ADVANCED BLOOD CELL ID: MORPHOLOGIC FEATURES OF REACTIVE LYMPHOCYTES

Educational commentary is provided for participants enrolled in program #259- Advanced Blood Cell Identification. This virtual blood cell identification program includes case studies with more difficult challenges. To view the blood cell images in more detail, click on the sample identification numbers underlined in the paragraphs below. This will open a virtual image of the selected cell and the surrounding fields. If the image opens in the same window as the commentary, saving the commentary PDF and opening it outside your browser will allow you to switch between the commentary and the images more easily. You will need Adobe Flash or the Microsoft Edge browser (included with Windows 10) to use this feature. Click on this link for the API ImageViewer™ Instructions.

Learning Outcomes

After completing this exercise, participants should be able to:

- Identify morphologic characteristics of normal peripheral blood leukocytes.
- Describe morphologic features associated with reactive lymphocytes.
- Compare morphologic characteristics of normal lymphocytes and reactive lymphocytes.

Case Study

A 19-year-old female was seen by her physician for a fever and sore throat. The CBC results are as follows: WBC=7.9 x 10^9/L, RBC=4.73 x 10^{12}/L, Hgb=13.9 g/dL, Hct=41.5%, MCV=87.7 fl, MCH=29.4 pg, MCHC=35.5 g/dL, RDW=13.9 %, Platelet=208 x 10^9/L.

Introduction

The patient presented in this testing event was diagnosed with infectious mononucleosis. The cells annotated for discussion represent normal peripheral blood cells, but also reactive lymphocytes and artifacts that are characteristically seen in this condition.

Educational Commentary

The cell identified in ABI-01 is a normal lymphocyte. Lymphocytes are variable in size. This particular lymphocyte is a medium-sized cell. Lymphocytes are generally round or oval with similarly shaped nuclei. The nucleus and cytoplasm of this lymphocyte were indented by red blood cells during the preparation of the blood smear; this is not a usual finding in normal lymphocytes. The nucleus is typically large in normal lymphocytes when compared to a scanty or moderate amount of cytoplasm. The chromatin is condensed and clumped, though this cell has some unusual lighter staining areas. The blue cytoplasm is characteristic.
The cell selected for **ABI-02** is a reactive lymphocyte. Sometimes these cells are described as variant or atypical. There can be such variety in the morphologic characteristics seen in reactive lymphocytes that there is no “typical” atypical lymphocyte. The morphologic variations in reactive lymphocytes reflect the heterogeneity of the immune response that results in their production. Reactive lymphocytes represent stimulated cells that are “reacting” or “responding” to an antigenic challenge, often a viral illness such as infectious mononucleosis. Although reactive lymphocytes display various morphologic characteristics, certain features are usually associated with these cells. They are generally large cells, often with moderate to abundant cytoplasm. The cytoplasm may appear deep blue, gray-blue, or gray. Sometimes, clear areas may be seen in the cytoplasm, as in this cell. Also note the darker blue cytoplasm where this particular cell interfaces with surrounding RBCs; this is a common finding in reactive lymphocytes. The skirting and molding of the cytoplasm by adjacent erythrocytes is, likewise, a usual finding in these cells. Cytoplasmic vacuoles and/or azurophilic granules may also be seen. Nuclei in reactive lymphocytes are large and may be round, oval, indented, or lobated. Nucleoli may or may not be present. The nuclear chromatin is generally fine, with minimal clumping, when compared to a normal lymphocyte, though there is variety in chromatin appearance as well. Parachromatin may be more distinct in reactive lymphocytes.

The cell annotated in **ABI-03** is another reactive lymphocyte. This cell was chosen to emphasize the variety of morphologic features that can be seen in reactive lymphocytes. This cell shares many characteristics of the cell in ABI-02, such as overall cytoplasmic color, deep blue cytoplasmic margins, vacuoles, nuclear shape, the presence of distinctive nucleoli, and chromatin that is minimally clumped. However, this reactive lymphocyte is a larger cell with more prominent cytoplasmic clearing. The cytoplasm is also unevenly stained.

Compared to the normal lymphocyte in ABI-01, the reactive lymphocytes in ABI-02 and ABI-03 are larger cells with irregular nuclear shape and nucleoli. The cytoplasm in both reactive cells is blue-gray and there is darker bluing at some of the margins.

The cell identified in **ABI-04** is an eosinophil. Eosinophils are medium-sized cells characterized by numerous, red-orange cytoplasmic granules. These copper-colored granules are large and uniform in size. The nucleus is often bi-lobed, as in this example, with condensed and clumped chromatin.

The cell chosen for **ABI-05** is a segmented neutrophil. Also a medium-sized cell, neutrophils feature small pink, tan, or violet cytoplasmic granules. The nuclei in segmented neutrophils are typically separated into two to five lobes. The lobes are usually connected by thin threads of chromatin. It is difficult to visualize strands of chromatin in this cell, but at least three lobes are present and the folding of
the lobes may obscure possible chromatin filaments. The nuclear chromatin is dense and clumped as is characteristic of segmented neutrophils.

The object in ABI-06 is a smudge or basket cell. These artifacts are white blood cells that have been damaged or distorted during the preparation of the blood smear. Most often, the cells are fragile lymphocytes. Lymphocytes are especially sensitive to disruption and this fragility is enhanced in certain disease conditions such as chronic lymphocytic leukemia or, as in this case study, infectious mononucleosis. This damaged cell either appears like a smear or smudge of chromatin or with strands of chromatin fanning out from the central nuclear mass, similar in morphology to a basket. The cell in this example has a smudge-like feature, but also openness spreading out from a central chromatin clump, suggestive of a basket. Clearly, however, there is no distinguishable cytoplasm to indicate this is a whole cell. Therefore, it is not possible to classify this cell as any specific WBC. Note that, when numerous, smudge cells should be reported.

The final cell selected for commentary, ABI-07, is a reactive lymphocyte. This cell is similar to the other reactive lymphocytes (ABI-02 and ABI-03). This reactive lymphocyte is large, just as the other cells discussed. Likewise, the cytoplasm is moderate in amount and blue-gray, although perhaps slightly more blue than gray. The cellular shape in all three reactive lymphocytes is uneven, though there appears to be more pronounced cytoplasmic skirting in the cells identified for ABI-02 and ABI-03. The nucleus in ABI-07 has only slight indentations whereas the nuclear creases in the other reactive lymphocytes (especially in ABI-03) are greater. Most notable, however, is the nuclear chromatin in ABI-07. The chromatin pattern in reactive lymphocytes is variable and this feature is particularly evident in ABI-07. The chromatin in this cell is more open and linear with clearing areas of distinctive parachromatin. In contrast, the nuclear chromatin in the reactive lymphocytes seen in ABI-02 and ABI-03, while not dense, is still not as open as the chromatin in ABI-07. Nucleoli are more evident in the lymphocytes in ABI-02 and ABI-03. Although not visible in any of these three reactive lymphocytes, the nuclear chromatin sometimes may appear even more loose and fine, resembling the pattern seen in blasts. The different nuclear chromatin features in the reactive lymphocytes selected for discussion underscore the variety of nuclear morphologic characteristics, in addition to cytoplasmic variations, that can be associated with these cells.

**Infectious Mononucleosis**

Infectious mononucleosis (IM) is a benign disorder caused by the Epstein-Barr virus (EBV) that is characterized by lymphocytosis and the presence of reactive lymphocytes in the peripheral blood smear. EBV infects B lymphocytes, but the reactive cells in the blood are primarily cytotoxic T lymphocytes produced as an acute immune response against the infection. Although complications may rarely occur,
most cases of IM are self-limiting. Since reactive lymphocytes may be seen in other viral illnesses, the laboratory evaluation of a patient suspected of having IM should include additional testing for antibodies, such as the IM heterophil antibody and EBV-specific antibodies, if indicated.

Summary

The patient presented in the case study for this testing event was diagnosed with IM. Lymphocytosis and an increase in reactive lymphocytes characterize this condition and are important for diagnosis. This exercise emphasizes the morphologic variety, or heterogeneity, associated with reactive lymphocytes. Laboratory professionals are key “first responders” in recognizing the presence of reactive lymphocytes on a peripheral blood smear. This observation requires additional morphology review and testing to confirm the disorder as IM.

References