Hairy Cell Leukemia: Cellular Characteristics Including Surface Immunoglobulin Dynamics and Biosynthesis

By Harvey Jay Cohen, Edward R. George, and William B. Kremer

Hairy cell leukemia (HCL) is caused by the proliferation and accumulation of morphologically characteristic cells whose cell line of origin (lymphocyte versus monocyte) has not been determined. In an attempt to determine the cell line from which these cells are derived, we studied the cells from the peripheral blood and spleens of 6 patients with HCL for their cytochemical, immunologic, surface, and functional properties. Clinically the patients were similar to others reported with HCL, with some beneficial responses to splenectomy. Both the peripheral blood cells and spleen cells from these patients had tartrate-resistant acid phosphatase and lacked nonspecific esterase. They failed to rosette with sheep red blood cells (SRBC), C3-coated RBC, or anti-D-coated SRBC. Intense surface fluorescence was produced by fluorescein-labeled anti-immunoglobulin reagents, and capping rapidly occurred. Reincubation following capping or trypsinization and restaining with anti-immunoglobulin reagents showed the reappearance of monoclonal immunoglobulin. The pattern of immunoglobulin synthesis and release was demonstrated directly by 3H-leucine incorporation into specific immune coprecipitable immunoglobulin and was similar to that of normal B lymphocytes. The cells had a low labeling index but had modest phagocytic ability. The unequivocal demonstration of endogenous immunoglobulin synthesis establishes these cells as B-lymphoid in nature. However, they may represent a particular B-lymphocyte subset originating in the spleen and having phagocytic ability.

Hairy cell leukemia (HCL, leukemic reticuloendotheliosis) involves proliferation and accumulation of abnormal mononuclear cells that produce a disorder of insidious onset, marked splenomegaly, pancytopenia of normal blood elements, and infiltration of the marrow. The cells characteristically have prominent cytoplasmic projections easily visible by phase microscopy, from which the name HCL is derived. The importance of distinguishing this entity from other leukemias is that most of these patients have shown little response or marginal response to chemotherapy, whereas many of them have benefited from splenectomy, with prolonged disease-free intervals. Thus a considerable amount of effort has been directed toward characterizing these cells more specifically. Their major distinguishing characteristic is the presence of a tartrate-resistant acid phosphatase in the cytoplasm. This particular enzyme has not been noted in other leukemic cells. The cell line from which these cells are derived has been a matter of controversy. Evidence has been presented in different instances favoring a monocytic origin or a lymphocytic origin or neither. However, most recent evidence has suggested that these cells are lymphocytic, mostly of a B-cell line, but...
of a subset possessing some unusual properties such as phagocytosis. The predominant evidence for a B-lymphocyte origin has included demonstration of surface immunoglobulin by immunofluorescence and by reappearance of antimmunoglobulin fluorescence after trypsinization, demonstration of the presence of immunoglobulin in the medium by radial immunodiffusion, and demonstration of incorporation of radiolabeled precursors into immunoglobulin. Others have not accepted these findings and have been unable to demonstrate endogenous immunoglobulin production by the cells.

We have studied 6 patients with HCL to obtain a profile of the clinical spectrum and cellular characteristics. This includes direct determination of new immunoglobulin synthesis in some instances. The data presented provide unequivocal evidence for the presence of surface immunoglobulin and immunoglobulin synthesis by these cells and would appear to establish them as B-lymphoid in nature, although perhaps belonging to a small subset of this line because of their unusual relationship to the spleen and other characteristics.

MATERIALS AND METHODS

Six patients with HCL were studied. The clinical and routine laboratory characteristics of these patients are presented in Table 1. The diagnosis was made clinically and morphologically by the presence of hairy cells on routine peripheral blood Wright stains and phase microscopy. The presence of tartrate-resistant acid phosphatase in the cells was required for confirmation. Bone marrow aspiration was difficult, but biopsy demonstrated typical histology, with large cells and abundant cytoplasm. In 5 of 6 patients therapeutic splenectomy was attempted, and the diagnosis was confirmed by histologic evidence of massive infiltration by hairy cells, especially within pulp cords. In these cases cells from both peripheral blood and spleen were studied. The sixth patient sustained traumatic rupture of the spleen, and material was not available for study. The studies were approved by the Durham VA Human Investigation Committee.

Cell Preparation

Peripheral blood mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation as previously described. Generally, no incubation with iron filings was performed prior to centrifugation for fear of removing phagocytic cells. In those instances in which the spleen was available for study, tissue was minced extensively, and single cell suspensions were prepared by tearing the tissue with steel brushes through a fine steel-mesh filter. All cell preparations in plastic tubes were then centrifuged at 400 g and washed four times in medium 199 (GIBCO, Grand Island, N.Y.). Viability at the end of cell preparation, tested by exclusion of trypan blue, was more than 95%. Patients 1, 2, 4, and 6 had more than 90% hairy cells, and patients 3 and 5 had 80% hairy cells by morphologic criteria, after separation. Surface immunoglobulin fluorescence was determined as described previously in detail using goat polyvalent and specific anti-immunoglobulin antisera obtained from Meloy Laboratories, Springfield, Va., with specificity verified by immunoelectrophoresis and Ouchterlony analysis, resulting in precipitin lines only with the appropriate human immunoglobulins, and not with fetal calf serum components. The reaction could also be specifically blocked by preincubation with the appropriate antigen. Initial incubations were performed in M199 at 4°C, and cells were examined for fluorescence with a Leitz Ortholux II microscope (Leitz, Rockleigh, N.J.) equipped with an HB200 mercury lamp, a Ploem vertical epi-illumination system, and FITC filters. Simultaneously a substage tungsten halogen light was used to count the total number of lymphocytes. To determine the redistribution of surface immunoglobulin, cells were incubated and washed as described, then incubated at 37°C in M199 and removed at specific time intervals, and the percentage of surface immunoglobulin-bearing cells exhibiting migration of fluorescence to a unipolar distribution in a cap arrangement was assessed.

Assessment of membrane immunoglobulin as an integral part of the surface membrane by anti-immunoglobulin fluorescence was approached in two ways. In the first method, 2 × 10⁶ cells/ml in incubation medium containing 5% fetal calf serum were incubated for 30 min with 2.5 mg/ml of twice-crystallized trypsin (viability > 80%). They were then washed four times and reincubated in
Table 1. HCL: Clinical Characteristics at Presentation

<table>
<thead>
<tr>
<th>Patient</th>
<th>WBC (cells/μl)</th>
<th>Hairy Cells (%)</th>
<th>Hct (%)</th>
<th>Platelets (cells/μl)</th>
<th>Chemistries*</th>
<th>Bone Marrow</th>
<th>Spleen</th>
<th>History Prior to Treatment</th>
<th>Infections†</th>
<th>Treatment</th>
<th>Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8,470</td>
<td>95</td>
<td>16.5</td>
<td>80,000</td>
<td>Normal</td>
<td>Areas of hairy cell infiltration and hypocellular areas</td>
<td>16 cm 1800 g</td>
<td>5 mo fatigue, Coombs test +</td>
<td>URI, sinusitis, gingivitis</td>
<td>Splenectomy + prednisone</td>
<td>Normal high, platelet: WBC still abnormal 2 yr postop</td>
</tr>
<tr>
<td>2</td>
<td>26.900</td>
<td>90</td>
<td>34</td>
<td>61,000</td>
<td>Packed with hairy cells</td>
<td>10.5 cm 2500 g</td>
<td>3 mo fatigue, weight loss, epistaxis, observed 6 mo Recurrent infections, fatigue for 4 mo</td>
<td>Pneumonia, sepsis, Recurrent URI, Neutropenia, neutropenia, pneumonia</td>
<td>Splenectomy, COP‡</td>
<td>Little response at 4 mo; little response, died 18 mo postop Progressive disease, complete response, normal bone marrow, 3½ yr postop</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7,000</td>
<td>75</td>
<td>37</td>
<td>60,000</td>
<td>Normal</td>
<td>Packed with hairy cells</td>
<td>13 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>18,600</td>
<td>92</td>
<td>29</td>
<td>51,000</td>
<td>Normal</td>
<td>100% replaced with hairy cells</td>
<td>3 cm 920 g</td>
<td>3 mo thrombocytosis, node enlargement, fatigue, bone pain, skin lesions, observed 1 yr</td>
<td>URI + UTI</td>
<td>Splenectomy</td>
<td>Transient improvement, than 1 WBC Good response</td>
</tr>
<tr>
<td>5</td>
<td>4,660</td>
<td>23</td>
<td>41</td>
<td>660,000</td>
<td>Normal</td>
<td>Areas of hairy cell infiltration and hypocellular areas</td>
<td>6 cm</td>
<td>Asymptomatic, diagnosis made because of chance WBC, observed 3 yr, than trauma and ruptured spleen</td>
<td>None</td>
<td>Splenectomy</td>
<td>Asymptomatic 1½ yr postop, 75% hairy cells</td>
</tr>
<tr>
<td>6</td>
<td>900</td>
<td>80</td>
<td>17</td>
<td>57,000</td>
<td>Normal</td>
<td>Packed with hairy cells</td>
<td>10 cm 2400 g</td>
<td>2 mo fatigue, headache, observed 2 mo, folate deficiency</td>
<td>None</td>
<td>Splenectomy + folate</td>
<td>Good response; later i platelets, partial response to prednisone; died 1 yr postop, intracranial bleed</td>
</tr>
</tbody>
</table>

*Chemistries include standard SMA-18 and serum protein electrophoresis. Spleen measurement is centimeters below the right costal margin, 10 cm from the midline.
†URI = upper respiratory infection, UTI = urinary tract infection.
‡COP = cyclophosphamide, vincristine, prednisone; B = BCNU.
medium for 6 hr. Cells were then reincubated with anti-immunoglobulin fluorescent antisera as described previously. Alternatively, cells were allowed to incubate with anti-immunoglobulin fluorescent antisera for 1 hr in M199. At the end of that time anti-immunoglobulin fluorescence could be seen to disappear from the cell surface, and it was not present on immediate restaining with anti-immunoglobulin fluorescent antisera. Cells were then allowed to incubate for 6 hr in medium and were restained with fluorescent-labeled antisera.

The determination of new immunoglobulin synthesis by these cells was performed by methods described in detail previously from this laboratory. Sterile isolated peripheral blood or bone marrow cells were washed twice in minimal essential medium (MEM) without leucine (GIBCO) and adjusted to \(5 \times 10^6\) cells/cc. For the determination of new protein synthesis \(^{1}H\)-l-leucine (46 Ci/\(\mu\)m) (New England Nuclear, Boston, Mass.) was added to the leucine-free medium at a final concentration of 5 \(\mu\)Ci/cc. Aliquots were then incubated in a humidified 5% CO\(_2\) incubator for selected periods up to 8 hr, removed from the incubator, and centrifuged at 1200 g for 5 min, and the supernatant was collected. Cell buttons were then washed twice with phosphate-buffered saline (pH 7.0) and digested with 0.75% deoxycholate (Sigma, St. Louis, Mo.) for 1 hr. Supernatants and cell digests were dialyzed for 48 hr at 4° C against phosphate-buffered saline (pH 7.0). The resulting dialysates were centrifuged for 10 min at 13,000 g in a Sorvall RC 2B centrifuge, and the supernatants were subsequently analyzed for labeled immunoglobulin.

Immune coprecipitation was performed as previously described. Briefly, aliquots of culture supernatants or cell extracts were incubated with carrier human immunoglobulins (polyvalent IgM, IgG, IgA) and goat antihuman immunoglobulin (GIBCO) at equivalence for precipitation. This antiserum had been previously absorbed and had reactivity against IgA, IgM, IgG, and light chains, but no other serum proteins. Simultaneously, incubations for the control of nonspecific precipitation were performed with carrier egg albumin and rabbit anti-egg albumin. The specific and control precipitates were both adjusted, at equivalence, to form 0.6 mg of precipitate so that counts nonspecifically trapped in the precipitate could be determined. Precipitation was carried out in phosphate-buffered saline (pH 7.0) in a total volume of 2 cc. Incubation was carried out at 37° C for 1 hr and then overnight at 4° C. Immune precipitates were washed three times with phosphate-buffered saline (pH 7.0) and solubilized with NCS tissue solubilizer (Amersham Searle, Arlington Heights, Ill.). Solubilizer was added to POPOP toluene phosphor at a ratio of 1 cc to 10 cc, and radioactivity was detected with a liquid scintillation counter. The counts per minute (cpm) detected in the control nonspecific precipitate were subtracted from the counts incorporated into the specific immunoglobulin-anti-immunoglobulin system to give the final specific immunoglobulin counts. These background counts were generally less than 15% of the total. The system had previously been shown to detect immunoglobulin with a high degree of specificity and sensitivity. Since the antiserum and antigens employed contained all immunoglobulin determinants, including both kappa and lambda light chains and heavy chains, the system will detect the total immunoglobulin synthesized.

The percentages of mononuclear cells forming rosettes with sheep red blood cells (E), C3-coated human red cells (EC), and human red cells coated with anti-D antibody (EA) were determined as described previously from this laboratory. Non-specific esterase, PAS, and tartrate-resistant acid phosphatase were determined as previously described, and the labeling index was assessed as described previously from this laboratory. The phagocytic ability of the mononuclear cells was determined by incubation of cells with \(1 \mu\) latex beads for 2 hr at 37° C and observation by phase-contrast microscopy and of Wright-stain blood films, as previously described.

RESULTS

Table 2 summarizes the findings and the cellular studies in these 6 patients. In four of six instances both spleen cells and peripheral blood cells were studied. The values shown are for the peripheral blood cells, but in all instances the numbers were virtually identical for spleen cells obtained by teasing the splenic tissue, as described in Materials and Methods. Histologically and morphologically the cells obtained from the spleen in each of these instances were hairy cells identical to those of the peripheral blood (Figs. 1A and 1B). All patients were positive for tartrate-resistant acid phosphatase as determined histochemically. All were negative for the non-specific esterase most widely used as a monocyte marker. One
Table 2. HCL: Cellular Characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Surface Ig Fluorescence</th>
<th>Rosettes</th>
<th>Cytoc hemistry</th>
<th>Phagocytosis</th>
<th>Labeling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poly Ig (IgM, IgG, IgA, Kappa and Lambda chains)</td>
<td>Capping (15 min)</td>
<td>E</td>
<td>EA</td>
<td>EC</td>
</tr>
<tr>
<td>1</td>
<td>86 75 64 99 IgM+</td>
<td>+</td>
<td>9 1 2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>92 89 2 85 IgG+</td>
<td>+</td>
<td>3 &lt;1</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>88 84 75 IgM+</td>
<td>+</td>
<td>6 &lt;1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>99 96 5 95 IgG+</td>
<td>+</td>
<td>2 0 3</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>98 75 75 IgM+</td>
<td>ND</td>
<td>5 ND 5</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>84 70 70 ND</td>
<td>ND</td>
<td>4 ND 2 ND</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*Details of all assays are given in Materials and Methods. Patients 1, 2, 4, and 6 had studies of peripheral blood and spleen performed; all spleens were essentially replaced by hairy cells. The numbers shown are for peripheral blood, but the splenic cells showed virtually identical characteristics in each case. Poly Ig antisera with specificity for IgM, IgG, IgA, and kappa and lambda chains; IgG and IgM studied with fluorescein-labeled antisera specific for gamma and mu chains, respectively.

1E = sheep red blood cells, EA = human group O red blood cells (RBC) coated with anti-D antibody, EC = human RBC coated with human C3 by interaction with cold agglutinin antibody.

NSE = nonspecific esterase, PAS = periodic acid Schiff, TRAP = tartrate-resistant acid phosphatase.

*Percentage of cells ingesting latex beads: plus indicates function performed by cells, minus indicates function not performed by cells, ND = not done.

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Fig. 1. (A and B) Phase microscopy views of typical hairy cells with large nuclei, abundant cytoplasm, and long filamentous projections (×1000). (C) Light microscopy view of a hairy cell with multiple latex beads attached in a unipolar distribution. In this view it is difficult to distinguish the ingested particles (×1000).
patient had PAS+ granules. These cells were negative for peroxidase and Sudan black as well. Urine or serum lysozyme was not present in the 2 patients with the extreme leukocytosis of hairy cells in whom this was studied (patients 2 and 4).

Small variable percentages of the hairy cells in most patients were capable of phagocytosis of latex beads. In many of these instances the beads appeared to become densely adherent to the cell surface and to accumulate in clusters. Such an appearance was not seen among normal peripheral blood lymphocytes or monocytes. Normal monocytes actively phagocytosed the beads without accumulating at the surface. Normal lymphocytes, on the other hand, generally had few beads adhere, and few were ingested. Among the hairy cells, subsequent internalization appeared to occur, but it was often difficult to gauge because of the surface coating. Small numbers of latex particles in these instances appeared to be internalized. It was of interest that in some instances the latex beads attached to the surface appeared to migrate to a unipolar description in a cap configuration (Fig. 1C). These surface dynamics will be described more extensively with the fluorescent antibody studies. The labeling index of these cells was extremely low in all cases studied, thus suggesting a low proliferative rate.

**Rosette Formation**

In all instances, very low percentages of cells formed rosettes with SRBC, thus indicating that a few normal T cells were remaining. EC3 rosettes performed under conditions in which antibody had been previously removed (i.e., using cold agglutinin antibody to attach complement) demonstrated a very low percentage of reacting cells. Previously this reagent had been shown to react with lymphocytes and some monocytes. EA were prepared by incubating human red cells with anti-D antibody, forming a low-density surface coating of erythrocytes such that monocytes and activated monocytes with high-affinity receptors would react and form rosettes, but very few normal lymphocytes do so. With this reagent a very low percentage of the hairy cells formed rosettes (Table 2). Fc receptors using high-density immunoglobulin coating or aggregated immunoglobulin were not assessed.

**Fluorescence Studies**

All patients exhibited extremely bright surface fluorescence with the polyvalent anti-immunoglobulin antiserum (Table 2 and Fig. 2A). This immunoglobulin was shown to be predominantly IgG in 2 patients and IgM in 3 patients. In two cases both IgG and IgM fluorescence were detected. However, following trypsinization, only IgM reappeared on the surface. In such instances, when cells were trypsinized the fluorescence was lost when the cells were reobserved immediately, but after reincubation for 6–8 hr immunoglobulin was found once again on the surface. The immunoglobulin was always of one heavy-chain class and when tested was of one light-chain type as well (Table 2); thus by this criterion the cells were capable of resynthesizing the surface immunoglobulin. The extremely bright fluorescence was of the order of magnitude previously described by us and others in B-cell lymphosarcoma cell leukemia. It tended to be brighter than normal surface immunoglobulin-containing peripheral blood cells. Following incubation at 37°C
for 15 min, the surface immunoglobulin fluorescence formed tight caps in 70%–99% of cells in all patients (Figs. 2B and 3). This is within the normal range for our laboratory and is similar to the phenomenon observed in lymphosarcoma cell leukemia, but it is quite distinct from the findings in chronic lymphocytic leukemia patients, in whom less than 20% of cells form surface immunoglobulin caps. Further incubation at 37°C following the formation of caps resulted in loss of fluorescent material from the surface. Whether this occurred by pinocytosis or...
sloughing could not be determined, although both processes seemed operant. Immediate reincubation of cells with fluorescein-labeled anti-immunoglobulin demonstrated no surface immunoglobulin. However, following incubation for 6 hr, the cells redeveloped surface immunoglobulin, as demonstrated by antiimmunoglobulin fluorescence. The specific immunoglobulin type reinserted in the membrane was the same as that seen after trypsinization. Thus, by this independent assessment, surface immunoglobulin was seen to be reformed by these cells.

Although the antisera used in these experiments were not F(ab')\textsubscript{2} fragments, the phenomenon observed could not be explained by Fc binding, since in the demonstration of the monoclonal nature of the immunoglobulin the various other anti-heavy-chain and anti-light-chain sera all possessing the same Fc fragments failed to bind, whereas the one anti-heavy-chain and anti-light-chain serum did. Moreover, the IgG in goat antisera used in these experiments has been shown to have very low affinity for the Fc receptors of human cells and to bind to the same number and same cells as F(ab')\textsubscript{2} fragments.\textsuperscript{31}

**Immunoglobulin Biosynthesis**

In 3 patients direct assessment of immunoglobulin synthesis and release by the hairy cells was performed by determination of \textsuperscript{3}H-leucine incorporated into protein specifically precipitable by anti-immunoglobulin serum by immune coprecipitation. In 2 of these patients more than 90% of the cells present were hairy cells by morphologic and histochemical criteria. In the third patient virtually all (99%) of the separated cells were hairy cells by these criteria. Cells were pulse-labeled with \textsuperscript{3}H-leucine, and samples were taken at specified time points. In addition, cells were incubated for 5 days in RPMI-1640 in the presence of pokeweed mitogen, then pulsed in a similar manner on day 5 with \textsuperscript{3}H-leucine. The results of pulse-labeling experiments performed using cells from the 3 patients with more than 90% hairy cells are shown in Fig. 4 and were quite similar. It can be seen that the newly synthesized immunoglobulin was detected within 1 hr and reached a peak within 2 hr. Small but detectable amounts of \textsuperscript{3}H-labeled immunoglobulin were detected in the extracellular fluid. After a 5-day incubation with pokeweed mitogen, a similar pattern was seen, although it was augmented slightly and had a more prolonged linear phase of cellular immunoglobulin production. The pattern is similar to that produced by other B lymphocytes that possess surface immunoglobulin, i.e., active

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**Fig. 4.** Incorporation of \textsuperscript{3}H-leucine into immunoglobulin as determined by specific immune coprecipitation. Incubations of unstimulated hairy cells (day 0) and cells after 6 days of culture with pokeweed mitogen (see Materials and Methods) are compared. Results are expressed as specific immunoglobulin cpm × 10\textsuperscript{-3} per 10\textsuperscript{6} hairy cells. Solid line with closed symbols = cellular immunoglobulin; dotted line with open symbols = extracellular immunoglobulin (released into medium). The 3 patients are represented by circles (patient 4), triangles (patient 2), and squares (patient 1).
immunoglobulin synthesis but not active secretion, as shown by previous studies from this laboratory.\textsuperscript{24,32} The presence of puromycin (50 mg/ml) (Sigma) during the incubation completely inhibited the incorporation of \textsuperscript{3}H-leucine into immunoglobulin.

**DISCUSSION**

The initial classic description of HCL\textsuperscript{1} and other reports of this entity used the name leukemic reticuloendotheliosis and suggested that these cells were derived from the reticuloendothelial or monocyte system.\textsuperscript{7-10} Other studies have suggested that these cells have characteristics more suggestive of a B-lymphoid line.\textsuperscript{11-13} Some of the features of these cells, however, remain somewhat atypical for lymphocytes and have allowed the controversy over the cell origin to continue.

In our studies, as also reported by others, the negative esterase reaction,\textsuperscript{3,15,28} the positive tartrate-resistant acid phosphatase,\textsuperscript{5} the absence of SRBC rosetting, the absence of low-density IgG receptors,\textsuperscript{33} and the lack of C3 receptors\textsuperscript{3,9} of these cells are findings consistent with a nonmonocytoid origin of these patients' cells. The presence of limited phagocytosis is not inconsistent with a lymphocyte origin, since lymphocytes have been shown to pinocytose membrane complexes\textsuperscript{21} and in other instances to be more actively phagocytic.\textsuperscript{34,35}

The question central to the issue of the B-lymphocyte nature of hairy cells is whether or not such cells have the capacity to produce immunoglobulin endogenously, since such a property has been considered to reside solely within this class of lymphoid cells. Previous studies have demonstrated the presence of surface membrane immunoglobulin on such cells, and by indirect methods some workers have demonstrated presumptive synthesis of this immunoglobulin.\textsuperscript{18,19} Using fluorescein-labeled specific antisera, we have likewise demonstrated the presence of the surface membrane immunoglobulin on these cells, and we have used several independent means for assessing the dynamics of the surface immunoglobulin on the hairy cells. The surface membrane immunoglobulin of these cells appeared extremely dense and bright on direct incubation, in similarity to that previously demonstrated by us and others for lymphosarcoma cell leukemia cells,\textsuperscript{22} and it was clearly distinct from the surface properties of chronic lymphocytic leukemia cells in this regard.\textsuperscript{21} In the patients reported here the surface membrane immunoglobulin was shown to be monoclonal, although on initial examination some of these cells appeared to have more than one immunoglobulin type. Probably because of the latter finding some authors have concluded that all of the surface immunoglobulins must be absorbed, and they have concluded that these are not surface immunoglobulin-bearing cells.\textsuperscript{8,15} However, at least one of the immunoglobulins appears to be truly endogenous, since following trypsinization in these instances surface immunoglobulin of only one heavy- and light-chain type reappeared after incubation. It is interesting that in each instance the immunoglobulin was of kappa light-chain type. The binding of the second immunoglobulin may be via Fc receptors (not tested in this study) or perhaps via surface IgM acting as antibody for other immunoglobulins, as reported by Preud'Homme and Seligmann in other lymphoproliferative disorders.\textsuperscript{36} The reappearance of surface immunoglobulin, as demonstrated by immuno-fluorescence following initial removal of the immunoglobulin by trypsinization, as reported here, is confirmatory of previous reports in the literature\textsuperscript{18,19} and
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represents one of the indirect methods demonstrating that the surface immunoglobulin of these cells is truly an integral part of the membrane.

In this study we report for the first time a quantitative assessment of the capping capability of these cells with respect to the surface membrane immunoglobulin. In this regard the hairy cells exhibit the ability to rapidly redistribute the surface membrane immunoglobulin into a unipolar distribution characteristic of capped cells. In this sense they behave as do normal peripheral blood B cells and cells from patients with B-cell lymphosarcoma cell leukemia, as we have previously reported,\(^\text{22}\) and they are in clear distinction from cells from patients with chronic lymphocytic leukemia.\(^\text{21}\) This active capping suggests that these cells maintain an intact microtubular-microfilament apparatus, unlike the cells of chronic lymphocytic leukemia, but like those of lymphosarcoma cell leukemia. The phenomenon of capping and subsequent removal of the fluorescing immunoglobulin material from the membrane allowed an alternative indirect method of demonstrating endogenous reinsertion of the surface immunoglobulin in the membrane. Indeed, following another 6–8 hr of incubation, the monoclonal surface immunoglobulin once again could be demonstrated.

In an attempt to demonstrate immunoglobulin synthesis more directly, two previous studies used immunoelectrophoresis of cell lysates and supernatants from hairy cells incubated with radiolabeled amino acids.\(^\text{13}\) These authors were able to show radiolabeling of immunoglobulin arcs in those instances, thus indicating new immunoglobulin synthesis by these cells, but they did not show any evidence pertaining to the kinetics of the process. Golde et al.\(^\text{20}\) reported immunoglobulin kinetic studies in 2 patients with HCL wherein cellular immunoglobulin synthesis and slow release were demonstrated. One of these patients also had a monoclonal gammopathy. On the other hand, Braylan et al.\(^\text{15}\) more recently have stated that they could not demonstrate active immunoglobulin synthesis by hairy cells in vitro. In our study we have provided direct quantitative and qualitative evidence for the biosynthesis of immunoglobulin by hairy cells. The kinetic analysis unequivocally indicates cellular production (inhibited by an inhibitor of protein synthesis) and accumulation of immunoglobulin, with a small amount being released into the medium. This kinetic pattern is the same as that seen with both resting and stimulated normal peripheral blood lymphocytes\(^\text{32}\) and is of approximately the same degree. It is not representative of the pattern seen with active immunoglobulin-secreting cells such as we have shown for mature plasma cells,\(^\text{24}\) wherein levels of extracellular immunoglobulin exceed intracellular levels within 6 hr. We have also demonstrated a similar nonsecretory pattern for plasma cells from patients with nonsecretory multiple myeloma.\(^\text{24}\)

The studies reported here cumulatively provide unequivocal evidence for a B-lymphocyte nature of these HCL cells, and although two cases of T-lymphocyte-derived HCL have been reported recently,\(^\text{37,38}\) these studies lend support to the contention that most HCL is B-lymphocyte-derived. These cells appear to originate from a subgroup having extremely bright anti-immunoglobulin surface fluorescence and the capacity for phagocytosis. In previous studies we have suggested the possibility that chronic lymphocytic leukemia cells are derived from a bone marrow lymphocyte subpopulation, since such infiltration characterizes this disease, whereas lymphosarcoma cell leukemic cells are derived from a lymph-node-based
subset with properties similar to those of the follicular center cell. Likewise, it is conceivable that HCL lymphocytes are derived from a splenic lymphocyte subpopulation with the properties already described. This suggests the need for careful studies of normal splenic lymphocytes to determine if a small population of similar cells exists therein.

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Hairy cell leukemia: cellular characteristics including surface immunoglobulin dynamics and biosynthesis

HJ Cohen, ER George and WB Kremer