Soluble Interleukin 2 Receptor Levels in Children with Type I Insulin-dependent Diabetes Mellitus*†

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ABSTRACT

Soluble interleukin 2 receptor (sIL-2R) levels reflect mononuclear cell activation and are elevated in a variety of autoimmune, neoplastic and infectious conditions. Several investigators have studied sIL-2R levels in patients with Type I diabetes mellitus (IDDM), but results have been conflicting. Our primary objective in this study was to compare sIL-2R levels of children and adolescents with newly diagnosed IDDM with those of age-matched controls. In addition, sIL-2R levels in a cohort of patients were followed longitudinally for 1 to 2 years after diagnosis. Serum sIL-2R levels of 38 IDDM children and adolescents (age <20 years) were compared with levels of 39 nondiabetic, age-matched controls. Mean sIL-2R levels declined with age (P < 0.000005), and there was no significant difference in the regression line relating age and sIL-2R levels between
patients and controls. The sIL-2R levels remained fairly consistent over 1–2 years of follow up. The presence of islet cell antibodies (ICA) had no apparent effect on sIL-2R levels in children with diabetes. The sIL-2R levels were similar in magnitude among first degree relatives of patients with IDDM compared to the range of unrelated subjects. It is our conclusion that sIL-2R levels are highest during infancy and decline throughout childhood. The sIL-2R levels do not appear to be clinically useful as a reflection of immune activation in patients with IDDM. Finally, there may be a genetic influence which partially regulates production of sIL-2R.

Introduction

Interleukin-2 (IL-2), along with its specific interleukin-2 receptor (IL-2R), is produced by T lymphocytes after antigenic or mitogenic stimulation. The interaction of IL-2 with IL-2R leads to clonal expansion of these activated T cells. Also, IL-2 has a role in the modulation of the immunological responses of cytotoxic T cells, natural killer cells, activated B lymphocytes and lymphokine-activated cells. Negative regulatory effects of IL-2 have been described and include the programming of mature T cells for apoptosis.

The effects of IL-2 are mediated through interactions with various isoforms of the IL-2 receptor. The three forms of IL-2R which are known to be expressed on human peripheral blood mononuclear cells are composed of various combinations of protein chains known as alpha, beta, and gamma chains. The high affinity IL-2 receptor is composed of all 3 chains (alpha, beta, and gamma) and is expressed by activated T lymphocytes. It is the interaction of IL-2R with this receptor isoform that leads to T cell proliferation and regulation of cytotoxicity and cytokine production. This high affinity IL-2 receptor also mediates the role of IL-2 in activated B lymphocyte and natural killer cell proliferation and function.

A soluble form of the IL-2R (sIL-2R) is also released and can be extracted from cell-free supernatant after in vitro T cell stimulation. Although the function of sIL-2R is not known, it appears to reflect immune system activation and may have a role in the regulation of IL-2 dependent cell function. Because sIL-2R is thought to be a sensitive marker of immune activation, there has been interest in quantifying levels in a variety of neoplastic, autoimmune, and infectious conditions. High levels of sIL-2R have been found in patients with human T lymphotropic virus Type 1-associated adult T cell leukemia, hairy cell leukemia, Hodgkins disease, non-Hodgkins lymphoma, and human immunodeficiency virus (HIV) infection. Autoimmune or inflammatory diseases in which sIL-2R levels have been evaluated include rheumatoid arthritis, Grave’s disease, systemic lupus erythematosi, multiple sclerosis, and diabetes mellitus. In some cases, sIL-2R levels have been found to reflect both the presence and intensity of disease activity when measured in a serial fashion.

Type I diabetes mellitus (IDDM) is an autoimmune disease with a prolonged preclinical phase characterized by gradual destruction of beta cells. Serum autoantibodies directed against islet cells and endogenous insulin have been used as markers of this diabetogenic process. Patients at risk for developing clinical diabetes have been identified and treated with immunosuppressive agents (i.e., cyclosporin A) and other forms of early intervention with variable degrees of success. Recent studies have sug-
gested that IL-2 may be an important mediator of the immune response in IDDM. Interleukin-2 receptor-targeted immunotherapy has been shown to extend the survival of major histocompatibility (MHC)-identical allogenic islet grafts in diabetic BB rats. The IL-2 receptor targeted fusion toxin has been shown to block diabetogenic autoimmunity in non-obese diabetic (NOD) mice. Human trials of related IL-2 receptor targeted therapies have been initiated.

Clearly, the ability to identify a marker of T cell activation would be useful in selection and follow up of patients for experimental therapies. Several authors have measured serum sIL-2R levels in patients with diabetes and prediabetes, but results have been conflicting.

Our aims in this study* were to compare sIL-2R levels of newly diagnosed children and adolescents with Type I IDDM with those of an age-matched control group and to follow longitudinally sIL-2R levels in a cohort of patients over the first 1 to 3 years after diagnosis, a time during which disease activity appears to change significantly.

Methods and Materials

SUBJECTS

Thirty eight children with newly diagnosed Type I IDDM were studied. Twenty females and 18 males with a mean (±SD) age of 9.3 ± 4.3 years were included in this group. Ten of these patients presented in diabetic ketoacidosis. Blood samples were obtained within the first few days after diagnosis in all patients and serum samples were frozen at −20°C until the assays were performed.

In 17 patients, serum samples which were obtained between 8 to 17 months after diagnosis were also frozen and later analyzed for serum sIL-2R levels. In 14 patients, samples drawn between 18 to 30 months after diagnosis were analyzed for sIL-2R levels. Islet cell antibodies (ICA) were determined in 8 patients and 17 first degree relatives (8 siblings and 9 parents).

The control group included 31 healthy children with either idiopathic short stature or treated, euthyroid, congenital hypothyroidism. There is no evidence that either of these conditions effects immune function. None of these children had known autoimmune conditions or other significant illnesses when examined during routine outpatient visits. Children were not excluded from the control group or the diabetes group owing to a history of recent minor illnesses. In a study of children with idiopathic nephrotic syndrome, Bock et al found that the presence of intercurrent minor illnesses did not effect sIL-2R levels. Eight siblings of patients with IDDM were also studied. None of these siblings had ICA. The mean (±SD) age of the control group was 7.7 ± 5.2 years. Seventeen females and 22 males were studied. Ten parents of 11 diabetic patients were also studied but were not included in the control group. The sIL-2R levels of 7 family groups, including 7 patients, 7 parents, and 7 siblings, were evaluated separately.

ASSAY

The Cellfree Interleukin-2 receptor test kit† is an enzyme linked immunosorbent assay (ELISA) which was used for determination of sIL-2R levels in this study. All samples were run in duplicate. The detection limit of this assay is 50

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* This study received prior approval of the Human Research Committee and the Institutional Review Board of the Department of Clinical Investigation, Walter Reed Army Medical Center, Washington, DC.

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U/ml IL-2R. The intra-assay coefficient of variation is <4 percent. The inter-assay coefficient of variation is <6 percent.

The presence of pancreatic islet cell autoantibodies was determined by indirect immunofluorescence using fresh frozen human pancreatic tissue. These studies were performed at the University of Florida at Gainesville.19

STATISTICAL ANALYSIS

Data were analyzed using the two tailed Student’s t test. Comparison of sIL-2R levels between the diabetics and controls was also examined using analysis of variance, with the covariate identified as age. The sIL-2R values were logarithmically transformed to satisfy assumptions for normality.

RESULTS

As shown in table I, there was no difference in mean ages or sIL-2R levels between patients with IDDM at the time of diagnosis compared to controls.

As shown in figure 1, sIL-2R levels declined with age (P < 0.000005, analysis of covariance). When age was included as a covariate for analysis, there was no significant difference between the sIL-2R levels of patients compared to controls.

| TABLE I |
| Mean Ages and Serum Soluble Interleukin 2 Receptor Levels of Patients with Type I Diabetes Mellitus and Controls |

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Range</th>
<th>Median</th>
<th>sIL-2R (U/ml)</th>
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</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>yr</td>
<td>Age (yr)</td>
<td>Mean ± SD</td>
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Diabetes
(n=38) 9.3±4.3 1.08–19.6 9.4 1106 ± 105

Control
(n=39) 7.7±5.2 0.08–19.6 8.2 1067 ± 99

There was no significant difference in the regression lines relating age and sIL-2R levels between patients and controls. There was no difference between sIL-2R levels in males vs. females in either the diabetic or control group. The mean sIL-2R level of males with diabetes was 1157 ± 497 U/ml compared to 1061 ± 754 U/ml for females. The mean sIL-2R level of male controls was 1139 ± 672 U/ml compared to a level of 974 ± 552 U/ml for females. Patients who presented in diabetic ketoacidosis (n = 10) had a mean sIL-2R level of 1368 ± 335 U/ml. This did not differ from the mean level of 1079 ± 104 U/ml obtained from 17 patients without DKA at diagnosis.

As shown in figure 2, sIL-2R levels appeared to remain fairly consistent over 1 to 2 years of follow up. There were no differences between mean levels at diagnosis of 1187 ± 145 U/ml and at 8 to 17 months of 1353 ± 227 U/ml or 18 to 30 months of 1040 ± 111 U/ml. As shown, individual sIL-2R levels appeared to be fairly consistent over time.

Of interest, family groups appeared to have sIL-2R levels which clustered fairly closely compared to the ranges of non-related subjects (figure 3). This observation was true for both patients and non-affected family members. No statistical analysis or attempt to control for age was performed on these data owing to the small number of families which were studied.

Finally, in the 8 patients with IDDM who were tested for ICA, 5 had antibodies present. The mean sIL-2R level of 907 ± 221 U/ml of the patients with antibodies did not differ from the level of 714 ± 218 U/ml of patients without antibodies. Islet cell antibodies were not found in any of the non-affected family members who were tested.

Discussion

A few studies have previously evaluated the usefulness of serum sIL-2R as a
Figure 1. The sIL-2R levels of patients with IDDM (O) and controls (•) vs age. Linear regression analysis showed r value of 0.64 in patients with IDDM (--.--.) and 0.48 in controls (-----).

marker of immune activation in IDDM.\textsuperscript{11,12,13} Results of these studies are conflicting in spite of the fact that similar enzyme-linked immunosorbent assays (ELISA) were used in each of the studies. Giordano et al\textsuperscript{11} and Keller et al\textsuperscript{12} used antibodies* which were identical to those used in our study. Vialettes et al\textsuperscript{13} used an ELISA kit purchased from Immunotech.\textsuperscript{t}

\* Obtained from T Cell Sciences, Cambridge, MA.
\textsuperscript{t} Purchased from Marseille-Luminy, France.

Giordano et al\textsuperscript{11} found that mean sIL-2R levels of a group of 35 newly diagnosed patients with Type I diabetes was significantly higher than the mean level of a normal control group. Although these investigators reported no correlation between age and sIL-2R level, the age range of the diabetic patients and the number of children included in each study group were not stated.

Our study and that of Keller et al\textsuperscript{12} confirm previous observations that sIL-2R levels in children decline with age.\textsuperscript{20} In contrast to the results of Giordano et al,\textsuperscript{11}

Figure 2. The sIL-2R levels in a cohort of patients with IDDM who were tested at diagnosis (N = 26), at 8 to 17 months (N = 17) and/or at 18 to 30 months (N = 14) after diagnosis.
However, Keller et al.\textsuperscript{12} found that that sIL-2R levels of patients with newly diagnosed IDDM were lower than age-matched controls. These investigators divided subjects into age-groups with small numbers of children in each group. For example, there were only 7 controls compared with 4 diabetics in the <3 years old. Although the mean ages of the various groups are given, the medians are not stated, and the control group has about twice as many children who are ≤5 years of age. If sIL-2R levels are naturally higher in infants and very young children, this method of analysis may lead to misleading results. Vialettes et al.\textsuperscript{13} found a correlation between age and sIL-2R levels only in controls but not in patients with IDDM. The sIL-2R levels in patients with IDDM tended to be lower than control levels, though differences were not significant. The numbers of patients in various age groups were again small, i.e., 3 diabetic patients were compared with 10 controls in the <5 years old age group.

Our study is the largest to date in children and confirms the presence of a very highly significant negative correlation between age and sIL-2R levels in children. Using two different statistical methods of analysis, and a similar ELISA method as other studies, no significant difference was found between the sIL-2R levels of diabetic and control groups. In contrast to Keller et al.\textsuperscript{12} the relationship between sIL-2R and age was maintained in both study groups.

The sIL-2R levels have been elevated in a variety of conditions which have an autoimmune basis. Patients with rheumatoid arthritis\textsuperscript{7} have sIL-2R levels which parallel changes in clinical parameters of disease activity. The fact that sIL-2R levels are not elevated in patients with IDDM and do not vary with ICA status suggests that a different mechanism of immune dysregulation may occur in IDDM. Hyposecretion of IL-2 has been reported in patients with type I diabetes.\textsuperscript{21,22,23} Further understanding of this abnormal IL-2 secretion may help to delineate further the pathogenesis of IDDM.

It is not clear to what extent the altered IL-2 production in patients with IDDM is genetically predetermined. In exploring this question, Kaye et al.\textsuperscript{22} compared
IL-2 levels of patients with IDDM with levels of their nondiabetic twins. Although IL-2 levels of patients were consistently lower, there was a positive correlation between the levels of individual patients and their twins. These results suggest that genetic factors may influence IL-2 production. The consistency of sIL-2R noted over time in our patients and those previously studied support the possibility of genetic contributions to the regulation of sIL-2R as does our observation that family members have sIL-2R levels which cluster in a narrow range. Further research is needed to elucidate any possible genetic set point involving regulation of sIL-2R.

In summary, it is concluded that sIL-2R levels are highest during infancy and decrease with increasing age throughout childhood. Serum sIL-2R measurements are neither higher or lower in children with IDDM than in controls and do not appear to be clinically useful as a reflection of immune activation in this group of patients. Finally, there may be a genetic influence which partially regulates the production of IL-2 and sIL-2R.

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