LABORATORY 13 - BLOOD CELL DEVELOPMENT

This lab has an associated homework assignment that is posted on Blackboard. If you have not done this assignment already, it would make this lab more efficient if you did so.

OBJECTIVES: LIGHT MICROSCOPY: Recognize the different stages of erythrocyte development: proerythroblast, basophilic erythroblast, polychromatophilic erythroblast, orthochromatophilic erythroblast and reticulocyte.
Recognize the different stages of granulocyte development: myeloblast, promyelocyte, neutrophilic and eosinophilic myelocytes, neutrophilic and eosinophilic metamyelocytes, and neutrophilic band and neutrophilic segmented cells. Recognize and understand the significance of the morphological changes that occur during development of these cells.

ELECTRON MICROSCOPY: See Section F

ASSIGNMENT FOR TODAY'S LABORATORY

GLASS SLIDES

SL 177 Peripheral blood smear from a patient with chronic myelogenous leukemia (CML), to be used for identifying early stages of granulocyte development - use tables and key at the end of this section.
SL 68 Normal bone marrow smear - Stages of development of erythrocytes and granulocytes use tables and key at the end of this section.
SL 22 Peripheral blood smear of baby's blood, reticulocytes
SL 39 Adult bone marrow
SL 41 Adult bone marrow

ELECTRON MICROGRAPHS See Section F

HISTOLOGY IMAGE REVIEW - available on computers in HSL
Chapter 6. Blood and Myeloid Tissue
Frames: 326-344

SUPPLEMENTARY ELECTRON MICROGRAPHS

Rhodin, J. A.G., An Atlas of Histology
Copies of this text are on reserve in the HSL.
Megakaryocytes pp. 77 and 78

BLOOD CELL DEVELOPMENT

Review the developmental stages of each cell type from the earliest form to mature cells by studying Figure 13-5 in Junqueira. Follow each cell type from the earliest recognizable stage to the mature cell type. In the erythroid series note the changes in color of the cytoplasm and the decrease in size and increase in the amount of heterochromatin of the nucleus. In the granulocyte series note the first appearance of granules (azurophilic) and subsequent appearance of specific granules (neutrophilic, eosinophilic or basophilic), with a general trend within the cytoplasm of neutrophils and eosinophils from basophilia to acidophilia. Note that maturation in the granulocyte series can be followed roughly on the basis of the shape of the nucleus. For both lines of cells, early developmental stages are indicated by cytoplasmic basophilia and a euchromatic nucleus that includes prominent nucleoli.

To begin your study of blood cell development, practice identifying selected cells from still images using the links in the table below. The unlabeled images have arrows to signify cells that should be readily identifiable. Most, if not all, of the remaining cells in each slide are more difficult to place into a specific stage of development. Using Figure 13-5 from Junqueira, and/or the tables and schema found at the end of this assignment, try to identify the cells indicated by the arrows in the unlabeled images. Check your answers using the corresponding labeled images.

Unlabeled Images	Labeled Images
Slide 1	Slide 1
Slide 2	Slide 2
Slide 3	Slide 3
Slide 4	Slide 4
Slide 5	Slide 5
Slide 6	Slide 6
Slide 7	Slide 7
Slide 8	Slide 8
Slide 9	Slide 9
Slide 10	Slide 10
Slide 11	Slide 11
Slide 12	Slide 12
Slide 13	Slide 13
Slide 14	Slide 14

Now that you have had some practice identifying clear-cut examples of blood cells in development using images in which the cells to be identified were indicated with an arrow, the next challenge is to try to determine whether a cell on a slide is readily identifiable, and, if it is, to place it into a specific stage. (Hint: most cells on your slides will fall into a category that is not definitive; either the cell is between stages, has characteristics of two stages, or is completely unrecognizable.)

1. SLIDE STUDY

As with the previous lab, in which we looked at mature formed elements, you will need to use your oil objective to discriminate between the different developing blood cell types. Note that lymphocyte development will be considered with the lymphoid tissue. Also, in contrast to the granulocytes, identification of stages in monocyte development requires special methods and in our preparations monocytes will not be identifiable with accuracy. Finally, the stages of basophil development are observed infrequently.

A. SL 177 - CHRONIC MYELOGENOUS LEUKEMIA (CML). Peripheral blood smear stained by Wright's or Giemsa's stain. Smear was obtained from a patient diagnosed with CML. In this pathologic condition, early developmental stages, mostly of neutrophils, are numerous in the circulating peripheral blood. Morphologically many of these cells they are similar to normal cells of the bone marrow and may be considered representative of normal. However, you should be aware that there are also many abnormal cells in which the nucleus and cytoplasm lack the synchronization that is found in normally developing blood cells.

1. First identify mature forms of red blood cells, lymphocytes and neutrophils.

2. Then find cells that are immature. As you are aware, although you are observing a smear of peripheral blood, none of these immature cells are present in a normal blood smear of peripheral blood. This means that many of these cells will have abnormal characteristics. However, the cells will contain the characteristics of the developing blood cells and one of your tasks while observing this smear is to acquire some familiarity with recognizing these characteristics. Try to identify the cells on the basis of the:

    a) presence or absence of azurophilic granules (blue to purple), or specific granules (neutrophilic or eosinophilic specific granules)

    b) presence or absence of nucleoli

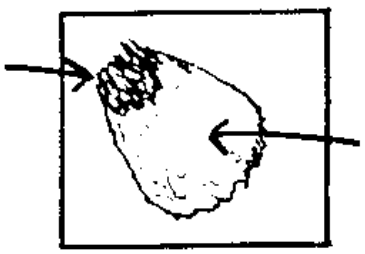
    c) degree of basophilia

    d) the shape of the nuclei.

Try to find as many of the cell types as possible: myeloblasts, promyelocytes, myelocytes, metamyelocytes, and band forms. Many slides show an increased number of basophils and occasional cells of the erythroid line.
Although it is possible, you will not have to distinguish myeloblasts from proerythroblasts unless given direct information about the cell in question (e.g. "Identify this cell that is from the red blood cell series.").

B. SL 68 NORMAL BONE MARROW SMEAR (W.3.11) - This slide was prepared from a small piece of aspirated bone marrow placed on a cover slip and spread by a second cover slip placed over it.

Dark area = Site where original specimen was placed. From observation of this region the concentration of cells can be determined.



Smear: Study are where **CELLS ARE FLATTENED AND FAIRLY WELL SEPARATED.**

1. Cells of the granulocytic series will not be as clearly identifiable as on SL 177. However, the different stages in development can still be determined on the basis of the types of granules, cytoplasmic staining and nuclear changes.

2. Scan over the smear identifying different stages in erythroid differentiation according to diagrams, schema and W. 3.17. Polychromatophilic erythroblasts and orthochromatophilic erythroblasts are most numerous. Only a few basophilic erythroblasts and rarely proerythroblasts will be found. You are not expected to differentiate between myeloblasts and proerythroblasts, unless you are provided with additional information or clues concerning the lineage of the cell. Megakaryocytes (Megakaryocyte A and Megakaryocyte B) (W. 3.16) of different stages of maturation are quite evident. Keep in mind that both plasma cells and mature lymphocytes are also present in bone marrow and you should be able to recognize them, although other incidental cells may not be identifiable.

C. BLOOD, BABY - SL 22 image from SL 22 Stained for Reticulocytes (W. 3.3). Note reticulocytes and some immature band neutrophils. The reticulocyte count of this blood is elevated. What is the normal reticulocyte (W. 3.3) and band neutrophil percentage in the adult?

D. ADULT BONE MARROW - SL 41, 39 (J. 13-3 to13-9, 13-13; W. 10.14). On slide 41 (low, high) note the general appearance of bone marrow. Most apparent is the hematopoietic compartment of developing cells. Sinusoids are identifiable only by seeing aggregates of RBC^s. The reticular stroma is not evident and fat is minimal. SL 39 (low, med) demonstrates a marrow with abundant fat cells. What is the difference in the distribution of red and yellow marrow in adults and children? J. pp. 251-252.

E. Note that in development and pathologic conditions hematopoiesis may occur in organs other than bone marrow. (e.g., J. p.249, 260).

F. Electron Microscopy
The details of every stage of development are too specialized at this time; however, from your knowledge of cell ultrastructure and the changes occurring in blood cell development you should be able to estimate the appearance one might see in early, middle and late stages of granulocyte and erythroid development. To aid in this see (J. 13-1, 13-20; W. 3.13, 3.16).

ERYTHROCYTE MATURATION							
CELL	**NUCLEUS**				**CYTOPLASM**		
TYPE AND SIZE	**SHAPE**	**CHROMATIN**	**NUCLEAR MEM.**	**NUCLEOLI**	**COLOR**	**GRANULES**	**RELATIVE AMOUNT**
Proerythroblast 10 – 20 :	Round	In thick strands, coarse, dark staining tendency to clump	Fine	Must have 2 – 3	Very basophilic & condensed	None	Moderate to scant
Basophilic erythroblast 10 – 15 :	Smaller round	Thick strands, darker than previous, tendency to clump in later stages	Fine	0	Basophilic & condensed	None	Moderate
Polychomato-philic erythroblast 12 – 15 :	Smaller round	Coarse, clumped, “checker board”	Fine	0	Light gray mixing with pink as Hb is synthesized	None	Increasing amount
Orthochromato-philic Erythroblast 7 – 10 :	Round	Mostly heterochromatic, a few small patches of euchromatin may remain, nucleus may be fragmented	None	0	Pink, almost full complement of Hb	None	Increasing amount
Reticulocyte 8 – 9 :	Nuc. absent Cell shape: round			0	Pink or acidophilic, special stain reticulum	None	--
Erythrocyte 6 – 8 :	Nuc. absent Cell shape: bi-concave				Acidophilic	None	--

GRANULOCYTE MATURATION							
CELL	**NUCLEUS**				**CYTOPLASM**		
TYPE & SIZE	**SHAPE**	**CHROMATIN**	**NUCLEAR MEM.**	**NUCLEOLI**	**COLOR**	**GRANULES**	**RELATIVE AMOUNT**
Myeloblast 10 – 20 :	Large round to slightly oval	Fine meshwork, sieve-like or dust-like appearance, stippled, basophilic (pale)	Fine	2 - 4	Basophilic & condensed	None	Scant
Promyel-ocyte 12 – 20 : some 25 :	Round, may become oval & eccentrically placed	Fine meshwork may become coarse	Indistinct	2 in early, 0 in later	Basophilic	Must have azurophilic gran., number varies, no specific gran.	Scant to moderate
Myelocyte (Last to divide) 12 – 20 :	Oval, usually & eccentric in position	Coarser chromatin network	Indistinct	0	Basophilic, then grades of pink	Azurophilic gran. decrease Specific gran. first appear, later filled with specific gran.	Moderate
Metamyel-ocyte 10 - 18 :	Indented or sausage	Coarse	Present	0	Pink, or acidophilic	Few azurophilic, filled with specific (neutrophilic or eosinophilic)	Moderate
Band or stab 10 - 15 :	Curved band, horseshoe	Coarse	Present	0	Pink or acidophilic	Mostly specific, occasional azurophilic	Moderate
Three segmented forms	Studied with blood, review						

SCHEMA FOR IDENTIFICATION OF DEVELOPMENTAL STAGES OF ERYTHROPOIESIS AND GRANULOCYTOPOIESIS

First determine A or B, then proceed.

A. Are there numerous granules present?
(either azurophilic or specific) - if so determine 1-4 and proceed.

1. Nucleus - round or oval.

   a. Round, has nucleoli, basophilic cytoplasm, azurophilic granules only – **Promyelocyte**

   b. Oval, no nucleoli, few to nearly filled with specific granules (some azurophilic granules may or may be evident) = **Myelocyte**

2. Nucleus - indented = **Metamyelocyte**

3. Nucleus - "horseshoe" = **Band**

4. Nucleus - Segmented = **Neutrophil**

*Stages between Myelocyte and mature forms for eosinophils and basophils are difficult to designate.

B. No granules present - if none, determine 1 or 2 and proceed.

1. Hemoglobin not evident, cytoplasm entirely basophilic.

   a. Nucleus, fine chromatin, nucleoli present - Cytoplasm light basophilic = **Myeloblast**

   b. Nucleus, coarse, clumped chromatin, nucleoli present, perinuclear halo, cytoplasm intensely basophilic = **Proerythroblast**

   c. Nucleus coarser chromatin nucleoli absent, basophilic cytoplasm = **Basophilic erythroblast**.

2. Hemoglobin evident in cytoplasm

   a. Nucleus checkerboard - cytoplasm mixed basophilia and acidophilia = **Polychromatophilic erythroblast**

   b. Nucleus mostly or fully condensed, a few small patches of euchromatic may be present, nearly full complement of hemoglobin, no basophilia in cytoplasm = **Orthochromatophilic erythroblast**

OBJECTIVES FOR LABORATORY 13: BLOOD CELL DEVELOPMENT (HEMATOPOIESIS)

1. Using the light microscope or digital slides, identify:

    - Bone marrow (or leukemia) smear
        - Stages of granulocyte development
            - Myeloblast
            - Promyelocyte
            - Myelocyte
                - Neutrophilic
                - Eosinophilic
                - Basophilic
            - Band neutrophil
            - Mature cells (neutrophil, eosinophil, basophil)
        - Stages in erythroid development
            - Proerythroblast
            - Basophilic erythroblast
            - Polychromatophilic erythroblast
            - Orthochromatophilic erythroblast
            - Reticulocyte
            - Mature red blood cell
        - Other cells you may see in a bone marrow smear
            - Megakaryocyte
            - Plasma cell
            - Lymphocyte
    - In sections of bone marrow
        - Red bone marrow
        - Yellow bone marrow

2. On electron micrographs, identify:

    - Megakaryocyte
    - Plasma cell (see posted EMs from lab 7)
    - Lymphocyte

REVIEW

1. What structural alterations are found in the cytoplasm and nucleus in the maturation of erythrocytes and granulocytes from myeloblast to mature cell?

2. Both erythrocytes and platelets are formed elements of the peripheral blood that lack nuclei. Compare their methods of development.

3. What is the significance of an increase,

   a) in reticulocytes in a blood smear?

   b) in band neutrophils?

4. In tissue sections can adipocytes be observed in red marrow?