Serum Soluble IL-2 Receptor as a Tumor Marker in Patients With Hairy Cell Leukemia

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Activated T cells synthesize and express a cell membrane-bound receptor for interleukin-2 (IL-2) and have recently been shown to secrete a soluble form of the same receptor. Hairy cell leukemia is a chronic disorder caused by expansion of a clonal population of an unusual mononuclear cell of B cell origin. These cells have previously been shown to express an IL-2 receptor on the cell membrane. The sera of 26 patients with hairy cell leukemia were examined for the presence of a soluble IL-2 receptor before and during therapy with either recombinant interferon alpha-2a or 2'-deoxycoformycin. Before therapy, all patients had markedly elevated levels of this soluble IL-2 receptor ranging from five to 60 times the highest level observed in normal control sera. In individual patients changes in the level during therapy correlated well with clinical assessments of tumor response: levels fell to near the normal range in patients responding to therapy. Patients not responding to interferon alpha had no significant change in the soluble IL-2 receptor level. These results suggest that hairy cells secrete a soluble IL-2 receptor and that serial measurements of the level of this receptor in the serum can be used as a noninvasive means to assess disease response to therapy.

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Hairy cell leukemia is a chronic lymphoproliferative disorder characterized by pancytopenia, splenomegaly, and by the absence of significant lymphadenopathy. Patients with the disease frequently have abnormal circulating mononuclear cells with characteristic fine cytoplasmic projections that on phase contrast microscopy give the cells their typical hairy appearance. These cells preferentially infiltrate the bone marrow and spleen resulting in unaspirable bone marrow and splenomegaly, respectively. Ferrokinetic studies have suggested that the pathogenesis of the pancytopenia in this disease is related to inadequate production of cells in the bone marrow, to excessive peripheral destruction of circulating cells in the spleen, or to a combination of both. Splenectomy frequently results in an improvement in peripheral blood counts that can be quite prolonged despite persisting marrow infiltration with hairy cells. Until recently, treatment of post-splenectomy patients who developed progressive pancytopenia was unsuccessful. Although small numbers of patients have been salvaged with high doses of combination or single agent chemotherapy, this approach results in prolonged, severe pancytopenia making it an unacceptably toxic form of therapy for the majority of hairy cell leukemia patients. Chronic administration of low doses of alkylating agents has improved platelet counts and hematocrits in some patients, though there appears to be an appreciable risk of early myelosuppression and fatal infection.

Alpha interferons including natural (leukocyte), recombinant, and lymphoblastoid types and 2'-deoxycoformycin have recently been shown to result in prompt improvement in peripheral blood counts, shrinkage of enlarged spleens, and clearing of malignant cells from the marrow of patients with hairy cell leukemia. Indeed, pathologically documented complete responses have been observed in some patients. Preliminary data suggest that interferon is probably not curative in this disease since discontinuation of interferon in complete responders has resulted in recurrence of the disease in some patients. Nonetheless, alpha interferon has become the therapy of choice, at least for patients who progress following splenectomy.

The malignant cell in the vast majority of patients with hairy cell leukemia is of B cell origin, since nearly all cases examined so far have had an identifiable immunoglobulin gene rearrangement. The B cell variety of hairy cell leukemia has been shown to express a cell membrane-bound receptor for interleukin-2 (IL-2) as recognized by the anti-Tac monoclonal antibody. These receptors are characteristically found on activated T cells or T cells infected with the type I human T cell leukemia virus (HTLV-I), though activated B cells and B cells infected with the Epstein-Barr virus express these receptors as well (reviewed in reference 17). Rubin et al showed that normal peripheral blood mononuclear cells and certain cell lines of B and T cell origin release a soluble form of the IL-2 receptor into the culture medium. Release of this soluble IL-2 receptor appears to be a consequence of cellular activation, requires RNA and protein but not DNA synthesis, and probably is not due merely to proteolysis of the membrane-bound receptor. The molecular weight of this soluble form of the IL-2 receptor is...
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approximately 10 kilodaltons (kd) smaller than the membrane bound form and it binds to anti-Tac. Low levels of this soluble receptor have been found in the serum of normal individuals and in theory, measurements of soluble IL-2 receptor levels might be useful in assessing the in vivo state of immune stimulation in normal individuals or the volume of disease in patients with malignancies that secrete the receptor. In this report, we examined the sera of patients with hairy cell leukemia both before and serially during therapy with either recombinant alpha interferon or 2'-deoxycoformycin to determine if elevated receptor levels were present in these patients and if changes in the receptor level correlated with tumor response.

MATERIALS AND METHODS

Patients. The patients evaluated in this study were examined, treated, and followed-up in the clinic of the Biological Response Modifiers Program, NCI. The diagnosis of hairy cell leukemia was made by the typical clinical presentation, histologic examination of peripheral blood and bone marrow in all cases, and in 13 cases, examination of splenic tissue. Recombinant interferon alpha-2a (Roferon; Hoffmann-LaRoche, Nutley, NJ) was administered subcutaneously at a dose of 3 million units daily for 6 months then three times per week. Deoxycytosine arabinoside (dCF) was supplied by the Cancer Treatment Evaluation Program, NCI, and was administered only to patients whose disease was resistant to alpha interferon therapy. Patients received 4 mg/m2 of dCF weekly for 3 consecutive weeks, then biweekly. Both the interferon and deoxycytosine arabinoside studies were approved by the investigational review boards of the Frederick Cancer Research Facility and the Clinical Oncology Program of the National Cancer Institute. All patients gave written informed consent prior to protocol entry.

Response criteria were similar to those reported previously. Briefly, complete responders had resolution of all signs and symptoms of the disease, including normalization of all peripheral blood counts and absence of hairy cells from both the peripheral blood and bilateral iliac crest bone marrow biopsies. Normal peripheral blood counts were defined as an absolute granulocyte count >1,500/μL, a platelet count >100,000/μL, and a hemoglobin >12.0 g/dL. Partial responders had normalization of peripheral blood counts and at least a 50% reduction of the hairy cell infiltrate in the bone marrow. Minor responders showed some improvement in the peripheral blood counts above baseline values.

Soluble IL-2 receptor assay. Serial serum samples were collected during the course of therapy from patients with hairy cell leukemia. Normal laboratory volunteers served as controls. This is a retrospective study, and not all patients had sera available at the same time points during the course of their treatments. Soluble IL-2 receptor levels were determined as described previously. Briefly, serial dilutions of each serum specimen were added to wells of a 96-well Immulon microtiter plate (Dynatech Laboratories, Alexandria, VA) previously coated with the monoclonal anti-IL-2 receptor antibody anti-Tac (generously provided by Dr T. Waldmann, NIH). Plates were incubated for two hours, washed, and incubated two hours further with antibody 7G7/B6 conjugated with fluorescein isothiocyanate (FITC). This antibody recognizes an epitope of the IL-2 receptor distinct from that recognized by anti-Tac. An alkaline phosphatase-conjugated rabbit anti-FITC antibody was then added and the mixture was incubated for one hour. The wells were washed and p-nitrophenyl phosphate (Sigma, St Louis, 1 mg/mL) was added, and absorbance of the wells was determined after 30 minutes at 405 nm by using a Titertek enzyme linked immunoassay (ELISA) recorder (Flow Laboratories, Rockville, MD). A reference reagent, consisting of the cell-free supernatant of a normal IL-2-dependent human T cell line four days after stimulation with 10% IL-2 (Cellular Products, Buffalo) was used in all these assays. The undiluted supernatant was assigned an arbitrary value of 1,000 IL-2 receptor U/mL, and the absorbance values as determined by the ELISA assay of serial dilutions of this supernatant were used to generate a reference curve. The absorbance of the test wells was then compared with the standard curve and was converted to a numerical value. Receptor levels in this study are expressed in units per milliliter.

RESULTS

Expression of the IL-2 receptor on the surface of hairy cells has been documented in a previous study in small numbers of patients. Of 26 patients in our study, 13 had sufficient numbers of circulating hairy cells to allow an adequate flow cytometric analysis of IL-2 receptor expression on their malignant cells. The malignant cells of all 13 patients expressed the Tac IL-2 receptor. The density of receptor expression on these cells as reflected in the mean fluorescence intensity of Tac-positive cells was increased twofold over normals (mean, 308, range, 264 to 350 in hairy cell patients vs mean, 159, range, 112 to 166 in the same patients after disappearance of morphologically identifiable hairy cells from the peripheral blood during therapy). Representative results of this type of analysis from a single patient before and during therapy with recombinant interferon alpha 2A are shown in Fig 1.

The serum level of soluble IL-2 receptors was determined before therapy in 24 patients with hairy cell leukemia (two patients did not have pretreatment serum specimens available for analysis). Before the institution of therapy, serum levels of this receptor were significantly elevated when compared with those of normal controls (Fig 2). There was no overlap of serum levels between hairy cell leukemia patients and normal controls. The lowest level observed in
hairy cell patients was fivefold greater than the highest level observed in controls. The mean level in patients was 20,465 (±3,055) U/mL, while in the controls it was 301 (±39) U/mL. There was no correlation between initial receptor level and response to interferon therapy.

The absolute serum level of the IL-2 receptor did not correlate with estimates of tumor burden such as numbers of circulating hairy cells (Fig 3) or spleen size (Table 1). In fact, the patient with the highest hairy cell count (76,000/μL) had one of the lowest IL-2 receptor levels (6,000 U/mL) (Fig 3). In addition, among the eight patients who had not previously undergone splenectomy (Table 1), the highest serum IL-2 receptor level (53,943 U/mL) was observed in a patient with a nonpalpable spleen and a relatively low absolute hairy cell count (2,694/μL).

Serial serum samples obtained during therapy were available in 25 patients (one patient did not have serial serum specimens available for analysis). In all 25 patients, changes in the serum level of the IL-2 receptor paralleled the response to interferon or 2'-deoxycoformycin treatment. All 20 patients who continuously responded to interferon had a progressive fall in IL-2 receptor level. Three patients who had transient responses to interferon were found to have initially decreasing soluble IL-2 receptor levels that began to rise when, despite continued interferon administration, the peripheral blood counts worsened. In two patients who failed to respond to interferon from the start, there was either no significant change or an increase in the serum IL-2 receptor level. Examples of changes in IL-2 receptor levels from these three types of patients are shown in Table 2 and Figs 4 and 5. As shown in Table 2, patient EM had progressive improvement in the circulating granulocyte count and rapid normalization of the platelet count concurrent with a fall in the IL-2 receptor level. Similar results were obtained with patient RM initially. Despite continued administration of interferon, however, the level of the IL-2 receptors began to rise at day 144 of therapy. Subsequently, the peripheral blood granulocyte count fell, and the spleen became palpable for the first time (day 236). Thus, in this patient a progressive rise in the IL-2 receptor level antedated clinical deterioration by approximately 3 months. The results shown in Fig 4 are from a patient whose peripheral blood counts failed to improve during 6 consecutive months of interferon administration. Soluble IL-2 receptor levels during this time rose progressively from 16,000 to 32,000 U/mL. The patient was subsequently treated with 2'-deoxycoformycin, which resulted in improvement in peripheral blood counts and a concurrent fall in the IL-2 receptor level toward the normal range. Figure 5 shows the results of soluble IL-2 receptor levels in the 20

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**Table 1. Relationship of Pretreatment Spleen Size and Absolute Hairy Cell Count to Soluble IL-2 Receptor Levels**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Spleen Size</th>
<th>AHCC (U/mL)</th>
<th>Soluble IL-2 Receptor Level (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM</td>
<td>17.5 cm</td>
<td>5,328</td>
<td>51,986</td>
</tr>
<tr>
<td>RD</td>
<td>5 cm</td>
<td>0</td>
<td>23,594</td>
</tr>
<tr>
<td>PV</td>
<td>4 cm</td>
<td>34</td>
<td>10,561</td>
</tr>
<tr>
<td>RM</td>
<td>NP</td>
<td>2,694</td>
<td>53,943</td>
</tr>
<tr>
<td>RT</td>
<td>NP</td>
<td>92</td>
<td>10,170</td>
</tr>
<tr>
<td>MF</td>
<td>NP</td>
<td>30</td>
<td>3,732</td>
</tr>
<tr>
<td>JE</td>
<td>NP</td>
<td>28</td>
<td>21,004</td>
</tr>
<tr>
<td>CO</td>
<td>NP</td>
<td>0</td>
<td>11,536</td>
</tr>
</tbody>
</table>

Abbreviation: AHCC, absolute hairy cell count; NP, not palpable.

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**Table 2. Change in Soluble IL-2 Receptor Levels With Clinical Response to Interferon-Alpha 2a**

<table>
<thead>
<tr>
<th>Day</th>
<th>Soluble IL-2R (U/mL)</th>
<th>AHCC (cells/μL)</th>
<th>AGC (cells/μL)</th>
<th>Platelets (no./μL)</th>
</tr>
</thead>
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<tr>
<td>EM</td>
<td>0</td>
<td>51,986</td>
<td>5,328</td>
<td>296</td>
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<tr>
<td></td>
<td>62</td>
<td>2,825</td>
<td>256</td>
<td>1,400</td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>2,312</td>
<td>280</td>
<td>1,812</td>
</tr>
<tr>
<td></td>
<td>139</td>
<td>1,675</td>
<td>174</td>
<td>1,856</td>
</tr>
<tr>
<td>RM</td>
<td>0</td>
<td>53,943</td>
<td>2,964</td>
<td>214</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>4,380</td>
<td>2,064</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>1,002</td>
<td>184</td>
<td>253</td>
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<td></td>
<td>109</td>
<td>800</td>
<td>80</td>
<td>592</td>
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<td></td>
<td>144</td>
<td>1,203</td>
<td>153</td>
<td>748</td>
</tr>
<tr>
<td></td>
<td>236</td>
<td>8,500</td>
<td>0</td>
<td>512</td>
</tr>
<tr>
<td></td>
<td>318</td>
<td>21,547</td>
<td>252</td>
<td>238</td>
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</table>

Abbreviation: AGC, absolute granulocyte count.
correlated well with response to therapy, continuously responding to recombinant interferon-alpha 2A. Thus, although changes in the soluble IL-2 were elevated in 17 at the time of the restaging evaluation.

Although the mean receptor level of this receptor was normalized in 19 responders, however, had normalization of the mean normal level deviations of response, there was extensive overlap of receptor levels between these two groups. In total, 19 patients had either a partial or minor response, and of these, IL-2 receptor levels were elevated in 17 at the time of the restaging evaluation. Thus, although changes in the soluble IL-2 receptor level correlated well with response to therapy, the absolute value

<table>
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<tr>
<th>Response Category</th>
<th>Number of Patients</th>
<th>Mean IL-2 Receptor Level (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>4</td>
<td>590 (444-947)</td>
</tr>
<tr>
<td>PR</td>
<td>4</td>
<td>684 (291-1,111)</td>
</tr>
<tr>
<td>MR</td>
<td>15</td>
<td>3,151 (234-9,000)</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete response; PR, partial response; MR, minor response.

for receptor level at restaging evaluation did not correlate well with standard clinical assessments of the degree of response to therapy. These data would indicate that it is not possible to distinguish complete, partial, and minor responders solely on the basis of soluble IL-2 receptor levels, though it is possible to determine if response is occurring to either interferon or deoxycoformycin by serial determinations of soluble IL-2 receptor levels.

**DISCUSSION**

This study shows that the serum of patients with hairy cell leukemia contains strikingly elevated levels of a soluble form of the IL-2 receptor. Although the level of this receptor varied significantly from patient to patient and even between patients with similar estimates of tumor burden, the change in receptor level in an individual patient correlated closely with response to interferon alpha. In one patient examined so far changes in the levels also correlated with response to 2'-deoxycoformycin. The serum level of this receptor appears to be a fairly sensitive measure of residual marrow involvement with hairy cells since 17 of 19 partial or minor responders with residual disease in the bone marrow had elevated levels of this receptor. Moreover, the four patients in this study who attained a complete response with interferon therapy had slight but definite elevations of the IL-2 receptor level at the time that a complete response was documented. A previous study has shown that interferon-treated hairy cell patients in complete remission probably have persisting disease in the bone marrow since many such patients develop recurrent disease following discontinuation of interferon. In addition, Southern blot analysis of bone marrow cell DNA from hairy cell leukemia patients felt to be in complete remission has shown a clonal rearrangement of the immunoglobulin gene in all five such patients so far evaluated (S. Giardina, personal communication, June, 1987), proving that such patients have persisting but microscopically undetectable marrow disease. The elevated IL-2 receptor levels in these patients might, therefore, be indicative of minimal but undetected marrow involvement. Indeed, further follow-up of these four apparent complete responders with additional bilateral iliac crest bone marrow biopsies has shown either recurrence or persistence of previously undetected disease in three of them. Thus, persistently elevated soluble IL-2 receptor levels in these patients might be an indication of minimal, persisting disease. Because 2'-deoxycoformycin appears to be capable of inducing durable complete responses and possibly cures in these patients, determination of IL-2 receptor levels in the sera of these patients and

**Fig 4.** Serial serum levels of soluble IL-2 receptors in patient DS. The patient had no detectable hematologic response to six consecutive months of interferon-alpha administration. 2'-deoxycoformycin was instituted as shown with resultant improvement in hematologic parameters and a decrease in the IL-2 receptor level.

**Fig 5.** Changes in serum IL-2 receptor levels in patients continuously responding to recombinant interferon-alpha 2A.
correlation with clinical outcome would be of interest. Such studies are now in progress.

On the basis of soluble IL-2 receptor levels, patients with complete, partial, or minor responses could not be distinguished from one another. This is despite the fact that patients in these response categories differ significantly in tumor burden. If one assumes that the serum IL-2 receptor is derived from the hairy cells themselves, this lack of discriminating ability must be due to differences in the rate of secretion of IL-2 receptors from the hairy cells, to differences in metabolism of the receptor, or to other factors. Thus, single, isolated measurements of serum IL-2 receptor levels in patients who have responded to interferon therapy would not be useful as an indicator of the extent of disease response. However, in all patients examined to date, changes in the soluble IL-2 receptor level compared with baseline correlated with response to therapy. Among 20 patients continuously responding to interferon alpha therapy, levels of the receptor fell progressively during treatment; patients progressing during therapy had progressive increases in the receptor level. Serial measurements of this receptor, therefore, appear to be a useful, noninvasive means of determining if a response is occurring but not to determine the degree of response. Thus, serial measurements of soluble IL-2 receptors in the serum of patients with hairy cell leukemia is analogous in many respects to the usefulness of serial carcinoembryonic antigen determinations in patients with colorectal carcinoma, and, indeed most other tumor markers.

The data presented here do not demonstrate that the origin of the elevated serum IL-2 receptor levels is the hairy cell itself. Indeed, those factors that determine the level of this receptor in normal individuals are at the moment unknown. It is conceivable that the elevated levels of receptor in hairy cell leukemia patients is due to an ongoing T cell mediated immune response to the malignant cell, a response that would be expected to lessen in magnitude as tumor volume reduction occurred in response to alpha interferon administration. However, there is no evidence for such an antitumor immune response in these patients. Clinically a functional T cell deficiency manifesting as a tendency to develop unusual opportunistic infections seems to be the rule in this disease. That this is a functional T cell deficiency and not necessarily due to reduced T cell numbers in these patients is suggested by the studies of Lauria et al and of Worman and Cowley, who found normal numbers of T cells in the peripheral blood of these patients. Thus, it appears that elevated levels of the soluble IL-2 receptor are likely the result of secretion of the receptor by the hairy cells themselves.

Because this soluble form of the IL-2 receptor is capable of binding IL-2, it is possible that the very elevated levels found in hairy cell leukemia patients might in part be responsible for the immune abnormalities observed clinically and in vitro in this disease. IL-2 is felt to be an important mediator in the immune response to pathogens. If IL-2 secreted by an activated T cell after exposure to a foreign antigen is absorbed by a soluble IL-2 receptor, functional immune paralysis and blunting or inhibition of an appropriate immune response might result. Clinically this might result in an enhanced susceptibility to infection by organisms ordinarily controlled or prevented by T cells. Among such organisms are atypical mycobacteria and Toxoplasma gondii, pathogens found in patients with hairy cell leukemia. The data mentioned above suggesting a functional T cell deficit in hairy cell leukemia patients is in keeping with this notion. However, there is as yet no direct evidence that this mechanism is important in vivo and further study of the immunologic consequences of high concentrations of this soluble IL-2 receptor are needed to determine if it plays a role in the pathogenesis of the immune dysfunction observed in patients with hairy cell leukemia.

ACKNOWLEDGMENT

Acknowledgment is made to the nurses, physicians, and clinic coordinator of the Clinical Research Branch of the Biological Response Modifiers Program, DCT, NCI-FCRF without whose assistance this study would not have been possible.

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