Rearrangement of Both Immunoglobulin and T-Cell Receptor Genes in a Prolymphocytic Variant of Hairy Cell Leukemia Patient Resistant to Interferon-Alpha

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We describe a patient with the so-called "prolymphocytic variant" form of hairy cell leukemia (HCL) resistant to treatment with interferon-α (IFN-α). Analysis of immunoglobulin (Ig) and T-cell receptor-β (TCRβ) gene rearrangements from peripheral blood mononuclear cell specimens (MNCs) confirmed not only the B-cell nature of the disease, but also the subsequent emergence of a morphologically indistinguishable population of cells with a clonal TCRβ rearrangement in addition to the original Ig gene rearrangement. With the exception of a transient increase in peripheral blood T cells during treatment with deoxycoformycin (DCF), the MNCs remained essentially constant throughout therapy with no evidence of a co-existing T-cell clone to account for the TCRβ rearrangement. Although MNCs from this patient bound significantly less IFN-α than did MNCs from other HCL patients, the binding was of high affinity with a kd similar to that of control cells. The number of IFN-γ receptors on our patient's MNCs was four times higher than the number of IFN-α receptors and was similar to the number of IFN-α receptors on MNCs from HCL patients responsive to IFN-α. While various treatments including IFN-α, DCF, chlorambucil, splenectomy, leukopheresis, and IFN-γ were not able to change the clinical progression of the disease, they may have provided an opportunity for the divergent TCRβ rearranged clone to expand and displace the initially dominant clone. This is a US government work. There are no restrictions on its use.

Hairy Cell Leukemia (HCL) is a rare lymphoproliferative malignancy that is seen predominantly in middle-aged males. It is characterized by the proliferation of cells with prominent cytoplasmic projections and possessing the tartrate-resistant isoenzyme 5 of acid phosphatase. Although the pathologic and clinical aspects of this neoplasm have been extensively described, only recently has immunoglobulin (Ig) gene rearrangement analysis of HCL patient cells led to the now generally accepted premise that HCL is typically of B-cell origin, although rare cases of T-cell leukemia with morphologic features of HCL have been reported. In addition, a prolymphocytic variant form of HCL has been described that, based on surface marker analysis, also appears to be of B-cell lineage. It is characterized by very high peripheral WBC counts, the absence of monocytopenia or neutropenia and, to date, a female predominance.

The majority of patients with HCL require treatment due to profound cytopenias. Typically, the initial therapy is splenectomy, and while successful in the majority of patients, approximately one third to one half of all patients require additional treatment. A number of studies have demonstrated significant responses in patients treated with interferon-α (IFN-α). Recent reports have also indicated that 2-deoxycytosine (DCF), an adenosine deaminase inhibitor, is also an extremely effective HCL treatment. The response to DCF appears to be rapid and patients who have failed to respond to IFN-α may respond to DCF.

In this report, we describe an unusual case of leukemia with features characteristic of what has been termed the "prolymphocytic variant" of HCL. The patient was essentially unresponsive to IFN-α, DCF, and splenectomy. This patient's treatment-resistant course and atypical features (high WBC count and absence of cytopenias) raised the suspicion that this case of variant HCL might be unusual in important ways. We, therefore, have systematically evaluated the malignant cells from this patient for their morphology, surface marker phenotype, Ig and T-cell receptor-β (TCRβ) gene rearrangement, and IFN receptor number and affinity. Laboratory evidence obtained from clinical samples over the course of a year indicates a unique form of HCL characterized by the emergence of a TCRβ gene rearranged subclone of the original B-cell HCL progenitor.

MATERIALS AND METHODS

Case history. The patient (BS) was a healthy 67-year-old Pakistani woman. In 1964 she was noted to have splenomegaly, mild lymphadenopathy, and a lymphocytic leukocytosis with a total WBC count of 30,000/μL. A presumptive diagnosis of chronic lymphocytic leukemia was made and she received a brief course of chlorambucil therapy without response. In 1979, re-evaluation led to the
diagnosis of HCL. Although she had a significant leukocytosis at that time, her absolute granulocyte count, platelet count, and hematocrit were essentially normal and splenectomy was not advised. She did well until May 1984 when she developed indurated skin lesions, a rising WBC count, and progressive anemia. She was treated with chlorambucil and prednisone experiencing a transient improvement in her peripheral blood counts followed by progression of her disease. She was then referred to the Biological Response Modifiers Program of the National Cancer Institute in December 1984. Physical examination was remarkable for splenomegaly with a spleen tip palpable 16 cm below the left costal margin, the absence of lymphadenopathy, and an enlarged liver with a total span of 19 cm. Bone marrow biopsy revealed an extensive, but focal, infiltrate with mononuclear cells compatible with HCL. Her WBC was 132,000/μL, HCT 35%, and platelet count of 92,000/μL. The differential cell count included 2% granulocytes and 98% mononuclear cells. While many cells resembled classic hairy cells, the majority of mononuclear cells (MNCs) had enlarged nuclei with prominent nucleoli. Binucleation was readily observed. The cells had abundant pale cytoplasm and prominent cytoplasmic projections. The abnormal peripheral blood cells were tartrate-resistant acid phosphatase (TRAP) positive. She was treated with recombinant IFN-α2a (IFN-α) (Hoffman-LaRoche, Nutley, NJ) at a dose of 3 million units daily from December 1984 until May 1985. During that time her WBC count rose slightly from 132,000 to 153,000 and her spleen increased in size from 16 cm below the left costal margin to 26 cm. The platelet count and hematocrit remained stable. She was then treated with DCF at a dose of 4 mg/m² administered weekly. Her WBC count fell over 15 weeks of this therapy from 183,000 to 22,000/μL. However, despite continued administration of DCF, her WBC count then progressively rose. Chlorambucil therapy was administered at 4 mg/d for ten days, but her WBC count continued to rise. Cyclophosphamide was administered at a dose of 1 g intravenously (IV) without a detectable response. Splenectomy was performed and the patient’s WBC count subsequently fell to 16,500/μL from a presurgical WBC count of 184,000/μL. However, this response lasted approximately 2 months when her WBC count began to progressively rise. The patient subsequently was treated with recombinant IFN-γ (Genetech, South San Francisco) at a dose of 0.1 mg/m² intramuscularly daily for five days, then with .25 mg/m² intramuscularly daily without effect. The patient received 3 million units of IFN-α daily for approximately 3 weeks; during this time her WBC count progressively rose from 187,000 to 298,000/μL. Alpha interferon was administered at progressively higher doses up to a total of 12 million units daily for approximately 1 month. However, her WBC count did not change in response to these escalating doses, and the patient subsequently was managed with repeated leukaphereses. Progressive leukocytosis terminated in an intracerebral hemorrhage in May 1986. Permission for an autopsy was denied.

Electron microscopy. The patient’s MNCs were centrifuged at 180 g for ten minutes and the cell pellet fixed with buffered 1.25% glutaraldehyde for two hours. The pellet was rinsed with 0.1 mol/L cacodylate buffer (pH 7.2) followed by post-fixation in 1% osmium in 0.1 mol/L cacodylate buffer for one hour. The osmium was replaced with a 4.5% sucrose solution and block staining was performed with a 1% aqueous solution of uranyl acetate for 30 minutes. The cells were dehydrated in graded series of ethanol solutions with three final changes of absolute ethanol propylene oxide. The pellet was then infiltrated with an equal volume of propylene oxide and epoxy resin (Lexi-112) overnight. The cells were embedded in pure epoxy resin in Beem capsules and allowed to cure for 48 hours at 60°C before cutting thin sections (70 nm). The sections were double-stained with uranyl acetate and lead citrate. The sections were observed and photographed with an Hitachi HU-12 electron microscope operated at 75 kV.

Surface marker analysis. Mononuclear cells from peripheral blood and spleen were isolated by separation on a Ficoll-Hypaque gradient. Cell surface markers were assessed by incubating 2 μg of commercially supplied antibodies with 1 × 10⁶ cells. Binding of antibodies was measured on a cytofluorograph 50H (Ortho Diagnostic Systems, Westwood, MA). Phycocerythin (PE) conjugated Leu M5 and FITC-conjugated anti-Leu 2a, -Leu 3a, -Leu 4, -Leu 10, -Leu 11, Leu12, -Leu 14, κ, λ, and HLA-DR were provided by Becton-Dickinson, Inc (Mountain View, CA). Anti-B-1 was purchased from Coulter Immunology (Hialeah, FL). Anti-β1 was provided by Dr T. Waldmann, Metabolism Branch, NCI.

Analysis of Ig and TCR receptor gene rearrangements. DNA was purified from peripheral blood mononuclear cells as described previously. All procedures for the Southern blot hybridization assay, as well as a description of the DNA probes, have been presented in detail elsewhere. Northern blot analysis was performed as previously described. The Jα Ig probe was obtained from Dr Philip Leder, Harvard School of Medicine, and the T-cell receptor B-chain probes were obtained from Dr Tak Mak, Ontario Cancer Research Center. Certain blots were analyzed by scanning analysis performed through the courtesy of Betagen Corp (Waltham, MA) and Automated Microbiology Systems (San Diego).

IFN-α binding assay studies. Peripheral blood mononuclear cells were isolated on a Ficoll-Hypaque gradient as previously described. The patient had >97% malignant cells in the peripheral blood throughout treatment. The other HCL patients had >70% malignant cells in their peripheral blood before treatment. Normal small resting T lymphocytes were prepared as described; the preparations were >95% T cells. The B lymphoblastoid Daudi cell line was cultured as described. rIFN-α (Hoffman-LaRoche, Nutley, NJ) and rIFN-γ (Biogen, Cambridge, MA) were iodinated as previously described. Binding assays with iodinated IFNs were performed as described except that 1 × 10⁶ cells were incubated with 0.7 ng ¹²⁵I-rIFN-α or 3 × 10⁶ cells were incubated with 0.25 ng ¹²⁵I-rIFN-γ. Nonspecific binding of ¹²⁵I-rIFN-α or ¹²⁵I-rIFN-γ, measured in the presence of a 500- and 3,000-fold excess of the respective unlabeled IFN, was subtracted from the total binding to give specific cpm bound. Nonspecific binding was 10% to 20% of the total binding in all cases except for approximately 50% nonspecific binding of ¹²⁵I-rIFN-α to the patient’s MNCs. All assays were performed in duplicate with <10% variation between duplicates. Competitive binding experiments were analyzed by the method of Scatchard with nonspecific binding subtracted, as previously described.

RESULTS

Pathology. The patient underwent splenectomy on October 7, 1985. The spleen weighed 2,900 g. Microscopic examination revealed diffuse infiltration by large lymphoid cells (15 to 30 μm) with round to oval vesicular nuclei, prominent nucleoli, abundant pale eosinophilic cytoplasm, and frequent mitotic figures. The red pulp was extensively expanded by this infiltrate and the white pulp essentially effaced. Blood lakes and pseudolymph nodes, findings associated with typical HCL, were not seen. Splenic hilar lymph nodes showed diffuse infiltration by a similar neoplastic lymphoid infiltrate. A wedge biopsy of the liver showed extensive sinusoidal infiltration by a similar process.

Peripheral blood and bone marrow. Peripheral blood smears at the time of splenectomy and thereafter showed moderate thrombocytopenia and a marked leukocytosis, with a differential of 2% neutrophils and 98% abnormal mononuclear cells.

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and were characterized by round, central nuclei with condensed chromatin and a single prominent small to medium-sized nucleolus in approximately 90% of the cells (Fig 1A). Binucleate cells were readily observed. The cells contained moderate amounts of pale blue cytoplasm and their cytoplasmic borders exhibited multiple fine hairy projections. A bone marrow biopsy obtained before splenectomy was moderately hypercellular with increased megakaryocytes and hyperplastic granulopoiesis and erythropoiesis with a myeloid to erythroid ratio of 1:2. There was focal infiltration with abnormal medium-sized lymphoid cells that had finely stippled nuclear chromatin and indistinct cytoplasmic borders with features as observed in peripheral blood. The cells were positive for TRAP. The morphologic characteristics of this cellular population were consistent with those previously described as HCL variant. Electron microscopic analysis clearly demonstrated the presence of prominent cytoplasmic projections (Fig 1B).

**Surface marker analysis.** The results of the surface marker analysis of the peripheral blood MNCs from our patient are summarized in Table 1. Most of the surface markers remained essentially constant throughout the course of the study. Strong immunofluorescence (IF) with anti-B-1 (CD 20), -Leu 10, and -Leu 12 (CD 19) confirmed the B-cell nature of the disease. Furthermore, the dominant lambda light chain IF suggested a single clone of cells. However, there was a transient increase in low intensity IF on IgG-

![Fig 1](image1.png)

**Fig 1.** (A) Morphologic examination of peripheral blood mononuclear cells. A peripheral blood smear reveals the presence of cells with irregular edges and a large centrally placed nucleus with condensed chromation consistent with variant HCL. (B) Electron microscopic analysis shows the presence of the cytoplasmic projections typical of HCL.

![Fig 2](image2.png)

**Fig 2.** Two-color staining of patient and normal peripheral blood mononuclear cells stained sequentially with FITC-conjugated α-Leu 14 and PE-conjugated α-Leu M5. Strong nonoverlapping IF is seen with normal peripheral blood mononuclear cells (A) while virtually all the cells from a typical HCL patient show intense dual staining (B). In contrast, no double IF was seen on the peripheral blood MNCs of our patient (C) underscoring the unique nature of the malignant cells.
bearing MNCs during IFN-α treatment and a second increase in high intensity IF at the start of DCF therapy. This transient change in MNC phenotype during DCF treatment was also seen with the T-cell associated markers Leu 2a (CD 8), Leu 4 (CD 3), and T101 (CD 5). A differential count at this time indicated that the total cell count had decreased to 26,400 and was composed of 74% HCLs, 15% lymphocytes, 8% segs, and 3% eosinophils. Since these transient phenotypic changes correlated with the appearance of normal peripheral blood MNCs, it would appear that this transient increase in T- and B-cell markers was due to normal rather than malignant MNCs. These changes completely disappeared by the time DCF administration was discontinued. The only persistent alteration in surface phenotype was on the IgM-bearing MNCs. At a point that also correlates with the initiation of DCF therapy there was a progressive decrease in the fluorescence intensity of the IgM-bearing cells, which remained dull even after DCF was discontinued. No significant IF was demonstrable with monoclonal antibody (MoAb) 7G7 (CD 25) or anti-TAC (CD 25) indicating the absence of detectable IL-2 receptor. Two-color staining with Leu 14 (CD 22) and Leu M5, usually seen with typical HCL, was absent (Fig 2). Thus, the peripheral blood cells did not exhibit the Leu 14\(^+\), Leu M5\(^+\), TAC\(^+\) phenotype characteristic of HCL. However, they were characterized as clonal B cells by virtue of their expression of surface Ig of a single light chain isotype and the B cell associated markers B1, Leu 10, and Leu 12.

Surface marker analysis of mononuclear cells isolated from the spleen demonstrated a phenotype characteristic of HCL. The cells expressed the B-cell associated antigens CD 19, CD 20, and CD 24 (>90%). The cells were positive with both anti-Leu M5, (CD 11c) and anti-TAC (CD 25), as previously described in HCL. Fewer than 5% of the mononuclear cells were T cells as determined by staining with Leu 4 (CD 3), T11 (CD 2), and 3A1 (CD 7). In contrast to B-chronic lymphocytic leukemia (CLL), the cells were negative with Leu 1 (CD 5) (data not shown).

**Determination of Ig and Tβ gene configurations for malignant peripheral blood hairy cells.** To confirm the lineage of the peripheral blood hairy cells, DNA was isolated from mononuclear cells at various times during therapy and the configuration of Ig and TCR\(_{β}\) gene DNA was determined. Ig heavy chain analysis detected the presence of rearranged bands in both the HindIII and BamH1 digests (Fig 3A). Although not apparent in the BamH1 digest there appeared to be an increase in the intensity of both rearranged bands in the HindIII digests suggesting an increase in the proportion of malignant cells. These results clearly establish the B-cell nature of the HCL cells.

Analysis of the BamH1 digested samples reveals the presence of a TCR\(_{β}\) gene rearrangement that is initially weak but increases in intensity during treatment (Fig 3B). On hybridization of patient and normal DNA digested with a number of different restriction enzymes with a probe containing only the constant region of the TCR\(_{β}\) gene, significant differences between the DNA samples could be detected with XbaI and BamH1 digestion (Fig 4A). A twofold increase in the intensity of some of the patient’s DNA fragments seen after digestion suggested the possibility of a TCR\(_{β}\) amplification and/or rearrangement. The amplified fragments corresponded to the Cβ2 region of the TCR

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**Table 1. Surface Marker Analysis of Patient Peripheral Blood Mononuclear Cells**

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>Subpopulation Identified</th>
<th>Treatment</th>
<th>Days in Study</th>
<th>Pre Release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IFN-α</td>
<td>DCF</td>
<td>IFN-γ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54 145</td>
<td>209 223 230</td>
<td>380 448</td>
</tr>
<tr>
<td>B1 20 B cells</td>
<td></td>
<td>95 (865)</td>
<td>87 (823)</td>
<td>81 (837)</td>
</tr>
<tr>
<td>Leu 10 — B cells, monocytes</td>
<td></td>
<td>95 (773)</td>
<td>92 (799)</td>
<td>87 (771)</td>
</tr>
<tr>
<td>Leu 12 19 B cells</td>
<td></td>
<td>NT</td>
<td>93 (341)</td>
<td>83 (389)</td>
</tr>
<tr>
<td>IgM (&gt;200) — B cells</td>
<td></td>
<td>98 (561)</td>
<td>97 (454)</td>
<td>84 (496)</td>
</tr>
<tr>
<td>IgG — B cells</td>
<td></td>
<td>6 (364)</td>
<td>1 (318)</td>
<td>68 (312)</td>
</tr>
<tr>
<td>IgD — B cells</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>x — B cells</td>
<td></td>
<td>44 (147)</td>
<td>17 (183)</td>
<td>18 (203)</td>
</tr>
<tr>
<td>λ — B cells</td>
<td></td>
<td>98 (421)</td>
<td>93 (450)</td>
<td>87 (457)</td>
</tr>
<tr>
<td>Leu 2 8 T suppressor</td>
<td></td>
<td>2 (368)</td>
<td>1 (336)</td>
<td>17 (381)</td>
</tr>
<tr>
<td>Leu 3 4 T helper</td>
<td></td>
<td>1 (348)</td>
<td>1 (327)</td>
<td>4 (356)</td>
</tr>
<tr>
<td>Leu 4 3 T cells</td>
<td></td>
<td>2 (432)</td>
<td>1 (405)</td>
<td>12 (425)</td>
</tr>
<tr>
<td>T101 5 T cells</td>
<td></td>
<td>3 (346)</td>
<td>2 (356)</td>
<td>17 (407)</td>
</tr>
<tr>
<td>Cells 10 Common acute lymphoblastic leukemia antigen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7G7 25 IL-2 receptor</td>
<td></td>
<td>1 (371)</td>
<td>1 (353)</td>
<td>3 (344)</td>
</tr>
<tr>
<td>TAC 25 IL-2 receptor</td>
<td></td>
<td>0</td>
<td>0</td>
<td>3 (542)</td>
</tr>
</tbody>
</table>

Immune flow cytometry of peripheral blood mononuclear cells from our patient. Mononuclear cells from several time points were isolated and prepared for IF analysis as described in Materials and Methods. Binding was assessed by incubating 2 μg of commercially supplied antibody or hybridoma supernatant with 1 x 10⁶ cells. The data are expressed as both the percentage of cells staining positively for each marker and the mean fluorescence intensity (MFI) of the positively staining cells. Transient increases in Ig and in the T-cell markers Leu 2, Leu 4, and T101 were noted at the initiation of DCF therapy. The remaining markers were essentially constant throughout the study. However, while the percentage of IgM-bearing cells remained high, there was a progressive decrease in the MFI, which appeared to begin with the initiation of DCF treatment.

Abbreviation: NT, not tested.

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Fig 3. Ig heavy chain and TCR$\beta$ chain gene rearrangement analysis of MNC DNA. Samples were analyzed from sequential time points during therapy and digested with either HindIII or BamHI. (A) Analysis with J$\alpha$ probe (The absence of DNA in lane 11 reflects loss of sample before loading of the gel; (B) TCR$\beta$ probe containing both constant and variable regions. BamHI digestion only. Rearranged bands are indicated with arrows. Normal, control, no treatment; lane 1, day 54, IFN-\(\alpha\); lane 2, day 54, IFN-\(\alpha\) (bone marrow MNCs); lane 3, day 145, IFN-\(\alpha\); lane 4, day 201, DCF; lane 5, day 209, DCF; lane 6, day 244, DCF; lane 7, day 272, DCF; lane 8, day 278, chlorambucil; lane 9, day 287, splenectomy; lane 10, day 348, post-splenectomy; lane 11, day 380, IFN-\(\gamma\); lane 12, day 405, IFN-\(\alpha\) (leukopheresis); and lane 13, day 448, pre-discharge.

Genome DNA. Ethidium bromide staining showed that the amount of DNA per lane was constant. In addition, stripping of the blot and rehybridization with a human IFN-\(\gamma\) genomic DNA probe resulted in hybridization of equal intensities in the lanes (Fig 4B) indicating that the increased hybridization seen with the TCR$\beta$ probe represents an alteration in gene structure. Northern blot analysis detected only the 1 kb message frequently seen in B cells (data not shown). While this supports the conclusion that the tumor cells are B cells, it also indicates that the rearrangement was nonproductive.
Table 2. Binding of $^{125}$I-IFN-α and $^{125}$I-IFN-γ to Patient MNCs During Treatment

<table>
<thead>
<tr>
<th>Date (day)</th>
<th>Treatment</th>
<th>Sp cpm $^{125}$I-IFN-α per 1 x 10^6 cells</th>
<th>Sp cpm $^{125}$I-IFN-γ per 3 x 10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>1,100</td>
<td>2,360</td>
</tr>
<tr>
<td>79</td>
<td>rIFN-α</td>
<td>420</td>
<td>NT</td>
</tr>
<tr>
<td>227</td>
<td>DCF</td>
<td>1,790</td>
<td>NT</td>
</tr>
<tr>
<td>272</td>
<td>DCF</td>
<td>1,680</td>
<td>NT</td>
</tr>
<tr>
<td>275</td>
<td></td>
<td>1,400</td>
<td>3,400</td>
</tr>
<tr>
<td>345</td>
<td>Post-splenectomy</td>
<td>1,530</td>
<td>NT</td>
</tr>
<tr>
<td>376</td>
<td>rIFN-γ</td>
<td>NT</td>
<td>3,800</td>
</tr>
<tr>
<td>387</td>
<td></td>
<td>740</td>
<td>3,350</td>
</tr>
<tr>
<td>397</td>
<td>Leukopheresis</td>
<td>770</td>
<td>NT</td>
</tr>
<tr>
<td>(MNCs from other HCL patients)</td>
<td></td>
<td>4,790 ± 930</td>
<td>860 ± 20</td>
</tr>
<tr>
<td>(MNCs from normal T lymphocytes)</td>
<td></td>
<td>1,950 ± 40</td>
<td>1,140 ± 20</td>
</tr>
</tbody>
</table>

Cells were incubated with either $^{125}$I-IFN-α or $^{125}$I-IFN-γ (as described in Materials and Methods) for two hours at 4°C. Nonspecific binding was subtracted from total binding to give specific binding. The data shown for normal T lymphocytes is the mean of the binding to purified T cells from four normal donors analyzed in parallel with the patient’s MNCs. The binding shown to pretreatment MNCs from other HCL patients is the mean from eight patients for IFN-α and five for IFN-γ.

When the blot was stripped and reanalyzed with a probe for the TCRγ gene, no rearrangement was detected (data not shown).

Based on these results it appears that while the clonal Ig gene rearrangement remained constant throughout the course of this patient’s therapy, a subpopulation of the malignant cells went on to rearrange the TCRγ gene. Over time this new population was able to expand and represent an increasing proportion of the malignant cells. However, histologic examination showed no discernible change in the morphology of the hairy cells from the time of entry into the study.

IFN-α and IFN-γ receptors. MNCs were assayed periodically during the course of treatment for the binding of $^{125}$I-IFN-α and $^{125}$I-IFN-γ. As shown in Table 2, and as previously described, pretreatment samples of MNCs from this patient bound significantly less $^{125}$I-IFN-α than pretreatment samples of MNCs from other HCL patients. During treatment with IFN-α, the ability of the cells to bind $^{125}$I-IFN-α further decreased, suggesting down regulation of IFN-α receptors on the cell surface. After conclusion of IFN-α therapy, the binding of $^{125}$I-IFN-α increased to a level somewhat higher than the binding before treatment, but it was still lower than the binding to MNCs from other HCL patients.

In contrast to the binding of $^{125}$I-IFN-α, MNCs from our patient bound more $^{125}$I-IFN-γ than normal T lymphocytes or MNCs from other HCL patients (Table 2). As shown in Table 3, although the number of IFN-α receptors on our patient’s MNCs was very low, the binding of IFN-α to MNCs from our patient was of high affinity with a Kd similar to that on the control cells. As determined by cross-linking studies the molecular weight of the IFN-α receptor on our patient’s MNCs was also similar to that on the highly responsive Daudi cell line and on normal T lymphocytes (data not shown).

The binding affinity of $^{125}$I-IFN-γ to our patient’s MNCs was also very high and was similar to that previously reported on the control cells (Table 3). It should be pointed out that the number of IFN-γ receptors on our patient’s MNCs was four times higher than the number of IFN-α receptors on these cells and was similar to the number of IFN-α receptors on MNCs from HCL patients responsive to IFN-α. The molecular weight of the IFN-γ receptor on the patient’s cells was similar to that on the Daudi cells and on T lymphocytes (data not shown). Thus, the resistance of this patient’s malignant cells to IFN-α therapy is not explained by the presence of low-affinity receptors for IFN-α. It is possible, however, that the low number of IFN-α receptors contributed to the clinical resistance to therapy. Unresponsiveness to IFN-γ therapy cannot be attributed to either a defect in IFN-γ receptor number or affinity.
assess what characteristics were associated with this individ-
ual's unusual clinical course.

Features that favor a diagnosis of HCL include the mor-
phology of the neoplastic cells and the presence of TRAP activity. The immunologic phenotype of the cells isolated from the spleen was also characteristic of HCL. Conversely, many of the pathologic features would be compatible with a diagnosis of prolymphocytic leukemia. Although the spleen was massively involved and replaced by the neoplastic process, splenic pseudosinuses or blood lakes, features highly characteristic of HCL, were absent. In addition, many of the cytologic features of the malignant cells such as large vesicular nuclei, prominent nucleoli, and frequent mitotic figures are more characteristic of prolymphocytic leukemia. While the prolonged clinical history would be compatible with HCL, the patient's age and sex, as well as marked leukocytosis without significant cytopenias, are unusual in this disease. Cases with these morphologic and clinical features were described by Catovsky et al as a "prolympho-
cytic" variant of HCL. However, one could question whether categorization as a "hairy cell variant" of proly-
ymphocytic leukemia would be more appropriate. The high peripheral WBC and nonresponsiveness to IFN-α are more characteristic of DCF therapy and did appear to begin at the approximate time of the emergence of the TCRβ rearranged subclone. The remainder of the surface markers remained essentially constant throughout the entire study.

The histology and phenotyping of the peripheral blood, bone marrow, and splenic lymphoid cells supported a diagnosis of the prolymphocytic variant of HCL. Sequential analyses of Ig and TCRβ gene rearrangement analysis further revealed the distinctive nature of the disease. The Ig gene rearrangement analysis confirmed the B-cell nature of the disease as well as indicated an increase in the percentage of malignant cells in the peripheral blood as the study progressed. When analyzed for TCRβ chain rearrangement using a probe containing the C region, an altered germ line pattern was seen with two restriction enzymes. Scanning analysis of the blot (kindly performed by Betagen [Waltham, MA] and Ambis Corp [San Diego]) indicated a twofold increase in the TCRβ hybridization but no increase in IFN-γ hybridization, suggesting a duplication of the TCRβ C region in the tumor cells. However, the intensity of one of the bands increased late in the therapy, which suggested a change in the cell population. The increasing intensity of this rearrangement over time without a reciprocal decrease in the Ig gene rearrangement suggests that a subclone of the B-cell tumor subsequently rearranged the TCRβ gene. Northern blot analysis failed to detect the 1.3 kb message typically seen in T cells, but did detect the 1.0 kb message that is also present in some B cells.

### DISCUSSION

HCL is a well characterized clinical entity. However, cases of variant HCL have been described that appear to be the result of the proliferation of a cell with features characteristic of both HCL and B-cell type prolymphocytic leukemia. In this report, we have described an unusual case of this disease. Cases with these morphologic and clinical features were described by Catovsky et al as a “prolympho-
cytic variant HCL.4 However, one could question whether categorization as a “hairy cell variant” of proly-
ymphocytic leukemia would be more appropriate. The high peripheral WBC and nonresponsiveness to IFN-α are more characteristic of DCF therapy and did appear to begin at the approximate time of the emergence of the TCRβ rearranged subclone. The remainder of the surface markers remained essentially constant throughout the entire study.

The histology and phenotyping of the peripheral blood, bone marrow, and splenic lymphoid cells supported a diagnosis of the prolymphocytic variant of HCL. Sequential analyses of Ig and TCRβ gene rearrangement analysis further revealed the distinctive nature of the disease. The Ig gene rearrangement analysis confirmed the B-cell nature of the disease as well as indicated an increase in the percentage of malignant cells in the peripheral blood as the study progressed. When analyzed for TCRβ chain rearrangement using a probe containing the C region, an altered germ line pattern was seen with two restriction enzymes. Scanning analysis of the blot (kindly performed by Betagen [Waltham, MA] and Ambis Corp [San Diego]) indicated a twofold increase in the TCRβ hybridization but no increase in IFN-γ hybridization, suggesting a duplication of the TCRβ C region in the tumor cells. However, the intensity of one of the bands increased late in the therapy, which suggested a change in the cell population. The increasing intensity of this rearrangement over time without a reciprocal decrease in the Ig gene rearrangement suggests that a subclone of the B-cell tumor subsequently rearranged the TCRβ gene. Northern blot analysis failed to detect the 1.3 kb message typically seen in T cells, but did detect the 1.0 kb message that is also present in some B cells. It would appear, therefore, that the TCRβ gene duplication/rearrangement was nonproductive. These findings underscore the importance of sequential molecular analysis of chronic malignancies as well as the necessity of using several restriction enzymes if a true determination of the nature of the malignant cell is to be obtained.

Recently, Williams et al studied the simultaneous pres-
ence of both Ig and T-cell receptor rearrangements in human lymphoid malignancies. They reported that TCRβ re-
arrangements, with or without Jβ rearrangement, occurred primarily in T-cell tumors with the single exception of a patient with small, noncleaved cell (Burkitt's) lymphoma. Grieser et al,21 in a study of 55 patients, noted that in non-Hodgkin's lymphoma TCRβ rearrangement was found in all 14 T-cell lymphomas and in two of seven B-cell lymphomas. However, an Ig gene rearrangement was not seen in any of the T-cell lymphomas but was present in all of the B-cell lymphomas. Others have also reported such rearrangements in B- and T-cell tumors. A study of 11 untreated HCL patients by Migone et al24 demonstrated that while a rearrangement of the Ig heavy chain locus was found in each case, the TCRβ gene was in the germ line configuration. The conditions used in that report were essentially identical to those in the present study. We believe, therefore, that our patient is the first instance of variant HCL where the emergence of a divergent Ig and TCRβ rearranged HCL clone has been detected using molecular techniques. The factors that allowed this new population of hairy cells to expand are unknown. What is clear is that this progression of events was not evident from either histologic or phenotypic evidence.

The usefulness of IFN-α in the treatment of HCL was first reported by Quesada et al in 1984 and confirmed in subse-
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quent trials.25,26 A small percentage of patients do not respond to IFN-α. Subsequent treatment with DCF can be effective in patients whose disease has progressed during IFN-α therapy.9 While evidence suggests that a particular patient's responsiveness to IFN-α therapy cannot be predicted by the degree of interaction between IFN and its receptor,11 the lack of response to IFN-α by our patient may be explained at least in part by the low number of IFN-α receptors. However, the reason for only a transient response to DCF as well as the failure of all subsequent treatment remains unclear. Reghauachar et al27 recently described the elimination of one clone in an apparent case of biclonal B-cell HCL suggesting that different HCL subpopulations in the same patient may have different sensitivities to IFN-α. The lack of response to IFN-γ, despite the presence of high numbers of high affinity IFN-γ receptors on the malignant cells suggests that as in typical HCL, variant HCL may also not respond to IFN-γ. Speculation as to the mechanism(s) of resistance must wait until the exact role of IFN-α and DCF in variant HCL is better defined.

In conclusion, by combining histologic, phenotypic, and genetic analysis we have been able to examine fundamental changes in a unique case of mature B-cell leukemia over a long period of therapy. While this case fulfills criteria for what has been termed the prolymphocytic variant of HCL, the low numbers of IFN-α receptors suggest that clinically these patients may require different approaches than classic HCL. While the phenotype of the cells in the spleen was typical of HCL, the cells in the peripheral blood demonstrated a variant phenotype, more characteristic of prolymphocytic leukemia. Genotypically, although the process was clearly a clonal B-cell proliferation, another unique feature was the late emergence of a TCRγ rearranged subclone. Based on the analysis of this one individual it is not clear which, if any, of the various traits associated with the malignant cell population may be predictive of therapeutic unresponsiveness to IFN-α. Such correlations await the extensive evaluation of a larger number of similarly unresponsive variant HCL patients.

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Rearrangement of both immunoglobulin and T-cell receptor genes in a prolymphocytic variant of hairy cell leukemia patient resistant to interferon-alpha [published erratum appears in Blood 1989 Feb;73(2):624]

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