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 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 the 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.

The idiopathic hypereosinophilic syndrome (HES) is a heterogeneous group of diseases of unknown pathogenesis defined according to criteria proposed by Chusid et al.1 HES 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 effect of tissue eosinophil infiltration.1 Some cases show no evidence of progressive organ-system dysfunction and show a more favorable course.2 In contrast, other cases have been regarded as malignant forms due to the presence of myeloproliferative or leukemic markers,1 the presence of chromosomal abnormalities in lymphocytes,3 or the association with the possible evolution into a lymphoblastic leukemia4 or a T-cell lymphoma.5 Various parameters have been used to discriminate between malignant (increased serum vitamin B12, abnormal leucocyte alkaline phosphatase scores, basophilia, thrombocytopenia)1,5 or nonmalignant forms of HES (elevated serum IgE levels, sensitivity to glucocorticoid therapy).5,7 Nevertheless, such criteria are not regularly found in HES patients and their significance in relation to the pathogenesis of HES remains to be clarified.

T lymphocytes are known to play a critical role in the induction of blood hypereosinophilia. Blood eosinophilia can precede, sometimes by several years,4 the onset of cutaneous T-cell lymphoma for which a retroviral origin is suspected.8 The role of T-cell lymphokines both in the control of eosinopoiesis or in eosinophil activation9,10 has previously led us to investigate the possible involvement of a T lymphotrophic transforming retrovirus in the pathogenesis of some malignant HES.11 Eosinophilia can be associated both with human T-cell lymphotropic virus type 1 (HTLV1)-induced adult T-cell lymphoma/leukemia12 or T-cell lymphoblastic lymphoma,13 which are both characterized by the presence of very elevated serum levels of soluble interleukin-2 receptor (sIL-2R).13,14 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 (normodense and hypodense eosinophils) that has previously allowed us to characterize immunologic15 and metabolic16 eosinophil heterogeneity. Predominantly activated hypodense eosinophil populations were found in HES patients having hematologic markers of malignancy or evident clinical abnormalities. The serum sIL-2R levels changed in close parallel with both the level of eosinophilia and the proportion of blood hypodense eosinophils in malignant HES after effective α-interferon (α-IFN) therapy. The association between clinical disease status and sIL-2R levels are studied, and the causes and consequences of blood hypereosinophilia are discussed.

MATERIALS AND METHODS

Patients. The study included 38 patients fulfilling the diagnostic criteria of the HES as previously defined by Chusid et al.1 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–cell purification procedure. The processing of blood samples for cell purification was the same for all patients. Mononuclear cells were separated from heparinized blood by Ficoll-Hypaque gradient centrifugation. Blood eosinophils were purified by centrifugation on discontinuous metrizamide gradients as previously described. Using this separation procedure, distinct eosinophil populations, i.e., eosinophil populations that sediment in fractions of low density (hypodense eosinophils in 20%, 22%, and 23% metrizamide solution), intermediate density (24% metrizamide solution), or a high-density zone (25% metrizamide solution), were collected. The degree of eosinophil purity and the yield of eosinophils recovered were evaluated for each fraction (cytocentrifuge smears and Giemsa staining). The total number of hypodense eosinophils was calculated as follows: total blood eosinophil count × yield (%) of eosinophils recovered in low-density fractions.

Flow cytometric analysis of cell surface CD25. After separation of mononuclear cells and washing, 2 × 106 cells in 100 μL phenol red-free Hank’s Balanced Salt Solution (HBSS) were stained using an indirect method. They were first incubated with 5 μg/mL of unlabeled antibody (anti-CD25; Becton Dickinson, Meylan, France) for 30 minutes at 4°C. After washing with HBSS, cells were incubated with fluorescein isothiocyanate (FITC)-labeled (Fab′), fragment of antimouse IgGl antibody (Silenus, Hawthorn, Australia) for 30 minutes at 4°C and were washed twice with HBSS. The cells were then analyzed using a flow cytometer (Epics Profile; Coulter Electronics, Hialeah, FL). The percentage of positive cells was determined by subtraction of the binding of an unrelated mouse antibody of the IgGl isotype. The present analysis was restricted to mononuclear cells using structural and size parameters.

Isolation and Northern blot analysis 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). After UV cross-linking, the blotted membrane was prehybridized for 2 hours at 42°C in hybridization solution (50% formamide, 5X SSPE) (1X SSPE is 0.02% sodium dodecyl sulfate [SDS], 180 mMol/L NaCl, 10 mmol/L sodium phosphate, 0.1 mmol/L EDTA), 5X Denhart’s solution, 200 μg/mL heterologous DNA, 200 μg/mL yeast RNA). The filters were then hybridized for 16 hours at 42°C in fresh hybridization solution containing a cDNA probe radiolabeled with 32P dCTP by random priming. After hybridization the filters were washed three times for 30 minutes at 65°C in 0.1% SDS, 0.1X SSPE and exposed to X-ray film (Kodak, Rochester, NY) at −70°C. The Ps5 IL-2R cDNA probe was the 0.9-kb EcoRI-EcoRI fragment of pIL-2-R19 generously provided by Dr W.C. Greene (Duke University, Durham, NC).

Statistical analysis. All data are reported as geometric mean ± SEM. The Student’s t-test was used to compare sIL-2R levels in individual groups. The relationships between sIL-2R levels and other biologic variables (logarithmically transformed laboratory data) were determined using the nonparametric Spearman’s rank correlation test (a P value less than .05 was considered significant).

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 ± 1,639.7 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 ($P < .01$).

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 leucocyte 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 $sIL-2R$
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.

Fig 3. Absolute number of blood hypodense eosinophils in various groups of HES compared with other eosinophilic diseases (HES-A, absence of detectable lesions; HES-B, visceral involvement; HES-C, malignant forms).

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 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-
Soluble IL2 receptor levels

Fig 4. Relationship between sIL-2R levels and, respectively, the number of blood eosinophils (A) or the number of blood hypodense eosinophils (B) in eosinophilic patients having T-cell lymphoma (logarithmically transformed data).

Fig 5. Serial measurements of (■) sIL2R levels compared with the changes of the total number of (□) blood eosinophils or (■) blood hypodense eosinophils in four eosinophilic patients with T-cell lymphoma (A, T-cell lymphoma, 6 × 10⁶ U α-IFN at the initiation of treatment and then 3 × 10⁶ U subcutaneously daily; B, cutaneous T-cell lymphoma, 6 × 10⁶ U α-IFN daily; C, cutaneous T-cell lymphoma, 6 × 10⁶ U α-IFN and then 18 × 10⁶ U daily; D, cutaneous T-cell lymphoma, corticoids cyclophosphamide adriblastin, eldesin, bleomycin).
elevated levels of blood hypodense eosinophils in eosinophilic diseases, including malignant HES or HES with visceral lesions. We have previously characterized the cytotoxic properties of hypodense eosinophils. Besides the morbidity and mortality related to the leukemic progress, clinical complications secondary to eosinophil toxicity have been also reported in malignant HES. Our data suggest a close relationship between mononuclear cell activation and eosinophil activation. From this perspective, the level of sIL-2R in serum could partially reflect the degree of eosinophil stimulation. It may thus represent a new risk factor, useful for the evaluation of possible widespread organ involvement, in eosinophilia-associated diseases.

The processes leading to blood eosinophilia in HES are not universal. In the case of malignant forms, they could be related to uncontrolled or constitutive secretion of cytokines, possibly by transactivation mechanisms or by cytogenic abnormalities. In acute lymphocytic leukemia with eosinophilia, a chromosomal translocation involving chromosome 5 and genes encoding GM-CSF, IL-3 and IL-5 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.

ACKNOWLEDGMENT

We thank Prof Piette (Service de Dermatologie, CHR Lille), Dr M. Bagot, Prof Revuz (Service de Dermatologie, Hôpital Henri Mondor, Créteil), Dr J.M. Mièlea (Service d'Hématologie, Hôpital Saint Louis, Paris), Prof A.B. Tonnel (Service de Pneumologie et Immonoallergologie, CHR Lille), and Dr P. Veyssier (Centre Hospitalier de Compiègne) for allowing us to study their patients, Dr W.G. Greene et al for their generous supply of essential reagents for this study. We also thank J.L. Neyrinck for data management assistance and C. Colson and M.F. Massard for their secretarial collaboration.

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