Host Defense Deficiency in Hairy Cell Leukemia and its Correction by Leukocyte Transfusion

By Evan M. Hersh, Samuel Murphy, Axel Zander, Karel Dicke, David J. Stewart, Hironobu Toki, and Jean Latreille

A similar defect in host defense mechanisms in hairy cell leukemia was defined in two patients. Surface-adherent monocytes were not detected in the peripheral blood nor were monocytes that mediate antibody-dependent cell-mediated cytotoxicity (ADCC) to isoantibody-coated human erythrocytes. In addition, lymphocytes of both patients failed to show blastogenic responses to concanavalin A (Con-A) and pokeweed mitogen (PWM) but showed a vigorous response to phytohemagglutinin (PHA). Other immunologic abnormalities were present but were either moderate in degree or were not present in both patients. In vitro lymphocyte blastogenic responses were fully restored by incubation of patients' leukocytes with a normal donor's adherent monocytes. One patient received daily allogeneic leukocyte transfusions for 4 days. This resulted in complete normalization of monocyte adherence and ADCC that persisted for several months after transfusion and was associated with hematologic improvement. Therapy in case 1 resulted in correction of the blastogenic responses to Con-A and PWM. Thus, a host defense defect in hairy cell leukemia has been defined in 2 patients and a preliminary result suggests that therapy with leukocyte transfusions may be useful in the postsplenectomy patient with an infectious complication and should be explored further.

Hairy cell leukemia (HCL) is a leukoproliferative bone marrow disorder characterized by pancytopenia, splenomegaly, a protracted course, lack of response but excessive toxicity to chemotherapy, and bizarre infections. The malignant cell, "hairy cell," is an undifferentiated leukocyte with cytoplasmic projections and with heterogeneous biologic characteristics. Most studies indicate that the hairy cell is a B cell possibly with phagocytic properties. However, monocytes and T cells have also been implicated as the cell of origin. Characteristically, the hairy cells have surface immunoglobulin that is resynthesized after trypsin digestion, and they phagocytize zymosan and polystyrene latex particles but not Ig-coated target erythrocytes. Usually the cells are positive histochemically for tartrate-resistant acid phosphatase and an α-naphthyl acetate esterase. The cells are glass adherent. They usually have variable numbers of Fc or C receptors.

The immunologic status of these patients has not been well studied. Serum immunoglobulin levels and the antibody response may be normal but have not been well studied. T-cell levels in the peripheral blood are often normal, while levels of cells with receptors for EA and EAC are variable but may also be low. Series of patients with normal and with impaired in vivo delayed hypersensitivity to recall antigens have been described. The in vitro responses of the peripheral blood lymphocytes of some patients to phytohemagglutinin is delayed, as in chronic lymphocytic leukemia, while in others, particularly among separated T cells, it is normal. These variable findings emphasize the heterogeneity of this disease.

The bulk of the data regarding impaired host defense mechanisms relates to the monocyte. Peripheral blood monocytes are decreased as is the serum muramidase level, monocyte exudation into inflammatory sites in the skin (as measured by the skin window technique) is markedly impaired, and there are circulating inactivators of monocyte chemotaxis. The peripheral blood mononuclear cells don't produce colony-stimulating factor (a monocyte product) on in vitro incubation.

Granulocyte mobilization from the bone marrow is also impaired and probably partially explains the prolonged granulopenia after chemotherapy. This is also associated with moderate impairment of granulocyte exudation into inflammatory sites in the skin.

The current study of two patients with hairy cell leukemia was prompted by the observation of a refractory atypical mycobacterial infection in one of them. This case supports the general observation that there is a relationship between hairy cell leukemia and atypical mycobacterial infection. Thus, there is a need to define the host defense defect so that corrective therapy might be applied. A complex defect involving impaired monocyte function was defined that was virtually identical in the two patients. In one patient, the defect was partially reversed by transfusions of peripheral blood leukocytes (predominantly mononuclear cells) from a normal donor. Most important, the correction of the defect persisted after the termination of the transfusions. In the other, a short course of intravenous C. parvum had a transient effect on the defect.
HOST DEFICIENCY IN HAIRY CELL LEUKEMIA

MATERIALS AND METHODS

Leukocyte Counts

Leukocyte counts were made on peripheral blood smears and on cytocentrifuge preparations stained with Wright-Giemsa stain. The presence of monocytes was confirmed by phagocytosis of latex particles, Sudan black stain, and nonspecific esterase stain done on whole blood and/or Hypaque-Ficoll density solution centrifuged mononuclear cells.

T cells were determined by the E rosette method;23 B cells were determined by fluorescence with SS rabbit anti-human IgM and SS rabbit anti-human Fab;24,25 C' receptor cells were determined by the EA rosette method26 and by incubation with fluorescein-labeled aggregated IgG.27 One-hundred cell differential counts were done on each preparation, and the results were reported as percent of cells or used to calculate the absolute cell count when appropriate.

Lymphocyte Blastogenesis

Lymphocyte blastogenic responses were measured utilizing the standard flat-bottom well microculture system as previously described.28 Cultures for each point were done in triplicate with 1.5 × 10^5 lymphocytes per well. Cells were cultured in RPMI 1640 medium with 20% normal AB serum in a total volume of 0.2 ml. After 64 hr in culture at 37°C in 5% CO_2 in air, 1 μCi of [3H] thymidine (specific activity, 1.9 Ci/mmole) was added for 8 hr, and the cultures were harvested with a MASH II harvester onto fiberglass filter disks. The incorporated radioactivity was counted in a liquid scintillation counter, and results were reported as counts per minute (cpm) per 1.5 × 10^5 lymphocytes.

Cultures for monocyte restoration of blastogenesis contained an estimated 5 × 10^5 monocytes from a normal donor in addition to 1.5 × 10^5 patient lymphocytes. To introduce that number of normal allogeneic monocytes into the wells, a Hypaque-Ficoll separated leukocyte suspension containing 10^5 monocytes and about 10^6 lymphocytes was introduced into the wells in 0.2 ml of RPMI 1640 with 20% AB serum. After incubation for 1 hr at 37°C, the nonadherent lymphocytes were washed out with jets of warm medium, and patients' leukocyte suspensions at 1.5 × 10^5 lymphocytes per well were added. Microscopic examination of these wells revealed that over 95% of the adherent cells were monocytes.

Lymphocyte culture additives included phytohemagglutinin (PHA, Difco), concanavalin A (Con-A, Nutritional Biochemicals), and pokeweed mitogen (PWM, Difco) at 0.02 ml of 1:10, 1:10, and 1:20 dilutions of the stock solutions, respectively.

Natural Killer (NK) Cells

NK cell activity was determined using Hypaque-Ficoll separated cells as effector cells and the CEM human T-cell leukemia cell line as the target. The target cells were labeled with 100 μCi ^51Cr in the conventional fashion. The effector:target cell ratio was 100:1 with 5 × 10^5 effector mononuclear cells in a total volume of 0.25 ml of RPMI 1640 and 10% fetal calf serum. Incubation of triplicate cultures for each experimental point was for 4 hr at 37°C. ^51Cr release and estimation of the percent specific lysis were calculated in the standard fashion as previously described.29

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

ADCC was determined among Hypaque-Ficoll separated cells using chicken erythrocytes coated with rabbit antibody and human erythrocytes coated with isoantibody (B, anti-B). The target cells were labeled with ^51Cr in the conventional fashion. The target:effector ratios were 3:1 and 10:1 with 5 × 10^5 effector mononuclear cells in a total volume of 0.70 ml of RPMI 1640 with 5% fetal calf serum. Incubation of triplicate cultures for each point was for 20 hr at 37°C, and ^51Cr release from labeled target cells was the measure of cytotoxicity as previously described.30

Monocyte Adherence

Monocyte adherence was measured by placing sufficient Hypaque-Ficoll separated leukocytes into wells of the standard microculture tray to give 2 × 10^5 monocytes per well in 0.2 ml of RPMI 1640 with 50% normal pooled AB serum. These were incubated for 1 wk. Nonadherent cells were washed out and the adherent cells released with 1% citric acid. The final step was counting the released nuclei and calculating the numbers of adherent cells per milliliter of blood. This method has been described previously.31

Delayed-Type Hypersensitivity (DTH)

DTH was measured to the recall antigens dermatophytin (Derm), variadise or streptokinase-streptodornase (Var), Candida (Cand), mumps, and purified protein derivative (PPD), using standard doses as previously described.32 Primary DTH was measured by the administration of 100 μg of keyhole limpet hemocyanin (KLH) intradermally and repeating this test 2 wk later.32 Responses were measured as the average diameter of induration at 48 hr.

CASE REPORTS

Patient

A 60-yr-old male presented with his third episode of fever and chills associated with hepatosplenomegaly in March 1978. A toxoplasmosis titer was 1:4096, PPD skin test was positive, but cultures of sputum were negative. A liver biopsy showed a diagnosis of hairy cell leukemia infiltration. A bone marrow was also diagnostic of hairy cell leukemia. The patient was referred to the M.D. Anderson Hospital. He was on daraprim and sulfa and had daily intermittent fever and sweats. Physical examination revealed a spleen at 8 cm below the left costal margin. Laboratory data revealed Hb 8.2 g/dl; platelets, 134,000; WBC 1200; polys, 56; lymphs, 37; monos, 7; alkaline phosphatase, 350; bilirubin, 1.5; LDH, 282; and SGGT, 24. The chest x-ray showed diffuse bilateral parenchymal infiltrates. Lung biopsy was compatible with pulmonary angitis and granulomatosis, and this was thought to be due to the sulfa drugs that were stopped. A toxoplasmosis titer performed twice was 1:256. The diagnosis of hairy cell leukemia was confirmed by light and electron microscopy, and 27% infiltrate of the marrow with leukemic cells was noted.

Cytochemistry revealed positive acid phosphatase resistant to tartrate. The patient was given prednisone 50 mg/day with no change in symptomatology. On May 1, 1978, he had a splenectomy. The spleen was diagnostic for hairy cell leukemia and also for tuberculosis. Large caseating granulomata were also present and hairy cells were seen in them. Prednisone was stopped. He was started on INH, streptomycin, and rifampin. Mycobacterium kansasii was cultured from the spleen. His WBC 26 days post-splenectomy had only risen to 3000 and stayed at that level until day 36. His marrow showed 25% leukemic cells at that time. Because of persistent fever, worsening of this chest x-ray, and the development of positive sputum, and because his defect appeared to be one of monocyte function, he was given (with informed consent) mononuclear cell transfusions from his son who was immunologically normal (see Results for details on numbers and differentials of cells administered).

His hematologic status gradually returned to normal, including a fall in leukemic cells to 4% in the bone marrow. Subsequently, his
infectious symptoms cleared. He remained in symptomatic and hematologic remission for 8 mo, after which he relapsed.

**Patient 2**

A 50-yr-old male was admitted to M.D. Anderson Hospital because of general weakness, sore throat, and fever over a few months prior to admission. His present illness dated to 1973, when he was found to have left neck lymph node swelling. This was biopsied and showed nonspecific reaction. In July 1974, biopsy of enlarged right axillary and left inguinal lymph nodes also showed nonspecific reaction. The diagnosis of hairy cell leukemia was made in May 1977, when he showed lymphadenopathy, splenomegaly, neutropenia, and thrombocytopenia. In January 1978, a splenectomy was performed. In April 1978, patient was treated at a local hospital because of pseudomonas infection of the mouth and pseudomonas sepsis, which resolved promptly with antibiotics. He was transferred to M.D. Anderson Hospital in May 1978. On admission, the patient did not show lymphadenopathy or hepatomegaly. Auscultation showed rales in the right lower lung compatible with a pneumonic infiltrate seen on chest x-ray.

The diagnosis of hairy cell leukemia was confirmed by several methods. The morphology of the bone marrow smear by Wright stain and the findings by electron microscopy were consistent with hairy cell leukemia. The cytochemistry revealed positive acid phosphatase resistant to tartrate. The histology of the spleen, lymph nodes, and bone marrow was reviewed and shown to be hairy cell leukemia. The hematologic data on admission were as follows: hemoglobin, 11.0 g/dl; hematocrit, 35.7%; platelet count, 235,000/cumm; WBC, 4100/cumm with polys 29, lymphs 67, and monos 4. Bone marrow smear showed 55.5% hairy cell leukemia infiltration. Kidney and liver functions were normal. Serum muramidase level was 0.7 µg/ml (normal 2.8-8.0).

After admission, the patient was treated successfully with iv. Ticarcillin and Bactrim for pseudomonas infection of the lung. The immunologic evaluations were performed 1 mo later when the pneumonia was completely resolved. Because we have recently observed that the relatively low monocyte adherence, the serum lysozyme and the peripheral blood leukocyte-mediated ADCC of cancer patients can be increased by i.v. C. parvum or i.v. MER (Hersh et al., unpublished), we felt that C. parvum was appropriate experimental therapy for this patient. The therapeutic trial of intravenous C. parvum immunotherapy was given with the informed consent of the patient.

**RESULTS**

The peripheral blood leukocyte counts and differentials, including cell surface and other markers, are shown in Table 1. Repeated examinations of the peripheral blood failed to show leukemic cells. Both patients were mildly leukopenic, had a moderate reduction in monocyte counts, and had absolute lymphocyte counts in the normal range. T-cell and B-cell levels were essentially normal. The percentage mononuclear phagocytes (for latex particles) were normal but at the lower end of the range. By one measurement (EA), Fc receptor positive cells were normal; whereas by another test (aggregated IgG binding), Fc receptor positive cells were quite low. There were no other severe abnormalities in leukocyte subpopulation numbers. Sudan black and nonspecific esterase stains confirmed the presence of low numbers of monocytes.

Table 2 shows the lymphocyte blastogenic responses of the two patients compared to two typical normals and a group of concurrent patient and normal controls. The patients had a characteristic and identical defect in blastogenic response with little or no response to Con-A and PWM, and at the same time, a vigorous response to PHA. Serum from patient 1 modestly suppressed the blastogenic responses of normal lymphocytes. The standard deviations of the blastogenesis data were 10% or less of the mean values when stimulated counts were greater than 5000 cpm.

Cell-mediated cytotoxicity was studied utilizing both the NK cell assay and ADCC to antibody-coated chicken and human erythrocytes (Table 3). NK activity was normal and was not affected by patients’ serum. ADCC to chicken erythrocytes was deficient but not absent. Serum from patients 1 and 2 suppressed the anti-chicken erythrocyte ADCC of normal cells to about 50% of control. This is at the borderline of significance. Both patients had an essentially complete absence of ADCC to isoantibody-coated human erythrocytes both without added human serum and in serum from patient and normal donors. All values were below 1% cytotoxicity.

Table 4 shows the initial monocyte adherence data. Adherent monocytes were completely absent from the peripheral blood of both patients compared to the

| Table 1. Distribution of Peripheral Blood Leukocyte Types and Subclasses in Hairy Cell Leukemia |
|-----------------------------------------------|---------|---------|---------|
| Cell Type | Patient 1 | Patient 2 | Normal  |
| WBC/cumm | 3,400 | 4,100 | 5,000–10,000 |
| Percent lymphocytes | 37 | 67 | 20–40 |
| Percent monocytes | 7 | 4 | 1–6 |
| Lymphocytes/sq mm | 1,256 | 2,747 | 1,000–4,000 |
| Monocytes/sq mm | 238 | 164 | 285–500 |
| Percent phagocytic cells in Hypaque-Ficoll separated leucocytes | 4 | 3 | 2–20 |
| Percent cell surface markers or receptors | | | |
| E | 82 | 70 | 60–80 |
| EA | 19 | 25 | 10–25 |
| Aggregated IgG | 2 | 0 | 8–25 |
| EAC | 5 | 4 | 8–20 |
| SIgM | 8 | 3 | 5–11 |
| SFab | 6 | 11 | 5–11 |

Abbreviations: E, sheep erythrocyte rosettes; EA, rosettes with antibody-coated erythrocytes; aggregated IgG, cells tagged with fluorescein-labeled heat aggregated; EAC, rosette formation with antibody and complement-coated erythrocytes; SIgM, cells tagged with fluorescein-tagged 5S rabbit anti-human IgM; SFab, same for 5S rabbit anti-human Fab. The normal ranges are those observed for the laboratory. See Materials and Methods section for details on these assays.
approximately 4–12 × 10⁴ adherent cells/ml of blood in cancer patients and normals.

Delayed hypersensitivity skin test results are presented in Table 5. Patient 1 reacted only to PPD, while patient 2 reacted to Candida and mumps. Neither patient developed DTH to KLH when retested after immunization.

The family of patient 2, including his son and five siblings, was studied. The three major impaired functions, namely monocyte adherence, ADCC, and lymphocyte blastogenesis, were studied in these six individuals. The son and one sister had low monocyte adherence and the son also had low ADCC (Table 6). This suggests the possibility of a genetic basis in this disease, or alternately, that monocyte adherence and ADCC are genetically determined.

After these initial studies, we attempted to restore immunologic reactivity, first in vitro and then in vivo. Blastogenesis was restored by adding normal allogeneic monocytes to the patients’ mononuclear cell suspensions, which were then stimulated with Con-A or PWM (Table 7). Addition of normal subjects’ monocyte to patient leukocytes in vitro increased blastogenesis to Con-A to 12,900 counts in patient 1 and 46,000 counts in patient 2, and the response to pokeweed mitogen to 8900 counts in patient 1 and 12,600 counts in patient 2. Thus, both Con-A and PWM responses were restored to the normal range by the addition of monocytes from a normal subject.

After recognition of the monocyte function deficiency, we gave transfusions of mononuclear leukocytes from the patient’s son, collected with the IBM 2997 blood cell separator. Cells were given daily for 4 days at doses of 2.9–5.8 × 10⁹ predominantly mononuclear cells per dose. The differentials on these preparations ranged from 4%–20% polys, 65%–89% lymphocytes, and 1%–15% monocytes. This was done in patient 1. In patient 2, monocyte activation was attempted by

Table 2. Lymphocyte Blastogenic Responses and the Effect of Patients’ Serum in Hairy Cell Leukemia

<table>
<thead>
<tr>
<th>Lymphocyte Stimulant</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Normal 1</th>
<th>Normal 2</th>
<th>Normal Controls (n = 35)</th>
<th>Patient Controls (n = 35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (Control)</td>
<td>159</td>
<td>248</td>
<td>228</td>
<td>358</td>
<td>1,130 ± 719</td>
<td>1,097 ± 962</td>
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<tr>
<td>Con-A</td>
<td>120</td>
<td>2,186</td>
<td>18,185</td>
<td>59,973</td>
<td>29,800 ± 19,400</td>
<td>11,400 ± 13,900</td>
</tr>
<tr>
<td>PHA</td>
<td>30,416</td>
<td>182,702</td>
<td>164,233</td>
<td>131,657</td>
<td>91,300 ± 48,700</td>
<td>38,900 ± 49,300</td>
</tr>
<tr>
<td>PWM</td>
<td>939</td>
<td>979</td>
<td>18,649</td>
<td>9,313</td>
<td>17,600 ± 14,200</td>
<td>8,500 ± 7,700</td>
</tr>
</tbody>
</table>

Data reported as cpm/1.5 × 10⁵ cells; cells cultured in 20% normal AB serum in microwells and harvested at 3 days. Normal 1 and 2 were individuals studied on the same day as patient 1 and 2, respectively. The 35 normal controls and 35 patient controls were studied during the same general time period as the patients with hairy cell leukemia. The 35 patient controls had advanced disseminated or metastatic malignancies, and 20 had received prior chemotherapy.

Table 3. Cell-Mediated Cytotoxicity Expressed as Percent Specific Lysis (%) in Hairy Cell Leukemia

<table>
<thead>
<tr>
<th>Assay</th>
<th>P₁</th>
<th>P₂</th>
<th>N₁</th>
<th>N₂</th>
<th>Normal Control</th>
<th>Patient Control</th>
<th>Additive</th>
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</thead>
<tbody>
<tr>
<td>NK</td>
<td>19.9</td>
<td>21.7</td>
<td>34.5</td>
<td>35.0</td>
<td>27.7 ± 14.4</td>
<td>24.8 ± 13.2</td>
<td>None</td>
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<tr>
<td></td>
<td>23.3</td>
<td>23.9</td>
<td>38.1</td>
<td>35.8</td>
<td>Patient serum</td>
<td>Normal serum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.7</td>
<td>19.8</td>
<td>37.7</td>
<td>38.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADCC</td>
<td>2.8</td>
<td>9.3</td>
<td>18.9</td>
<td>19.4</td>
<td>22.6 ± 9.6</td>
<td>35.5 ± 16.7</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>2.1</td>
<td>12.3</td>
<td>11.1</td>
<td>Patient serum</td>
<td>Normal serum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>1.9</td>
<td>17.1</td>
<td>16.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADCC</td>
<td>0.2</td>
<td>0.9</td>
<td>16.3</td>
<td>11.6</td>
<td>19.5 ± 10.7</td>
<td>33.8 ± 20.6</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.2</td>
<td>12.5</td>
<td>12.5</td>
<td>Patient serum</td>
<td>Normal serum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.2</td>
<td>12.9</td>
<td>7.6</td>
<td></td>
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</tr>
</tbody>
</table>

Table 4. Peripheral Blood Monocyte Adherence in Hairy Cell Leukemia (Adherent Cells/ml Blood × 10⁶)

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>Serum Source</th>
<th>N</th>
<th>P₁</th>
<th>P₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>2.09</td>
<td>1.93</td>
<td>2.89</td>
</tr>
<tr>
<td>Normal controls</td>
<td></td>
<td>14.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Patient controls</td>
<td></td>
<td>0.90–72.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P₁</td>
<td></td>
<td>2.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P₂</td>
<td></td>
<td>0.00–48.00</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

The single normal (N) was run on the same day as the two patients. The normal controls and patient controls are as described in previous tables. There were 92 patients and 46 normals from which the means shown were obtained.
administration of C. parvum daily for 4 days at doses of 0.50, 1.0, 1.5 and 2.0 mg/sqm/day on days 1–4, respectively.

The immunologic and related data are shown in Table 8. In patient 1, there was an increase in the total leukocyte and absolute granulocyte counts during transfusion that persisted for several months after the 4 days of treatment. Marrow leukemic cell percent declined from 25% to 5%. It can be seen that monocyte adherence and ADCC were completely restored in patient 1 who received the leukocyte transfusion. The only change noted in patient 2 was a slight increase in marrow leukemic cell percent during which time he had normal peripheral leukocytes. Patient 1's hematologic and immunologic improvement lasted for 8 mo during which time he had normal peripheral blood and normal immunologic tests. He then began to progress slowly with a return of leukemic cells in the marrow to 30%–40% but has been symptomatically normal and has not received antileukemic therapy.

Table 5. Other Immunologic Studies in Hairy Cell Leukemia

<table>
<thead>
<tr>
<th>Skin Test Antigen</th>
<th>P1</th>
<th>P2</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTH (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermatophytin</td>
<td>0</td>
<td>0</td>
<td>49*</td>
</tr>
<tr>
<td>Varidase</td>
<td>0</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>Candida</td>
<td>0</td>
<td>18.5</td>
<td>82</td>
</tr>
<tr>
<td>Mumps</td>
<td>0</td>
<td>20.0</td>
<td>50</td>
</tr>
<tr>
<td>KLH</td>
<td>0</td>
<td>0</td>
<td>90*</td>
</tr>
<tr>
<td>PPD</td>
<td>0</td>
<td>32.0</td>
<td>29</td>
</tr>
</tbody>
</table>

Abbreviations: DTH, delayed-type hypersensitivity; PPD, purified protein derivative; KLH, keyhole limpet hemocyanin.

*Percent normal subjects with positive reaction to test antigen.
†Postimmunization.

Immunologic status declined associated with recurrence of disease.

DISCUSSION

Hairy cell leukemia is a clinicopathologic diagnosis characterized by pancytopenia, splenomegaly, marrow infiltration with abnormal hematopoietic cells, and frequent bizarre infections. However, the patients have a relatively long untreated survival. The hairy cells are heterogeneous but are predominately phagocytic B cells.2 The two patients in this study were similar in that they did not have circulating hairy cells when studied and the disease was predominantly limited to the bone marrow. This is unusual, and most series show only a small fraction without circulating hairy cells. For example, some recent series have shown 1/8, 2/5, 1/7.21 The defects defined in these patients might not be manifested in patients with other clinical presentations.

Immunologically, the disease has not been well defined, but the bulk of evidence suggests a quantitative and qualitative monocyte defect including low monocyte levels, poor monocyte tissue mobilization, low serum muramidase levels, absent mononuclear cells CSF production, and increased serum levels of monocyte chemotactic inhibitor.17 21

In the current study, two patients with hairy cell leukemia were noted to have identical defect of low but present monocyte levels, absent monocyte adherence, absent ADCC to human erythrocytes, and low to absent Con-A and PWM blastogenic responses with a vigorous PHA response. All three effects are monocyte related, including blastogenesis. ADCC to human erythrocytes is known to be a monocyte function and is abolished by removal of adherent cells.30 We have confirmed this observation in our laboratory (Hersh, unpublished observations) using both glass adherence and also carbonyl iron methodology. Lymphocyte...
HOST DEFICIENCY IN HAIRY CELL LEUKEMIA

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patients
quent
morphologically.
Further exploration
explain
normal macrophages could
possible, although over
population is
nism. Also, restoration by an adherent lymphocyte
plastic-adherent cells by these methods are mono-
and
response. This is in agreement with the known role of
and PWM responses with a relatively
normal PHA
activation might be clinically beneficial. These
would
attack both the deficient monocyte number and defi-
cent monocyte function problems.
The monocyte defect
in our patients was both quan-
titative and qualitative. Thus, the monocyte count was
low but not absent, mononuclear cell phagocytosis was
normal, while in addition to the above described
defects, and perhaps related to them, was a low to
absent level of circulating cells with Fc receptors for
aggregated IgG but not for EA. The overall findings
suggest that there is inadequate monocyte production
and that the monocytes produced are immature and
have only a fraction of the normal receptors and
functions. Impaired monocyte number and function
would be sufficient to explain the development of
infections, particularly with mycobacteria and fungi.

We observed deficient but in vitro restorable Con-A
and PWM responses with a relatively normal PHA
response. This is in agreement with the known role
of monocytes in blastogenic response as reported in some
papers33,34 but not in others where a role for monocytes
in PHA responses also seems to be mandatory for
optimal responses.35,36 Further exploration in subse-
quent patients will be necessary to define the mecha-
nism. Also, restoration by an adherent lymphocyte
population is possible, although over 95% of the glass-
and plastic-adherent cells by these methods are mono-
cytes morphologically. One might also argue that an
MLC between the patient lymphocytes and the
normal macrophages could explain the finding or that

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>Therapy</th>
<th>WBC/cu mm x 10^4</th>
<th>Monocyte Adherence Cells/ml x 10^4</th>
<th>ADCC to HRBC % Lysis (E:T-1:3)</th>
<th>Blastogenesis (cpm/1.5 x 10^5 cells)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>5/15</td>
<td></td>
<td>2.3</td>
<td>0</td>
<td>120</td>
<td>939</td>
</tr>
<tr>
<td></td>
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8. Effect of Therapy on Hematologic and Immunologic Parameters in Hairy Cell Leukemia

Table 8. Effect of Therapy on Hematologic and Immunologic Parameters in Hairy Cell Leukemia

blastogenic responses to mitogens and antigens are
also known to require monocytes.33 However, it should
be noted that a defect in adherent lymphocyte sub-
populations could also explain some of the findings.
The monocyte defect in our patients was both quan-
titative and qualitative. Thus, the monocyte count was
low but not absent, mononuclear cell phagocytosis was
normal, while in addition to the above described
defects, and perhaps related to them, was a low to
absent level of circulating cells with Fc receptors for
aggregated IgG but not for EA. The overall findings
suggest that there is inadequate monocyte production
and that the monocytes produced are immature and
have only a fraction of the normal receptors and
functions. Impaired monocyte number and function
would be sufficient to explain the development of
infections, particularly with mycobacteria and fungi.

We observed deficient but in vitro restorable Con-A
and PWM responses with a relatively normal PHA
response. This is in agreement with the known role
of monocytes in blastogenic response as reported in some
papers33,34 but not in others where a role for monocytes
in PHA responses also seems to be mandatory for
optimal responses.35,36 Further exploration in subse-
quent patients will be necessary to define the mecha-
nism. Also, restoration by an adherent lymphocyte
population is possible, although over 95% of the glass-
and plastic-adherent cells by these methods are mono-
cytes morphologically. One might also argue that an
MLC between the patient lymphocytes and the
normal macrophages could explain the finding or that
any residual normal lymphocytes (from the macro-
phage donor) could have responded. These are
unlikely because (1) the cultures are harvested at 72
hr before the MLC becomes prominent, (2) there was
minimal 1H-thymidine incorporation by the adherent
cells stimulated by Con-A and PWM, and (3) the
appropriate control (patient leukocytes + normal
adherent cells without mitogen stimulation) showed
little 1H-thymidine incorporation.

Our finding of absent monocyte adherence and
absent monocyte-induced ADCC as well as impaired
blastogenic responses extend the work of others in
which impaired monocyte tissue infiltration was
observed. Indeed, the restoration of blastogenesis to
Con-A and PWM by in vitro addition of allogeneic
monocytes confirms the monocyte defect in this
disease. These findings in total suggested that
attempts at monocyte administration or monocyte
activation might be clinically beneficial. These would
attack both the deficient monocyte number and defi-
cent monocyte function problems.

Mononuclear cell administration did indeed restore
monocyte adherence, ADCC, and also blastogenesis.
This was associated with an increase in the patient's
peripheral blood count, fall in leukemic cell count in
the marrow, and immunorestitution that persisted for
several months after the transfusion. Grafts from
leukocyte transfusions have been described previously
and could have occurred in this case.37 However, this
seems unlikely, since some T-cell functions were intact
in these patients. We could not test this cyogeneti-
cally because both donor and recipient were male. Release of a colony-stimulating factor from the transfused cells with subsequent activation of bone marrow and production of mature monocytes seems more likely. The results with C. parvum were equivocal at best. However, macrophage activation with better defined nonviable microbial adjuvants should also be explored as a therapy for this disorder.

The relationship between the mononuclear cell transfusions and the hematologic improvement in case one is speculative. It is possible that the improvement was the result of the control of the infectious process, although it was worsening at the start of the hematologic improvement. To further explore this point, we have started a leukocyte transfusion protocol for the research management of hairy cell leukemia.

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