Serum Soluble Interleukin-2 Receptor Is Associated With Clinical and Pathologic Disease Status in Hairy Cell Leukemia

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Hairy cell leukemia is a chronic lymphoproliferative disorder of B-cell lineage, whose malignant cells express the interleukin-2 (IL-2) receptor. A soluble form of the IL-2 receptor is released by these cells in culture, and the sera of patients with hairy cell leukemia contain elevated levels of this soluble receptor. Four hundred twenty-seven serum samples from 101 patients were analyzed for soluble IL-2 receptor (sIL-2R). The clinical status of patients appeared to be associated with the serum level of sIL-2R. The hairy cell index (a measure of tumor cell burden) was correlated with the square root of the serum sIL-2R level ($r = .77$).

Improved clinical status was associated with decreasing serum sIL-2R levels, whereas disease relapse was associated with increasing levels. Notably, every patient who responded to therapy had a decline in serum sIL-2R level, and every patient with disease progression had an increase in serum sIL-2R level. This phenomenon was observed for several different treatments, including standard-dose interferon, low-dose interferon, and deoxycoformycin. The predictive reliability of this test is currently being prospectively evaluated.

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HAIRY CELL LEUKEMIA (HCL), a disease characterized by splenomegaly without adenopathy, by pancytopenia, and by circulating malignant cells with prominent cytoplasmic projections requires therapy in most cases.[1,2] The manifestations of HCL result from the combined effects of (a) leukemic infiltration of the red pulp of the spleen by hairy cells, which leads to hypersplenism,[3] and (b) leukemic infiltration of the bone marrow, which leads to decreased production of bone marrow elements and to peripheral blood cytopenia(s). Splenectomy has historically been the primary treatment in most patients with progressive pancytopenia and/or severe splenomegaly,[3] and although hematologic improvement postsplenectomy has been common, these hematologic partial remissions have not been durable.

Chemotherapy, specifically chlorambucil, has had limited value in the management of HCL,[4] and it was not until the introduction of interferon that effective systemic therapy was available for this disease.[5,6] Although response to alpha-interferon can be demonstrated in over 90% of patients with HCL, this treatment is not curative, and alternative therapies have been evaluated. Pentostatin (2'-deoxycoformycin) recently has been found effective in the treatment of HCL, especially in those cases resistant to alpha-interferon.[7,8] However, quantifying the extent of disease to determine response and/or relapse during treatment with any of the above regimens has required repetitive bone marrow biopsies and calculation of the hairy cell index.

HCL cells express the receptor for interleukin-2 (IL-2)[9] and a soluble form of this receptor (sIL-2R) is released by activated human lymphocytes and hairy cells in vitro.[10,11] The sera of patients with HCL contain elevated levels of sIL-2R,[11-13] and these levels have been reported to decrease in many patients treated with alpha-interferon.[11-13] Although sIL-2R production is not unique to hairy cells,[10,14] we sought to better define its relationship to clinical status. Thus, we retrospectively analyzed 427 serum samples from 101 HCL patients to determine whether the level of serum sIL-2R was associated with clinical and pathologic parameters of disease.

MATERIALS AND METHODS

Patients. All 101 patients in this retrospective study were diagnosed with HCL, and this diagnosis was pathologically confirmed at the University of Chicago. Serum samples, collected at the same time that peripheral blood counts were tested, were stored in 1-mL aliquots at $-90°C$. A total of 427 unique samples, collected since 1980, were available for this analysis. In this retrospective study, patients were variously managed by observation, chemotherapy with chlorambucil (4 mg daily), recombinant interferon alpha-2b (IFN), and/or deoxycoformycin (dCF). IFN was administered thrice weekly by subcutaneous injection at a dose of either 2.0 mU/m^2 or 0.2 mU/m^2. Deoxycoformycin was administered as an intravenous infusion at a dose of 4 mg/m^2 every 2 to 4 weeks. Interferon and dCF were administered as part of Investigational Review Board–approved treatment protocols, and all patients gave informed written consent.

Response criteria. Response criteria for this analysis were as follows. Complete response: fewer than 5% hairy cells in the bone marrow biopsy, hemoglobin > 12 g/dL, platelet count > 100,000/μL, absolute granulocyte count > 1,500/μL, and absence of circulating hairy cells. Partial response: at least a 50% decrease in the bone marrow hairy cell index (HCI) and normalization of peripheral blood counts as defined for complete response. Minor response: normalization (as defined for complete response) of at least one hematologic abnormality. Progressive disease: appearance of a new peripheral blood abnormality or worsening of a preexistent abnormality in the peripheral blood or bone marrow. Pretreatment was defined as presence of disease before systemic therapy (ie, other than splenectomy or radiotherapy). On each date from which a serum sample was available, patients were retrospectively assigned to one of the preceding disease status/response categories by one individual, based on the preceding hematologic and pathologic criteria. Patients were assigned to categories without knowledge of the results

of the serum sIL-2R assays. HCI was calculated as the product of the bone marrow cellularity (%) and the fraction of leukemic cells in the bone marrow divided by 100.

sIL-2R assay. sIL-2R levels in serum samples were determined using the Cellfree IL-2R Bead Assay (T-Cell Sciences, Cambridge, MA). Samples were codified and results were reported without knowledge of the disease status associated with the sample. The principles of this assay have been described previously.[9,10] Briefly, serum samples were incubated with plastic beads coated with monoclonal antibody (MoAb) (anti-Tac) directed against one epitope on the P55 moiety of the IL-2 receptor molecule and with a horseradish peroxidase–linked second MoAb (7G7) directed against a second epitope on the P55 moiety of the IL-2 receptor molecule. Following a 1-hour incubation at room temperature, the beads were washed and incubated with substrate solution for 30 minutes. The reaction was terminated with stopping solution and the optical density at 492 nm was determined. All samples were tested in duplicate and converted to units of sIL-2R/mL by comparison with simultaneously assayed standards. All assay results are expressed as U sIL-2R/mL serum. The mean sIL-2R level in the serum of 174 healthy donors was 273 μ/mL with a standard deviation of 204 μ/mL.

Statistical methods. This article is based on a retrospective analysis of 427 serum samples obtained serially from 101 patients with HCL. Stored frozen serum samples were thawed and analyzed for level of soluble IL-2 receptor in conjunction with a retrospective assessment of clinical status of the patient at the time of the sample.

Where a best response could be assessed after therapy for HCL, a percent change in sIL-2R level from the baseline level was calculated. Percent change was defined as the ratio of the difference between the pretreatment level and the level at first observation of best response, to the pretreatment level, multiplied by 100. A three-way comparison of percent change among complete response, partial response, and minor response patients was performed by analysis of variance; pairwise comparisons were performed by t tests.[15]

Linear regression was performed to describe the relationship between HCI and sIL-2R level. A Pearson correlation coefficient (r) was used to assess strength of linear association.[15]

RESULTS

At the times that serum samples were obtained, patients were categorized as pretreatment, complete response, partial response, minor response, or relapse, based on the criteria defined in Materials and Methods. The level of serum sIL-2R subsequently determined was then linked to the appropriate category by the patient's disease status on the date of the serum sample. As illustrated in Table 1, the mean pretreatment levels of sIL-2R were the highest of any category. As expected for a test that reflects the status of disease, the categories of minor, partial, and complete response seemed to

Table 1. Serum sIL-2R Levels in Patients With HCL

Response	No.	Units sIL-2R/mL Serum		
		Mean	SD	Range
Pretreatment	123	19,310	13,360	1,726-81,042
Minor response	79	9,807	10,761	703-56,368
Partial response	145	3,195	3,538	467-17,413
Complete response	15	1,000	1,337	383-5,544
Relapse	65	18,793	14,206	3,586-79,050

Patients were categorized for response at the time the serum sample was collected, as defined in Materials and Methods.
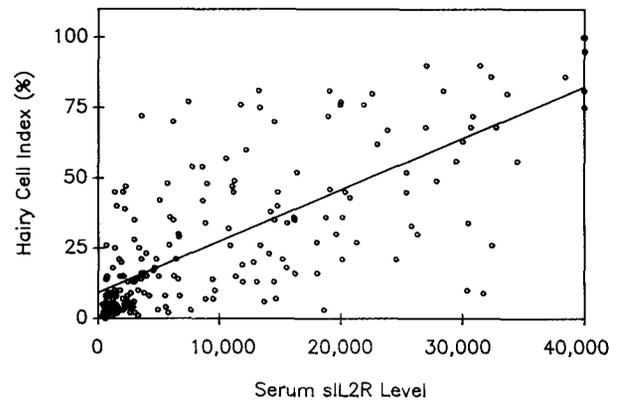

Fig 1. The serum sIL-2R level in U/mL was plotted against percent hairy cell index. The least-squares regression line is defined by HCI = 0.0012 (sIL-2R) + 14.5 (r = .71).

reflect a progressive decrease in mean sIL-2R levels. Additionally, the magnitude of the mean sIL-2R level in the relapse category was strikingly similar to that of all pretreatment samples.

Because the determination of the bone marrow HCI is a reliable measure of disease in HCL, we investigated the relationship between the serum sIL-2R level and this disease parameter. Bone marrow biopsies performed within 1 week after serum samples were taken were available for 207 serum sIL-2R values. The sIL-2R values were plotted against the HCIs calculated from the bone marrow biopsies (Fig 1). Multiple manipulations failed to improve the correlation achieved by regressing (sIL-2R)$^{1/2}$ on HCI and described by the equation HCI = 0.35 (sIL-2R)$^{1/2}$ − 3.07 (r = .77, $P < .001$).

A change in sIL-2R level was significantly associated with a change in disease status. Both pre- and posttreatment sera were available from 40 responding patients, and the percent change in serum sIL-2R level was calculated between the last pretreatment sample and the sample obtained at the first, documented, best response. The mean decrease in serum sIL-2R level was 83.1%. As shown in Table 2, the magnitude of this decrease in serum sIL-2R was significantly associated with the clinical status of disease. Patients who achieved a minor response had only a 68.6% decline in serum sIL-2R level, whereas patients who achieved a complete response had a 95.1% decline in serum sIL-2R level, and those who achieved a partial response had an intermediate (87.5%) decline in serum sIL-2R level. A statistically significant difference was identified among the responder groups in a three-way comparison. Pairwise comparisons showed marginal differences between the categories of CR and PR ($P = .01$) and PR and MR ($P = .03$), but a strong difference between CR and MR was noted ($P = .005$). Notably, every patient who responded to treatment had a decline in serum sIL-2R level.

Since most patients with HCL respond to treatment, few of the patients in this retrospective study were classified as nonresponders. However, pretreatment sera were available from five patients who never achieved a response. Pretreatment serum sIL-2R values declined only 16.1% on the

Table 2. Percent Change in Serum sIL-2R Levels Relative to Clinical Status of HCL

	No.	% Change in sIL-2R Mean	SD
Total responders	40	−83.1	18.4
Complete response	4	−95.1	1.5
Partial response	25	−87.5	13.2
Minor response	11	−68.6	24.5
Nonresponders	5	−16.1	22.6
Relapse	22	666.8	50

average and one patient had a slight increase in serum sIL-2R level. During the follow-up period of this retrospective study, 22 patients had sufficient progression of disease to be classified as progressive disease. Twenty of these patients had concurrent bone marrow biopsies that revealed a mean increase in HCI of 237.4% associated with their disease progression. Using the lowest serum sIL-2R level observed during the best response as a baseline, the mean increase in serum sIL-2R level at the time of disease progression was 666.7%.

In 20 progressive disease patients with serum sIL-2R and HCI, an analysis was performed to evaluate the correlation between these variables. A model was generated that related the ratio of HCI at disease progression and HCI at best response to the ratio of sIL-2R at relapse and sIL-2R at best response. This linear correlation was not statistically significant ($P = .10$). In 34 responsive patients with sIL-2R and HCI levels, a model defined by

$$\frac{\text{HCI at best response}}{\text{HCI at pretreatment}} = 0.15$$

$$+ 0.90 \frac{\text{sIL-2R at best response}}{\text{sIL-2R at pretreatment}}$$

provided a weak but statistically significant linear correlation ($r = .41, P = .02$) between these two ratios.

A subset of patients had both bone marrow biopsies and frequent serial serum sIL-2R levels determined in conjunction with systemic therapy. In these patients it is possible to compare the serum sIL-2R level, HCI, and clinical status of disease, and illustrate the apparent relationship between serum sIL-2R level and both HCI and clinical disease status.

Figure 2 illustrates the response of a single patient to low-dose (0.2 mU/m^2) IFN administered over a 6-month period. Twenty-four weeks into the IFN therapy the patient had sufficient improvement in peripheral blood counts to be considered a minor response. Four weeks later the patient was classified as having a partial response, and a bone marrow biopsy demonstrated a decrease in HCI from 0.57 to 0.21, a 62% decrease. This decrease in HCI was apparently paralleled by a progressive decrease in serum sIL-2R level from 10,538 to 4,785 U/mL (a 55% decrease) over the treatment interval.

HCL generally responds more dramatically to higher doses of IFN than the dose depicted in Fig 2.[5,6] Figure 3 illustrates the response of a patient with HCL to IFN administered at a standard dose (2.0 mU/m^2). The first bone marrow biopsy and serum sIL-2R level shown were obtained
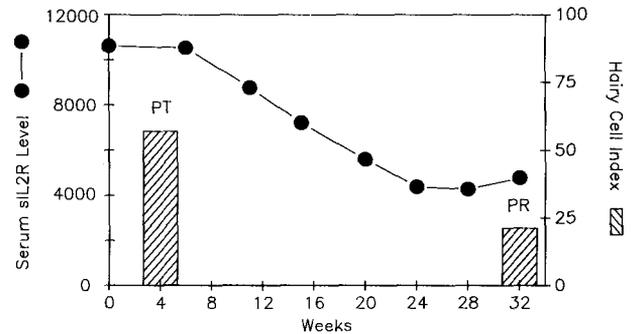

Fig 2. Response of HCL to low-dose alpha-2b-interferon in a single patient. Serum sIL-2R levels are indicated by solid circles and HCI by cross-hatched bars. Alpha-2b-interferon (0.2 mU/m^2) was administered subcutaneously thrice weekly, and was begun on week 4. Clinical status at the time of bone marrow biopsy is indicated above HCI. PT, pretreatment; PR, partial response.

immediately before initiating a 12-month treatment with IFN. Bone marrow biopsies performed at 9 and 12 months revealed a marked decline in HCI from 42 to 4.5 and 5.0, respectively. The decline in the percent HCI was paralleled by a decline in serum sIL-2R from 12,903 to 1,046 U/mL (a 92% decrease), and the serum sIL-2R level remained less than 1,000 U/mL for the remainder of the IFN treatment. Three months after discontinuing IFN treatments (month 15), HCL had progressed. A bone marrow biopsy revealed an HCI of 12.5%, and this coincided with an increased serum sIL-2R level of 5,027 U/mL (a 425% increase).

One final case illustrates the apparent association of serum sIL-2R level with the clinical and pathologic status of patients with HCL (Fig 4). A patient refractory to IFN therapy was begun on dCF. The bone marrow HCI decreased from 35% to 2.3% after 10 weeks of treatment and the patient was classified as having partial response due to the persistence of circulating hairy cells. At week 22 it was possible to classify this patient as having a complete response with an HCI of 0. The patient's response to therapy was apparently accurately charted by the progressive decline in serum sIL-2R from 16,170 U/mL initially, to 5,786 U/mL (a 64% decrease) at week 10, and finally 882 U/mL (a 95%
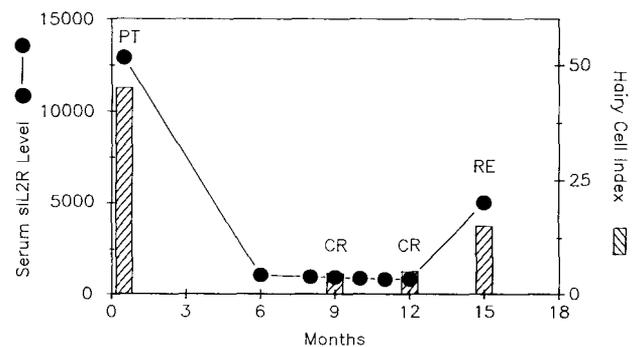

Fig 3. Clinical course of a patient with HCL treated with standard-dose interferon. Serum sIL-2R levels are indicated by solid circles and HCI by cross-hatched bars. Alpha-2b-interferon (2.0 mU/m^2) was administered subcutaneously thrice weekly. Clinical status at the time of bone marrow biopsy is indicated above HCI. PT, pretreatment; CR, complete response; RE, relapse.
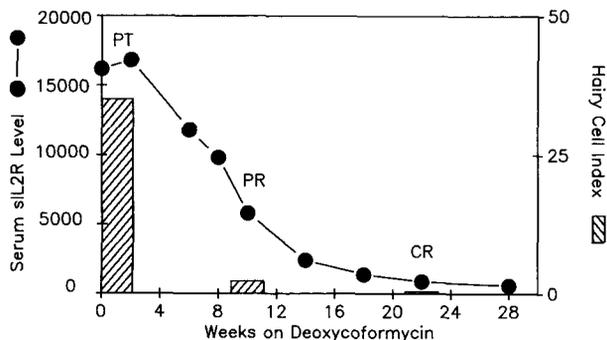
Fig 4. Treatment of HCL with deoxycoformycin (dCF) in a single patient. Serum sIL-2R levels are indicated by solid circles and HCI by cross-hatched bars. Deoxycoformycin (4 mg/m²) was administered intravenously on days 1 and 15 of each month. Clinical status at the time of bone marrow biopsy is indicated above HCI. PT, pretreatment; PR, partial response; CR, complete response.

decrease) at week 22. This case also illustrated that the relationship of serum sIL-2R level to disease status and HCI was not limited to treatments using IFN.

DISCUSSION

Comparing serum sIL-2R levels with clinical disease status in the population of patients with HCL suggests a correlation, but the range of sIL-2R values associated with each category (Table 1) does not allow categorization of a patient based on the absolute sIL-2R levels. Comparison of sIL-2R with the standard method of pathologic staging (ie, HCI) yielded a relationship defined by the equation HCI = $0.35 \, (sIL-2R)^{1/2} - 3.08$, but the correlation coefficient was only .77. Several factors may be responsible for the lack of a better correlation between HCI and sIL-2R. The serum level of sIL-2R at any given time is the result of both its production and elimination. Data regarding the elimination of serum sIL-2R are not available. However, it is probable that production of sIL-2R is subject to significant interpatient variation. This conclusion is supported by in vitro studies demonstrating that hairy cells derived from different patients express widely different amounts of cell surface IL-2R[9] and secrete different amounts of sIL-2R into culture media.[10] Thus, the unpredictable rate of sIL-2R production per leukemic cell may introduce significant interpatient variability in sIL-2R level and result in the weak interpatient correlation between serum sIL-2R level and HCI.

If both the rate of production of sIL-2R per leukemic cell and the serum sIL-2R elimination rate remained constant in an individual patient, then a correlation between serum sIL-2R level and HCI would be expected in a single patient. Although no single patient had a sufficient number of simultaneous serum sIL-2R levels and bone marrow biopsies

to test this hypothesis, the data illustrated in Figs 2 through 4 are consistent with a direct correlation between these two parameters and further suggest that this relationship is not dependent on the use of interferon therapy.

If sIL-2R level and HCI are linearly related in an individual patient, then changes in sIL-2R level should closely correlate with disease status. This relationship is illustrated in Table 2. A decrease of 50% or more was observed in 38 of 40 (95%) treatment responders and in 0 of 5 (0%) nonresponders. Although definitive prediction of response based on serum sIL-2R level may not be drawn from this retrospective study, a percent decrease of 50% or more may be useful in monitoring clinical response. The potential role of serum sIL-2R in monitoring treatment response is further supported by the statistically significant linear correlation between the ratios of serum sIL-2R and HCI.

No statistically significant correlation was observed between sIL-2R and HCI for cases of progressive disease. This lack of correlation may lie in the fact that disease relapse was frequently associated with dramatic increases in serum sIL-2R (see Table 2) but only modest increases in HCI. The possibility that the leukemic cells at relapse may produce more sIL-2R per cell cannot be excluded and is consistent with our results. Despite the lack of correlation between sIL-2R and HCI in cases of disease progression (Table 2), an increase of 60% or more was observed in 22 of 22 (100%) relapse cases and in 0 of 45 (0%) cases categorized as responder or nonresponder. Although definitive prediction of disease progression based on serum sIL-2R may not be drawn from this retrospective study, an increase of 60% or more may suggest progressive disease.

We have previously described the use of neutrophil alkaline phosphatase (NAP) and residual bone marrow hairy cells (HCI) at the completion of interferon therapy for the prediction of relapse.[16] In this retrospective study, the sIL-2R level at the completion of therapy was not related to the duration of time to relapse. As noted previously, the wide interpatient variability in the serum sIL-2R level may be responsible for this lack of correlation in the population. The possibility that the rate of change in serum sIL-2R level following treatment may have prognostic value could not be tested with our retrospective data, but will be addressed in the future in combination with other parameters (eg, NAP, HCI).

Others have noted that the level of sIL-2R is elevated in HCL and have proposed sIL-2R as a tumor marker for HCL.[11-13] Our data not only corroborate that assertion in a large population, but suggest that serum sIL-2R level may be a quantitative measure of leukemic cell burden in individual patients, independent of treatment regimen. The potential role of sIL-2R for monitoring and predicting response and/or progression is supported by this study and is currently being prospectively tested.

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