Biochemical, Morphological, and Immunological Observations of Leukemic Reticuloendotheliosis

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SUMMARY

This study reports the biochemical, immunological, and morphological characteristics of the abnormal mononuclear cells from four patients with leukemic reticuloendotheliosis. Two patients had a homogeneous population of mononuclear cells with elevated thymidine incorporation, normal uridine incorporation, suboptimal or delayed response to phytohemagglutinin and a characteristic ultrastructure. Glucose utilization by these cells was somewhat greater than that by normal lymphocytes, but considerably less than that by normal monocytes and macrophages. The leukemic cells from both of these patients had surface-bound immunoglobulin. In contrast, the disease of the other two patients was characterized by a heterogeneous population of mononuclear cells with normal thymidine and uridine incorporation, normal or increased response to phytohemagglutinin, and a paucity of surface-bound immunoglobulin. All four patients had low levels of serum muramidase. The aforementioned two patients had elevated thymidine counts while the latter two patients had normal or low values. Attempts to establish long-term cultures were unsuccessful. All four patients appeared to have lymphoproliferative processes.

INTRODUCTION

Leukemic reticuloendotheliosis (hairy cell leukemia, reticulohistiocytic leukemia, lymphohistiocytic leukemia, malignant reticuloendotheliosis) is an uncommon form of leukemia, but it engenders considerable interest because the neoplastic leukocytes have unique "hairy" projections. The clinical and hematological features of the disease have been well documented (1, 3, 8). The light, phase, and electron microscopic features have been described (1, 4, 10, 12, 17, 18, 22), as has the histochemistry of the cell (11, 22, 24). Motility studies have been performed on the cell in vitro (15). Studies of the functional capabilities of the hairy cell have been carried out on isolated cases (17, 18, 22, 24). Little is known about the metabolism of the hairy cell except for the content of enzymes. Rubin et al (17) found low rates of DNA and RNA synthesis in the 1 case studied.

This study was undertaken to delineate the biochemical, immunological, and morphological features of these leukocytes. Leukocytes from 2 patients had elevated rates of thymidine incorporation. This group of patients presented with clinical, morphological, and immunological characteristics that were different from those diseased patients whose cells had normal rates of thymidine incorporation. These alternate presentations of the disease have not previously been recognized. Further evidence is offered that this leukemia is a lymphoproliferative disease.

MATERIALS AND METHODS

Cell Preparation. Clinical summaries of the patients studied are included as an "Addendum." The leukocytes were isolated from the heparinized peripheral venous blood by dextran sedimentation of red cells, as described previously (2). Leukocyte pellets were formed by centrifugation of the leukocyte-rich plasma (300 g) for 8 min. After being washed with PBS, pH 7.3, the leukocytes were suspended in MEM (Grand Island Biological Company, Grand Island, N. Y.), supplemented with glutamine, penicillin G (200 units/ml), streptomycin sulfate (0.2 mg/ml), and 20% fresh autologous serum. For the PHA stimulation studies, the red cells were sedimented without dextran and the medium was supplemented with autologous plasma rather than serum. White cell counts were performed in a hemocytometer after staining with Turk's stain, and viability counts were performed with 0.5% trypan blue. Both Wright-stained blood smears and the final cell suspensions were examined, and differential counts were performed. The percentages of abnormal mononuclear cells were confirmed by phase microscopic examination of the cell preparations.

Thymidine and Uridine Incorporation. Sterilized, disposable glass culture tubes (16 x 125 mm) containing 4 X 10^6 viable leukocytes and isotopes in 2 ml of medium were incubated at 37°C after being flushed with 5% CO_2-95% air. The isotopes used were thymidine-C^3H_3 (5 µCi; 14,000 dpm/pmol), for estimation of DNA synthesis, and uridine-5'-H (5 µCi; 60,000 dpm/pmol) (New England Nuclear, Boston, Mass.), for estimation of RNA synthesis. These were present in the respective tubes during the entire incubation period. After 4 hr, the cells were washed and the nucleic acids were

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The abbreviations used are: PBS, phosphate-buffered saline; MEM, Eagle's minimal essential medium; PHA, phytohemagglutinin; PAS, periodic acid-Schiff; CLL, chronic lymphocytic leukemia; IPPD, intermediate strength purified protein derivative.
precipitated with trichloroacetic acid as previously described
(21). The washed residue was dissolved in Soluene 100 (Packard Instrument Co., Downer's Grove, Ill.), and the
radioactivity was determined with a Packard 3003 scintillation
spectrometer. All counts were corrected for quenching, and
results were expressed as pmoles of nucleoside incorporated
into nucleic acid per hr per 10^6 cells.

Glucose Utilization. Glucose utilization studies were carried
out in siliconized 50-ml center-well Erlenmeyer flasks containing
50 X 10^6 viable leukocytes and glucose-1-14C (1 μCi)
(New England Nuclear) in 5 ml of medium. Because of the
wide variation in glucose utilization by various types of
leukocytes (20), only cell preparations with greater than 85%
abnormal mononuclear cells were used for this portion of the
study. Incubations were performed at 37° in an Eberbach
water-bath shaker (80 strokes/min). Respiratory carbon
dioxide (CO2) was collected in 3 N NaOH introduced into the
center well of the closed flask just prior to disruption of the
cells with 35% perchloric acid. Isolation of the products by
sequential extractions, including partition and ion-exchange
chromatography have been described previously (19).

Mitogen Stimulation. For the PHA studies, 1.5 X 10^6
mononuclear cells were incubated for 3 or 5 days with 25 μl of
PHA-P (Difco Laboratories, Inc., Detroit, Mich.) in a volume
of 1.5 ml of medium. This amount of PHA-P gave maximal
stimulation of thymidine incorporation by normal lympho-
cytes in the same system. Four hr prior to termination of the
experiment, the cultures were pulsed with 1 μCi thymidine-
C3H3. Results are expressed as cpm per tube. These stimulation
studies and the previous thymidine incorporation studies were
done in independent research laboratories and the results are
expressed, in each case, as is usual for that laboratory.

Extended Culture Technique. Leukocytes from all patients
were cultured in McCoy's 5A medium supplemented with 20%
heat-inactivated fetal calf serum. The initial cell count was 1 X
10^6 mononuclear cells per 1 ml of medium. At weekly
intervals the medium was changed, and the cell count was
readjusted to 1 X 10^6 cells/ml.

Muramidase (Lysozyme). Muramidase activity was de-
determined on the sera of the 4 patients by a spectrophotometric
method (20).

Electron Microscopy. Forty to 50 X 10^6 leukocytes in
MEM were incubated with or without latex particles (25 μl of
Difco Bacto Latex, 0.81 μm), at 37° for 45 min as a test for
phagocytic ability. The cells were fixed in a freshly made cold
mixture of glutaraldehyde and osmium tetroxide and stained
with uranyl acetate by the method of Hirsch and Fedorko (7).

Immunofluorescence. Blood was collected in a plastic
syringe containing heparin (20 units/ml), and the erythrocytes
were allowed to sediment at room temperature. After
centrifugation of the leukocyte-rich plasma, the cells were
resuspended in a media containing 50% autologous plasma in
MEM. After 1.5 hr of incubation, the media (which contained
a majority of lymphocytes and hairy cells and not over 15%
neutrophils and monocytes) was decanted into a tube and
lightly centrifuged. After 4 washings in PBS, pH 7.2, the cell
pellet was treated with 0.83% ammonium chloride for 10 min
at room temperature to lyse the erythrocytes.

Monospecificity of commercial fluorescein-conjugated anti-
human IgM and IgG (Hyland Laboratories, Los Angeles, Calif.)
was determined by immunoelectrophoresis. The antisera was
then diluted 1:8 with PBS, pH 7.2. Nonspecific activity was
eliminated by (a) deaggregation of the antisera by passage
through a Millipore filter (0.4 μm) and (b) adsorption with
viable cultured human lymphoblastoid cells (Epstein-Barr
virus-negative Raji cells). After this adsorption, no membrane
activity was seen on these cells, which served as controls for
evaluation of nonspecific staining.

Immunofluorescent staining was carried out by suspension
of 5 X 10^6 washed mononuclear cells and the conjugate for 15
min at room temperature and then for 15 min at 37°. After 3
washes with PBS, approximately one-half of the cell suspen-
sion was examined immediately by UV microscopy, while the
remainder of the cells was spread thinly over a glass slide and
allowed to dry at room temperature. These cells were then
fixed with acetone for 10 min, counterstained with aqueous
Evans blue, and mounted in 50% buffered glycerol. This
procedure made it possible to preserve the specimen for later
observation. Cells were considered negative if they failed to
display any fluorescence or exhibited only an occasional small
dot of fluorescence on the surface. This latter type of staining
was infrequent.

Histochemistry. Blood smears on a coverglass were stained
for peroxidase by the method of the Kaplan (9) and for PAS
by the method of Hayhoe et al. (6).

RESULTS

Biochemical Studies. Thymidine incorporation by the
leukocytes of each patient was determined on 2 occasions
(Table 1). Patients could be divided into 2 groups by rate of
thymidine incorporation. Patients S. R. and D. R., who had

<table>
<thead>
<tr>
<th>Leukocyte source</th>
<th>Date of experiment</th>
<th>Thymidine Incorporationa of 14C thymidine</th>
<th>Uridine Incorporationa of 14C uridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. R.</td>
<td>Initial</td>
<td>65.5</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>1 mo. laterb</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>D. R.</td>
<td>Initial</td>
<td>29.9</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>3 mo. later</td>
<td>20.2</td>
<td>1.1</td>
</tr>
<tr>
<td>J. R.</td>
<td>Initial</td>
<td>7.5</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>2 wk later</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>H. H.</td>
<td>Initial</td>
<td>6.1</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>2 mo. later</td>
<td>5.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Normal lymphocytesc</td>
<td></td>
<td>8.0 ± 0.8</td>
<td>3.3 ± 1.0</td>
</tr>
</tbody>
</table>

| a Expressed as pmoles of nucleoside incorporated into nucleic acid per hr per 10^6 leukocytes; mean of triplicate determinations.
| b After chemotherapy.
| c Determinations (mean ± S.E.) performed on the lymphocytes from 4 normal volunteers. The lymphocytes were separated by means of the glass bead column technique (14).
the highest percentage of abnormal mononuclear cells by light and phase microscopy, exhibited a significantly elevated thymidine incorporation. In 1 subject, the rate of thymidine incorporation decreased after therapy. Patients H. H. and J. R. had fewer abnormal mononuclear cells and normal rates of thymidine incorporation. Uridine incorporation, as a measure of RNA synthesis, was normal in both groups. The highest value observed, however, was in the cell preparation from the patient with the highest thymidine incorporation.

The leukocytes from the patients with the highest percentage of abnormal cells utilized glucose at rates of 0.85 and 1.09 µmoles/hr/10^8 cells. These values were corrected for the normal granulocytes that were present in the cell suspension. Glycogen turnovers were 0.056 and 0.044 µmole/hr/10^8 cells.

In Patients S. R. and D. R., most (85 to 90%) of the cells, which were predominantly abnormal mononuclear cells, and a few cells had large, strongly PAS-positive, globular-shaped cytoplasm masses. Many cells contained 1 or 2 strands of endoplasmic reticulum which were agranular (smooth) in most cases. Phagolysosomes and autophagocytic vacuoles were not seen.

Mitogen Stimulation. The results of PHA stimulation are shown in Table 2. The cells from 2 patients failed to stimulate normally. The cells from S. R. had only a minimal increase in thymidine incorporation at Days 3 and 5. Cell preparations from D. R. showed a delayed response with suboptimal stimulation at Day 3. Stimulation at Day 5 was increased, compared with that on Day 3, and was within the range of normal, had it occurred at Day 3. The stimulation of the mononuclear cells from the other 2 patients was either greater than normal (J. R.) or was normal (H. H.) on Day 3. After 5 days, the cells from these latter 2 patients incorporated thymidine at a much lower rate than on Day 3.

Histochernistry. Stains of the blood smears from all patients were negative for peroxidase activity. PAS stains revealed a diffuse slight positivity of the cytoplasm of most mononuclear cells, and a few cells had large, strongly PAS-positive, globular-shaped cytoplasm masses.

Membrane Immunofluorescence Studies. The results of these studies are summarized in Table 2. Two distinctively different immunofluorescent staining patterns were seen in the 4 patients studied. In Patients S. R. and D. R., most (85 to 90%) of the cells, which were predominantly abnormal mononuclear cells, stained only for surface-bound IgG. The fluorescence was, in many cells, concentrated in a patch at 1 side of the cell forming a "cap." Only occasionally were IgM-bearing cells detected.

In Patients J. R. and H. H., fewer cells stained with either anti-IgG or -IgM conjugates. The immunoglobulins on the surface of these cells were distributed uniformly in a ringed pattern, although a few "caps" were noted; in Patient J. R., 5 and 20% of the cells stained with IgM and IgG antiserum, respectively. In H. H., only 5% of the lymphoid cell population possessed surface IgG and only about 1% stained for IgM. Table 3 shows the results of quantitative serum immunoglobulins. There appears to be no association between the amount of surface immunoglobulins and the quantity of corresponding serum immunoglobulins.

Light Microscopy. A smear (Wright's stained) of the peripheral blood of Patient S. R. (Fig. 1) and bone marrow of J. R. (Fig. 2) demonstrated abnormal mononuclear cells with frayed edges. A section of the spleen of J. R. is also shown (Fig. 3).

Electron Microscopy. By means of transmission electron microscopy, the mononuclear cells were classified into 3 general types, on the basis of cytoplasmic organelles and the nuclear chromatin pattern.

Cell Type I (Figs. 4 and 5). These were moderately large cells (7 to 12 µm in diameter) with numerous cytoplasmic projections. A mean of 16 projections was seen on the sections of the cells, and they were up to 3.4 µm in length. The latter are presumably the electron microscopic equivalent of the "hairs" seen on phase microscopy and the frayed edges seen on light microscopy. The projections are rod shaped, triangular, or (less often) club shaped. The cytoplasm contains numerous mitochondria (mean, 12/cell) and vesicles. Some cell sections visualized centrioles, larger clear vacuoles, multiple vesicular bodies, Golgi apparatus (well developed, when present), and lipid bodies. Only a few electron-dense granules were seen. Many cells contained 1 or 2 strands of endoplasmic reticulum which were agranular (smooth) in most cases. Phagolysosomes and autophagocytic vacuoles were not seen. The matrix (ground substance) was electron transparent but contained free ribosomes and polyribosomal aggregates. A few cells contained fibrillar material in approximation to and parallel with the nuclear membrane. Langerhans-like granules [such as were described in 1 previous case (12)] were not found. Generally, the organelles did not extend past the base of the cytoplasmic projections. The nucleus was usually irregular and

<table>
<thead>
<tr>
<th>Patient</th>
<th>Thymidine incorporation (cpm/tube)</th>
<th>% of cells containing surface-bound immunoglobulins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 5</td>
</tr>
<tr>
<td>S. R.</td>
<td>850</td>
<td>13,000</td>
</tr>
<tr>
<td>D. R.</td>
<td>770</td>
<td>56,150</td>
</tr>
<tr>
<td>J. R.</td>
<td>1,440</td>
<td>240,880</td>
</tr>
<tr>
<td>H. H.</td>
<td>1,220</td>
<td>112,280</td>
</tr>
<tr>
<td>Normal lymphocytes</td>
<td>630</td>
<td>123,740</td>
</tr>
</tbody>
</table>

a Without PHA.
b Mean of lymphocyte preparations from 4 normal persons determined simultaneously with those from patients.
indented. The nuclear chromatin was finely dispersed with only minimal condensation at the nuclear membrane. The latter was sharply defined and trilaminar. A single nucleolus (rarely 2) was frequently seen and had a well-defined nucleolonema and pars amorpha.

Cell Type II. The 2nd type cell had features of a more mature leukocyte (Fig. 6). These cells were somewhat smaller (diameter, 6 to 8 \( \mu \)m) than type I cells, and the cytoplasm projections were less numerous (average, 7/cell) and shorter (rarely over 1 \( \mu \)m). The cytoplasm contained fewer mitochondria (mean, 4/cell), less endoplasmic reticulum and other organelles, except for free ribosomes, and a somewhat greater electron density of the ground substance. Occasional granules were seen in the cytoplasm. The nuclear chromatin was condensed toward the periphery to a greater extent than in type I, but the type II cell was just as likely to contain a nucleolus.

Cell Type III. This cell was similar to type II, but it exhibited only a normal number of short cytoplasmic projections. Its morphology is compatible with that of a normal lymphocyte, which it probably represents.

None of the 3 cell types contained phagocytosed latex particles. This is in contrast to the polymorphonuclear leukocytes, which invariably contained the ingested latex particles.

The blood of Patients S. R. and D. R. contained predominantly type I mononuclear cells, while J. R. and H. H. had an admixture of types (Table 4).

### Table 3

**Quantitative serum immune globulins**

<table>
<thead>
<tr>
<th>Patient</th>
<th>IgG (mg/100 ml)</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. R.</td>
<td>340</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>D. R.</td>
<td>1775</td>
<td>315</td>
<td>75</td>
</tr>
<tr>
<td>J. R.</td>
<td>1775</td>
<td>295</td>
<td>210</td>
</tr>
<tr>
<td>H. H.</td>
<td>3600</td>
<td>430</td>
<td>118</td>
</tr>
<tr>
<td>Normal</td>
<td>570–1920</td>
<td>60–330</td>
<td>50–150</td>
</tr>
</tbody>
</table>

DISCUSSION

This biochemical, morphological, and immunological study of the characteristic cell of leukemic reticuloendotheliosis suggests that the disease entity may present in 2 ways. Previous studies have been done on the leukocytes from individual patients. The larger clinical studies have not included simultaneous biochemical, morphological or immunological studies; therefore, this diversity may not have been appreciated.

Patients S. R. and D. R. presented in a similar manner. Both patients had an elevated WBC, and Patient S. R. had a clinical course suggestive of acute leukemia. The leukocytes from S. R. and D. R. had elevated thymidine incorporation but had normal uridine incorporation. Both had abnormal stimulation of the thymidine incorporation by their mononuclear cells in response to PHA. The leukocytes from S. R. had suboptimal stimulation at Days 3 and 5. Perhaps these would have stimulated to a greater degree if we had continued the experiment to Days 7 to 9. D. R. had a delayed response. This delay in PHA response has been reported for lymphocytes from CLL (5) and also in 1 previous case of leukemic reticuloendotheliosis (17). The accumulating mononuclear cell is of fairly consistent morphology (cell type I), and its ultrastructure has many immature features. It has morphological similarities to both normal blood lymphocytes and monocytes.

### Table 4

**White blood counts and differentials at time of metabolic studies**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date of study</th>
<th>WBC</th>
<th>Abnormal mononuclear cells (^a)</th>
<th>Lymphocytes</th>
<th>Other (^b)</th>
<th>Ultrastructural differential (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. R.</td>
<td>Initial</td>
<td>24,000</td>
<td>87</td>
<td>7</td>
<td>6</td>
<td>99.0 0.0 1.0</td>
</tr>
<tr>
<td></td>
<td>1 mo. later (^d)</td>
<td>5,400</td>
<td>14</td>
<td>15</td>
<td>71</td>
<td>97.3 1.8 0.9</td>
</tr>
<tr>
<td>D. R.</td>
<td>Initial</td>
<td>26,600</td>
<td>90</td>
<td>8</td>
<td>2</td>
<td>52.0 38.0 10.0</td>
</tr>
<tr>
<td></td>
<td>3 mo. later</td>
<td>19,100</td>
<td>90</td>
<td>6</td>
<td>4</td>
<td>55.5 30.6 13.9</td>
</tr>
<tr>
<td>J. R.</td>
<td>Initial</td>
<td>3,900</td>
<td>45</td>
<td>36</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 wk later</td>
<td>4,800</td>
<td>52</td>
<td>37</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>H. H.</td>
<td>Initial</td>
<td>6,600</td>
<td>47</td>
<td>36</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 mo. later</td>
<td>6,200</td>
<td>36</td>
<td>44</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Percentage abnormal mononuclear cells in final cell preparation was confirmed by phase microscopy. A differential count of smears of the cell preparations was similar.

\(^b\) Granulocytes, monocytes, and unclassified cells.

\(^c\) Roman numerals refer to cell type (see text). Granulocytes are excluded. Performed on cell preparations.

\(^d\) After chemotherapy.
Patients J. R. and H. H. represent an alternate presentation of the disease. These patients had normal or low WBC and a much higher percentage of lymphocytes with normal morphology by light microscopy. Unlike the cells from the other form of the disease, these leukocytes incorporated thymidine at a normal rate and when stimulated by PHA, they responded normally. Morphologically, these mononuclear cells are an admixture of cell types I, II, and III. The clinical manifestations of the disease and the biochemical properties and immunological capabilities of the leukocytes from these patients may depend on the relative percentages of each type.

The abnormal mononuclear leukocytes from all patients had common features, suggesting a lymphoid rather than reticuloendothelial origin. (a) Ultrastructure: The aggregation of the nuclear chromatin in clumps, its tendency to condense near the nuclear membrane, the small number of mitochondria, the paucity of endoplasmic reticulum and the infrequent granules, all characteristics of the type II cell, are suggestive of a lymphocyte. The type I cell combines features of both normal lymphocytes and monocytes. The absence of autophagic vacuoles and phagolysozomes would be unusual for a monocyte. (b) Ultrastructure after culture: After 10 days in culture, type I cells developed the features of the type II cell. (c) Lack of phagocytosis: Unlike reticuloendothelial cells, the mononuclear cells of the patients uniformly failed to phagocytose latex particles. (d) Muramidase: The low values of muramidase in the serum of all patients is characteristic of the lymphoproliferative diseases (20). (e) Mitogen stimulation: The response to PHA was normal (H. H. and J. R.), suboptimal (S. R.), or delayed (D. R.). In all cases, the stimulation was greater than that which could result from normal lymphocytes (type III cells) present. (f) Glucose utilization: The values from this study can be compared to those obtained by Stjernholm et al. (20), who used exactly the same method. The glucose utilization rate was somewhat higher than that of normal lymphocytes (0.28 to 0.50 μmole/hr/10⁸ cells) and pathological lymphocytes (0.14 to 0.50 μmole/hr/10⁸ cells), but it was considerably less than that of normal monocytes and monoblasts (5.0 to 6.0 and 2.0 to 4.5 μmole/hr/10⁶ cells, respectively). Cells with a major phagocytic function have high rates; for example, guinea pig peritoneal macrophages utilize glucose at a rate of 33.6 μmole/hr/10⁶ cells (23). Similarly, the glycerogen turnover values are intermediate between normal lymphocytes and monocytes but are nearer values characteristic of the lymphocyte. (g) Histochemistry: The negative peroxidase and positive PAS stains are typical of lymphocytes.

It is tempting to postulate that the hairy cells from Patient S. R. are of B-cell origin, since these cells contain detectable immunoglobulins on their surface and stimulate poorly with PHA. The “capped” pattern of fluorescence seen on these cells has also been described for lymphocytes from CLL (13). The lymphoid cells from Patient D. R. are also similar to cells from CLL in respect to their delayed response to PHA. The cells from S. R. might also have displayed this delay if we had cultured them for a longer time. Thus, it appears that the hairy cells from these patients resemble, at least in part, the lymphocytes found in CLL.

To the contrary, the lymphoid cells from Patient H. H. and J. R. resemble more closely those of T-cell origin. In support of this, only a small percentage of these cells are immunoglobulin bearing, and their response to PHA is within the normal range, with maximal stimulation occurring at Day 3 instead of Day 5. Both of these observations are consistent with what is known about T-cells, at least in the murine system (16). This may represent abnormal accumulation of T-cells, which would truly be a unique situation. If this were the case, there would seem to be 2 alternate forms of the disease; an accumulation of abnormal cells of B-cell origin (Patients S. R. and D. R.) and one of T-cell origin (Patients H. H. and J. R.).

An alternate explanation of the observations of these 4 patients identifies the type I cell as the single abnormal type cell seen in all patients with leukemic reticuloendotheliosis. It has high thymidine incorporation, surface immunoglobulins, and an abnormal response to PHA; therefore, it is possibly of “B” lymphocyte origin. The biochemical, morphological, and immunological characteristics of the cell preparation from a patient with this disease would then depend upon the percentage of abnormal cells admixed with normal lymphocytes. If the latter is the correct interpretation, it is not clear what the type II cell represents. In addition, the mitogen stimulation of Patients J. R. and H. H. should be less than normal, since the preparations from these patients contained greater than 50% abnormal mononuclear cells (type I).

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ADDENDUM

Case Reports

1. S. R. A 41-year-old white man who first developed easy fatigability, sore throat, fever, and abdominal pain in October 1971. He had pneumonitis 2 years prior to admission and mild easy bruisability for 1 year. When examined at the University of Iowa Hospitals, he had hepatomegaly and massive splenomegaly. There were palpable bilateral small inguinal lymph nodes and a 1- x 1-cm left axillary lymph node. The results of his laboratory tests were: hematocrit, 30%; hemoglobin, 10.1 g/100 ml; mean corpuscular volume, 86 cu Mm; mean corpuscular hemoglobin concentration, 33%; reticulocyte count, 1.8%; WBC, 20,100/cu mm, with 12% polymorphonuclear neutrophils and 88% mononuclear cells. The latter were 7 to 15 μm in diameter with an often eccentric round, oval, dumbbell-shaped, or folded nucleus. The nuclear chromatin was stringy and relatively immature appearing, and only an occasional nucleolus was seen. The cytoplasm was basophilic and foamy with a “ragged” perimeter and many projections. Only a few cells having the usual morphological characteristics of lymphocytes were seen. Examination with phase microscopy revealed 92% of the mononuclear cells had hairy projections. These were thought to represent the typical cell of leukemic reticuloendotheliosis. Platelet count was 100,000/cu mm, alkaline phosphatase 156 milliunits/ml (normal, 30 to 85), lactate dehydrogenase, 385 milliunits/ml (normal, 100 to 225), serum glutamic oxalpyruvic transaminase, 43 milliunits/ml (normal, 7.5 to 40). Other tests including uric acid, heterophil agglutination titer, stool guaiac, prothrombin time, partial thromboplastin time, electrocardiogram, and chest X-ray were normal. The bone marrow aspirate was hypercellular with 85% of the nucleated cells

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appearing like the mononuclear cells seen in the peripheral blood. Mumps and Candida albicans skin tests were negative.

On the basis of the clinical picture and the high thymidine incorporation (vide supra), the patient was treated with a combination regimen consisting of cytosine arabinoside (15 mg/kg) by i.v. push on Days 1 to 5; vincristine (0.025 mg/kg) i.v. on Days 1, 8, and 15; and prednisone (1.0 mg/kg) in divided doses on Days 1 to 21. The WBC decreased to a nadir of 600, at which time about one-half of the leukocytes were abnormal mononuclear cells. The therapy was complicated by increased thrombocytopenia and anemia, coliform sepsis, and a drug reaction. He was discharged (taking prednisone, 20 mg daily) on the 43rd hospital day, at which time his WBC was 5000/cu mm with 36% mononuclear cells; hemoglobin, 13.8 g/100 ml; and platelets, 62,000/cu mm. His bone marrow contained 35% abnormal mononuclear cells. Since discharge he has felt well, and his prednisone has been tapered to 15 mg/day. His blood counts are about the same as at discharge and his spleen is barely palpable.

2. D. R. A 49-year-old white man who was admitted to his local hospital in August 1970, with an 11-day history of nonproductive cough, fever, and pleuritic chest pain. In retrospect, he recalled increasing easy fatigability and emotional irritability for 6 to 9 months. After receiving a course of tetracycline and 3 days of treatment with chlorambucil, he was transferred to the Iowa City Veterans Administration Hospital with a diagnosis of acute lymphocytic leukemia. On examination, he had a barely palpable spleen but no hepatomegaly. There was a 2-component friction rub and inspiratory rales over the right lower lung field. His WBC was 3400/cu mm with 67% mononuclear cells (similar to that of Patient S. R.) and 33% neutrophils; hemoglobin was 11.2 g/100 ml; hematocrit, 35%; reticulocyte count, 1.2%; and platelets, 135,000/cu mm. A rare nucleated RBC was seen. Chest X-ray revealed a right lower lobe pneumonia which responded to cephalothin and gentamicin. Numerous cultures and serological titers failed to reveal a causative organism. Mumps, IPPD, 2nd strength purified protein derivative, and histoplasmin skin tests all were negative. Bone marrow aspirate was hypercellular, containing 83% mononuclear cells with the same morphology as those seen in the blood.

In May 1971, D. R. was readmitted for biopsy of a lymph node that had appeared in the left axilla. On histological examination, it was normal. The hepatomegaly was again noted and the WBC was 21,800/cu mm with 99% mononuclear cells; hemoglobin, 13 g/100 ml; and platelets, 73,000/cu mm; the bone marrow was infiltrated (90% of the nucleated cells) with mononuclear cells.

When last seen in November 1972, he was doing well without therapy. At that time, the WBC was 27,200/cu mm with 84% abnormal mononuclear cells, 14% lymphocytes, and 2% neutrophils. His hemoglobin was 13.4 g/100 ml; platelets, 115,000/cu mm.

3. J. R. A 41-year-old white truck driver, who was referred to the Iowa City Veterans Administration Hospital in February 1972. J. R. had been admitted to his local hospital 2 weeks earlier with colicky abdominal pain and microscopic hematuria. Because of leukopenia, a bone marrow aspirate had been done and was interpreted as acute undifferentiated leukemia. On admission in Iowa City, his spleen was enlarged to 12.5 cm below the costal margin. The laboratory values included a hemoglobin of 14.9 g/100 ml; hematocrit, 43; platelets, 100,000/cu mm; and WBC, 3700/cu mm, with 33% mononuclear cells (similar to those of Patient S. R.), 43% lymphocytes, 1% eosinophils, 1% basophils, and 22% polymorphonuclear leukocytes. The mumps skin test was reactive (3 cm) and the IPPD test was negative. A bone marrow aspirate revealed 39% mononuclear cells (as in Patient S. R.) and 32% small lymphocytes. Megakaryocytes and myeloid precursors were slightly decreased. Treatment was not instituted at that time, but in September 1972, he underwent a splenectomy because of left upper-quadrant pain and persistent thrombocytopenia. The spleen weighed 2560 g, and on histological section revealed extensive infiltration with lymphocyte-like cells which varied in size, shape, and staining properties (Fig. 3). When last seen, he felt well and his WBC was 5500/cu mm; hemoglobin, 11.8 g/100 ml; platelets, 315,000/cu mm; and his peripheral smear showed 24% abnormal mononuclear cells.

4. H. H. This patient was first admitted to the University of Iowa Hospitals in October 1970, with easy fatigability, dyspnea on exertion, and easy bruisability. Anemia was first noted in April 1970. There was little benefit from p.o. iron and splenectomy. He was receiving 2 to 3 units of blood every 6 to 8 weeks. On physical examination, he had pallor and a small, palpable lymph node in the left axilla. The laboratory results included: hemoglobin, 9.2 g/100 ml; hematocrit, 28%; mean corpuscular volume, 97 cu µm; mean corpuscular hemoglobin concentration, 33%; reticulocyte count, 1.1%; and WBC, 4900/cu mm with 48% polymorphonuclear leukocytes, 2% monocytes, 1% bands, and 49% abnormal mononuclear cells thought to be mostly lymphocytes. Platelet count was 250,000/cu mm; stool guaiac, 1+; serum iron, 88 µg/100 ml; total iron-binding capacity, 345 µg/100 ml. Bone marrow aspirate was dilute, and a biopsy showed infiltration with a mononuclear cell thought to be typical of leukemic reticuloendotheliosis. Mumps, Candida albicans, and IPPD skin tests were negative. A review of the histology of the spleen showed infiltration with a similar cell, and there was no extrafollicular hematopoiesis.

After discharge, H. H. continued to receive periodic blood transfusions. In February 1971, a prostatic biopsy revealed adenocarcinoma, but the acid phosphatase was 0.5 units, and there was no radiological evidence of metastasis. A liver scan revealed only hepatomegaly. He was started on chlorotrianisene. When last seen in the clinic, his hemoglobin was 10.1 g/100 ml; platelets, 78,000/cu mm; and WBC, 6200/cu mm; with 36% abnormal mononuclear cells.

REFERENCES


Fig. 1. Patient S. R. Peripheral blood smear showing abnormal mononuclear cells. Wright's stain, X 1,000.
Fig. 2. Patient J. R. Bone marrow smear showing abnormal mononuclear cells. Wright's stain, X 1,000.
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Fig. 3. Patient J. R. Spleen section showing disruption of normal architecture and infiltration with pleomorphic mononuclear cells. H & E, × 400.

Fig. 4. Electron micrograph of abnormal mononuclear cells (type I, see text). × 9,120.
Fig. 5. Electron micrograph of abnormal mononuclear cell (type I). N, nucleus; M, mitochondrion; G, Golgi body; C, centriole. X 26,300.
Fig. 6. Electron micrograph of abnormal mononuclear cell (type II). X 15,390.
Biochemical, Morphological, and Immunological Observations of Leukemic Reticuloendotheliosis

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