High Serum Levels of Soluble Interleukin 2 Receptor in Patients With B Chronic Lymphocytic Leukemia


By using an enzyme-linked immunosorbent assay, the presence of the soluble form of the interleukin-2 receptor (sIL-2R) was evaluated in the peripheral blood of 54 patients with B cell chronic lymphocytic leukemia (B-CLL). Serum levels of sIL-2R were correlated with clinical features, relevant hematologic and immunologic data, and in some cases, with in vitro functional studies. In 51 patients (94.4%), the levels of sIL-2R were increased as compared with normal age-matched controls (1.781 U/mL ± 231 v 276 U/mL ± 26, respectively; P < .001). Although this increase was observed in all stages of the disease and independently of several hematologic and immunologic parameters, a trend toward lower levels of sIL-2R was documented in patients with a less-invasive disease. When the values were correlated with the functional status of the residual T cell population, it was found that patients with the lowest levels of sIL-2R showed the best mitogenic response and helper capacity. It is suggested that in B-CLL patients the high levels of serum sIL-2R, capable of binding to its ligand, may block the T cell–produced IL-2, thus contributing toward a defective physiological action by this lymphokine. In turn, this defective availability of IL-2 may play a part in the abnormal immunoregulation that is implicated in the hypogammaglobulinemia, susceptibility to infections, and incidence of second neoplasias often observed in this disease.

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B CELL CHRONIC LYMPHOCYTIC leukemia (B-CLL) is a lymphoproliferative disorder characterized by the accumulation of relatively mature monoclonal B lymphocytes. Although the residual T cell population in this disease does not belong to the neoplastic clone, as indicated by the lack of chromosome abnormalities and by the heterozygosity for glucose-6-phosphate dehydrogenase, it has been shown to exhibit a number of abnormalities. These include an increased absolute number, a reversed CD4/CD8 ratio that often correlates with the stage of the disease, an abnormal T cell subset redistribution between peripheral blood (PB) and bone marrow, and several functional impairments. The latter include a defective proliferative response to mitogens and allogeneic cells, a reduced colony formation, and a depressed helper and cytotoxic activity. Although most of the studies point toward multiple functional defects, the precise origin and significance of these T cell abnormalities in B-CLL is unexplained. It is still under discussion whether they represent a primary or a secondary event.

The receptor for interleukin-2 (IL-2R), which is defined by the CD25-related monoclonal antibodies (MoAbs), is present on the cell membrane of T lymphocytes, B lymphocytes, and macrophages when these cells are activated or when they belong to a discrete stage of their ontogeny. In B-CLL patients, both residual T lymphocytes and neoplastic B cells have been shown to be capable of expressing IL-2R. It has been recently demonstrated that, in particular in vitro and in vivo conditions, IL-2R may be released from the cell surface in a soluble form (sIL-2R). High serum levels of sIL-2R have been demonstrated in several diseases including hairy cell leukemia and Hodgkin’s disease.

In view of the fact that IL-2R may be detected on the surface of neoplastic B cells and that exogenous IL-2 may play a direct role in the proliferation of normal and leukemic B lymphocytes, it is tempting to hypothesize that, in some pathological conditions such as B-CLL, alterations of the normal levels of sIL-2R may affect the balance of the autocrine system represented by IL-2 and IL-2R molecules. In turn, this may downmodulate the immune response and lead to defective immunoregulation, which may help to explain the T cell abnormalities reported in B-CLL. To test this possibility we evaluated the presence of sIL-2R in 54 patients with B-CLL. In all of them the levels of sIL-2R were correlated with the clinical stage, the relevant hematologic and immunologic data, and in some cases, with functional in vitro studies.

MATERIALS AND METHODS

Patients. The study included 54 patients with B-CLL, 32 men and 22 women, with a mean age of 66 years. The diagnosis was made on the basis of established hematologic criteria and confirmed in all cases by phenotypic analyses on PB cell suspensions. Patients were graded according to Rai’s staging system as follows: stage 0, 14 stage I, 17 stage II, 9 stage III, and 6 stage IV. The total lymphocyte count ranged between 16 × 10^9/L and 240 × 10^9/L. Thirty-seven patients never received treatment, and 17 patients had been previously treated with low doses of alkylating agents, but at the time of this study they had not been treated for at least 16 weeks. The mean serum immunoglobulin levels, as determined by radial immunodiffusion, were IgG, 904 mg/mL ± 141 (control range, 800 to 1,680 mg/mL); IgA, 102 mg/mL ± 27 (control range, 60 to 400 mg/mL); IgM, 52 mg/mL ± 13 (control range, 50 to 250 mg/mL).

Twenty-five healthy volunteers in the same age range were used as controls.

Detection of sIL-2R. sIL-2R levels in the serum of B-CLL...
patients and of normal controls were determined by using a sandwich enzyme immunoassay (Cellfree, T Cell Sciences, Inc, Cambridge, MA) as described by Rubin et al. Briefly, the IL-2R available in the test samples or in the standards binds to the polystyrene microtiter wells previously incubated with 100 μL anti-Tac equivalent MoAb (1 μg/mL). A horseradish peroxidase-conjugated anti-IL-2R MoAb directed against a second epitope on the IL-2R molecule binds to the IL-2 captured by the first antibody and completes the sandwich. After washing to remove the unbound enzyme-conjugated anti-IL-2R MoAb, a substrate solution is added to the wells. The reaction is then stopped and the absorbance determined at 490 nm. A standard curve is prepared from four IL-2R standards. The IL-2R standard was the cell-free supernatant obtained from phytohemagglutinin (PHA) in vitro–stimulated T cells and was assigned a value of 1,000 IL-2R U/mL.

Lymphocyte separation and T cell purification. Mononuclear cells were separated by a Ficoll-Hypaque (F/H) density gradient from freshly drawn heparinized PB and washed three times in sterile phosphate-buffered saline. Adherent cells were removed after incubation at 37°C for 45 minutes in plastic Petri dishes. T lymphocytes were purified from non-T cells by rosetting with neuraminidase (Sigma Chemical Co, St Louis)-treated sheep RBC (nSRBC) followed by repeated F/H gradient separations as previously described. T cells were recovered after SRBC lysis with 0.83% ammonium chloride and resuspended in RPMI 1640 (GIBCO, Grand Island, NY) at a concentration of 10^6/mL. The purified fractions always contained more than 97% and 90% E-positive cells (in controls and B-CLL groups, respectively), whereas the contamination with surface immunoglobulin–positive cells never exceeded 2%. The viability, assessed by trypan blue exclusion, was always greater than 95%.

Surface marker analysis. The following MoAbs, designated according to the Boston workshop, were used to characterize the phenotype of the enriched T cell populations. OKT3 (CD3) MoAb (Ortho Pharmaceutical Corp, Raritan NJ) defines T cells. OKT4 (CD4)-positive cells include helper/inducer lymphocytes, and cells reactive with OKT8 (CD8) MoAb include cytotoxic/suppressor cells. In some cases the CD4/CD8 ratio was also determined directly by double staining using reagents labeled with the fluorochromes phycoerythrin and fluorescein as previously described. Similar results were consistently found.

Response to mitogens and helper function. The response of enriched T cells to PHA was evaluated as already reported. Briefly, triplicate cultures of 2 x 10^5 T cells were performed in 200 μL RPMI 1640 medium (GIBCO) supplemented with 20% fetal calf serum (Microbiological Associates, Walkersville, MA), 2 mmol glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and graded concentrations of PHA-M (Difco, Detroit) ranging from 0.5 to 6 μg/mL. Cultures were incubated at 37°C in a 5% CO2 atmosphere for 72 hours. One microcurie of [3H]-thymidine (CEA IRE, Sorin Biomedica, Saluggia, Italy; specific activity, 26 mCi/ mmol/L) was added over the last six hours of culture and the incorporation measured in counts per minute with a liquid scintillation counter. The results are expressed as the mean cpm/10^5 lymphocytes of triplicate cultures at the optimal dose (1.5 μg/mL).

The effect of total enriched populations on the in vitro pokeweed mitogen (PWM)-driven B cell differentiation was evaluated in a mixture of T cells depleted of cells bearing Fc receptors for immunoglobulin G (Fc) and normal B cell populations (5 x 10^5 and 10 x 10^5 cells/well, respectively). The detailed procedure and the method for removal of Fc cells have already been described. The results are expressed as the absolute number of viable plasma cells per well.

Analysis of data. All data are presented as means ± SEM; comparisons between values were carried out by using either the Cochran-Cox test or Spearman's rank correlation test.

RESULTS

As shown in Fig 1 the mean value of sIL-2R in the serum of patients with B-CLL taken as a group was statistically higher than that obtained in age-matched controls (1.781 U/mL ± 231 v 276 U/mL ± 26; P < .001). When analyzed independently, the sIL-2R levels fall within the normal range in only three cases (5.5%). No statistically significant difference was observed between values detected in treated and untreated patients. Although patients with the lowest levels of sIL-2R were observed in stage 0, a direct correlation between the different stages of the disease and the sIL-2R values was not always found (Fig 2). The only significant difference was observed between stage 0 and stage IV values (932 U/mL ± 170 v 3,103 U/mL ± 1,021, respectively; P < .05).

The levels of sIL-2R in our patients were also compared in all cases with the number of WBC, in 47 cases with the T cell subset distribution, and in 49 cases with the serum immunoglobulin levels (Table I). Although no statistically significant differences were observed between each subgroup because the sIL-2R levels were almost always higher than in normal controls, a general trend pointing to overall lower values in patients with less-invasive disease was observed. This was further confirmed by the rank correlation test (Table I), determined in all patients taken as a group, that showed that the serum levels of sIL-2R correlated significantly with WBC counts and the levels of serum immunoglobulins (r = .29 and .33, respectively; P < .05); no significant correlation was demonstrated with the T cell subset distribution. When a correlation was made between sIL-2R levels and the T and B lymphocyte numbers, the statistical signifi-
cells
patterns observed in patients tested for their proliferative analysis of sIL-2R levels and functional in vitro studies were correlated with in vitro functional T cell activities. The cancer did not differ from that observed comparing sIL-2R levels and this in vitro function was not always observed (Fig 2. Serum levels of sIL-2R in B-CLL patients according to the different stages of the disease defined by Rai’s system. The only significant difference was found between stages 0 and IV (P < .05). The shaded area indicates the control range).

As illustrated in Fig 3, the levels of sIL-2R were also correlated with in vitro functional T cell activities. The analysis of sIL-2R levels and functional in vitro studies were performed at the same time. Panel A illustrates the different patterns observed in patients tested for their proliferative response to PHA. Although in a few patients the enriched T cells displayed a normal or near-normal differentiation capacity did not differ from that observed comparing sIL-2R levels and the whole number of WBC.

In this study we documented that patients with B-CLL display serum levels of sIL-2R that are significantly higher than in normal, age-matched controls. This finding confirms and extends a recent observation by Greene et al. The presence in the serum of these patients of high quantities of sIL-2R may, in turn, play a role in the establishment of a number of functional abnormalities that have been described within the residual T cell population in B-CLL. Although the increase in sIL-2R was found in all stages of the disease, the lowest levels of sIL-2R were nonetheless observed in patients in stage 0, with a WBC count lower than $25 \times 10^9/L$, with a CD4/CD8 ratio greater than 1.1, and with serum immunoglobulin values higher than 15 g/100 mL. Furthermore, when the sIL-2R was correlated with the functional status of B-CLL T lymphocytes, it was found that patients with the lowest levels showed the best response to PHA and helper capacity.

Although the exact function of sIL-2R remains to be clarified, the ability of this molecule to bind to IL-2 suggests a potential role in the regulation of IL-2-dependent cell functions. The increased levels of sIL-2R found in B-CLL, associated with the evidence that B-CLL cells are capable of expressing IL-2R and absorbing variable amounts of IL-2, may play a role towards removing the available IL-2. This would represent a reasonable explanation for some of the immunologic functional abnormalities reported in this disease, which are basically mediated by IL-2. In line with our suggestion, it has been demonstrated that the addition of exogenous IL-2 is capable of partially correcting the in vitro T cell proliferative response to mitogens, of inducing a cytotoxic activity, and of improving T cell colony growth.

A major problem that remains is the definition of the type of IL-2 receptors in B-CLL. It has been demonstrated recently that different affinity forms of the IL-2 receptor exist, notably high- and low-affinity receptors. Only the high-affinity receptors seem to mediate the growth-promoting response to IL-2, whereas the function of low-affinity receptors remains undefined. We presently do not know the precise form and the actual function of the sIL-2R present in B-CLL sera. However, preliminary studies performed in our laboratory point to the immunoregulatory role of sIL-2R in these patients. In fact, B-CLL sera were able to inhibit the proliferative in vitro response of normal lymphocytes to PHA, the highest inhibitory capability being provided by sera with the highest levels of sIL-2R. The inhibi-

### Table 1. sIL-2R Values in Different Groups of B-CLL Patients

<table>
<thead>
<tr>
<th>Different Groups of B-CLL Patients</th>
<th>n</th>
<th>sIL-2R Values (U/mL)</th>
<th>r,†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with WBC ($x 10^9$/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>14</td>
<td>1,157 ± 229</td>
<td></td>
</tr>
<tr>
<td>25-49</td>
<td>20</td>
<td>1,937 ± 495</td>
<td>.29 (P &lt; .05)</td>
</tr>
<tr>
<td>50-75</td>
<td>11</td>
<td>1,896 ± 382</td>
<td>(n = 54)‡</td>
</tr>
<tr>
<td>&gt; 75</td>
<td>9</td>
<td>2,264 ± 603</td>
<td></td>
</tr>
<tr>
<td>Patients with CD4/CD8 ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1.1</td>
<td>17</td>
<td>1,257 ± 217</td>
<td></td>
</tr>
<tr>
<td>1.1-0.5</td>
<td>19</td>
<td>2,049 ± 491</td>
<td>.23 (P = NS)</td>
</tr>
<tr>
<td>&lt; 0.5</td>
<td>11</td>
<td>1,708 ± 354</td>
<td>(n = 47)‡</td>
</tr>
<tr>
<td>Patients with serum Ig (mg/100 mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 15</td>
<td>14</td>
<td>1,073 ± 161</td>
<td>.33 (P &lt; .05)</td>
</tr>
<tr>
<td>15-8</td>
<td>24</td>
<td>2,006 ± 324</td>
<td>(n = 49)‡</td>
</tr>
<tr>
<td>&lt; 8</td>
<td>11</td>
<td>2,597 ± 796</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SE. The mean values among different groups were not statistically significant.
†Correlation between sIL-2R and different hematologic parameters. Spearman’s rank correlation test was used.‡All patients taken as a group.
HIGH SERUM LEVELS OF sIL-2R IN B-CLL

A possible explanation for the lack of a strict correlation between serum levels of sIL-2R and relevant clinicobiologic features (Fig 2 and Table 1) is that the suggested defective availability of IL-2 may interfere with a large number of immunologic functions. As a result, the complex balance of immunoregulatory circuits makes it difficult to correlate strictly the serum levels of sIL-2R with individual immunologic parameters. The defective availability of IL-2 due to the presence of increased levels of sIL-2R may affect both the residual T and B cell populations in B-CLL patients, ultimately leading to defective immunoregulation, which may account for the hypogammaglobulinemia, susceptibility to infections, and incidence of second neoplasia often observed in this disease.6,36-39

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High serum levels of soluble interleukin 2 receptor in patients with B chronic lymphocytic leukemia

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