Hairy-Cell Leukemia With T-Cell Features

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A case of clinically and hematologically typical hairy-cell leukemia has been presented in which, at the various times of testing, 52%-95% of peripheral blood and 73% of splenic mononuclear cells formed spontaneous sheep erythrocyte (E) rosettes. Many of the rosetting cells were shown to be typical morphologic hairy cells by light and electron microscopy. It was found that 70%-75% of peripheral blood mononuclear cells stained with an anti-T antiserum, and this antiserum also abolished E-rosette formation. A variable percentage of peripheral blood mononuclear cells was also shown to bear surface (IgDK) and internal (IgMK and IgGK) immunoglobulins. Additional B-cell features demonstrated included possession of the P29/34 La-like antigen and formation of mouse rosettes. It was demonstrated by a variety of blocking and inhibition studies that the E-rosette formation was not attributable to chance antigen specificity of the surface membrane immunoglobulin. These marker studies suggest that this is a case of true hybrid cell HCL. Despite these unusual marker characteristics, the patient showed no distinctive clinical or hematologic features.

The immunologic surface marker characteristics of hairy cells (HC) of hairy-cell leukemia (HCL) or leukemic reticuloendotheliosis have been intensively investigated in recent years, but no cases have been described in which the HC possessed T-lymphocyte features.

In the present paper we report a patient with typical HCL in whom the HC consistently expressed T lymphocyte phenotypic markers. A detailed morphologic and immunologic marker study has been performed in an attempt to define the nature and significance of this phenomenon.

Case Report

This 51-yr-old white man presented in December 1976 with general malaise and marked (10 cm) splenomegaly, minimal hepatomegaly (2 cm), and no lymphadenopathy. Blood count at presentation showed a hemoglobin of 12.2 g/liter, a white blood cell count of 4.6 × 10^9/liter, and a platelet count of 50 × 10^9/liter. Differential count revealed approximately 20% typical HC and the marrow was shown to be extensively infiltrated with similar cells; a proportion of the HC contained tartrate-resistant acid phosphatase. In a differential count of 500 cells, no monocytes were seen either in Romanowsky- or esterase-stained preparations. Electrophoresis revealed no evidence of paraproteinemia, and individual serum immunoglobulin levels were normal by radial immunodiffusion.

Shortly after presentation, the hemoglobin fell to 10.2 g/dl and the white cell count to 2.8 × 10^9/liter, while the platelet count remained unchanged. The patient remained pancytopenic until
splenectomy 5 wk later, when the spleen was found to be diffusely infiltrated by HC both macro-
scopically and microscopically. Apart from a wound infection, he made an excellent postoperative
recovery, and the pancytopenia rapidly disappeared. At the time of this writing, the patient
remains very well with a hemoglobin of 14 g/dl, white count of 5.0 x 10^9/liter (again with
some 20% morphologic HC), and platelet count of 200 x 10^9/liter. The patient at no time re-
ceived chemotherapy.

Other Patients
For comparison, an additional nine patients with clinically and hematologically typical HCL
were studied; in six of these cases spleen cells were also examined.

For control of the blocking studies described later, a patient with chronic lymphocytic leu-
kemia (CLL) was studied whose abnormal cells possessed surface membrane immunoglobulin
(Smlg) with antibody specificity for unsensitized ox erythrocytes.

 Morphologic and Cytochemical Studies
Romanowsky-stained bone marrow and peripheral blood smears, in addition to splenic impres-
sion smears, were examined by routine microscopy. Standard techniques were used to examine
blood and spleen cells by phase-contrast and electron microscopy.12

The mononuclear cells of the blood and spleen were also examined for the presence of acid
phosphatase13 (with and without tartrate14) and α-naphthyl butyrate esterase (with and with-
out sodium fluoride inhibition15).

 Mononuclear Cell Isolation
Mononuclear cells were separated from heparinized peripheral blood by centrifugation over
Ficoll–Hypaque and were washed as described below.

A splenic single-cell suspension was obtained by forcing several pieces of tissue from different
areas of freshly removed spleen through a stainless steel mesh into a Hepes-buffered Hanks’
balanced salt solution (HBSS). The cell suspension was kept on ice for 3 hr until its return to
the laboratory, where the cells were washed in HBSS and mononuclear cells were again separated
by centrifugation over Ficoll–Hypaque. The cells were then washed three times in HBSS plus 0.2% bovine serum albumin (BSA) to a final concentration of 2 x 10^6 cells/ml.

In all experiments viability by trypan blue dye exclusion was greater than 95%.

 Rosette Methods for Surface Markers
E rosettes. Sheep erythrocyte (E) rosettes were usually performed according to the method of
Kaplan and Clark.16

EA (IgG) rosettes. Ox erythrocytes were coated with rabbit IgG as previously described.17

EA (IgM) rosettes. Ox erythrocytes were sensitized with rabbit IgM according to the method
described by Burns et al.17

Yeast–C3 rosettes. The modified18 method of Mendes et al.19 was employed.

Smlg rosettes. The method of Ling et al.20 which employs chromic chloride coupling of puri-
fied sheep anti-Smlg antibody to indicator ox erythrocytes, was used. The purity of these antisera
was confirmed by cross agglutination tests with antigen-coupled erythrocytes. Sheep IgG does not
readily bind to the Fc receptor of human cells,21 but this was controlled in every test by the
inclusion of indicator cells coupled to nonimmune sheep IgG; in all instances rosette formation
with this reagent was less than 1%. The purified antisera20 were a kind gift of Dr. Noel Ling
of the Department of Experimental Pathology, University of Birmingham.

Before testing by this method, the leukocytes were preincubated for 60 min at 37°C in HBSS
to allow removal of any exogenous antibody attached to the Fc receptor.22

Mouse erythrocyte (M) rosettes. The method of Gupta et al.23 using fresh washed unsensi-
tized CBA mouse erythrocytes, was employed.

In all rosette tests, the indicator cells were washed three times and resuspended to 1% in HBSS
(with 0.2% BSA) before testing. The percentages of rosetting cells were assessed by the fluorescein
diacetate method of Ramasamy.24
Fluorescent Methods for Surface Markers

SmIg. Direct immunofluorescence using purified rabbit anti-individual SmIg antisera conjugated with fluorescein isothiocyanate (FITC) was employed. The control was normal, conjugated rabbit serum. All antisera were ultracentrifuged immediately before use. These monospecific antisera were a kind gift of Dr. John Smith of the Tenovus Research Laboratory, Southampton General Hospital. Again, leukocytes were in all instances preincubated at 37°C for 60 min before testing.

Cytoplasmic Ig. Cytocentrifuge preparations of washed splenic mononuclear cells, which had been preincubated for 60 min at 37°C to remove extrinsic Ig, were fixed in acetone at -20°C overnight and stained with the individual anti-heavy and anti-light chain antisera conjugated with FITC. A normal rabbit serum used as a control was completely negative in each test.

Epstein-Barr nuclear antigen (EBNA). An indirect fluorescent method was used to stain aceton-fixed leukocytes.

Anti-P29/34. Rabbit antiserum raised against purified P29/34 B cell membrane antigen was employed in an indirect method using FITC-coupled goat anti-rabbit serum. This test was carried out either on washed viable cell suspensions when the antisera were ultracentrifuged immediately before use, or on acetone-fixed cells from cytocentrifuge preparations in order to exclude further Fc binding of the antisera. Results were identical using either method. The anti-P29/34 antiserum was a kind gift of Dr. Tim Springer of the A.R.C. Institute of Animal Physiology, Babraham, Cambridge.

Anti-T. An indirect method using extensively absorbed anti-monkey thymic cell serum (kindly given by Dr. G. Janossy of the ICRF Membrane Immunology Laboratory, Lincoln's Inn Fields, London) followed by goat anti-rabbit serum was employed. Again, all antisera were ultracentrifuged immediately before use.

RESULTS

Morphologic and Cytochemical Studies

In all experiments, the Ficoll preparations were cytocentrifuged and stained to exclude granulocyte contamination (always less than 5%), and the absence of monocytes was confirmed.

The percent peripheral blood mononuclear cells (11.1.76) showing the HC esterase pattern was approximately 65%, while some 28% possessed the single-dot pattern characteristic of normal T lymphocytes. The finding of a higher percentage of HC by cytochemistry than by simple morphology has been consistent in HCL and was observed in all of our other nine patients with the disease.

In the splenic cell suspension, 92% of cells contained acid phosphatase activity, of which some 10% showed tartrate-resistant positivity. In the esterase stain, 90% of the cells showed the HC pattern, while 3% showed the normal T-lymphocyte pattern.

Transmission electron microscopy of the peripheral blood showed many morphologically typical HC, and a proportion of these were demonstrated to contain the ribosome–lamellar complexes typical of the disease. The almost total replacement of splenic tissue by HC was confirmed at the ultrastructural level and some of the HC were again seen to contain ribosome–lamellar complexes.

T-Cell Markers

The percentage of cells forming E rosettes varied but it was consistently high (Table 1). This finding was in marked contrast to the other nine cases of
Table 1. Surface Markers (%) on the Patient’s Isolated Peripheral Blood Mononuclear Cells

<table>
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<th>Date</th>
<th>E Rosettes</th>
<th>SmIg Rosettes</th>
<th>EA (IgG) Rosettes</th>
<th>EA (IgM) Rosettes</th>
<th>M Rosettes</th>
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<tr>
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<td></td>
<td>30</td>
<td>34</td>
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<tr>
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<td>62</td>
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<tr>
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<td>52, 4</td>
<td>65, 1</td>
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<tr>
<td>26/2/77†</td>
<td>52</td>
<td>77(12), 37, 72(14)</td>
<td>70(22), 16(18)</td>
<td>78, 66</td>
<td></td>
</tr>
<tr>
<td>12/3/77</td>
<td>84</td>
<td>33</td>
<td>34</td>
<td>47</td>
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</tbody>
</table>

*Not tested.
†Figures in parenthesis represent percent rosettes before incubation for 1 hr at 37°C to remove extrinsic immunoglobulin.

HCL studied, in which E-rosette formation ranged from 15% to 45% with a mean of 30%. Examination of cytocentrifuge preparations of the patient’s E rosettes showed that approximately 50% of the rosetting cells were hairy cells; this was confirmed at the ultrastructural level.

In the spleen, 73% of the patient’s cells formed rosettes with sheep erythrocytes. In contrast, in splenic cell suspensions prepared in a similar way from six other patients with HCL, 3%-22% (mean 13%) of mononuclear cells formed E rosettes.

Staining of the patient’s peripheral blood leukocytes with anti-T antiserum was carried out on two occasions, and 75% and 70% of cells stained at a time when E-rosette formation was 79% and 52%, respectively. Staining with the cells from a different case of HCL, which formed 31% E rosettes, yielded 24% fluorescing cells with this anti-T antiserum.

B-Cell Markers

SmIg. The results are shown in Tables 1 and 2. In the six other HCL patients studied for SmIg, 57%-85% (mean 66%) of peripheral blood leukocytes possessed SmIg of a single light chain class.

The patient’s mononuclear cells were also tested for SmIg after 1 wk in culture, when 68% of the cells expressing SmIgD before culture continued to do so. However, most of the other heavy and light chain SmIg classes were no longer expressed. We cannot account for this imbalance between heavy and light chain
expression after culture, but heavy–light chain imbalance is a feature of both cultured B cells and myeloma cells. No morphologic transformation or differentiation into plasma cells was observed during this period of culture even when sheep erythrocytes were included in the culture.

The percentage of splenic cells bearing SmIg is shown in Table 2. By both the rosette method and immunofluorescence, approximately 80% of cells possessed IgD SmIg. In the two other patients with HCL whose splenic cells were examined for SmIg, 80% and 85% of cells were positive for SmIg of a single light chain type (one case studied by fluorescence and one case studied by SmIg rosettes).

Intracytoplasmic Ig. The results are shown in Table 2.

Anti-P29/34. The anti-P29/34 antiserum stained 79% of the patient’s peripheral blood mononuclear leukocytes (26.2.77) and 90% of splenic cells. In the only other patient with HCL studied with the anti-P29/34 serum, 75% of splenic leukocytes were positive. Negative controls included a T-cell line and cells from a case of Sezary’s syndrome; positive controls were cells from several established B-cell lines.

EBNA. The present patient and all other patients with HCL studied were negative for EBNA.

Yeast–C3. The present patient and all other patients with HCL studied by this method were negative for the C3b receptor.

Ea (IgG) rosettes. The percent peripheral blood and splenic mononuclear cells bearing a receptor for the Fc of IgG are shown in Tables 1 and 2. In the other HCL patients studied for this Fc receptor, 44%-83% (mean 60%) of peripheral blood mononuclear leukocytes (nine patients) and 78%-94% (mean 88%) of splenic mononuclear cells (three patients) formed Fc rosettes.

M rosettes. As shown in Table 1, only a small percentage of peripheral blood mononuclear cells from the patient formed rosettes with mouse erythrocytes, as was also true of the other four cases of HCL in which peripheral blood mononuclear cells were studied for M-rosette formation (0%-17%; mean 4%).

The percent splenic HC forming M rosettes was somewhat higher both in the patient reported (47%) and the other three HCL spleens tested (4%-30%; mean 18%).

HC Markers

EA (IgM) rosettes. Table 1 shows the percent of the peripheral blood cells forming EA (IgM) rosettes at the different times of study. These figures are to be compared with the other nine patients with HCL in whom peripheral blood EA (IgM)-rosette formation was studied (20%-78%; mean 45%).

Blocking and Inhibition Studies

E rosettes. In order to determine whether the E-rosette formation by HC in the patient could be attributed to SmIg with specificity for sheep erythrocytes, blocking with antiserum against individual heavy and light chains was attempted. In each instance, E-rosette formation was shown to be unaffected under conditions shown to abolish totally the corresponding SmIg rosette.
SmIg was also capped off with the individual antisera (that capping had taken place was confirmed by direct immunofluorescence), and again this was shown to have no effect on E-rosette formation.

As a control, a case of CLL in which the CLL cells formed spontaneous rosettes with unsensitized ox cells as a result of possessing SmIg (IgMK) with activity for an ox erythrocyte antigen was studied and ox rosette formation was shown to be virtually abolished by removal of SmIg by capping with anti-IgM and anti-K antisera.

The effects of other blocking agents on E-rosette formation by the HC of the patient were examined. Anti-T antiserum almost completely abolished E-rosette formation, whereas anti-P29/34, although shown by indirect immunofluorescence to stain the HC at this concentration, did not affect E-rosette formation.

A variety of other agents and conditions known to affect E-rosette formation by normal T lymphocytes was studied. In all instances the effect on the patient’s E-rosette formation resembled that on T lymphocytes from a normal individual. Thus, like E-rosette formation by normal T lymphocytes, the anti-T serum totally abolished E rosettes. Again, the HC-rosette formation resembled E-rosette formation by normal T cells in that it was markedly inhibited by cytochalasin B (20 μg/ml), sodium azide (10⁻¹ M), and temperature and centrifugation alterations known to affect E-rosette formation by normal T cells. Treatment of the HC by papain, anti-P29/34 serum, or puromycin (2.5 μg/ml) did not affect E-rosette formation.

The specificity of the E receptor on the patient’s HC was demonstrated by testing the mononuclear cells for spontaneous rosette formation with a variety of heterologous erythrocytes. Thus no rosette formation was observed with human GpA, ox, rat, guinea pig, donkey, and rabbit erythrocytes.

**DISCUSSION**

No cases of T-cell HCL have yet been reported, although CLL, a more common B-cell neoplasm, has in a number of instances been reported to possess T-cell features. Therefore, it is perhaps not surprising that HCL should also occasionally display T-cell features and, indeed, the occurrence of such cases has been predicted.

In the present paper we have reported a case of HCL in which the HC were shown to have T-cell features. The patient was clinically and hematologically typical in that he showed marked splenomegaly without substantial lymphadenopathy or hepatomegaly and displayed peripheral pancytopenia. The HC were typical morphologically (both by light and electron microscopy), possessed ribosome-lamellar complexes, and displayed both the butyrate esterase pattern and the tartrate-resistant acid phosphatase characteristic of HCL. The HC also possessed the Fc receptors characteristic of HCL.

However, a high percentage of the patient’s peripheral blood and splenic mononuclear cells (many of which were morphologically typical HC) both formed E-rosettes and stained with anti-T cell antiserum. In addition, formation of these E rosettes required conditions known to be necessary for E-rosette formation by normal T lymphocytes, and it was specifically blocked by the...
anti-T antiserum, but not by the anti-P29/34 antiserum (which was, however, also shown to stain the HC).

The patient's HC also possessed a number of B-cell features. The HC possessed surface membrane (IgM and IgG) and cytoplasmic (IgGK and IgG) immunoglobulin, probably also secreted small amounts of immunoglobulin (IgM and IgG), and stained with antiserum against the Ia-like or B-cell-associated P29/34 antigen. In addition, the splenic and a low percentage of peripheral blood HC formed M rosettes, the formation of which has been a feature of certain normal and pathologic B cells. The Fc(γ) receptor demonstrated on our patient's HC has been a general feature of HCL and has been regarded as evidence for the B-cell or monocyte nature of the HC, but this conclusion is not necessarily correct since a small population of T cells also bear this receptor.

Because the HC possessed SmIg, the possibility exists that SmIg was causing apparent E-rosette formation by a chance specificity for a sheep erythrocyte antigen, since this phenomenon has been described in a single case of CLL. This possibility was thoroughly investigated and discounted for the following reasons. First, the conditions required for E-rosette formation by our patient's HC resembled those necessary for true E-rosette formation by T lymphocytes rather than those required for antibody–antigen interaction. In addition, the inhibition of rosette formation by azide and cytochalasin B indicated that this was true E-rosette formation rather than passive antigen–antibody combination. Second, E-rosette formation, although blocked by anti-T serum, was not blocked by anti-SmIg antibody. Even complete removal of SmIg by papain treatment or by capping and shedding did not inhibit E-rosette formation. For comparison, a case of CLL in which the SmIg had specificity for an antigen of ox erythrocytes was studied and, under identical conditions of capping and shedding, rosette formation was virtually abolished with the relevant anti-SmIg antisera. Third, the specificity of the E receptor of our patient's HC, like that of T lymphocytes, was, of the species tested, restricted to sheep erythrocytes.

Analysis of our marker studies showed that several T-cell and B-cell features were present on the same cell since the sum of cells possessing features of both usually greatly exceeded 100%. This patient's HC therefore appeared to be true hybrid cells.

The existence of a leukemia with true hybrid lymphoid cells bearing both an E receptor and SmIg is not without precedent. Hsu et al have reported a case of lymphosarcoma cell leukemia in which sequential studies of leukemic T lymphocytes revealed an increasingly large proportion of cells generating SmIg. In addition, Brouet and Prieur have reported a case of CLL that may have been similar since it expressed SmIg of restricted light chain type and rosetted with sheep erythrocytes, but no blocking studies were reported.

Other cases of T-cell CLL have lacked SmIg, although they, and a variety of other lymphoproliferative disorders, have occasionally been shown to express less definitive B-cell markers such as receptors for Fc and C3. Such T-cell CLL has been associated with unusual clinical features such as massive splenic enlargement and skin involvement. However, the case of HCL under discussion had no unusual clinical or hematologic features; in particular, there was no thymic enlargement and no skin involvement.
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