Presence of Epstein-Barr Virus Viral Interleukin-10 in the Serum of Patients With Non-Human Immunodeficiency Virus-Related Diffuse Large-Cell Non-Hodgkin's Lymphomas

To the Editor:

Three series have reported increased serum interleukin-10 (IL-10) in patients with non-Hodgkin's lymphoma (NHL). In our initial report, total (ie, human and Epstein-Barr virus [EBV]) IL-10 (h-vIL-10) was detectable in the serum of 47 of 101 (46%) patients with intermediate- or high-grade NHL, and correlated to overall and progression-free survival. In the series by Stasi et al, 42 of 105 (40%) patients with aggressive lymphoma were found to have detectable serum human IL-10. In a recent publication, serum human IL-10 (hIL-10) was found increased in 30 of 52 (58%) patients with diffuse large-cell lymphomas (DLCL). There are several differences in terms of serum IL-10 levels and their prognostic significance in these three series. (1) In our initial series and in the series of Stasi et al, respectively, 12 of 24 (50%) and 15 of 37 (40%) patients with DLCL were found to have serum IL-10 levels greater than 100 pg/mL, compared with 5 of 52 (10%) in the report by Cortes et al. (2) Serum IL-10 correlated to progression-free and overall survival in univariate and multivariate analysis in our initial report. In the report by Stasi et al, serum IL-10 was found to be an independent prognostic factor to the risk of failure of induction treatment in a multivariate analysis in a series of 105 patients treated according to the same regimen. In the report by Cortes et al, serum IL-10 was not correlated to progression-free or overall survival in a cohort of 52 patients with DLCL treated with the same regimen.

We have analyzed the prognostic value of serum IL-10 in a larger series of patients with DLCL and obtained results that may help us to understand the discrepancies between these results. Serum IL-10 levels were determined in a series of 73 previously untreated human immunodeficiency virus (HIV)-negative patients with DLCL who received regimens combining cyclophosphamide, doxorubicin, vincristine/vindesine, bleomycin, and methotrexate between 1984 and 1994. Using the Shering-Plough (SP) enzyme-linked immunoassay (ELISA), which detects both hIL-10 and EBV viral (vIL-10), serum h-vIL-10 was detectable (ie, >100 pg/mL) in 23 of the 73 (32%) patients of this series. Serum hIL-10 levels were then measured with the immunoassay used in the report by Cortes et al (Cytoscreen; Biosource International, Camarillo, CA) that is specific for hIL-10 in 40 patients of this series. With this assay, only 1 (2.5%) patient had serum IL-10 levels greater than 100 pg/mL, compared with 10 (25%) with the SP assay in the same group (Fisher's exact test, P = .006). Using a regression analysis with a linear model, the values of IL-10 measured with the two assays were not found to be significantly correlated (r = .29, P = .11).

The serum levels of 40 patients were then tested with a third immunoassay (level of sensitivity, 3 pg/mL) that recognizes both human and viral IL-10 (IL-10 EIA; Dianova, Immunotech, Marseille, France). With this assay, 9 (23%) patients had serum IL-10 levels greater than 100 pg/mL, compared with 10 (25%) with the SP assay (Fisher's exact test, P > .9). Using a regression analysis with a linear model, the values of serum IL-10 measured with the Dianova and the SP assays were found to be highly correlated (r = .69, P = .0001). Serum IL-10 levels measured with the Dianova and Cytoscreen assay were not found to be correlated (r = .19, P = .26).

The correlation observed between the two assays recognizing both hIL-10 and vIL-10 (Shering-Plough and Dianova) as well as the discrepancies with the assay specific for hIL-10 (Cytoscreen) suggested that vIL-10 could be present in the serum of these patients. vIL-10 is a cytokine and the product of an EBV gene termed BCRF1. vIL-10 is produced by EBV lymphoblastoid cell lines in vitro and expresses biologic properties that are largely similar to those of hIL-10, in particular concerning its immunosuppressive properties and B-cell growth factor activity. Serum vIL-10 levels were measured using a specific ELISA that was previously reported. The limit of sensitivity of this ELISA is 50 pg/mL. hIL-10 at 10 pg/mL does not react with the vIL-10 ELISA. Nonspecific positive serum samples were excluded as in the initial reports. Nine of the 73 (12%) patients with DLCL (1 T-NHL, 6 B-NHL, and 2 undetermined) were found to have detectable vIL-10 in serum (Fig 1), ie, 39% of the 23 patients with detectable h-vIL-10 with the SP assay. In contrast, vIL-10 was undetectable in the serum of the 42 normal controls tested (Fisher's exact test, P = .02) and of 44 patients with nonhematologic tumors (metastatic melanoma [n = 27] or renal cell carcinoma [n = 17], Fisher's exact test, P = .02). Viral IL-10 was detectable in none of the 27 patients with DLCL in complete remission (CR) nor in the 14 patients in partial remission (PR). vIL-10 was detectable in 3 of 9 (33%) patients with untreated DLCL in relapse, indicating that serum vIL-10 is detectable only at an active phase of the disease with this assay (Fig 1). All patients with detectable vIL-10 had a positive EBV serology. In this series, the percentage of patients with detectable h-vIL-10 or vIL-10 was not significantly different according to stage, lactate dehydrogenase (LDH) levels, age, performance status, B2 microglobulin levels, or the presence of B symptoms (P > .2 by x2 analysis). These results indicate that vIL-10, the product of an EBV gene, is produced in vivo in a subset of patients with DLCL at an active phase of their disease.
CORRESPONDENCE

Fig 2. Overall survival from initial diagnosis according to pretreatment serum h-vIL-10 levels as determined with the SP assay. (1) Patients (n = 50) without detectable h-vIL-10 (n = 58); (2) Patients with detectable vIL-10 (n = 9). (3) Patients with detectable h-vIL-10 (n = 23). All patients in group 2 are also included in group 3. Survival is indicated in months.

The prognostic value of serum IL-10 was then evaluated. With a median follow-up of 53 months, both overall (Fig 2) and progression-free (data not shown) survival were found to be significantly inferior in the 23 patients who had detectable h-vIL-10 with the SP assay as compared with other patients (log rank = 6.99, \( P = .008 \); log rank = 4.46, \( P = .03 \); respectively). The overall survival of patients with detectable vIL-10 was found to be marginally different from that of patients without h-vIL-10 (log rank = 3.55, \( P = .058 \), see Fig 2). In addition, patients with detectable h-vIL-10 had a marginally higher risk of failure of induction treatment; respectively, 4 of 43 (9%) evaluable patients with undetectable h-vIL-10 and 6 of 22 (27%) evaluable patients with detectable h-vIL-10 failed to achieve CR or PR after induction (Fisher's exact test, \( P = .07 \)). Three of the 6 (50%) patients with both \( \beta_2 \) microglobulin levels greater than 3 mg/L and detectable h-vIL-10 did not achieve CR or induction treatment (Fisher's exact test, \( P = .02 \)), in agreement with the observations of Stasi et al.\(^2,3\)

The outcome of the 40 patients in whom serum IL-10 was measured with the ELISA specific for hIL-10 (CytoScreen) was investigated. In contrast with the results obtained with the SP assay,\(^2,3\) the outcome of patients with serum IL-10 levels greater than 8 pg/mL (n = 8) was found to be less favorable but not significantly different from that of patients with serum IL-10 levels less than 8 pg/mL (n = 32) in terms of overall survival (log rank = 2.6, \( P = .10 \)) and progression-free survival (log rank = 1.96, \( P = .15 \)). The only patient with a serum IL-10 level greater than 100 pg/mL, with this assay died of tumor progression 4 months after the initial diagnosis. The CR rate was lower but not significantly different in patients with serum hIL-10 levels greater than 5 pg/mL with this assay (Fisher's exact test, \( P = .11 \)). These results are comparable to those reported with the same assay by Cortes et al.\(^4\)

The presence of vIL-10 in 39% of patients with detectable h-vIL-10 with the SP assay is likely to have contributed to the discrepancies between the series of Cortes et al and ours.\(^2,4\) In addition, because the sensitivity of the vIL-10 assay is only 50 pg/mL, one cannot exclude that actually a higher proportion of patients with DLCL may have circulating vIL-10 and contribute to the discrepancies between the assays.

In conclusion, vIL-10, the product of an EBV gene, is detectable in the serum of a subset of patients with DLCL only at an active phase of the disease. Previous reports have shown the presence of EBV DNA in NHL tumor cells of patients without immunodeficiency.\(^7\) In vivo in humans, vIL-10 expression has been reported in tissue samples of oral hairy leukoplakia in acquired immunodeficiency syndrome patients.\(^8\) These observations indicate that the EBV vIL-10 gene is also produced also in patients with DLCL in vivo. In view of the discrepancies between the results of different ELISA for IL-10, a standardization of the assays would be required if this parameter was to be used as a prognostic tool. Nevertheless, these results suggest that both EBV viral and human IL-10 may play an important role in the clinical course of NHL and that inhibition of IL-10 production may have a therapeutic value in non-HIV NHL as well as in HIV NHL.\(^8\)

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REFERENCES


Response

Blay et al had published a report showing that serum IL-10 levels correlated with prognosis in a heterogeneous group of non-Hodgkin’s lymphoma patients. More recently, we showed that, in a homogeneous group of diffuse large-cell lymphoma patients treated in a uniform manner, IL-10 levels did not correlate with prognosis. We pointed out that the differences between the two studies may be attributable to the patient groups assessed or to differences in the assays used. Our ELISA was more sensitive than that used by Blay et al. In addition, our ELISA measured only human IL-10, whereas that used by Blay et al measured both human and viral IL-10.

In the accompanying letter, Blay et al show that, in a more homogeneous group of patients with diffuse large-cell lymphoma, the assay measuring both human and viral IL-10 gives results that correlate with prognosis. Results of the assay measuring only human IL-10 do not correlate with prognosis, confirming our analysis. These findings are of interest as they indicate that a standard assay measuring both viral and human IL-10 is needed if IL-10 is to be considered as a prognostic factor. In addition, the role of viral IL-10 in this disease may be of pathogenic importance.

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REFERENCES

Presence of Epstein-Barr virus viral interleukin-10 in the serum of patients with non-human-immunodeficiency-virus-related diffuse large-cell non-Hodgkin's lymphomas [letter; comment]

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