Hairy Cell Leukemia: Functional, Immunologic, Kinetic, and Ultrastructural Characterization

By L. Debusscher, J. L. Bernheim, E. Collard-Rongé, A. Govaerts, R. Hooghe, F. J. Lejeune, M. Zeicher, and P. A. Stryckmans

A diagnosis of hairy cell leukemia was made by optic microscopy, phase-contrast microscopy, electron microscopy, phase-contrast microscopy, and histochemistry of the abnormal blood cells. In vivo these cells were found to have a half-time in the blood of ~150 hr. In vitro they had the capacity to adhere firmly to plastic, making it possible to obtain a pure population of hairy cells. Neither T-rosette formation nor phytohemagglutinin (PHA) transformation could be demonstrated in these cells. On the other hand, the presence of immunoglobulins on the surface of the hairy cells (HC) by immunofluorescence, and the synthesis and secretion by these cells of IgM type λ-chains shown by radioimmunodiffusion, were in favor of their B-type lymphocyte origin. Similarities to chronic lymphocytic leukemia were apparent.

Hairy Cell leukemia is a rare type of chronic leukemia. The nature and characterization of the pathologic cells seen in the blood, spleen, bone marrow, and lymph nodes in this disease have been a matter of argument for several years. For some time, they were considered to be primitive reticulum cells, but then were said to synthesize immunoglobulin and to carry immunoglobulins on their surface. These observations favored a lymphocytic origin. More recently, in two cases of hairy cell leukemia, a high percentage of the hairy cells in the spleen were found to have membrane receptors for cytotoxophilic antibody, suggesting that they belonged to the monocyte–histiocytic series. Our present studies on the characterization of these cells in vivo, and in vitro in pure cell suspensions, however, favor their being lymphocytic in origin.

CASE REPORT

A male patient, age 51 when first admitted in 1971, had been exposed for the last 10 yr to trichlorethylene and perchlorethylene. He had been in good health until 1968, when he developed pneumonia. A few months later appendicectomy had to be performed, followed by fever for several weeks and by progressive fatigue and weakness. In October 1970 he was admitted to another hospital for fever of unknown origin. He was readmitted for fever in May 1971, and the diagnosis of chronic lymphocytic leukemia was made. Treatment with leukeran (2.5 mg/day) was started in September 1971. When he was admitted to our Institute in December...
1971, he complained of fever, fatigue, and anorexia. Physical examination showed only pallor, a low-grade fever, and instability of vestibular origin attributed to prolonged gentamicin treatment. There was no lymphadenopathy or hepatosplenomegaly. The hemoglobin was 11.1 g/100 ml, hematocrit was 33.7%o, RBC count was 3,120,000/cu mm, mean corpuscular volume was 111 cu μ, platelet count was 104,000 cu mm, WBC count was 4800 with 84% lymphocytes 10% of which were atypical, serum vitamin B₁₂ was 225 pmole/liter, and serum folate acid was 4 μg/liter (N, 14–18). Kidney function and urine sediment were normal. The thymol turbidity test was 9 Mac Lagan U (N, 0–4), and alkaline phosphatase was 250 1 mu. The serum proteins were 7.6 g/100 ml with a normal electrophoretic pattern. The immunoglobulins were IgA, 212 mg/100 ml (N, 120–240); IgG, 1501 mg/100 ml (N 800–1800); and IgM 60 mg/100 ml (N, 80–170). The immunoelectrophoresis was normal. Scanning of the spleen showed moderate splenomegaly. Bone marrow aspiration was repeatedly unsuccessful, the aspirated material being hypocellular with only a few erythroblasts and megakaryocytes. Seventy-five per cent of the cells were monocellular cells, 20% of which were of the reticular type. Fever disappeared spontaneously. The level of hemoglobin decreased too rapidly to be explained only by arrest of production. In order to rule out tuberculosis of the spleen (the tuberculin skin test with 10 U was strongly positive) and malignant lymphoma, a laparotomy with splenectomy and liver biopsy was performed on January 4, 1972. Liver and spleen were infiltrated with atypical cells similar to those found in the blood and marrow. The spleen was only slightly enlarged (460 g). After splenectomy, the anemia persisted but did not necessitate blood transfusions for over 1 yr. The WBC count, however, increased progressively and reached 73,000/cu mm in November 1973 with anemia and thrombocytopenia. During prednisone administration, 60 mg/day for 1 mo, an initial additional increase (110,000/cu mm) was observed, and thereafter a return to lower values (~20,000/cu mm) when the patient felt considerably improved. The patient entered the hospital with a severe lung infection in February 1974 and died in septicemic shock (Staphylococcus aureus) within a few hours.

MATERIALS AND METHODS

Morphologic and Cytochemical Examinations

Bone marrow and blood smears stained with May-Grünwald-Giemsa were examined by routine microscopy. Standard techniques were used to examine the blood cells by phase microscopy, electron microscopy, and scanning microscopy.

The mononuclear cells of the blood were also examined for the presence of alkaline phosphatase, acid phosphatase, tartrate-resistant acid phosphatase, nonspecific esterase, peroxidase, and PAS-positive material. The method described by Katayama to detect acid phosphatase was used on material processed for electron microscopy.

Isolation of Mononuclear Cells

An almost pure population of mononuclear cells was obtained by a slight modification of the Ficoll-Hypaque technique. In order to prepare a suspension of adherent mononuclear cells, those adherent to the bottom of 9-cm-diameter plastic petri dishes were harvested by washing with Versene, 0.25%, in phosphate-buffered saline (PBS) after the collection of non-adherent cells by five flushings with PBS.

In order to prepare a mononuclear cell suspension depleted of adherent cells, 16 ml of defibrinated blood were sedimented for 1 hr at room temperature with 4 ml of Plasmagel (Lab. Roger Bellon, Neuilly, France). The leukocyte-rich supernatant was poured into a vertically fixed syringe containing a PBS-soaked sterile nylon filter. This column was kept 1 hr at 37°C until the effluent cell suspension was collected.

Phagocytosis

The mononuclear cells obtained with the Ficoll-Isopaque mixture were resuspended in TC 199 with 15%, human AB serum and incubated for 2 hr at 37°C in the presence of approximately 10⁷
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S. aureus per ml or of carbon particles (% India-ink solution). These cells were then washed and processed for electron microscopy.

**Lymphocyte Blastic Transformation**

A modification of the method described by Dubois was used. Mitogenic stimulation was induced with 0.5 and 1 μg of phytohemagglutinin-P (PHA, purified phytohemagglutinin, Wellcome) in 10 μl PBS. Blast transformation was expressed as the ratio between stimulated and unstimulated cultures.

**Sheep Red Blood Cell Rosettes**

Various leukocyte preparations, suspended in fetal calf serum (FCS) at a concentration of 10⁶ cells per ml and sheep red blood cells (SRBC) at a concentration of 10⁸ cells per ml in PBS were mixed in equal volumes. 0.2 ml samples of this mixture were incubated for 10 min at 37°C. After 5 min of centrifugation at 150 g, the pellet was kept 60 min at 0°C. The cells were very gently resuspended by rotating the tubes, and the percentage of rosette-forming cells (RFC) was immediately observed under the microscope. Only lymphocytes having four or more SRBC attached were counted as RFC. Two hundred to one thousand cells were screened by this method. Normal donors had a mean of 54.3 ± 9.9% RFC.

**Surface Immunoglobulins**

Direct immunofluorescent staining was carried out according to the technique of Pernis. The mononuclear cells resulting from Ficoll-Isopaque flotation and containing mostly hairy cells as could be seen under phase-contrast microscopy were incubated at room temperature and at 4°C with monospecific γ, μ, and α fluorescein-conjugated swine antihuman sera. The cells were washed three times, put onto slides, and then examined with a Leitz Ortholux microscope using an Hg 200 W lamp and a Leitz vertical illuminator. Cells showing at least three spots on their surface were scored as fluorescent. Two hundred cells were counted for each score.

**Immunoglobulin Synthesis**

The method used was described by Moroz. Approximately 10⁵-10⁶ adherent cells were collected, washed twice, and preincubated for 15 min at 37°C with incomplete Eagle’s basal medium, i.e., containing nonradioactive aminoacids at 1/100 of the concentration in complete Eagle’s basal medium. Thereafter, 16 μCi of uniformly ¹⁴C-labeled aminoacid mixture obtained by hydrolysis of Chlorella protein (Amersham, England) were added to each milliliter of medium. Cells were incubated at 37°C for 10 hr, and the culture medium was then carefully removed and stored at –20°C. The adherent cells were lysed with a nonionic detergent (Nonidet P-40, Shell Corporation), 0.5%, in cold PBS containing iodoacetamide, 0.5 M, and Trasylol (Bayer), 1000 kallikrein inactivator units per ml. The lysate was centrifuged at 2000 g for 4 min, and the supernatant was dialyzed against PBS for 24 hr at 4°C. The culture medium and the dialyzed supernatant were then concentrated on Minicon and immediately tested by radioimmunoelectrophoresis. Rabbit anti-immunoglobulin, sheep anti-mu, horse anti-gamma, and horse anti-alpha sera were purchased from the Nederlandse Rode Kruis (Amsterdam, The Netherlands), and porcine anti-mu, horse anti-mu, swine anti-gamma, and rabbit antitotal sera were provided by Nordic (Tilburg, The Netherlands). The rabbit anti-light chain sera were produced by the Centre Départemental de Transfusion Sanguine (Bois-Guillaume, France). After immunoelectrophoresis, the dried plates were placed in contact with Kodak Royal X Pan film and developed after an exposure of 10 days.

**Kinetic Studies**

The fate in the circulation of in vitro labeled blood hairy cells was studied using a technique previously described for patients with chronic lymphocytic leukemia. This study was performed 11 mo after splenectomy.
RESULTS

Morphology, Adherence, and Phagocytosis

The blood smears and the imprints of bone marrow, spleen, and lymph node biopsies showed typical large mononuclear cells with abundant grayish-blue cytoplasm and a dark-stained nucleus (Fig. 1A). On phase microscopy (Fig. 1B) the blood cells appeared as typically "hairy" with numerous slender hair-like cytoplasmic projections, confirmed by electron microscopy which showed...
haired processes (h) are seen on the cell surface. The cytoplasm shows a well-developed golgi apparatus (g), lysosome-like bodies (arrows), several myelinic figures, and swollen mitochondria (Mit). Two ribosome-lamellar complexes (RLC) are present. Inset: Cytoplasmic inclusion in a hairy cell showing a positive reaction for the acid phosphatase assay according to Katayama.

ribosome-lamellar complexes (Fig. 1C). The cells were also examined by scanning electron microscopy (Fig. 1D).

The results of the cytochemical examinations are summarized in Table 1. Acid phosphatase according to Katayama was positive as seen in lysosome-like bodies (Fig. 1C, inset). All the mononuclear cells adherent to plastic were found to be exclusively hairy on phase microscopy. These cells did not phago-
cytize *S. aureus* or carbon particles, whereas the PMN seen in the buffy coat phagocytosed intensely (Fig. 1E).

**Response to PHA and SRBC Rosette Formation**

Figure 2 indicates that the blastic transformation by 1 γ of PHA is maximum on day 2.6 and is almost absent on days 5 and 7. It shows moreover that the mononuclear cells of the patient transformed considerably less than the control.

The fact that on day 2.6 the $^{3}$HTdR uptake was nearly four times higher for mononuclear cells depleted of adherent cells (20% instead of 70%) by passage over a nylon column suggested that the adherent cells did not participate in the transformation.

A pure population of hairy cells adherent to plastic dishes did not show any PHA response (Table 2). The nonadherent cells, on the other hand, showed definite $^{3}$HTdR incorporation independently of whether or not these cells had been EDTA treated, as were the adherent cells. Their subnormal response probably reflected a 20% contamination by nonresponding HC. Similar results were obtained with the suboptimal dose of 0.5 μg PHA.

The whole mononuclear population contained 14% RFC. The adherent cells contained no RFC and the nonadherent 35%–43% RFC.
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Fig. 1E. Leukocytes incubated for 1 hr with staphylococci. Two polymorphonuclears (PM) have phagocytosed many bacteria. The six hairy cells (HC), even in contact with bacteria (S), have not phagocytosed.

Table 1. Cytochemistry of Blood Hairy Cells

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<table>
<thead>
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<tbody>
<tr>
<td>Acid phosphatase</td>
<td>+</td>
</tr>
<tr>
<td>Tartrate-resistant acid phosphatase</td>
<td>+</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>–</td>
</tr>
<tr>
<td>Nonspecific esterase</td>
<td>–</td>
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<tr>
<td>Periodic acid Schiff</td>
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<td>Peroxidase</td>
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Fig. 2. The PHA blastic transformation (expressed in $^{3}$H-thymidine uptake) as a function of time.

Table 2. Response to PHA and SRBC Rosette Formation

<table>
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<tr>
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<th>PHA Transf. Ratio on Day 3</th>
<th>Per Cent SRBC Rosette Formation</th>
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<tr>
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<td>Patient</td>
<td>Control</td>
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<tr>
<td>Whole mononuclear cell suspension</td>
<td>64</td>
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<tr>
<td>Plastic-adherent cells after versene release</td>
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<tr>
<td>Plastic nonadherent cells</td>
<td>129</td>
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<tr>
<td>Plastic nonadherent cells treated by versene</td>
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Table 3. Surface Immunoglobulins (Per Cent)

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<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
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<tr>
<td>HC leukemia (Jan. 74)</td>
<td>1</td>
<td>—</td>
<td>89</td>
</tr>
<tr>
<td>Normal controls</td>
<td>0</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>4</td>
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</tr>
<tr>
<td>2</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>CLL</td>
<td>2</td>
<td>5</td>
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</tr>
<tr>
<td>0</td>
<td>5</td>
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<td>17</td>
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<tr>
<td>0</td>
<td>17</td>
<td>25</td>
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Surface Immunoglobulins

The results are shown in Table 3 and compared to those obtained in normal and CLL individuals.

Immunoglobulin Synthesis by the HC

With the cell lysate, the polyvalent serum directed against human globulins revealed two radioactive precipitation lines, one identical to the IgM line, the other behaving like an alpha-globulin.

Both culture medium and cell lysate showed one single immunoprecipitation line with each of the three different antihuman mu-chain sera used. No precipitation line was found either with anti-gamma or with anti-alpha serum. With anti-light chain sera, a precipitation line was observed with anti-lambda and not with anti-kappa.

Since all adherent cells were HC, as far as could be microscopically ascertained, it can be tentatively supposed that the neoplastic cells synthesized IgM molecules. We have no evidence for the presence of either monomeric or pentameric IgM at the moment.

The Kinetics of HC in the Blood

The labeling index of the blood HC determined on 1000 cells after incubation with $^3$H TdR was zero. Figure 3 shows the fate in the blood of $^3$H-cytidine-labeled hairy cells. After rapid disappearance of two-thirds of the labeled cells from the circulation, a slow exponential decrease with a half-time of 150 hr was observed. The overall aspect of this curve was quite similar to the one, shown below, of the labeled lymphocytes of a patient with CLL. The rapid initial

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Fig. 3. Disappearance curves of $^3$H-cytidine-labeled cells in HCL (on top) and in CLL (bottom).
decrease may have resulted from the redistribution of the labeled cells between a vascular and an extravascular "readily accessible" pool similar to the one described for CLL lymphocytes.25

DISCUSSION

Approximately 100 cases of hairy cell leukemia (HCL) have been described. Our case showed the main clinical characteristic features of the disease, i.e., splenomegaly, absence of adenopathy, fever, pancytopenia, a dry tap on bone marrow aspiration, and a chronic evolution. Our case also presented all the morphologic and cytochemical features of this disease: unusual abundant and pale cytoplasm on Giemsa-stained smears, very characteristic slender cytoplasmic projections on phase-contrast and electron microscopy (EM), and tartrate-resistant acid phosphatase. Ribosome-lamellar complexes were seen in EM (Fig. 1C). They have been described in almost 50% HC leukemias,26 in three cases of CLL,27,28 and one case of malignant lymphoma.29 The appearance of the blood cells on scanning EM was another argument for the diagnosis of HCL.30

The functional aspects of these leukemic hairy cells have not been found to be identical in all the cases described, as summarized in Table 4. This is the case for phagocytosis, adherence to glass and plastic and PHA responsiveness. The discrepancies found in the literature may have several explanations. First, the great differences relative to PHA responsiveness may result from a different percentage of HC in the blood of different patients. PHA transformation could in fact reflect the response of normal lymphocytes contaminating more or less the HC population. This hypothesis could explain why in the present study no 3HTdR incorporation in the presence of PHA was seen, since a pure population of HC could be obtained taking advantage of the reproducible adherence of HC to plastic. Secondly, the differences in the methods used for phagocytosis could

<table>
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<tr>
<th>Adherence</th>
<th>Phagocytosis</th>
<th>PHA Transformation</th>
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<tr>
<td>N</td>
<td>Techniques</td>
<td>Results</td>
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<tr>
<td>1</td>
<td>Gelatin-cotton separation</td>
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<tr>
<td>1</td>
<td>Nylon fibers</td>
<td>-</td>
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<tr>
<td>1</td>
<td>Siliconized glass beads</td>
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<tr>
<td>4</td>
<td>Glass slides</td>
<td>+</td>
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<tr>
<td>1</td>
<td>Siliconized glass beads</td>
<td>+</td>
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<tr>
<td>5</td>
<td>Glass tubes</td>
<td>±</td>
</tr>
<tr>
<td>1</td>
<td>Plastic dishes and nylon fibers</td>
<td>+</td>
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explain the discrepancies in the literature. Finally, it is impossible to rule out that the differences in the literature are due to the existence of different sorts of HCL.

The possibility has been raised that the cells seen in leukemic reticuloendotheliosis are of monocytic type. The half-time of the HC in the blood (140 hr) observed in the present study is not compatible with this hypothesis since it is approximately 7 hr for the normal monocytes and less than 100 hr in chronic monocytic leukemia. The absence of phagocytosis in our case, as in all series except one, is an additional argument against the monocytic nature of these cells.

The inability of the HC to transform in the presence of PHA and to form SRBC rosettes excluded them from the class of T-lymphocytes. In order to test the hypothesis of a B-type lymphocyte-derived leukemia, immunofluorescence studies were performed on the blood cells of this patient. It was shown that over 89% of these cells had μ-chains and therefore presumably had IgM on their surfaces. The presence of immunoglobulins on the surface of HC was also found in other studies. However, surface immunofluorescence does not allow a distinction between IgM synthesized by the hairy cells or extrinsic molecules sticking on the hairy cell surface. The uptake of radioactive amino acids demonstrated the synthesis of IgM molecules with λ-chains. The observation of the adherent cells in the phase-contrast microscope made it possible to ascertain that the cells responsible for synthesis and secretion of the IgM λ-type immunoglobulins were the hairy cells and not a few contaminating lymphocytes. Rubin et al. found synthesis of IgG type κ and λ by the blood cells of a patient with HCL. These authors did not demonstrate this finding on a pure suspension of HC since they did not separate HC from lymphocytes. The adherence property of the HC of our patient is not against their B-lymphocyte origin since B-lymphocytes have been found to adhere to nylon fibers. Although receptors for Fc, for C3, and for Epstein–Barr virus were not searched for, the present study provides evidence for the B-lymphocyte nature of the hairy cells of our patient.

The nature of the synthesized immunoglobulin is compatible with a monoclonal neoplastic process which could be closely related to CLL, as suggested by the disappearance curve of blood HC very similar to that observed in CLL and by the increase of these cells in the blood after administration of prednisone. The question remains open whether the peculiar morphology of these cells represents merely an unusual expression of the neoplastic process of B-lymphocytes or if it represents the expression of a special function of a lymphocyte subclass or differentiation stage. The latter, too small to be recognized in normal individuals, would then be revealed only when undergoing a neoplastic hypertrophy.

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