Clinical and Hematologic Fluctuations in Hairy-Cell Leukemia: A Sequential Surface-Marker Analysis

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A sequential surface-marker study was performed on the peripheral blood of ten patients with hairy-cell leukemia. All patients were studied for at least a year, and it was shown that changes in the predominant cell population occurred in six and that these changes were not related to variations in the absolute leukocyte count. In four cases, no major change in peripheral populations was observed, and these patients had consistent anemia, neutropenia, monocytopenia, and thrombocytopenia. In the remaining six patients, there was a complete reversal in the major peripheral blood cell type from Slg 

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IgG (γFc) and IgM (μFc) were detected by rosette methods employing ox erythrocytes coated with rabbit anti-ox IgG or IgM as described. Surface immunoglobulin (Slg) was sought by a direct antiglobulin rosette method employing nonspecific sheep antisera (against human κ, λ, μ, δ, γ, α isotypes) coupled by chromic chloride to ox erythrocytes. The method, the specificity of the antisera, and important technical aspects of the test are considered in detail in previous publications. T-cell subpopulation analysis. The subpopulations of T cells with receptors for the Fc of IgM (Tμ) and IgG(Tγ) and those with neither receptor (To) were identified after culture. Mononuclear cells were cultured for 12 hr at 37°C in TC199 containing 10% fetal calf serum and the washed cells tested for the appropriate Fc receptors by rosette formation with the reagents described above. In some experiments, E'Slg* cells were first enriched by removing Slg-rosetting cells (κ and λ) over Ficoll-Hypaque and by retaining those cells remaining at the interface. With these methods, in our laboratory, normal enriched T lymphocytes are composed of 56% Tμ, 23% Tγ, 21% To cells (mean of 5 individuals).

Fluorescent staining. Details of the heteroantiseras employed for the anti-T and anti-la staining have been given previously.

Intracytoplasmic Staining

Fluoresceinated, affinity-purified, sheep IgG preparations monospecific for individual human heavy (μ, δ, α, α) and light chains were used in a direct technique on preincubated, washed, and acetone-fixed cytocentrifuged preparations. The purity and specificity of the antisera has been described previously, but in addition to these checks, several confirmatory tests were performed. Anti-IgD staining of the E'Slg* cells (see Results) could be blocked with an unlabeled, independently prepared, sheep IgG preparation monospecific for the Fc of human IgD. This alternative anti-IgD preparation also stained the cells in an indirect technique. Similar preparations of IgG directed against other Ig classes did not block the staining reaction. Also, tests on normal PB mononuclear cell preparations demonstrated that the anti-IgD preparations did not possess fortuitous anti-T-cell properties, since the T cells from five normal donors did not stain.

RESULTS

Fluctuations in the Mononuclear Cell Populations of Peripheral Blood

In all the 10 patients, morphologically typical HC had the Slg*γFc*E* phenotype characteristic of HC. Frequently, the number of cells with this phenotype was higher than that of HC estimated on morphological criteria alone. However, evidence presented elsewhere (and see Table 1) indicates that mononuclear cells with the Slg*γFc*E* phenotype, but without the appearance of HC, are involved in the neoplastic proliferation; in particular, the Slg of these mononuclear cells is of the same single light chain type as that on the HC, and virtually no Slg* cells of the opposite light chain type are observed. This pattern of monoclonal Slg expression was observed in all our cases of HCL and, for the interpretation of the data presented in Fig. 1, the number of Slg* cells provides an estimate of the neoplastic B-cell population in the PB. The number of γFc* cells provides a similar, and sometimes more reliable, estimate of neoplastic cells, since these may be γFc*Slg* and since monocytes are usually absent throughout the course of the disease. However, interpretation of this marker is

| Table 1. Estimates of HC, Monoclonal B Cells, and T Cells in HCL During the Slg*γFc*E* Stable Phase and the E'Slg* T-Cell Phase |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Percent Neoplastic B Cells | Percent T Cells |
|                 | Morphological HC† | TRAP-Positive Cells‡ | Slg* Cells | E-Rosetting Cells | T Cells by Esterase | Comment |
| Patient         | Months*          |                 |               |                 |                           |          |
| K.D.            | 11.5             | 57              | 50             | 86 (x)          | 5                          | 11        | Stable phase |
|                 | 14.5             | 52              | 55             | 83 (x)          | 10                         | 18        |             |
| F.B.            | 16               | 18              | 12             | 24 (x)          | 37                         | 11        | Stable phase |
|                 | 19               | 44              | 78             | 30 (x)          | 43                         | 21        |             |
| A.B.            | 2                | 37              | 53             | 84 (x)          | 5                          | 3         | Stable phase |
|                 | 4                | 24              | 42             | 13 (x)          | 40                         | 30        |             |
|                 | 16.5             | 44              | 64             | 52 (x)          | 23                         | 26        |             |
| D.C.            | 17               | 1               | 5              | 3 (x)           | 77                         | 26        | T-cell phase |
| R.H.            | 18.5             | 0               | 3              | 6 (x)           | 87                         | 44        | T-cell phase |
| P.S.            | 19               | 28              | 36             | 40 (x)          | 45                         | 29        | Stable phase |
|                 | 22               | 19              | 28             | 30 (x)          | 54                         | 32        |             |
|                 | 28.25            | 21              | 38             | 22 (x)          | 75                         | 19        | T-cell phase |
| H.D.            | 17.5             | 14              | 38             | 43 (x)          | 40                         | 37        | Stable phase |
|                 | 21               | 18              | 32             | 9 (x)           | 74                         | 17        | T-cell phase |
| F.W.            | 11.5             | 35              | 29             | 59 (x)          | 8                          | 12        | Stable phase |
|                 | 12.5             | 29              | 43             | 5 (x)           | 63                         | 22        | T-cell phase |
|                 | 19               | 9               | —              | 40 (x)          | 25                         | 25        | Stable phase |

*Relates to months after 0 on relevant graph (Fig. 1).
†Scores made from whole blood films and expressed as a percent of mononuclear cells by allowance for polymorphs.
‡All cells containing significant activity scored; cells not necessarily of HC morphology.

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Fig. 1. (A) Sequential surface-marker analysis for patients K.D., F.B., A.B., and F.W. (—) Total leukocyte count and (□) percent HC of total leukocytes. (Δ–Δ) Fc receptor-bearing cells, (○—○) E-rosetting cells, (x—x) maximum Slg-rosetting cells. Rosetting cells graphed as percentages of mononuclear cells (upper graph for each patient) and in absolute values (lower graph for each patient). (B) Sequential surface-marker analysis for patients D.C., R.H., P.S., and H.D. (—–) Total leukocyte count and (□—□) percent HC of total leukocytes. (Δ–Δ) Fc receptor-bearing cells, (○—○) E-rosetting cells, (x—x) maximum Slg-rosetting cells. Rosetting cells graphed as percentages of mononuclear cells (upper graph for each patient) and in absolute values (lower graph for each patient).
FLUCTUATIONS IN HAIRY-CELL LEUKEMIA

D.C. (13.12.76.—12.5.78.)

RH. (18.9.75.—28.4.78.)

PS. (30.9.76.—2.2.78.)

H.D. (29.10.76.—9.2.78.)
complicated by the fact that some T cells possess a γFc receptor. 14

In four patients (K.D., F.B., A.B., A.D.), the neoplastic B-cell population in the PB remained conspicuous throughout the period of study (Fig. 1A; AD not presented graphically because of incomplete marker data).

In four patients (D.C., R.H., P.S., H.D.), a major sustained change was observed in the predominant mononuclear cell population in the PB. Thus, initially, Slg'γFc′E- cells (with many morphological HC) predominated, but later, HC became reduced or absent and the major cell population was comprised of E-rosette-forming Slg- T cells (Fig. 1B).

In two patients (F.W., Fig. 1A, and N.W.) a transient, but marked, E′Slg- phase was observed as the neoplastic B-cell population became greatly reduced.

Clinical and Hematologic Correlations

The four patients (K.D., F.B., A.B., A.D.) in whom the neoplastic B cells remained the predominant mononuclear population of the PB were symptomatically reasonably well throughout the period of study. However, all remained neutropenic (<2 × 10^9/liter) and three (F.B., A.B., A.D.) were consistently thrombocytopenic (≤100 × 10^9/liter) and mildly anemic (10–12 g/dl), while in K.D. the platelet count was consistently between 100 and 150 × 10^9/liter. Like the marker results, these hematologic values did not significantly alter during this period despite the fact that two patients (A.B., A.D.) underwent splenectomy (transient fluctuations did occur in the immediate post-splenectomy period, but did not involve a change in the predominant PB population), two patients received variable amounts of steroid (Fig. 1A), and one patient (F.B.) had a severe chest infection requiring hospitalization. K.D. received no therapy of any kind.

Three of the four patients (D.C., R.H., P.S.) in whom a major sustained change in the predominant mononuclear cell population was observed (Fig. 1B) also showed a marked clinical and hematologic change. Thus, having had severe general malaise, anemia requiring repeated transfusion, and symptomatic thrombocytopenia (<50 × 10^9/liter), the patients became well and were no longer anemic or thrombocytopenic. Neutropenia persisted in all patients.

The circumstances of the clinical and hematologic improvement varied in the three cases. In D.C., the improvement occurred some 5 wk after splenectomy (complicated by chest and wound infection) at a time when the patient was on a maintained dose of 30 mg/day of prednisone; at the same time, the major PB mononuclear cell population changed from Slg′γFc′E- HC to E′Slg- lymphoid cells. The patient has since remained clinically well and hematologically and immunologically stable on a reducing dose of steroid.

In R.H., a similar sustained clinical and hematologic improvement occurred, but took place in the first week after splenectomy (complicated by chest infection). Marker analysis was not performed at this time, but subsequent testing revealed that the same reversal of predominant phenotype had occurred (Fig. 1B).

In P.S., some symptomatic and hematologic benefit followed the introduction of steroids, and progressive clinical improvement has continued after splenectomy (followed by tailing of steroids) 1 mo later. However, although the morphological HC population became greatly reduced at this time, the neoplastic B-cell population remained predominant until some 20 mo later when the reversal to the E′Slg- lymphoid population was again observed. Since this time, the patient has remained in excellent health.

Patient H.D., who has not undergone splenectomy, has remained well, but has been consistently anemic and thrombocytopenic. The reversal in predominant PB population (Fig. 1B) has not been accompanied by any clinical or hematologic change. The change to an E′Slg- lymphoid phenotype occurred about the time of a modest increase in steroid dosage, but a similar previous increase had produced no such change.

In two patients, a transient, but marked, E′Slg- phase was observed. One patient (N.W., not shown graphically), having been persistently unwell with leukopenia (WBC count approximately 1 × 10^9/liter), neutropenia (0.1–0.3 × 10^9/liter), and a predominant Slg′γFc′E- population, temporarily entered a phase in which E′Slg- cells predominated in the peripheral blood. During this E′Slg- phase, he became well, the leukopenia disappeared (WBC 5–12 × 10^9/liter) and the neutrophil count rose (1–3 × 10^9/liter). Prednisone was being discontinued at this time and splenectomy had been performed some 6 mo previously. Subsequently, the Slg′γFc′E- HC population reappeared and the patient progressively deteriorated and died. In the other patient (F.W., Fig. 1A), similar marked clinical and hematologic improvement was seen in association with a transient reversal of predominant PB phenotype (Fig. 1A). The patient had been requiring monthly transfusions for some 12 mo and had general malaise. In this case, however, the transient phenotypic change has been associated with continued clinical improvement and, although neutropenia continues, no transfusion has been required since this time. The transient change in the PB populations...
was not associated with any drug treatment or infection, and splenectomy had been performed 3 yr before.

Nature of the E'Slg' Lymphocyte Phase

The major mononuclear cell population in normal PB is composed of E'Slg' T lymphocytes. Therefore, the change in the predominant cell population to the E'Slg' phenotype, together with the disappearance of HC, seemed to indicate that a normal T-cell population had emerged. This and the accompanying clinical improvement, suggested remission in five of our HCL patients. However, several observations make it clear that, even during this phase, the PB remained abnormal in a number of respects. Thus, all the patients continued to be neutropenic and monocytopenic and the leukocyte alkaline phosphatase score remained high (>200). Moreover, in the three patients whose bone marrow was examined during the "T-cell phase," aspiration was difficult and recognizable hairy cells persisted.

Intracytoplasmic Ig staining performed on the PB cells of three patients in the T-cell phase (P.S., D.C., R.H.) showed weak diffuse, but definite, staining for IgD and very weak diffuse staining of a single light chain type in the majority of lymphoid cells (>70%); that the E' cells were staining for IgD was confirmed in cytocentrifuge preparations of E rosettes. No other immunoglobulin classes were detected in these cells. The E'Slg' cells of patient H.D. (who had shown no clinical improvement) were negative when stained for intracytoplasmic Ig. Also, in the four stable cases not showing an E'Slg' phase, both the HC and any T lymphocytes present were negative for intracytoplasmic IgD, even when the HC possessed IgD at the surface (K.D.).

The E'Slg' lymphocytes also differed from normal T lymphocytes in that only a minority gave the dot esterase pattern of Tp cells (Tables 1 and 2) and, unlike in normal T cells, there was no concordance between numbers of cells giving dot esterase and acid phosphatase staining. In contrast, as is also shown in Table 2, there was a closer agreement between estimates of T cells by E rosetting and by esterase staining during the "stable phase" in which the Slg'-Fc'E neoplastic cells predominated. Furthermore, the E'Slg' cells frequently showed a distinctive nuclear indentation visible both by light and electron microscopy (Fig. 2) (this was often striking in the anti-IgD fluorescent preparations described above). In three patients (D.C., N.W., H.D.) after depletion of Slg' (k and l) cells and subsequent overnight culture, only a minority of cells possessed receptors for the Fc of IgG (γFc) or IgM (μFc) (Table 2). Despite these abnormal findings, both patients showed apparently normal phytohemagglutinin (PHA) transformation. Furthermore, the cells, in contrast to morphological HC but like normal T cells, stained with an anti-T serum but not with antiserum to the Ia-like p29,34 B-cell antigen.

### Table 2. Subpopulation Analysis of Cultured E'Slg' Cells From PB

<table>
<thead>
<tr>
<th>Patient</th>
<th>Percent Rosette Formation</th>
<th>Tp Esterase Staining (Tc)</th>
<th>Ty Esterase Staining (Tc)</th>
<th>TpE Esterase Staining (Tc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.C.</td>
<td>92</td>
<td>0</td>
<td>22</td>
<td>&gt;70</td>
</tr>
<tr>
<td>N.W.</td>
<td>87</td>
<td>12</td>
<td>16</td>
<td>&gt;50</td>
</tr>
<tr>
<td>H.D.</td>
<td>100</td>
<td>15</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>Normal</td>
<td>90 ± 6†</td>
<td>56 ± 12</td>
<td>23 ± 12</td>
<td>21</td>
</tr>
</tbody>
</table>

* T cells negatively enriched by removal of plastic-adherent and Slg-positive cells were cultured for 20 hr in TC199 + 10% fetal calf serum.† Estimate of T cells lacking both μFc and γFc receptors.‡ Mean ± SD of 5 normal individuals tested with the same reagents.

**DISCUSSION**

Although T-cell variants have been described,15,23-25 HCL is usually a B-cell lymphoproliferative disorder.12,16,19,20 Thus, morphological HC typically express monoclonal surface immunoglobulin (Slg), almost invariably possess a strong γFc receptor, and do not form spontaneous rosettes with sheep erythrocytes (E)—they therefore have the surface phenotype Slg'γFc'E.12,16,19,20 Surface-marker analysis of the
Peripheral blood in HCL frequently reveals that many more cells with this phenotype are present than are morphological hairy cells; the restricted light chain expression of the SIg of these lymphoid cells indicates that they are also involved in the monoclonal proliferation.

In the present study, these general observations are confirmed, but sequential surface-marker studies allowed analysis of changes in the lymphoid cells (both nonmorphological HC and E-rosetting T cells) present in the PB.

In four of our ten patients, there was no substantial change in the phenotype of the majority cell population, and SIg⁺γFc⁺E⁻ cells remained predominant throughout the period of study. In addition, although these patients remained symptomatically reasonably well, they were consistently anemic, neutropenic, and thrombocytopenic. Two of these patients had consistently high-normal or elevated total leukocyte counts with >50% morphological and immunologic hairy cells, while the other two were either leukopenic or had low-normal leukocyte counts with <40% morphological hairy cells (but a considerably higher percent of SIg⁺γFc⁺E⁻ cells was present). Two of these stable patients had undergone splenectomy and had been followed for at least 12 mo after operation. One had received continuous steroid throughout the period of follow-up, one received a single dose of steroid at splenectomy and had been on an unsuccesful attempt to improve the persistent asymptomatic neutropenia, both had received a short course of lithium carbonate several weeks before. In one patient, the reversal of predominant cell phenotype (which differed in a number of respects from that seen in the other five patients, see below) was observed without any accompanying clinical or hematologic improvement; he had not undergone splenectomy and the change occurred apparently spontaneously, although he had been on continuous steroid for a long period.

In summary, therefore, marked clinical and hematologic improvement was always associated with the reversal of the majority PB population to the E⁺SIg⁺ phenotype. This reversal was not clearly associated with chemotherapy but splenectomy appears to play a central role, since clinical and hematologic improvement immediately followed the procedure in some patients and was not observed in patients who had not undergone splenectomy. However, there is no simple direct relationship between splenectomy and the clinical, hematologic, and immunologic change, since this may occur some years after operation. The role of infection is not clear because it was associated with splenectomy followed by immediate improvement but, equally severe infection did not always induce the change, and change occurred without infection.

The nature of the E⁺SIg⁺ predominant PB population in the “remission” or “T-cell phase” of HCL requires some discussion. With the exception of the only patient showing no clinical improvement, the emergence of this T-cell phase was associated with a modest elevation above normal of the absolute count of E-rosetting cells (>2.8 × 10⁹/liter). In addition to these quantitative abnormalities, several features suggested that these E⁺SIg⁺ cells did not simply represent a reemergence of the normal T-cell population of PB. Thus, despite possessing some characteristics of normal T lymphocytes (PHA transformation, anti-T⁺, anti-1a⁺), the T cells were strikingly notched, most failed to express receptors for the Fe of either IgM or IgG after culture, and reduced numbers displayed the dot-esterase-staining pattern characteristically present in the majority of normal peripheral T lymphocytes. Furthermore, unlike normal T lymphocytes, but like some lymphomatous T cells, there was lack of concordance between the numbers of T cells giving a dot pattern with esterase and acid phosphatase stains. Of special interest was the finding of intracytoplasmic IgD in most of the E⁺SIg⁺ lymphocytes; this was of the same restricted light chain type as the SIg present on the HC before the E⁺SIg⁺ phase. Intracytoplasmic IgD was only present in the T cells of those patients showing clinical and PB “remission.”

Extensive controls, including blocking studies, checks of the specificity of the antisera, and the absence of intracytoplasmic staining in normal T cells and in the T cells of HCL patients in the “stable
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phase," all make it unlikely that the intracytoplasmic IgD in the T-cell phase is attributable to technical artifact. This unexpected finding therefore implies clonal T-cell involvement in this phase of the disease.

With regard to clonal T-cell involvement in HCL, the existence and nature of cases of HCL with T-cell features may be relevant. In certain respects, these T-cell cases are comparable with the patients described here in the T-cell phase. For example, the HC in some cases of T-cell HCL transform with PHA, are anti-T, and anti-Ia. Moreover, the HC in some of these T-cell cases express hybrid E'Slg' phenotypes and may fluctuate between E'Slg' and E'Slg'' in different organs and at different times of study, during the E'Slg'' phase of these hybrid cases, many of the cells may be of lymphoid appearance, but contain the ribosome-lamella complex of HC and possess intracytoplasmic IgD of the same light chain type as the monoclonal Slg of the hybrid HC.

Whatever the complex relationship between the E'Slg'' phase described in this article in B-cell HCL and that observed in T-cell HCL, transformation to this phase is clearly a general feature of HCL and is associated with improvement in the clinical and hematologic status of the patient.

REFERENCES

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JC Cawley, GF Burns, CP Worman, BE Roberts and FG Hayhoe