DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

Week 6

ASSIGNMENT, OBJECTIVES, AND CASE STUDY

TOPIC OF THE WEEK: HEMATOPOIETIC SYSTEM/LYMPH NODES AND SPLEEN

REQUIRED READING:

Cotran, Kumar, Collins: Robbins’ PATHOLOGIC BASIS OF DISEASE, 6th Edition,
White Cells, Lymph Nodes, and Spleen (Chapter 15, pp. 645-675; 688-693) 
(Leukemias, pp. 675-688 for review only)

Red Cells and Bleeding Disorders (Chapter 14, pp. 601-642)
(This material covered in the Hematology Course; please review only)

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REQUIRED STUDY FOR SMALL GROUPS

CASE BASED STUDY Small Group Sessions

ASSIGNMENTS:
- Laboratory Medicine Case Book Chapter 2, 23 OR
- Laboratory Medicine Case Set CD ROM Chapter 3, 33
- Printed Case 1 (attached)

OBJECTIVES:
1. Case Book, Chapter 2, OR, Case Set, Chapter 3
   - Pathogenesis, clinical course, and treatment of sickle cell anemia
   - Laboratory evaluation in sickle cell anemia:
     - Ravel: Clinical Laboratory Medicine, pp. 42-45;
     - Raskova, Shea, Skvara, and Mikhail: Laboratory Medicine Case Book, p.19
   - Complications of sickle cell anemia
   - Serum bilirubin as a test (understanding, interpretation, and diagnostic use)
     - Cotran, Kumar, Robbins: Pathologic Basis of Disease, pp. 837-838;
     - Ravel: Clinical Laboratory Medicine, pp. 309-311
   - Urine bilirubin and urobilinogen as a test (understanding, interpretation, and diagnostic use)
     - Ravel: Clinical Laboratory Medicine, pp. 311-312
2. Case Book, Chapter 23 OR, Case Set, Chapter 33
   - Clinical course of acute myeloblastic leukemia (AML)
   - Classification of AML:
     Raskova, Shea, Skvara, and Mikhail: Laboratory Medicine Case Book, p.247
   - Laboratory evaluation of the bone marrow and of peripheral blood in acute leukemias:
     Ravel: Clinical Laboratory Medicine, pp. 67-71

3. Printed Case (Attached)
   - Pathogenesis of the diagnosed problems
   - Immunophenotyping of lymphocytes as a laboratory procedure (understanding, interpretation, diagnostic use):
     Ravel: Clinical Laboratory Medicine, pp. 65-66
   - Understanding of problems raised by questions for homework and discussion

PATHTALK Small Group Sessions
ASSIGNMENTS:
- Projection slides on carousels in the Media Library, labeled by weekly topic and subject
- Slide Manual (pp. 48-60)
- Journal Club Article (see your Course Book)

OBJECTIVES:
- Correlations of histopathology, gross pathology, and laboratory findings
- Review of pathophysiology

ADDITIONAL MATERIAL (Optional, unless indicated otherwise)

- SELF-STUDY MATERIAL, MATERIAL FOR SELF EVALUATION and VISUAL AND AUDIOVISUAL MATERIAL

See your Course Book (page 4) for a complete listing.
DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

“PRINTED” CASE
CASE STUDY #1

CLINICAL SUMMARY:

A 70-year-old male presented with the following findings (see lab data) in his peripheral blood. There was no lymphadenopathy or hepatosplenomegaly. A kodachrome of a peripheral blood smear can be seen on the poster.

**Hematology**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>30,000/mm³</td>
</tr>
<tr>
<td>RBC</td>
<td>4.35 million/mm³</td>
</tr>
<tr>
<td>HGB</td>
<td>14.2 gm/dl</td>
</tr>
<tr>
<td>HCT</td>
<td>41.8</td>
</tr>
<tr>
<td>Plts</td>
<td>150,000</td>
</tr>
</tbody>
</table>

**WBC differential count:**

- 95% Lymphocytes

**Comment:**

A flow cytometric analysis was performed.* The scattergram** shown below reveals two cell clusters, lymphocytic (1) and granulocytic (2) Only the lymphocytic cell cluster is analyzed. The quantitation of lymphocytes (expressed as percentage of total lymphocytic population) is shown in the results and interpretation*** below.

Scattergram
**Description of specimen and procedure:**

The specimen labelled "peripheral blood" approximately 10ml was received in an EDTA vacutainer. Red cells were lysed and the remaining cells were stained with monoclonal antibodies and submitted for flow cytometry.

**RESULTS:**

**QUANTITATION OF B AND T LYMPHOCYTES**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45</td>
<td>99.5</td>
</tr>
<tr>
<td>CD14</td>
<td>0.1</td>
</tr>
<tr>
<td>CD3 (all cells of T lineage)</td>
<td>19.9</td>
</tr>
<tr>
<td>CD5 (usually a T-cell marker)</td>
<td>96.7</td>
</tr>
<tr>
<td>T helper /inducer CD4</td>
<td>13.4</td>
</tr>
<tr>
<td>T suppressor /cytotoxic CD8</td>
<td>3.0</td>
</tr>
<tr>
<td>B-cells CD20</td>
<td>64.3 (dim)</td>
</tr>
<tr>
<td>B-cells CD19</td>
<td>79.3</td>
</tr>
<tr>
<td>CD23</td>
<td>73.2</td>
</tr>
<tr>
<td>Cells expressing HLA-DR antigen</td>
<td>68.3</td>
</tr>
<tr>
<td>CD10</td>
<td>0.0</td>
</tr>
<tr>
<td>Cells expressing kappa light chain</td>
<td>72.5 (dim)</td>
</tr>
<tr>
<td>Cells expressing lambda light chain</td>
<td>3.2</td>
</tr>
<tr>
<td>Cells co-expressing CD5/CD19</td>
<td>76.3</td>
</tr>
</tbody>
</table>

Interpretation: This is an abnormal pattern. Majority of cells are of mature ??? lineage (positive for CD20, CD19, HLA-DR, SIg+; negative for CD10). They also coexpress CD5 and CD23 They appear to be of ??? clonality. This pattern is suggestive of ???.

*FLOW CYTOMETRY*

Flow cytometry is a technology which measures multiple characteristics of cells in suspension as they flow through a measurement region. The parameters that can be measured by flow cytometry are divided into INTRINSIC, such as cell size or cytoplasmic granularity, or EXTRINSIC, such as the presence of a surface antigen, for which specific reagents like fluorescent antibodies are needed.

The principle of the method is simple: individual cells pass through a laser beam. Each cell absorbs and scatters light and also, if labelled with fluorescent antibody to some cell surface marker, emits color. These signals are electronically analyzed and interpreted.

Initially the effect of light scatter is used for the determination of cell size, shape, internal structure, etc. On this basis the flow cytometer "separates" cells into "clusters" with similar properties to produce the scattergram. The presence or absence of a fluorescent marker can be then analyzed within the selected "cluster" (gating) and enables us to quantitate "positive" cells within this cluster.

**Recommended reading:**

- [http://pleiad.umdnj.edu/hemepath/immuno/immuno.html](http://pleiad.umdnj.edu/hemepath/immuno/immuno.html)
**SCATTERGRAM**

A histogram (or better, "scattergram") of cells included in this case represents the visual separation of cells (WBC) by light scatter only into lymphocytes and granulocytes. The cells are not stained with any fluorescent antibody. “FS” stains for forward scatter and means the light scattered in a forward direction. It correlates with cell size. “LSS” stands for light side scatter and means the light scattered at right angles to the laser beam. It correlates generally with cytoplasmic texture or granularity.

***REPORT***

The report included gives a quantitative measurement of extrinsic features of different cells within "lymphocytic" cluster only. The cells have been stained with individual (or with a combination of) monoclonal antibodies, and the percentage of cells staining with that antibody is reported.

**QUESTIONS FOR DISCUSSION**

1. What abnormalities are present in the CBC and/or WBC differential count?
2. What are the normal proportions of T and B lymphocytes in the peripheral blood?
3. Which markers signify T and B cells in the flow cytometric report? Other markers?
4. Do you see any evidence of restricted clonality (a proliferation of cells originating from a single cell) in either T or B cell populations? Try to fill in portions of the report marked "???".
5. What is the diagnosis and how do you support it?
6. Are this patient's lymphocytes functionally normal and what clinical course do you expect for this patient?
7. What would the gene rearrangement (Southern blot) studies show in this case?