PERIPHERAL BLOOD

Peripheral blood is defined as the blood contained in blood vessels and heart rather than in the hematopoietic tissue of bone marrow. It is sometimes classified as a variety of connective tissue, the obvious difference between blood and other connective tissues being that the intercellular substance surrounding blood cells is fluid, i.e., plasma. The purpose of today’s laboratory is to introduce you to the morphology of the different cell types in blood, primarily as seen in a routine, Wright’s stained, peripheral blood smear. Blood-forming (hematopoietic) tissues, e.g., red marrow of bone, will be studied later.

The examination of properly prepared peripheral blood smears allows one to assess red cell morphology, to estimate platelet number, and to determine (approximately) the percentage of each of the several types of leukocytes (white blood cells). A disadvantage is that only a small and not necessarily representative area of the smear is suitable for microscopic examination (e.g., leukocytes tend to be displaced to the sides and tail of the smear).

ERYTHROCYTES (RED BLOOD CELLS; RBCs)

Red blood cells are the most common cells in a blood smear. Normal, undistorted, adult human red blood cells will be in the 7-8 µm diameter range. (In histological sections of paraffin embedded tissues the diameter of RBCs is in the range of 6-7 µm due to some shrinkage artifact.) Normal erythrocytes are biconcave discs in order to increase surface area for gas exchange. There are no internal organelles left in a mature RBC.

One should note any abnormalities of the erythrocytes. Megaloblastic anemia will result in large RBCs, while iron deficiency will result in erythrocytes that are too small. A significant variation in shape of the erythrocytes should also be noted; this might include spherocytic changes, stippling or irregularly shaped cells (including sickle cells). When the area of central pallor occupies more than two-thirds of the cell, hypochromia is likely, and abnormalities in hemoglobin production should be suspected (such as iron deficiency). You will become acquainted with these and other less frequent abnormalities of erythrocytes when you study hematology.

Immature red blood cells, called reticulocytes, can be seen with certain stains in peripheral blood smears. About 1% of RBCs are reticulocytes. They usually remain as reticulocytes for about one day, and nearly 1% or RBCs are turned over each day.

WHITE BLOOD CELLS (WBC’s, LEUKOCYTES):

- neutrophils (polymorphonuclear leukocytes or “polys”)
- eosinophils
- monocytes
- lymphocytes
- basophils (rare)

Neutrophils have multi-lobed nuclei. They are phagocytic, being drawn to areas of acute inflammation. Their numbers increase in bacterial infections, for example. Immature neutrophils, with a horseshoe-shaped nucleus are called “band” (“stab”) cells. An increase in band cells suggests that more neutrophils are being produced and is called a “left shift” in neutrophil count.

Eosinophils have obvious red cytoplasmic granules and bi-lobed nuclei. These release a variety of vasoactive compounds and increase in allergic reactions and protozoal infections.

Basophils are so rare that you will probably not encounter one, but, if you do, it will have very dark cytoplasmic granules, obscuring the nucleus.
Monocytes are the largest white cells often with bean-shaped nuclei and agranular cytoplasm. These comprise only a small percent of WBCs, but may increase in chronic inflammatory conditions. These cells are attracted into tissues that, where they become phagocytic macrophages.

Finally, lymphocytes are the smallest WBC, slightly larger than the RBCs, and tend to be round with round nuclei that fill up almost the entire cell leaving scant cytoplasm. There are several types of lymphocytes, though they cannot be distinguished without staining for special cell surface markers. They are heavily involved in humoral and cell-mediated immune reactions and will be studied later in the module on immune system.

In practice, a search should be made for any abnormalities in the leukocytes, including such things as: hyper-lobulation and hypergranulation of the neutrophils; inclusion bodies; abnormal lymphocytes; the presence of immature cells (such as a high percentage of band cells); etc.

**PLATELETS**

Platelets can also be seen as small particles in between RBCs. Occasionally these are clumped together. When successive high power fields contain fewer than four platelets, and they are not present elsewhere on the slide in clumps, the platelets are said to be decreased. This will be confirmed with platelet counts.

What are platelets? What is their function? Where/how are they formed? What is the normal count range in a µl of blood?

**CHECK LIST**

Be able to identify and know the function of all cells and cellular components found in blood:
- erythrocytes
- granular leukocytes
  - neutrophils
  - eosinophils
  - basophils
- agranular leukocytes
  - monocytes
  - lymphocytes
- platelets

Define:
- rouleaux
- reticulocyte
- crenation
- hematocrit
HEMATOPOIESIS and BONE MARROW

Because of their finite life span, the blood cells in the peripheral stream must constantly be replenished. Most types of blood cells are highly differentiated and do not divide. A continuous supply of new blood cells is produced by the hematopoietic tissues (Gk., hemato, blood; poiein, to make), which are two special types of connective tissue derived from mesenchyme. An additional important function of the hematopoietic tissues is the elimination of old and/or defective blood cells by macrophages.

The first type of hematopoietic tissue is red bone marrow, alternatively known as myeloid tissue (Gr., myelos, marrow). This vital hematopoietic tissue is the essential source of most kinds of blood cells. The second hematopoietic tissue is lymphoid (lymphatic) tissue, which is chiefly involved in the production and maturation of lymphocytes. We will consider this tissue later with the immune system.

In the adult, red bone marrow (myeloid tissue) is largely confined to the medullary cavities of certain flat bones, such as ribs, sternum and pelvic bones, and in the vertebral column. For bone marrow transplants, the iliac crest or sternum are usually tapped under local anesthesia. The yellow marrow typical of adult long bones normally stores fat instead of producing blood cells. The microscopic organization of red marrow is not easily appreciated in routine histological sections. Basically the tissue consists of two major regions: a stroma, made up of CT cells supported by a delicate network of reticular fibers that holds a heterogeneous population of blood forming cells at various stages of differentiation; and abundant wide vascular channels called sinusoids by which newly formed blood cells enter the general circulation. The functional state of the blood forming cells of red marrow is routinely assessed by making smears of aspirated red marrow or by making sections of punch (core) biopsies of red marrow.

Venous sinusoids are identified by the aggregations of mature RBCs in their lumens.

Megakaryocytes. Giant cells with single large polyploid nuclei that send protoplasmic extensions into the venous sinusoids. These fragment to become platelets.

Erythroblastic (erythroid) islands. Clusters of developing red blood cells, the erythroid islands, can be found in the stroma of the bone marrow. These are in close proximity to the marrow sinusoids. A macrophage is typically found in the midst of the cluster, but may be difficult to distinguish. These are the sites of RBC production.

CHECK LIST

Understand the basic architecture of the red marrow slides, including:
- erythroid islands
- megakaryocytes
- marrow sinusoids
- lipocytes (fat cells)

With the marrow smear consider elements of the erythroid series:
- proerythroblasts
- basophilic erythroblasts,
- polychromatophilic erythroblasts
- orthochromatophilic erythroblasts
- reticulocytes

Understand the progression of the granulocyte series:
- myeloblasts
- promyelocytes
- myelocytes (neutrophilic; eosinophilic only)
- metamyelocytes (neutrophilic; eosinophilic only)
- band cells (neutrophilic; eosinophilic only)
- mature neutrophils & eosinophils
RESPIRATORY SYSTEM

For descriptive purposes the respiratory system is divided into conducting and respiratory portions. The conducting part includes all passageways through which air passes to reach the areas of the lung where gaseous exchange may occur between blood and air. These passageways include the nasal cavity and nasopharynx, the larynx, trachea, bronchi, and smaller bronchioles. The respiratory portion includes those areas of the lung, which possess alveoli. These two portions of the respiratory system overlap one another in the lung.

The trachea is characterized by C-shaped **hyaline cartilage**, which provides semirigid support for the wall of the trachea. The open part of the "C" is bridged by dense fibroelastic connective tissue (continuous with perichondrium) and contains smooth muscle of the **trachealis muscle**. It is lined by pseudostratified, ciliated columnar epithelium with goblet cells (respiratory epithelium). The epithelium is supported by a thick basement membrane and a lamina propria containing an abundance of elastic fibers. The looser connective tissue external to the lamina propria is considered the submucosa. **Mixed sero-mucous glands** are usually found in the submucosa.

Bronchi, accompanied by pulmonary arteries and the smaller bronchiole arteries continue into the lung. The pulmonary veins run slightly separate from the bronchi and arteries. The figure below shows the progression of the various elements of the wall of the respiratory tree. Note that cartilage and goblet cells are not present in the lining of the bronchioles. The lining cells of the bronchioles are cuboidal in shape. Also note that the smooth muscle becomes more prominent feature of
the wall of bronchioles.

Some bronchioles have accumulations of macrophages in their walls. These are easily identified because of their accumulated dense foreign particles. Diffuse aggregations of T- and B-lymphocytes are also common in the walls of bronchi and bronchioles (“BALT”, bronchus-associated lymphoid tissue).

Respiratory bronchioles are the first level where gas exchange can actually take place. These passages have some areas with cuboidal epithelium and some with outpouchings of squamous epithelium (alveoli). Alveolar ducts are passages lined by a series of alveoli with no intervening cuboidal epithelium.

Alveoli have a wall of squamous alveolar cells (Type I pneumocytes), and great alveolar cells (Type II pneumocytes). They often contain alveolar macrophages (“dust cells”), and have pulmonary capillary endothelium, elastic fibers, occasional mast cells and fibroblasts in the septal wall between alveoli. The type II pneumocytes produce pulmonary surfactant. Remember that while Type I cells constitute only about 40% of the cells in the alveolar wall, they cover >90% of the surface area. It may thus be difficult to discriminate between the nuclei of these very attenuated cells and those of the capillary endothelial cells or fibroblasts, which also reside in the alveolar septa. The blood-air (gas) barrier, at its minimum consists of the type I pneumocyte and the capillary endothelium, with intervening basement membrane.

Diagram of the respiratory subdivisions in the lung showing a respiratory bronchiole, alveolar ducts, and subdivisions. Smooth muscle (dark cells) ends in the alveolar ducts. The atria (circled) are spaces bounded by the termination of the alveolar duct on one end, and the openings of the alveolar sacs on the other. In addition, major features of the alveolar walls are presented.

The outer surface of the lung is covered with pleura, covered with a mesothelium (simple squamous epithelium).
CHECK LIST

Understand the progressive changes in the morphology of the airway from trachea to alveoli. Know the components of the conducting and respiratory systems.

Define:
- hyaline cartilage
- trachealis muscle
- seromucous glands
- smooth muscle
- trachea
- bronchus
- BALT (bronchus-associated lymphoid tissue)
- bronchioles
  - primary bronchiole
  - terminal bronchiole
  - respiratory bronchiole
- alveolar duct
- alveolar sac
- alveoli

Know the structure (LM and EM) and function of the following respiratory cells/structures:
- ciliated columnar cells
- goblet cells
- Clara cells
- alveolar macrophages (“dust cells”)
- Type I pneumocytes
- Type II pneumocytes
- pulmonary capillary endothelial cells
- components of the blood-gas barrier

INTEGUMENT

The integument consists of the skin, and a variety of skin derivatives. In humans, these include hair, nails and several types of glands. In other mammals, these cutaneous appendages include scales, quills, spines, claws, hooves, and horns. The skin consists of a superficial epithelial component, the epidermis, and an underlying layer of connective tissue, the dermis. Together these layers cover virtually the entire external body surface. The skin appendages derive from the epidermis and are therefore morphologically and functionally related to this epithelium. However, as you will see, in the adult some of these structures may have invaginated deeply into the dermis. Keep in mind that the epidermis and its derivatives are avascular; thus they depend on the underlying highly vascularized and innervated dermis for nutritional support.

The mammary glands (breast) will be examined in this lab since they are derived from, and reside within, the skin of the thorax.

Important cutaneous nerve receptors that are found in the skin will also be studied in this laboratory.

SKIN

The skin shows topographical diversity, ranging from thick to thin, rough to smooth, hairy to seemingly hairless (glabrous). In all cases the epidermal component consists of stratified squamous epithelium of varying thickness and degrees of cornification. Four different kinds of cells comprise the epidermis: keratinocytes, melanocytes, Langerhans cells, and Merkel cells.
A distinct basement membrane separates the epidermis from the underlying connective tissue dermis. The interface between these two strata, the **dermoepidermal junction**, varies over the body from relatively smooth in thin skin to highly corrugated in thick skin. Based on the organization of the various connective tissue fibers, especially collagen, the **dermis can be divided into two layers**: an outer or **superficial papillary layer** and an inner or **deep reticular layer**. The papillary layer tends to be loose, irregular fibrous connective tissue, while the reticular layer is dense, irregular fibrous connective tissue.

Beneath the dermis is a loose connective tissue layer of variable thickness and composition but usually containing considerable white adipose tissue. Its boundary with the overlying dermis is often indistinct. This subcutaneous layer, which corresponds to the superficial fascia of gross anatomy, is called the **hypodermis**.

**SOMATOSENSORY RECEPTORS**

Somatosensory receptors are abundant in skin (hairy and hairless). Most of these receptors are minute and require special staining methods to demonstrate. Two of the large encapsulated types (Meissner’s and Pacinian corpuscles) may be seen in routine H&E preparations. A summary of the various types of cutaneous receptors follows:
Meissner’s corpuscles: These consist of sensory nerve endings within a layered and encapsulated sensory organ in the dermal papilla.

Pacinian corpuscles. These structures, usually found in the deep dermis/hypodermis, are quite large. They have a single sensory axon that is encased in concentric layers of connective tissue (much like an onion).

GLANDS OF THE SKIN

The glands of the skin include sweat glands and sebaceous glands. Two types of sweat glands are seen, eccrine glands and apocrine glands. Apocrine sweat glands are more likely to be found in the axilla and perineum. Sebaceous glands are typically associated with a hair follicle.

MAMMARY GLAND (Breast)

The mammary glands are also developmental appendages of the skin. Each develops from about 20 cords of epidermal epithelial cells that penetrate into underlying connective tissue. Each cord develops into a lobe comprised of the rudiments of a compound tubular exocrine gland, which empties through its own lobar (lactiferous) duct at the nipple. After puberty, each lobar duct gives rise to several groups of smaller interlobular ducts. The development of the duct system and accumulation of fat account for enlargement of the mammary gland at this time. No further mammary gland development occurs until pregnancy. During pregnancy, the smaller interlobular ducts give rise to the many secretory portions of the glands, called alveoli, responsible for lactation. There are sebaceous glands (glands of Montgomery) the open onto the areola, providing lubrication.
CHECK LIST

Understand the morphology and components of thin and thick skin as well as its location on the body and the morphology of the layers of skin:
- epidermis
- dermoeipidermal junction
- dermis
- hypodermis (subcutis) (superficial fascia)
- papillary layer
- reticular layer

Learn the characteristics and contents of the epidermal strata:
- basale (germinativum)
- spinosum
- granulosum
- lucidum
- corneum

Recognize both the structure and function, in both LM and EM images, of the four types of cells of the epidermis:
- keratinocytes
- melanocytes
- Langerhans cells
- Merkel cells

Recognize the terms below related to pilosebaceous units:
- hair follicle
- hair bulb
- hair shaft
- dermal matrix
- dermal papilla
- sebaceous glands
- arrector pili muscle

Know the structure, location, secretory pattern and mode of secretion of:
- sebaceous glands
- eccrine (merocrine) sweat glands
- apocrine sweat glands

Know structure and function of:
- Meissner corpuscles
- Pacinian corpuscles

Understand the morphology of the mammary gland (breast) in resting and lactating tissue.