Elevated Serum Levels of Soluble Interleukin-2 Receptor: A Marker of Disease Activity in the Hypereosinophilic Syndrome

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We report here the presence of very high serum levels of the soluble interleukin-2 receptor (sIL-2R) in patients with blood hypereosinophilia with or without detectable markers of malignancy or signs of visceral involvement. The highest sIL-2R levels were observed in 16 eosinophilic patients with T-cell lymphoma (3,440 to 79,500 U/mL). Elevated levels of sIL-2R were also present (1,330 to 22,500 U/mL) in sera from 38 patients with the hypereosinophilic syndrome (HES) without detectable T-cell lymphoma. In this group of patients, the highest levels were noted in patients with the malignant form of HES. Significantly lower levels were measured in sera of patients with hypereosinophilia associated with parasitic diseases, allergic disorders, or other miscellaneous diseases. Elevated serum sIL-2R levels were not closely paralleled by changes in the number of CD25-positive peripheral blood mononuclear cells as assessed by flow cytometric analysis. However, expression of IL-2R messenger RNA was detected in blood mononuclear cells collected from HES patients. In eight eosinophilic patients with T-cell lymphoma, the serum sIL-2R levels were significantly correlated with the eosinophil counts, and with the total number of blood hypodense eosinophils. α-Interferon (α-IFN) therapy resulted in both a dramatic clinical improvement and a rapid decrease in sIL-2R levels and blood hypereosinophilia. Similar beneficial effects of α-IFN were noted in patients with malignant HES who lacked a detectable T-cell lymphoma. Our data indicate that HES is associated with elevated serum IL-2R levels. The highest levels were observed in the most severe forms of HES with hematologic markers of malignancy or evident visceral involvement. Serum levels of sIL-2R might represent a useful indicator for the management of HES patients. In addition, the respective changes of sIL-2R and blood eosinophilia might reflect distinct processes of mononuclear cell activation affecting the eosinophil lineage.

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The hypereosinophilic syndrome (HES) is a heterogeneous group of diseases of unknown pathogenesis defined according to criteria proposed by Chusid et al. It is characterized by a blood eosinophil count greater than 1.5 × 10^9/L for at least 6 months without detectable causes of eosinophilia and with visceral involvement, thought to be related to a toxic effects of tissue eosinophil infiltration. Some cases show no evidence of progressive organ-system dysfunction and show a more favorable course. In contrast, other cases have been regarded as malignant forms due to the presence of myeloproliferative or leukemic markers, the presence of chromosomal abnormalities in lymphocytes, or the association with the possible evolution into a lymphoblastic leukemia or T-cell lymphoma. Various parameters have been used to discriminate between malignant (increased serum vitamin B12, abnormal leucocyte alkaline phosphatase scores, basophilia, thrombocytopenia) or nonmalignant forms of HES (elevated serum IgE levels, sensitivity to glucocorticoid therapy). Nevertheless, such criteria are not regularly found in HES patients and their significance in relation to the pathogenesis of HES remains to be clarified.

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T lymphocytes are known to play a critical role in the induction of blood hypereosinophilia. Blood eosinophilia can precede, sometimes by several years, the onset of cutaneous T-cell lymphoma for which a retroviral origin is suspected. The role of T-cell lymphokines both in the control of eosinophilosis or in eosinophil activation has previously led us to investigate the possible involvement of a T lymphotropic transforming retrovirus in the pathogenesis of some malignant HES. Eosinophilia can be associated both with human T-cell lymphotropic virus type 1 (HTLV1)-induced adult T-cell leukemia/lymphoma or T-cell lymphoblastic lymphoma, which are both characterized by the presence of very elevated serum levels of soluble interleukin-2 receptor (sIL-2R). It has been shown that sIL-2R levels provide valuable information for the management of hematologic neoplasias.

In the present study, we report the presence of high levels of sIL-2R in HES patients with or without detectable markers of malignancy. In these patients, separate eosinophil subpopulations were selected by a density difference centrifugation assay. Two eosinophil subpopulations were selected by a density difference centrifugation assay. Two eosinophil subpopulations were selected by a density difference centrifugation assay. Two eosinophil subpopulations were selected by a density difference centrifugation assay. Two eosinophil subpopulations were selected by a density difference centrifugation assay. Two eosinophil subpopulations were selected by a density difference centrifugation assay.

MATERIALS AND METHODS

Patients. The study included 38 patients fulfilling the diagnostic criteria of the HES as previously defined by Chusid et al. These patients (11 women and 27 men, mean age, 41.5 years) were classified according to various clinicopathologic situations as fol-
of each total RNA was separated under denaturing conditions by cesium chloride method as previously described. Ten micrograms was determined by subtraction of the binding of an unrelated HES patients was isolated using the guanidine isothiocyanate-Coulter Electronics, Hialeah, FL). The percentage of positive cells restricted to mononuclear cells using structural and size parameters.

Isolation of total cellular RNA. Total RNA from mononuclear cells purified from the different groups of HES patients was isolated using the guanidine isothiocyanate-cesium chloride method as previously described. Ten micrograms of each total RNA was separated under denaturing conditions by electrophoresis through a 1.2% agarose/6% formaldehyde gel and transferred to a nylon membrane (Hybond N, Amersham, UK).

RESULTS

Serum levels of sIL-2R in hypereosinophilic patients. The highest sIL-2R values were found in 16 patients with T-cell lymphoma associated with blood hypereosinophilia (geometric mean, 12,111 U/mL). These levels were significantly higher than those observed either in patients with HES or in patients with other hematologic malignancies who lacked blood eosinophilia (T-cell lymphoma, 3,785 ± 3,300 U/mL; various hematologic neoplasias, 1,652.2 ± 791 U/mL) (Fig 1). All patients having different forms of HES possess serum levels of sIL-2R (1,330 to 22,500 U/mL, n = 38) significantly higher than those observed in controls or in patients with eosinophilia of a well-defined etiology (Fig 1). Patients with hypereosinophilia associated with parasitic diseases had intermediate levels of sIL-2R (663.4 U/mL, n = 24). These levels were clearly elevated above the levels of sIL-2R measured in the healthy controls but lower than the levels observed in patients with HES. In patients with allergic disorders (374.8 U/mL, n = 19) or with transient eosinophilia associated with other causes (446 U/mL, n = 6), the serum sIL-2R levels were not significantly different from those noted in healthy control individuals (436.4 U/mL, n = 17).

The level of sIL-2R correlated with various subgroups of patients with HES. More elevated values were found in HES (C) or the malignant forms of HES (8,021 ± 1,639 U/mL). These values were significantly higher than those observed in patients with HES (B) or HES patients with clinical complications (3,345.4 ± 938 U/mL; P < .02). The latter group also had significantly higher sIL-2R levels than patients with idiopathic HES (A) lacking organ system dysfunction (1,493.6 ± 461 U/mL; P < .02).

Analysis of blood mononuclear cells in HES patients. To investigate whether the blood mononuclear cells from HES patients were the major source of serum sIL-2R, we studied the presence of membrane p55 IL-2R subunit on mononuclear cells and the expression of IL-2R messenger RNA (mRNA) in purified blood mononuclear cells. First, elevated sIL-2R levels were not closely paralleled by changes
in the number of CD25-positive mononuclear cells, as detailed in Table 1. No significant correlation between these parameters was shown in any case of HES ($R = .164$).

As illustrated in Fig 2, IL-2R mRNA expression was detected in blood mononuclear cells from patients with malignant HES having or lacking a detectable T-cell lymphoma. Northern blot analysis also showed expression of IL-2R mRNA in blood mononuclear cells collected from some patients with idiopathic HES.

**Eosinophil distribution in HES patients.** In all eosinophilic patients, we evaluated the distribution of peripheral blood eosinophils on metrizamide gradients by a highly reproducible cell purification procedure. As illustrated in Fig 3, the absolute number of hypodense eosinophils collected in 20%, 22%, and 23% metrizamide solutions was significantly higher in 27 eosinophilic patients with associated hematologic malignancies, or in 10 idiopathic HES patients presenting evident clinical abnormalities, than those observed in other patients with idiopathic HES ($P < .02$) or various eosinophilic diseases ($P < .01$).

**sIL-2R levels related to variable parameters.** The levels and changes in serum sIL-2R were related to the levels and changes of blood leukocyte counts (mononuclear cells, granulocytes) in HES patients. A significant correlation was only shown between sIL-2R and blood eosinophil counts ($R = .76; P = .014$) and hypodense eosinophil counts ($R = .70; P = .038$) and this solely in eight eosinophilic patients having T-cell lymphoma (Fig 4). Only the untreated patients were analyzed (8 of 16). Corticosteroids and/or hydroxyurea or other cytotoxic agents (vincristine sulfate, thioguanine, cytosine-arabinoside, cyclophosphamide, adriamycin) have been used according to various protocols in all cases of malignant HES with or without detectable T-cell lymphoma. Six cases (cutaneous T-cell lymphoma [$n = 3$], T-cell lymphoma with adenopathy and hepatosplenomegaly [$n = 1$], HES with myeloproliferative markers [$n = 2$]) were unresponsive to conventional chemotherapy. This result led us to use α-IFN therapy (3 to $18 \times 10^6$ U subcutaneously daily). A dramatic clinical response was noted in all patients and sIL-2R levels changed closely in parallel with the level of total blood eosinophils and hypodense eosinophils. Representative data are illustrated in Fig 5 for three eosinophilic patients having T-cell lymphoma (Fig 5A, B, and C). In the latter case, responsive to conventional chemotherapy, no strict relation was found between sIL-2R levels and blood eosinophilia. In addition, a transient high increase in the sIL-2R

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**Table 1. Comparative Serum sIL-2R Levels and Numbers of CD25-Positive Blood Mononuclear Cells in HES Patients**

<table>
<thead>
<tr>
<th>Serum sIL-2R Levels (U/mL)</th>
<th>CD25-Positive Mononuclear Cells %</th>
<th>Cells/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>HES patients (A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>830*</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>750*</td>
<td>7</td>
<td>176</td>
</tr>
<tr>
<td>(B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,800†</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>1,150</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>3,200</td>
<td>28</td>
<td>700</td>
</tr>
<tr>
<td>(C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,050‡</td>
<td>1</td>
<td>170</td>
</tr>
<tr>
<td>9,800</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>3,650</td>
<td>2</td>
<td>39</td>
</tr>
<tr>
<td>6,050</td>
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<td>29</td>
</tr>
<tr>
<td>5,500</td>
<td>11</td>
<td>51</td>
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<tr>
<td>Eosinophilia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16,700</td>
<td>3</td>
<td>51</td>
</tr>
<tr>
<td>T-cell lymphoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,100</td>
<td>23</td>
<td>520</td>
</tr>
<tr>
<td>4,580</td>
<td>26</td>
<td>312</td>
</tr>
<tr>
<td>3,440</td>
<td>8</td>
<td>96</td>
</tr>
</tbody>
</table>

*HES (A): patients without clinical complications.
†HES (B): evident visceral involvement.
‡HES (C): malignant HES.
HIGH SERUM LEVEL OF sIL-2R IN HES

Fig 2. IL-2R mRNA expression in purified blood mononuclear cells collected from eosinophilic patients with T-cell lymphoma (eosinophilia/T-cell lymphoma), malignant form of HES (C), and idiopathic HES having (B) or lacking (A) visceral lesions. Mononuclear cells stimulated with (+) or without (−) anti-CD3 monoclonal antibody for 2 hours were used as positive and negative controls.

level was noted (Fig 5D). This increase might conceivably be attributable to tumor lysis during the treatment.

DISCUSSION

T lymphocytes are known to play a critical role in the induction of blood hypereosinophilia. Consequently, it seemed important to evaluate T-cell activation in patients with HES at the onset or during the progress of the disease. Previous work has clearly shown that the serum sIL-2R level is a sensitive and reliable quantitative marker of mononuclear cell activation in a wide spectrum of human diseases. Our results show different degrees of elevation of serum sIL-2R with distinct forms of HES. This might reflect variable processes in the dysregulation of IL-2R gene expression and might be informative on the physiopathologic mechanisms involved.

In idiopathic HES without detectable markers of malignancy, we found unexpectedly increased levels of sIL-2R. This finding was associated with IL-2R mRNA expression detectable in peripheral blood mononuclear cells. Previous data have shown a particular sensitivity to IL-2 in HES patients. Indeed, T cells from eosinophilic patients produce IL-5 after IL-2 stimulation in vitro. In addition, IL-2 treatment in cancer therapy can lead to increased levels of blood-hypodense, activated eosinophils. In all these cases, the exact mechanisms of the induction of blood eosinophilia after IL-2 activation remain to be clarified.

Blood eosinophilia can precede, sometimes by several years, the onset of lymphoma or leukemia. Patients with malignant HES with leukemic or myeloproliferative markers had higher serum sIL-2R levels than patients with other hematologic malignancies examined. The patient selection and/or the stage of hematologic disorders might explain the fact that somewhat higher sIL-2R levels have been noted in previously reported studies. It has been shown that sIL-2R levels provide valuable information for the management of hematologic neoplasias although the precise etiologic mechanisms are not known.

Markedly elevated levels of sIL-2R could be the result of the release, by proteolytic cleavage, of cell surface IL-2R constitutively expressed on malignant transformed T cells. Highly elevated levels have been previously reported in HTLV1-induced T-cell leukemia, which is sometimes associated with blood eosinophilia. We found, similarly, high levels of sIL-2R in eosinophilic patients with T-cell lymphoma. Using the polymerase chain reaction technique, we have previously detected the HTLV1/tax proviral DNA sequence in peripheral blood mononuclear cell DNA from three of these patients. It is known that the tax protein induces IL-2R α-chain mRNA expression by transactiva-
Elevated serum sIL-2R values generally correlated with clinical evolution of T-cell lymphoma after chemotherapy. Nevertheless, the real incidence of retroviral infection in these disorders remains controversial and requires more extensive studies.

In six cases of malignant HES with or without detectable T-cell lymphoma, α-IFN therapy resulted in a dramatic beneficial clinical response associated with a rapid decrease in sIL-2R levels and blood hypereosinophilia. Similar therapeutic effects of α-IFN on sIL-2R levels have been recently described in hairy cell leukemia. The beneficial action of α-IFN has also been shown previously in two cases of malignant HES. Further studies are now underway to define the mechanisms involved in this response and to evaluate the true efficacy of such alternative therapy in HES patients.

Until now, there has been no evidence to suggest that IL-2R can be secreted in a form lacking the transmembrane domain. Because high serum sIL-2R levels can be associated with low or undetectable IL-2R expression on the blood mononuclear cell surface, the generation of sIL-2R in localized tissue sites may contribute to serum levels. Both the low level of expression of CD25 protein on the cell surface and the spontaneous expression of IL-2R mRNA noted in each group of HES patients might be also related to the rapid turnover of the p55 subunit. Peripheral blood mononuclear cells might thus be involved in the release of the soluble form of IL-2R. Alternatively, other cellular sources of sIL-2R can be postulated. We have recently shown that blood eosinophils with the hypodense phenotype can spontaneously express the IL-2R p55 α-chain on the cell surface. In addition, IL-5 has the ability to induce the release of the IL-2R p55 subunit from the cell surface of murine B lymphocytes. Because the presence of IL-5 in sera of HES patients has been recently shown, it would be of interest to investigate the effects of IL-5 on CD25-positive human eosinophils.

Elevated serum sIL-2R values generally correlated with
have been shown. In progressive lymphoma, high serum sIL-2R could be the result of T-cell reactivity against malignant lymphoid tumor cells. In most other cases, elevated levels might reflect hyperactivation of mononuclear cells by factors that remain undefined. It is, however, certain that monitoring of sIL-2R should provide useful information for the diagnosis and the follow-up of patients with HES.

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