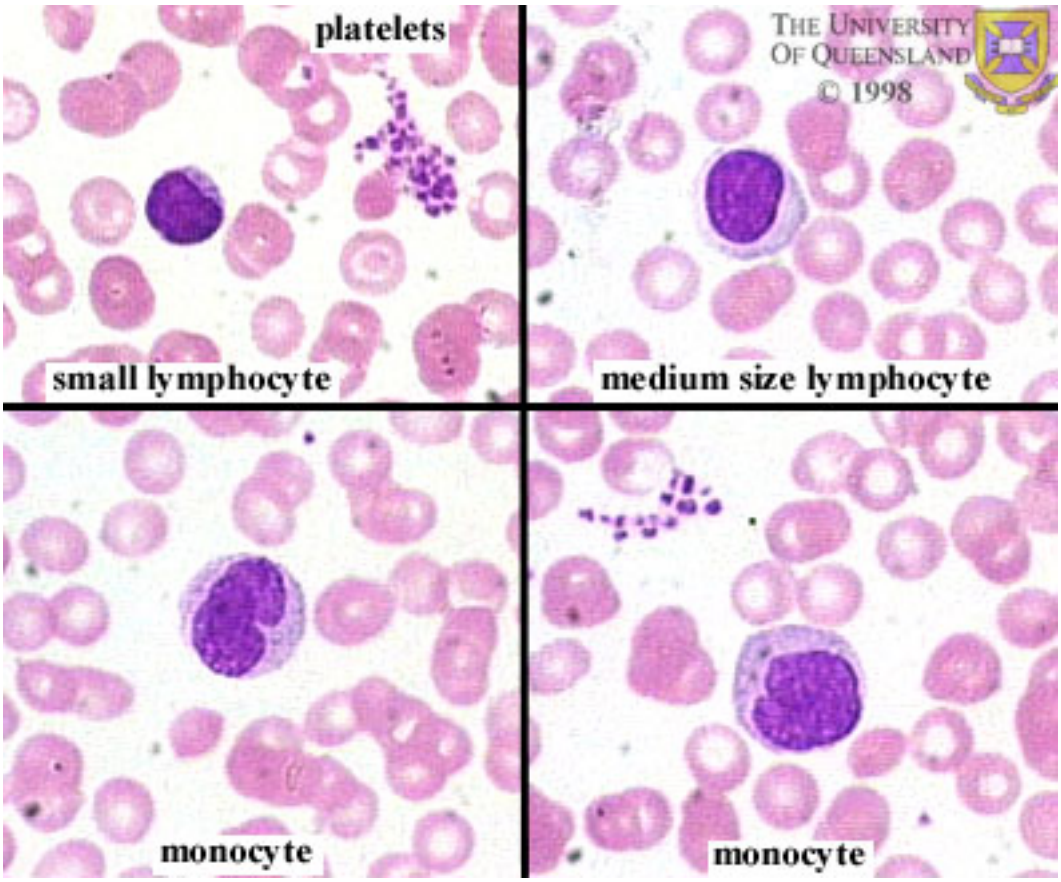
Eosinophils: cytoplasm with large, eosinophilic granules, bi-lobed nucleus, ~12-17μm.

Basophils: cytoplasm with large, dark-blue granules, bi-lobed to S-shaped nucleus (often obscured by the specific granules), ~10-12μm. Very few in number (<0.5% of white cell count).
Granulocytes have a short life span in the blood (about 8-12 hours) before migrating into connective tissues.

What are the functions of <u>each</u> of these granulocytes?

Agranulocytes

Agranulocytes are characterised by the absence of visible granules. However, a few purple-stained azurophil granules may be present in the cytoplasm.



Chan, 2004

Monocytes: largest of the white cells, ~20μm, irregular to indented nucleus. Monocytes also have a short life span in the blood. They migrate into connective tissues where they differentiate into macrophages.

Lymphocytes: smallest of the white cells, ~8μm, round, dark (clumped chromatin) nucleus with a thin rim of cytoplasm. Medium and large lymphocytes (~12-14μm) may be present. These have more cytoplasm and a round to slightly oval nucleus which may be slightly indented. The "lymphocyte" is a heterogeneous population of cells. Most of them are short- or long-lived (memory) T and B cells.

Haemopoietic Tissue

Haemopoietic tissue may be classified into two categories, myeloid and lymphoid tissue. In postnatal life, myeloid tissue produces erythrocytes, granular leucocytes and platelets whereas the lymphoid tissue produces most of the agranular leucocytes.
Haemopoietic (lymphoid and myeloid) tissue consists of a framework or stroma of reticular cells and fibres and sinusoids. Sinusoids are blood 'vessels' or spaces whose walls are composed of reticular cells. These reticular cells are lining cells and they produce and maintain the reticular fibres. Phagocytosis is performed by macrophages.

A primitive stem cell called the pluripotent or totipotent stem cell gives rise to unipotent stem cells, one for each subdivision of haemopoiesis (erytho-, granulo-, lympho-, monocyto- and thrombopoiesis). These unipotent cells are now committed to one fate and proceed to produce recognisable precursor cells. The progenitor cells are sometimes termed colony-forming units or CFUs.
Having originated in one site, they can pass by the bloodstream to another site and seed it with stem cells e.g. progenitor lymphocytes leave the bone marrow, travelling to the thymus where they complete their maturation into T lymphocytes.

Totipotent Cell → Lymphoid Stem Cell →Lymphocyte series
(Hemocytoblast) → Non-lymphoid (Myeloid) Stem Cell →everything else

The nomenclature of various haemopoietic growth factors derives from the concept of the CFU i.e. G-CSF, M-CSF, GM-CSF (CSF- colony stimulating factor).

The CFU develops from mesenchymal cells in blood islands of the yolk sac of the embryo and for a time the proliferation of the stem cells of the blood occurs only in the yolk sac. Stem cells formed in the yolk sac migrate to the liver. Haemopoiesis in the yolk sac is soon superseded by that occurring in the liver, and subsequently the spleen, thymus, lymph nodes, and bone marrow are seeded by stem cells from the liver. From 3 to 7 *m.i.u.*, haemopoiesis occurs primarily in the spleen and liver. Granulocyte and thrombocyte formation begins in the bone marrow cavities of the bones as they develop in the fourth and fifth *m.i.u.*. Erythropoiesis begins here in the 7th month and by birth, the bone marrow is the primary site of haemopoiesis. After birth, the bone marrow and spleen become the chief sites where the population of stem cells is maintained.
From birth till adulthood, haemopoiesis diminishes in the bone marrow, finally leaving only the skull, ribs, sternum, vertebrae, pelvis and proximal ends of the femurs, although all bone marrow can become active (retains haemopoietic potential) when needed. The spleen and liver may also become active in times of need.
Actively haemopoietic bone marrow is red in colour. Thus it is termed Red Marrow. Inactive bone marrow is filled with adipose tissue, so that it appears yellow. This is Yellow Marrow.

At birth, red marrow fills all the marrow space so infants have no reserve haemopoietic capacity, so any increased demand means that the volume of bone marrow must be expanded. This may result in the skeletal deformities in severe dyserythropoietic states e.g. thalassemia.
Extramedullary haemopoiesis occurs in the adult when bone marrow is unable to meet the demand e.g. when metastatic carcinoma deposits occupy most of the bone marrow space. Haemopoiesis reverts to the foetal sites of spleen and liver.

Bones are supplied by several nutrient arteries, which enter the medullary cavity and branch to form capillary networks. These drain into venous sinuses where new blood cells join the circulation from the bone marrow.
The walls of venous sinuses have three layers:
- Vascular endothelium
- Discontinuous basement membrane
- Macrophages

The reticular cells have long processes that divide the bone cavity into compartments occupied by blood cell precursors.
Each precursor type occupies a specific position and forms colonies. Megakaryocytes are next to the sinuses. Their processes project directly into the lumen of the sinus, so as the platelets are formed by fragmentation of the cytoplasm, they are washed away into the circulation. Erythroblastic islands are found with a 'nurse' macrophage and are also close to the venous sinuses. Granulocyte and monocyte precursors are deeper within the compartment. With maturity, these cells develop motility and 'crawl' toward the sinuses.

Look for sinusoids. Adipocytes are scattered throughout the marrow in some of these preparations. Identify megakaryocytes and draw. Make sure you can distinguish them from osteoclasts. Megakaryocytes are large cells with multi-lobed nuclei; osteoclasts are large cells with multiple nuclei.

Erythropoiesis (Red Blood Cell Formation)

The mass of RBCs remains fairly constant under normal conditions. Erythropoietin mediates a feedback system that adjusts the rate of erythropoiesis with respect to the oxygen demands of the body.
The entire mass of all erythroid cells (progenitors and mature) is termed the erythron, a concept that allows the cellular components of erythropoiesis to be viewed as a tissue. As in every cell line, the earliest erythroid progenitors cannot be identified histologically.
The unit of erythropoiesis is the erythroblastic island in the bone marrow. It consists of 1 or 2 'nurse' macrophages surrounded by erythroid progenitor cells. The islands are fragmented by the aspiration techniques used to sample bone marrow.
The nurse macrophages have long cytoplasmic processes or 'arms' which enclose the developing cells. As the erythroid cell matures, it migrates outward along the arms. Finally, it contacts the sinusoids, extrudes its nucleus (which is phagocytosed by perisinusoidal macrophages) and enters the bloodstream.

Erythropoiesis is characterised by 4 processes that occur throughout the maturation of the cells:
- Decrease in cell size;
- Production of haemoglobin (this produces an increase in eosinophilia or pinkness in the cytoplasm, the blue colouration of the earliest precursors is due to cellular organelles);
- Loss of cellular organelles (the blue staining is lost gradually with maturation);
- Condensation and extrusion of the nucleus.

The stages of erythropoiesis are proerythroblast, basophilic erythroblast, polychromatophilic erythroblast, orthochromatic/eosinophilic erythroblast and reticulocyte. Only the last couple may be identified with any certainty.

As immature reticulocytes, the RBCs are released into the circulation. These are slightly larger than mature RBCs, with enough organelles remaining to complete the remaining haemoglobin synthesis in the next 1-2 days. Normally, they constitute a very small % of circulating RBCs. A reticulocyte count gives an indication of bone marrow activity. Reticulocytosis is an abnormally high number of reticulocytes in the circulation (left shift).

Anaemia is a decrease in the functional mass of circulating RBCs. It commonly results from loss of blood, or increased destruction or decreased production of cells. Anaemias are described by cell size or haemoglobin content (macrocytic, microcytic or hypochromic)

Granulocytes and monocytes appear to derive from the same CFUs.

Granulopoiesis (Granulocyte Formation)
The various stages are very similar for eosinophils, neutrophils and basophils.

Granulopoiesis is characterised by:
- Increase in proportion of cytoplasm to nucleus;
- Condensation and lobulation of the nucleus;
- Increase in specific granules in the cytoplasm.

The stages are myeloblast, promyelocyte, myelocyte, metamyelocyte and stab or band cells. The granules become apparent at the metamyelocyte stage, and that and the band cells can be identified with certainty.

Monopoiesis (Monocyte Formation)

Lymphopoiesis and monopoiesis are similar:
- Decrease in cell size;
- Compaction of chromatin;
- Disappearance of nucleoli.

Differentiation of these precursors is difficult as they do not display either than granules or the nuclear changes of the other lineages.

Lymphopoiesis (Lymphocyte Formation)

Lymphopoiesis takes place in two stages.

I Lymphoid stem cell differentiates to form mature antigen-committed lymphocytes in primary lymphoid organs, with deletion of self-recognising cells.
T-lymphocytes in thymus
B-lymphocytes in foetal liver and adult bone marrow

These naïve lymphocytes migrate in the blood to colonise peripheral lymphoid organs.

II Antigen-dependent proliferation and development of lymphocytes in secondary lymphoid organs (spleen, lymph nodes and MALT)

In foetal life, the stem cells form in haemopoietic tissue. Some migrate to the thymus where they become T-lymphoid stem cells (pre-T cells). As they journey through the thymus, they differentiate into mature T-cells.

The stages are lymphoblast or monoblast, and prolymphocyte or promonocyte.

Levels of white cells below normal are referred to as –paenic.
(thrombocytopaenic, neutropaenia etc)

Leukaemias are common and important malignant diseases of white blood cells. They may be chronic (slowly progressing, with some normal bone marrow function) or acute (rapidly progressing, with bone marrow failure). Examination of the blood smear and bone marrow are important in diagnosing these leukaemias. An accurate diagnosis is essential for correct treatment. Polycythaemia rubra vera is a neoplastic proliferation of RBCs. It, with chronic myeloid leukaemia and myelofibrosis, is a myeloproliferative disorder. These diseases may undergo blast transformation into acute myeloblastic leukaemia.

Plasma cells form from B-lymphocytes, and are responsible for an important bone marrow tumour, the myeloma, which may be solitary (plasmacytoma) or multiple (multiple myeloma).

Thrombopoiesis (Platelet Formation)

The megakaryoblast is a very rare cell. Megakaryocytes tend to accumulate toward the edges of a bone marrow aspirate slide.
Megakaryoblasts display endomitosis (replication of cytoplasmic and nuclear content without cellular division) giving increased ploidy and lobulation of the nucleus, eventually producing the huge (10-100µm) megakaryocyte.

ACTIVITIES TO BE COMPLETED

Try examining this blood smear slide first.
[you will need to be signed in (UQ single sign on) to access external websites]
There is an annotated layer. Look at the labels on the left hand side. Some of these are testing your knowledge!

Also, scan the blood smear under medium magnification to locate an area where the smear is spread thin and evenly (no overlapping of cells). Note that in the dense areas of the slide, cells will be in different focal planes so the digital image may be blurry. Go to an area of the smear with fewer cells for a better focus.

Identify the blood cells using the following key features:
-presence or absence of cytoplasmic granules
-size and staining of granules
-nuclear characteristics
-cell size and shape

Now apply this to **Slide BT29 and Slide 79.**

You should be able to identify erythrocytes, neutrophils, lymphocytes, eosinophils and monocytes. Basophils may be difficult to locate as there are few of these in a normal healthy blood smear.

Note that animal (Slide 29) and human blood (Slide 79) differs.
Human blood has fewer target cells than animal blood (erythrocytes that appear as targets with a darker area inside the pale centre). Many target cells in human blood may indicate disease.

Animal blood smears often have more crenated red blood cells than human blood smears (erythrocytes that are spiky in appearance, as they have shrunk slightly and wrinkled, usually as a result of the collection and processing of the blood).

Examine this bone marrow smear.
http://histologyguide.com/slideview/MH-034ahr-bone-marrow-smear/08-slide-1.html?x=0&y=0&z=-1&page=1

Can you identify normal mature blood cells? Can you now identify the immature cells? Try to find band cells, orthochromatic erythroblasts, and megakaryocytes. Other immature cell types are difficult to identify with certainty as they look very similar. If positive identification is necessary (for example, to identify a type of leukaemia), electron microscopy or immunohistochemistry is used.

If you are particularly interested in Bone Marrow - Chapter 8 Hematopoiesis on the Histology Guide Website is an excellent resource. Bear in mind that you are not required to identify immature blood cells for this class, just the mature ones in a normal blood smear, and the megakaryocyte on the bone marrow smear.

Now, examine Slides 12, 13 and 46. These are sections of bone containing bone marrow in situ (not smeared). These are also stained with H&E not a Romanovsky stain. The purpose of examining these is for you to see what the normal architecture of

bone marrow (with sinuses and adipocytes) looks like, rather than just cells smeared on a slide.

White blood cells are typically in the blood stream in transit. They leave the blood stream and enter tissues in order to work. Now, examine some slides where blood cells are in tissues, and are working.

Slides 88 and 270 demonstrate neutrophil invasion in acute injury.

Slide 88 Acute Appendicitis, Human, H&E

In this slide, observe the large numbers of neutrophils infiltrating throughout the wall of the appendix. Neutrophils can also be observed in the lumens of the larger blood vessels in the outer (serosa) wall.

Slide 270 Heart Recent Infarction, Human, H&E

In this slide, observe the neutrophils that are moving into the infarcted area of cardiac muscle (remember that necrotic cells will be more intensely stained, and somewhat shrunken, when compared with normal myocytes).

Slides 47 and 280 contain examples of lymphocytes and plasma cells. Slide 47 also has epithelioid cells (macrophages) present.

Slide 47 Lymph Node with tuberculosis/granuloma, Human, H&E

This lymph node is infected, and reacting. Many lymphocytes are present - you can see these as the purple areas of the tissue at low power. The large pink/eosinophilic areas are caseous necrosis. Around the outside of these, look for the epithelioid cells / activated macrophages. The most identifiable of these are when a group of them fuse together to form a giant cell (Langhan's cell). Even on low to medium power, these are visible as giant, eosinophilic multinucleate cells just at the outside of the homogenous pink centres of the lesions.

Slide 280 Joint Synovium, Rheumatoid arthritis

This is a chronic inflammatory arthritis. In this slide, using low to medium power, identify the purple appearing regions. When you examine these with higher magnification, you will observe lymphocytes.

Slide 298 Skin, Pilonidal Sinus, Human, H&E

This is an example of a reaction to a foreign body (hair). Examine the material around the pieces of hair shaft, and identify macrophage/giant cells, with multiple nuclei and distinctly eosinophilic cytoplasm. They are larger than surrounding cells and can be identified at low to medium magnification.

FURTHER RESOURCES and SOURCES OF IMAGES

Chan K (2004) An atlas and practical guide to histology. School of Biomedical Sciences, The University of Queensland. CD ROM.

Eroschenko, VP (2017) Atlas of histology with functional correlations 13th ed. Wolters Kluwer, USA. [available in print through the library]
Hematopoietic tissue

Kerr, JB (2010) Functional Histology 2nd edition Mosby Elsevier [available in print through the library]
Chapter 3 Blood

Mescher, AL (2018) Junqueira's Basic Histology: Text and Atlas, 15th Ed, McGraw-Hill Education [accessible online via the library website]
Chapter 12 Blood

Ross MH & Pawlina W (2016) Histology: A text and atlas with correlated cell and molecular biology. 7th ed Wolters Kluwer, USA. [available in print through the library]
Chapter 10: pages 306-309 *

Young B, O'Dowd G & Woodford P (2014) Wheater's functional histology: A text and colour atlas (6th Edition). Churchill Livingstone/Elsevier, USA. [accessible online via the library website]
Chapter 3 Blood, haematopoiesis and bone marrow

Websites
Histology Guide by Sorenson, RL and Brelje, TC, http://www.histologyguide.com/index.html
Blue Histology http://www.lab.anhb.uwa.edu.au/mb140/