EDUCATIONAL COMMENTARY – BLOOD CELL IDENTIFICATION

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Learning Outcomes
After completion of this exercise, the participant will be able to:

- identify morphologic features of normal peripheral blood leukocytes and platelets.
- describe characteristic morphologic findings associated with reactive lymphocytes.
- compare morphologic features of normal lymphocytes, reactive lymphocytes, and monocytes.

Photograph BCI-01 shows a reactive lymphocyte. The term “variant” is also used to describe these cells that display morphologic characteristics different from what is considered normal lymphocyte appearance. Reactive lymphocytes demonstrate a wide variety of morphologic features. They are most often associated with viral illnesses, so it is expected that some of these cells would be present in the peripheral blood of this patient. This patient had infectious mononucleosis that was confirmed with a positive mononucleosis screening test.

An increased number of reactive lymphocytes is a morphologic hallmark of infectious mononucleosis.

Some generalizations regarding the morphology of reactive lymphocytes can be made. These cells are often large with abundant cytoplasm. Cytoplasmic vacuoles and/or azurophilic granules may also be present. Reactive lymphocytes have an increased amount of RNA in the cytoplasm, which is reflected by an associated increase in cytoplasmic basophilia. The cytoplasm may stain gray, pale-blue, or a very deep blue and appear patchy. The cytoplasmic margins may be indented by surrounding red blood cells and appear a darker blue than the rest of the cytoplasm. Likewise, the nuclei in reactive lymphocytes are variably shaped and may be round, oval, indented, or lobulated. The chromatin is usually finer with minimal clumping when compared to a non-reactive lymphocyte. Parachromatin may be more evident. Nucleoli may be visible and prominent.

Some participants identified the cell in BCI-01 as a monocyte. When compared to the monocyte in BCI-07, it may be seen that the cell in BCI-01 has blue edges at the RBC interface as well as lighter areas around the nucleus. This uneven appearance to the cytoplasm is more consistent with reactive lymphocytes. The monocyte in photograph BCI-07 is more consistently ground-glass looking or grainy by the nucleus and a lighter color at the RBC interface. The nucleus in this cell is also less homogeneous.
and more uneven. Likewise, the cell is more "pushy" against the RBC. These features are more suggestive of a monocyte.

The cell depicted in BCI-02 is a segmented neutrophil. Note the fine pink or tannish cytoplasmic granules. Also notice the characteristic nuclear lobes. Segmented neutrophils usually have 2 to 5 nuclear lobes that are connected by thin threads of chromatin. The nuclear chromatin is dense and clumped. Some participants identified the cell in BCI-02 (as well as the cell in BCI-05) as an eosinophil. Although the granules are pink in these photographs, they are not prominent enough to be those of eosinophils. Eosinophilic granules are larger and more intensely colored as orange. The granules in a neutrophil are less distinct, smaller, and less colorful (a dull pink). Furthermore, eosinophilic granules have been referred to as "copper pennies" because of their large, colorful appearance. In addition, eosinophils are typically bi-lobed. The cell in photograph BCI-02 has 3 lobes. For comparison, see the eosinophil in the 2007 3rd Test Event Blood Cell Identification case study labeled BCI-18. (This photograph can be accessed by clicking on ASCP Commentaries on the left side of the home page, then choosing "Blood Cell ID: Acute Lymphocytic Leukemia").

Picture BCI-03 shows a normal lymphocyte. Lymphocytes are variable in size. The one depicted here is a small lymphocyte. The nucleus in this example is round, but shape variations to include oval or slightly indented nuclei are also possible. The nuclear chromatin is condensed and clumped. Note the thin, barely visible rim of blue cytoplasm.

While some participants reported the cell in photograph BCI-03 to be a reactive lymphocyte, this cell is too small and the nucleus to cytoplasm ratio is too large (too little cytoplasm) to be classified as such. It is helpful to compare this cell to the reactive lymphocytes in the other photographs.
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The arrows in image BCI-04 are pointing to normal peripheral blood platelets. Platelets are usually small and though considered cells, they are actually not cells because they lack a nucleus. However, platelets originate from bone marrow cells called megakaryocytes. They represent fragments of the cytoplasm from megakaryocytes. Platelets stain a blue-gray or light purple. They may vary in shape, but are generally round or oval. Often, a central, more-grainy core is surrounded by a less grainy halo. Sometimes the cellular margins also appear frayed.

The cell identified in photograph BCI-05 is a band neutrophil. Notice that the size of this cell is similar to the segmented neutrophil shown in BCI-02. Bands represent the stage of maturation immediately before the segmented neutrophil. Band neutrophils are the earliest precursors of neutrophil maturation that can normally be seen in the peripheral blood. On a differential cell count from a normal adult, approximately 2% to 6% of the cells may be band neutrophils.

Bands typically have a nucleus that is shaped like a band, a sausage, or the letters “U” or “C.” However, they are a maturing cell, as indicated by the clumped and dense chromatin. Also, as seen in the previously discussed segmented neutrophil, the cytoplasm in bands features numerous pink or tannish granules.

The band cell shown in this image is a classic example. Sometimes, though, it can be difficult to distinguish bands from segmented neutrophils. Note that the nuclear lobes in the segmented neutrophil are connected by thin filaments. In contrast, the nuclear lobes in the band are joined by a bridge, with no constriction. When the classification stage of a cell is in doubt, the cell should be identified as the more mature stage.
There is considerable debate about the identification of the cell in photograph BCI-06. Some participants reported this cell to be a reactive lymphocyte; however, many participants reported it to be a normal lymphocyte. Although the nucleus of this cell is larger than a normal-sized RBC, even normal lymphocytes can be variable in size (small, medium, large). While the cytoplasm in this cell is somewhat darker, again, even normal lymphs can be variable in cytoplasmic color. The chromatin is also dense and clumped.

This cell differs morphologically from the reactive lymphocyte illustrated in BCI-01. Several other images in this testing event also present examples of reactive lymphocytes (BCI-04, BCI-05, and the top left cell in BCI-06). It is expected that an increased number of reactive lymphocytes be seen on the blood smear from the patient in this testing event. In fact, 90% of the lymphocytes from the differential cell count for this young woman were reported as reactive. Although reactive lymphocytes may be seen on normal peripheral blood smears, they usually represent less than 10% of the total number of lymphocytes. In infectious mononucleosis, reactive lymphocytes may exceed 50% of the total population of lymphocytes.

The variety of morphologic appearances among the reactive lymphocytes presented in this testing event is also characteristic of a benign lymphoproliferative disorder such as infectious mononucleosis. The cell identified in image BCI-06 may be considered reactive based on several features. When compared to the normal lymphocyte in picture BCI-03, it is larger and has a darker blue cytoplasm. Likewise, though the reactive features in the lymphocytes in BCI-04, BCI-05, and the additional lymphocyte in BCI-06 are variable, it is this heterogeneity that defines these cells as “variant.” The lymphocyte in BCI-04 has diffuse azurophilic granules in a pale-blue, uneven-stained cytoplasm. In addition, vacuoles are sometimes seen in reactive lymphocytes. The nuclear chromatin in this lymphocyte is also less dense and clumped than in the normal cell depicted in BCI-03. Parachromatin is not as evident in a normal lymphocyte. The cells in BCI-05 and the top left in BCI-06 are good examples of reactive lymphocytes that have a sprawling cytoplasm with cellular margins indented by surrounding RBCs.

Sometimes reactive lymphocytes may be mistaken for blast cells. There are several similarities. Blast cells are often large with a high nuclear to cytoplasmic ratio. The nuclei are oval or round with a loose and open chromatin pattern. The cytoplasm is generally very basophilic. However, a careful evaluation of blasts reveals several differences from reactive lymphocytes. Nucleoli are usually more visible and prominent in blasts. The cytoplasmic margins in blast cells are generally not indented and more uniform. Likewise, vacuoles and granules are not as commonly seen in blasts when compared to reactive
lymphocytes. Furthermore, blast cells within any peripheral blood sample are morphologically homogeneous. That is, all the blasts appear similar in overall size, nuclear features, and cytoplasmic characteristics. The morphologic heterogeneity typical of reactive lymphocytes is not seen.

The last image in this testing event, **BCI-07**, is a normal monocyte. Monocytes are large cells with abundant, blue-gray cytoplasm. There are often vacuoles present in the cytoplasm. Note that vacuoles can also be seen in segmented neutrophils. In both monocytes and neutrophils, vacuoles can represent phagocytized and digested organisms or debris. Sometimes faint purple or lilac granules may also be seen in monocytes, as in this example. The cytoplasm often appears uneven and the cytoplasmic margins are sprawling. The nuclear shape in monocytes varies and may be round, oval, indented, or lobulated. No visible nucleolus can be seen within a fine, minimally clumped cytoplasm.

Monocytes too may be confused with reactive lymphocytes. Although both cells are large, cytoplasmic and nuclear differences can be observed. Usually the cytoplasm of monocytes is more extended, vacuolated, and uneven or rough in appearance when compared to reactive lymphocytes. The cytoplasm of monocytes is not as readily indented by surrounding erythrocytes as in the cytoplasm of reactive lymphocytes. In addition the cytoplasmic margins of reactive lymphocytes are frequently a darker blue at the interface with red blood cells. The nuclear chromatin structure in reactive lymphocytes is variable, though the cells in this case study patient have moderately condensed chromatin. The chromatin structure in monocytes generally shows minimal clumping. Nucleoli are more likely seen in reactive lymphocytes than in monocytes.

The table on the next page compares morphologic features of normal lymphocytes, reactive lymphocytes, and monocytes.
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TABLE. Characteristics of Lymphocytes, Monocytes, and Blasts.

<table>
<thead>
<tr>
<th></th>
<th>Small Lymph</th>
<th>Reactive Lymph</th>
<th>Monocyte</th>
<th>Blast</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell Size</strong></td>
<td>8-12 µm</td>
<td>Variable, can be large</td>
<td>Large (15-18 µm)</td>
<td>Large (10-20 µm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(9-30 µm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nuclear Shape</strong></td>
<td>Round</td>
<td>Irregular (lobulated, oval, notched)</td>
<td>Horseshoe, round, folded</td>
<td>Round, oval; may be indented or folded</td>
</tr>
<tr>
<td><strong>Nuclear Chromatin</strong></td>
<td>Clumped; parachromatin not evident and more lavender</td>
<td>More fine (but not like a blast); parachromatin more evident and white</td>
<td>Lacy, loose strands, brain-like</td>
<td>Fine, lacy</td>
</tr>
<tr>
<td><strong>Nucleoli</strong></td>
<td>Usually absent</td>
<td>May be prominent</td>
<td>Absent</td>
<td>One or more; often prominent</td>
</tr>
<tr>
<td><strong>Cytoplasm Amount and Composition</strong></td>
<td>Scant</td>
<td>Abundant; vacuoles and granules may be present</td>
<td>Abundant; vacuoles and granules may be present</td>
<td>Scant; no granules</td>
</tr>
<tr>
<td><strong>Cytoplasm Color</strong></td>
<td>Blue</td>
<td>Often deep, intense blue with darker edges at contact points with other cells; may stain patchy</td>
<td>Light bluish gray; uneven or rough</td>
<td>Very blue</td>
</tr>
</tbody>
</table>

Summary
The images presented in this testing event represent classic examples of reactive lymphocytes, as may be seen in infectious mononucleosis. This case also highlights the challenges that sometimes exist when distinguishing normal lymphocytes, reactive lymphocytes, and monocytes. In addition to basing classification of cells on morphologic criteria related to cell size, nuclear features, and cytoplasmic characteristics, it can also be useful to review the results of a complete blood count (CBC). As a reminder, when viewing actual stained peripheral blood smears, several microscopic fields should be systematically evaluated and correlated with the available information.

Suggested Reading

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