High Serum Interleukin-2 Receptor Levels Are Related to Advanced Disease and a Poor Outcome in Childhood Non-Hodgkin’s Lymphoma


The clinical usefulness of serum interleukin-2 receptor (IL2R) measurements was determined in 59 children with non-Hodgkin’s lymphoma (NHL) and six with B cell acute lymphoblastic leukemia (B-ALL). Levels of the receptor showed a clear relationship to disease stage, as follows: B-ALL > stage III or IV diffuse small noncleaved-cell NHL > stage III or IV lymphoblastic NHL > stage I or II NHL. Soluble IL2R levels were also closely correlated with serum lactic dehydrogenase levels (r = 0.7, P = .0001), a recognized indicator of tumor cell burden. Children with higher soluble IL2R levels (>1,000 U/mL) were significantly more likely to fail treatment (P = .001). Even when the analysis was limited to those with more advanced disease: stages III and IV NHL and B-ALL (P = .02). In a multivariate analysis, soluble IL2R level was found to have greater predictive strength than either serum lactic dehydrogenase level or disease stage. Thus, the measurement of soluble IL2R in children with NHL could be expected to improve existing methods of risk assignment in this disease.

INTERLEUKIN 2, originally termed T cell growth factor, is synthesized and secreted by antigen- or lectin-activated T lymphocytes in the presence of macrophage-derived interleukin-1 and must interact with specific high-affinity membrane receptors to exert its biological effects. The interleukin-2 receptor (IL2R or Tac antigen) is not present on the surface of resting T or B lymphocytes but is rapidly expressed following activation. IL2R has also been found on certain B or T cell malignancies including Burkitt’s lymphoma, hairy cell leukemia, and human T-cell leukemia virus (HTLV)-I-associated adult T cell leukemia.

Using monoclonal antibodies directed against different epitopes of human IL2R, Ruben et al detected a soluble form of IL2R that is released by activated normal peripheral blood mononuclear cells and synthesized in large amounts in vitro by HTLV-I-infected leukemic cell lines. The soluble IL2R is smaller than its cellular counterpart but retains the ability to bind interleukin 2. Subsequent studies disclosed comparable levels of soluble IL2R in cord blood and peripheral blood from normal adults. Increased serum levels of IL2R have been found in patients with certain B or T cell malignancies, including HTLV-I-associated adult T cell leukemia, Sézary syndrome, Hodgkin’s disease, chronic lymphocytic leukemia, and hairy cell leukemia.

Because of the role of IL2R in T and B cell proliferation and the possibility that serum levels of the receptor influence responsiveness to chemotherapy, we measured soluble IL2R in a relatively large group of children with non-Hodgkin’s lymphoma (NHL) or B cell acute lymphoblastic leukemia (B-ALL). The receptor was present in serum samples from all patients, was found in higher levels in those with more advanced disease, and proved to have independent prognostic significance.

MATERIALS AND METHODS

Patients. From 1979 to 1986, 99 consecutive children with NHL or B-ALL were admitted to three clinical trials at St Jude Children’s Research Hospital, depending on the histologic features and stage of their disease. Serum samples taken before the start of chemotherapy were available for 65 of these patients. The 41 boys and 18 girls with NHL ranged in age from 1.8 to 17.9 years (median, 10.7 years). The three boys and three girls with B-ALL were 2.3 to 10.2 years old (median, 5.8 years). In each case the diagnosis was based on a combination of clinical, anatomic, histologic, and cytologic criteria, with addition of immunologic and cytogenetic studies in some instances. The Working Formulation was used to classify cases into diffuse small noncleaved-cell, lymphoblastic, or large cell (noncleaved, cleaved, and immunoblastic) types. The first category encompasses not only Burkitt’s tumor but also lymphomas that have been designated as undifferentiated non-Burkitt’s type. The diagnosis of B-ALL was made from the presence of surface immunoglobulins on bone marrow blast cells with L3 morphology according to French-American-British (FAB) criteria.

A stage was assigned to each case of NHL with use of a previously described system. Children with localized NHL in favorable sites have stage I or II disease. Stage III includes disseminated disease on both sides of the diaphragm, extensive unresectable intraabdominal disease, and all primary epideral or anterior mediastinal tumors without bone marrow or CNS involvement. Stage IV is defined by initial CNS and/or bone marrow involvement (>25% blasts) in addition to other tumor sites. Cases with >25% malignant B cells in the bone marrow were classified as B-ALL, representing advanced B cell NHL in a phase of leukemic evolution. In this study, 30 patients had diffuse small noncleaved-cell NHL (stage I in eight, II in ten, III in 11, and IV in one); 22 had lymphoblastic NHL (stage I in one, II in two, III in 14, and IV in five); seven had large cell NHL (stage I in three, and II in four) and six had B-ALL.

Soluble IL2R was also measured in 12 children with otitis media (OM) who were otherwise normal and ranged in age from 1 to 6 years (median, 3 years).

Treatment. Children with stage I or II NHL were treated according to a Pediatric Oncology Group (POG) protocol that uses three cycles of cyclophosphamide, doxorubicin, vincristine, and prednisone for remission induction and consolidation therapy and 6
months of 6-mercaptopurine and methotrexate for continuation therapy. Children with stage III or IV small noncleaved-cell NHL or B-ALL received 6 months of intensive treatment with alternating courses of either high-dose fractionated cyclophosphamide followed by vincristine and doxorubicin or coordinated high-dose methotrexate and cytarabine. Patients with stage III or IV lymphoblastic NHL were treated on a protocol designed for high-risk ALL in which teniposide plus cytarabine was added to an otherwise conventional regimen of therapy. Informed consent was obtained for all patients, and the investigation was approved by the institution's clinical trials committee.

**Determination of soluble IL2R.** Soluble IL2R was measured with a sandwich enzyme immunoassay available as the CELLFREE Interleukin-2 Receptor Test Kit (T-cell Science, Inc, Cambridge, MA). In brief, a mouse monoclonal antibody to human IL2R was first adsorbed onto a polystyrene microtiter well. A patient's sample or standard was added to the antibody-coated well and unreacted sample components were removed by washing. A second horseradish peroxidase-conjugated murine monoclonal antibody to human IL2R was then used to bind a second epitope on the molecule captured by the first antibody. After removal of unbound enzyme-conjugated anti-IL2R by washing, a substrate solution was added to the well. After terminating the reaction by a stop solution, the absorbance at 490 nm was measured. The average value of absorbance from duplicate samples was then plotted on a standard curve and converted to a numerical value. Serum levels of IL2R are expressed in units per milliliter. A reference preparation of 1,000 U/mL of supernatant from phytohemagglutinin-stimulated peripheral blood lymphocytes was used as a standard. The normal serum IL2R values in healthy adult donors range from 50 to 500 U/mL (mean, 260 U/mL).

**Determination of serum lactic dehydrogenase.** The total activity of serum lactic dehydrogenase (LDH) was measured with the Monitor Kinetic AMB-610 assay on the KDA analyzer (American Monitor Corp, Indianapolis). Samples with enzyme activities >700 U/L were diluted and reassayed; the values obtained were then multiplied by the dilution factor. The normal values for our laboratory range from 30 to 300 U/L.

**Statistical analysis.** The Kruskal-Wallis test was used to compare soluble IL2R or serum LDH levels among different subgroups of patients with NHL. The Pearson product-moment correlation (r) and t test analyses were used to determine the association between soluble IL2R and serum LDH levels. Time-to-failure curves were constructed by the Kaplan-Meier procedure with differences analyzed by the log-rank test. Time to failure was defined as the interval between achievement of remission and relapse or death due to any cause. Patients who did not enter remission were assigned a failure time of zero. The influence of potentially significant prognostic factors on time to failure was estimated with the Cox proportional-hazards model, which permits comparison of treatment outcome for two or more subsets of patients while simultaneously adjusting for the effect of other factors (covariates) in the model. Final selection of factors for the model depended on whether or not the P value was <.10 after adjustment for other variables already in the model, using a forward stepwise procedure. An IL2R value of 1,000 U/mL was chosen as the dividing point between high and low receptor levels because it coincided well with clinically distinct groups of diseases and treatment outcome.

**RESULTS**

All patients had detectable soluble IL2R levels (Fig 1), including the 12 children with OM (404 to 942 U/mL; median, 615 U/mL). The highest values were found in sera from patients with B-ALL (1,030 to 17,725 U/mL; median, 3,283 U/mL). Children with stage III or IV small noncleaved-cell NHL had soluble IL2R levels of 329 to 5,335 U/mL (median, 1,832 U/mL), significantly higher than the
correlation between serum IL2R and LDH levels in this study (Fig 2).

Higher soluble IL2R levels were associated with a poorer treatment outcome. Nine of 18 patients with levels above 1,000 U/mL, compared with only six of 47 with lower levels, have failed therapy (Fig 3A, $P = .001$). Even when patients with stage I or II disease were excluded, high levels of soluble IL2R were still associated with a poor outcome (Fig 3B, $P = .02$). Because of the possible interrelationships among clinical and biologic risk factors in our patients, a Cox regression analysis was used to assess the relative importance of each factor after adjustment for the effects of other covariates. As shown in Table 1, both soluble IL2R level and serum LDH level were found to have independent prognostic value.

**DISCUSSION**

The most reliable prognostic factors in childhood NHL have been the stage of disease at diagnosis and serum LDH level.\textsuperscript{12,16,20,21} We report here that high soluble IL2R levels predict a poor treatment outcome even after adjustment for these two factors. The increased serum IL2R levels in our patients could reflect greater release of the receptor from either malignant cells or activated normal lymphocytes. We favor the first explanation because soluble IL2R levels not only correlated with disease stage but also showed a linear relationship with serum LDH levels, a reliable indicator of the total body burden of malignant cells in both ALL\textsuperscript{22} and NHL.\textsuperscript{12,20,21}

**Fig 2.** Comparison of log serum IL2R levels and log serum LDH levels. The straight line represents the least-squares regression fit to the data.

376 to 3,390 U/mL (median, 808 U/mL) for patients with stage III or IV lymphoblastic NHL ($P = .02$). The latter concentrations were in turn greater than those found in stage I or II NHL, 100 to 1,143 U/mL (median, 477 U/mL; $P = .001$). Only one of the 28 subjects with low-stage disease had a soluble IL2R level above 1,000 U/mL. The distribution of serum LDH levels among the various subgroups of patients showed the same pattern as was noted for IL2R: B-ALL > stage III or IV diffuse small noncleaved-cell NHL > stage III or IV lymphoblastic NHL > stage I or II NHL (data not shown). There was, in fact, a strong positive correlation between serum IL2R and LDH levels in this study (Fig 2).

**Fig 3.** Comparison of time-to-failure rates according to IL2R for (A) all patients with NHL and B-ALL; (B) patients with stage III or IV NHL or B-ALL. Significantly worse treatment results were evident for patients with higher levels (>1,000 U/mL) in both comparisons.
Table 1. Relationship of Serum IL2R Level and Other Presenting Features to Time to Failure in 65 Children With NHL or B-ALL

<table>
<thead>
<tr>
<th>Feature</th>
<th>Category</th>
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<tr>
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*From the likelihood ratio test.
†Comparison of time to failure, with use of the Cox proportional-hazards model, between patients with the better v worse risk feature for relapse in the indicated category, without adjustment for the effects of other variables.
‡As above, except that stepwise regression analysis was used to identify the best predictors of treatment outcome, taking into account the competing effects of all covariates entered in the Cox model.

The function of cellular IL2R in lymphoid malignancies has not been fully elucidated. Several cases of common, pre- or T cell ALL have been induced to express IL2R after in vitro activation and, in some cases, interleukin 2 stimulated subsequent colony formation of neoplastic progenitor cells in vitro. Leukemia cells from some patients with T cell chronic lymphocytic leukemia were shown to have the receptors and a good proliferative response to exogenous interleukin 2. However, HTLV-1 associated adult T cell leukemia constitutively expressed high levels of cell surface IL2R but had no (or very poor) proliferative responses to interleukin 2. Even less is known about the functional significance of soluble IL2R. Since soluble IL2R is capable of binding interleukin 2, it may have an immunoregulatory role by competing with cellular IL2R for the ligand and thus down-regulating the immune response. In this regard, the soluble IL2R has been suggested to be a "blocking factor" produced by the malignant cells to inhibit the host's immune response to the tumor.

Children with OM were found to have elevated soluble IL2R levels compared with that reported for healthy adults. In fact, their levels were higher than those of patients with stage I or II NHL (P = .02). These increased levels were probably the result of lymphocyte activation in response to infection. In view of this finding, one could question the usefulness of soluble IL2R levels as indicators of residual tumor, as suggested by Rubin et al.

We conclude that the level of soluble IL2R in children with NHL has independent prognostic significance, higher levels being associated with more advanced disease, greater tumor burden, and a poorer outcome. Understanding the biological significance of soluble IL2R will assume increased importance with wider use of biologic response modifiers in cancer therapy.

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