Serum Interleukin-10 in Non-Hodgkin’s Lymphoma: A Prognostic Factor

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Serum interleukin-10 (IL-10) was measured retrospectively in 153 patients with a fully documented history of non-Hodgkin’s lymphoma (NHL) using an enzyme-linked immunosorbent assay (ELISA) detecting both human IL-10 and the Epstein-Barr virus (EBV) molecule BCRF1/viral IL-10. IL-10 was detectable in 47% of the 101 patients with active NHL, 3 of 52 (6%) patients in first partial or complete response, and none of the 60 healthy blood donors. Serum IL-10 was detectable with a similar frequency in all subtypes of NHL and in all clinical stages, as well as in EBV-seropositive and EBV-negative patients. In patients with intermediate or high-grade NHL, the presence of detectable serum IL-10 at diagnosis was correlated to a significantly shorter overall (P = .025) and progression-free (P = .030) survival. Patients with stage IV disease and detectable serum IL-10 had a particularly poor prognosis (4 years of survival: 0%). Multivariate analysis showed that IL-10 was an independent prognosis factor. These results indicate that IL-10 is detectable in a subgroup of patients with active NHL and correlates to a poor survival in patients with intermediate or high-grade NHL.

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served between serum IL-10 and the duration of the storage (r = .05; P = .65). Median IL-10 level was 377 pg/mL in patients diagnosed before 1984 (range: 115 to 2134) and 301 pg/mL in patients diagnosed after 1984 (range: 103 to 1655). Thus, there is no evidence of loss of IL-10 on storage at −80°C. The ELISA used for the quantification of IL-10 in the serum recognizes both human and viral IL-10 and was previously reported. IL-10 standard consisted of Chinese hamster ovary (CHO)-derived purified human IL-10 obtained from Shering-Plough Research Institute (batch 27785-82) (Bloomfield, NJ). The concentration of the standard solution of IL-10 was determined using amino acid quantification technique. The 12G8 and 9D7 antibodies were kindly provided by Dr J. Abrams, DNAX, Palo Alto, CA. 12G8 and 9D7 recognize both human and viral IL-10. The detection level for IL-10 was 100 pg/mL. The intra assay coefficient of variation (CV) ranged from 3.04% to 14.9% and interassay CV ranged from 2.3% to 15.3% in the reproducibility analysis of nine samples in replicates of two in four runs. To avoid nonspecific binding, rat serum was added during a saturation step (5%) and the incubation of the samples (5%). Moreover, a nonrelated biotinylated monoclonal antibody (MoAb) (anti-interferon gamma, B27) was used as second MoAb for each sample. When a binding of the unrelated B27 antibody was observed, the serum samples was excluded from the analysis. In addition to the 153 patients, four patients (two at diagnosis and two at relapse) were excluded from the analysis because of false-positive serum samples.

**Statistical Analysis**

Statistical analysis were performed using the procedures of the BMDP package (BMDP Statistical Software, Cork, Ireland): Yates corrected X² and the Fisher's exact test (two-tailed). Survival curves were computed according to the method of Kaplan-Meier. Survival was measured from the day of the initial treatment to either the day of progression or the date of death. The log rank test was used to compare the survival of the different subgroups of patients. The Cox proportional hazard model was used to study the effect of different variables on survival. The prognostic factors introduced in the model are commonly accepted and have been previously reported by the international NHL prognostic factor study on a large series of patients: age (<60 v >60 years), stage (I, II v III, IV), number of extranodal sites (0 or 1 v >1), performance status (PS) (Eastern Cooperative Oncology Group [ECOG] 0.1 v >1.0), serum lactate dehydrogenase (LDH) (normal v abnormal), and serum IL-10 (undetectable v detectable). Backward regression procedure was used to determine whether or not IL-10 was an independent prognostic parameter in addition to a model in which all the above-mentioned prognostic factors were maintained in the model.

**RESULTS**

**Serum IL-10 in Patients With NHL**

Serum IL-10 levels were measured in 153 patients with NHL and in 60 healthy blood donors (Fig 1). IL-10 was detectable in 47 of the 101 patients (46.5%) with active NHL compared with none of the 60 healthy blood donors (P < 10⁻⁸), 2 of 23 (8%) patients in first partial remission (P = .0004), and 1 of 29 (3%) patients in complete remission (P = .0003). The two patients in PR had residual masses less than 2 cm in diameter on CT scan at the date of the sample: one achieved CR and relapsed 14 months later, and the other never achieved CR and progressed 2 months later. The patient in CR with detectable serum IL-10 has not relapsed 11 years after the initial diagnosis. Within the 101 patients with evolutive NHL, serum IL-10 was detectable in 39 of the 86 (45%) patients tested at diagnosis and 8 of the 15 (52%) patients at relapse (Fig 1). In the 47 patients with active NHL and detectable IL-10, the mean and median concentrations of IL-10 were 517 and 378 pg/mL, respectively, with a range of 105 to 2,134 pg/mL. Serum IL-10 levels were determined sequentially in 10 patients: in all patients with detectable serum IL-10 at diagnosis, IL-10 decreased under the detection limit at remission but was detectable at relapse (Fig 2).

**Serum IL-10 in Patients at Diagnosis**

**IL-10 and clinicobiologic markers.** The percentage of patients with detectable serum IL-10 was comparable in the different histologic subtypes as well as in B and T NHL (Table 1). Similarly, the percentage of patients with high IL-10 levels was not significantly different in patients with stages I, II, III, and IV disease (Table 1). Serum IL-10 did not persist in the serum samples of patients with CR at diagnosis. 

**Statistical Analysis**

Statistical analysis were performed using the procedures of the BMDP package (BMDP Statistical Software, Cork, Ireland): Yates corrected X² and the Fisher's exact test (two-tailed). Survival curves were computed according to the method of Kaplan-Meier. Survival was measured from the day of the initial treatment to either the day of progression or the date of death. The log rank test was used to compare the survival of the different subgroups of patients. The Cox proportional hazard model was used to study the effect of different variables on survival. The prognostic factors introduced in the model are commonly accepted and have been previously reported by the international NHL prognostic factor study on a large series of patients: age (<60 v >60 years), stage (I, II v III, IV), number of extranodal sites (0 or 1 v >1), performance status (PS) (Eastern Cooperative Oncology Group [ECOG] 0.1 v >1.0), serum lactate dehydrogenase (LDH) (normal v abnormal), and serum IL-10 (undetectable v detectable). Backward regression procedure was used to determine whether or not IL-10 was an independent prognostic parameter in addition to a model in which all the above-mentioned prognostic factors were maintained in the model.
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not correlate to other previously reported\textsuperscript{25,26} prognostic factors; that is, age, performance status (PS), number of extranodal sites, LDH, and tumor mass over 10 cm (not shown). Serum IL-10 was detectable in 35 of 75 EBV seropositive (46\%) and in 4 of 11 seronegative (36\%) patients ($P > .5$).

\textit{Prognostic value of serum IL-10.} The prognostic value of serum IL-10 was studied in the 70 patients with high- or intermediate-grade NHL. Patients with detectable serum IL-10 had a significantly shorter overall ($P = .025$) and progression-free ($P = .030$) survival (Fig 3). Serum IL-10 correlated to a poor overall survival both in patients treated before ($P = .03$) and after 1984 ($P = .02$). Of note, five of the six patients with detectable IL-10 treated before 1984 died of lymphoma progression; this accounts for the fact that only one patient is followed more than 70 months in this subgroup. Although serum IL-10 is correlated to survival, the CR rate to induction treatment in patients with and without detectable IL-10 levels was not different (64\% vs 79\%, respectively; $P = .18$). There was no level of IL-10 beyond which 100\% of the patients failed to achieve remission or relapsed. Of note, IL-10 was associated with a particularly poor progression-free ($P = .0002$) and overall survival ($P = .00004$) in patients with stage IV disease (Fig 4). Stage IV patients with detectable IL-10 have a projected 5-year survival of 0\% compared with 85\% in stage IV patients with undetectable IL-10. A multivariate analysis of prognosis factors using Cox model was performed on the series of 70 patients with intermediate or high-grade lymphoma at diagnosis. The variables introduced in the Cox regression model were the prognostic parameters recently reported in the international NHL prognostic factor study\textsuperscript{26}; that is, performance status (0.1 v 1.0), age ($<60$ v $>60$), clinical stage according to the Ann Arbor staging system (I, II v III, IV), number of extranodal tumor sites (0.1 v 1.0), and serum LDH (normal v abnormal). Using a model in which all these factors were maintained, IL-10 remained an additional independent prognostic factor with a significant prognostic value for progression free survival ($X^2$ improvement: 4.03, $P = .04$); IL-10 was also an additional independent factor of marginal significance for overall survival ($X^2$ improvement = 2.89, $P = .09$).

\section*{DISCUSSION}

Two major points can be concluded from this study. First, serum IL-10 was detected in half of the patients with active NHL, whereas it was mostly undetectable in more than 90\% of patients who achieved PR or CR as well as in all healthy blood donors tested. Most importantly, there was no correlation between the duration of storage and IL-10 level and thus no evidence of loss of IL-10 on storage at $-80^\circ$C. These results as well as the follow-up of IL-10 levels in 10 patients at diagnosis, remission, and relapse highly suggest that detectable serum IL-10 is associated with the presence of an active disease. However, serum IL-10 did not precisely reflect tumor burden or disease dissemination, because it was not correlated to serum LDH, to the presence of a bulky tumor, or to clinical stage.

The second point is the poor survival of patients with detectable serum IL-10 at initial diagnosis in patients with intermediate- or high-grade lymphoma. The prognostic value of serum IL-10 was not restricted to a subgroup of patients, because it was observed in adults, children, and patients with diffuse mixed and large cell lymphoma (not shown). The most dramatic observation concerning this point is the absence of long-term survivors at 4 years among stage IV patients with detectable IL-10. Conversely, the projected 4-year survival of stage IV patients with undetectable IL-10 was over 75\%. These observations are in contrast with a recent report showing that serum IL-10 is increased in early stage but not advanced multiple myeloma.\textsuperscript{27}

Although all these patients received anthracyclin-based regimens, it was conceivable that the improvement of chemotherapy efficiency may have interacted with the prognostic value of IL-10. However, IL-10 correlates to survival both in patients diagnosed before and after 1984 suggesting that there were probably few if any interaction between treatment era and the prognostic value of IL-10. Serum IL-10 is also an independent prognostic factor in multivariate analysis. It is inversely correlated to both progression-free and overall survival in patients with intermediate- or high-grade NHL. This suggests that IL-10 is not redundant with other commonly accepted prognostic parameters for NHL.

\begin{table}[h]
\centering
\caption{Histology, Immunophenotype, and Clinical Stage of NHL in Patients at Diagnosis}
\begin{tabular}{|c|c|c|}
\hline
\textbf{Working Formulation*} & \textbf{Range of Serum IL-10 Levels (pg/mL)} & \textbf{Number (%) of Patients With Detectable IL-10 (total 39/86 [45\%])} \\
\hline
\textbf{A} & 4 & <100-1,457 \hspace{1cm} 2 (50) \\
\textbf{B} & 6 & <100-654 \hspace{1cm} 3 (60) \\
\textbf{C} & 6 & <100-116 \hspace{1cm} 2 (33) \\
\hline
\textbf{Low grade (total)} & & 7 (43) \\
\hline
\textbf{D} & 1 & 322 \hspace{1cm} 1 (100) \\
\textbf{E} & 5 & <100-808 \hspace{1cm} 2 (40) \\
\textbf{F} & 8 & <100-1,362 \hspace{1cm} 4 (50) \\
\textbf{G} & 24 & <100-2,134 \hspace{1cm} 12 (50) \\
\textbf{H} & 9 & <100-892 \hspace{1cm} 4 (44) \\
\textbf{I} & 3 & <100-792 \hspace{1cm} 1 (33) \\
\textbf{J} & 20 & <100-1,508 \hspace{1cm} 8 (40) \\
\hline
\textbf{Intermediate/high grade (total)} & & 32 (46) \\
\hline
\textbf{Clinical stage (Ann Arbor) of the 86 patients studied at the diagnosis:} & & \\
\hline
\textbf{I} & 9 & <100-984 \hspace{1cm} 4 (44) \\
\textbf{II} & 15 & <100-892 \hspace{1cm} 9 (60) \\
\textbf{III} & 25 & <100-1,016 \hspace{1cm} 11 (44) \\
\textbf{IV} & 34 & <100-1,457 \hspace{1cm} 14 (41) \\
\textbf{Unknown} & 3 & <100-2,134 \hspace{1cm} 1 (33) \\
\hline
\textbf{Immunophenotype} & & \\
\hline
\textbf{T} & 10 & <100-892 \hspace{1cm} 4 (40) \\
\textbf{B} & 48 & <100-1,655 \hspace{1cm} 18 (39) \\
\textbf{Not determined} & 28 & <100-2,134 \hspace{1cm} 17 (80) \\
\hline
\end{tabular}
\end{table}

\textsuperscript{*} A, small lymphocytic; B, follicular small cleaved; C, follicular mixed; D, follicular large; E, diffuse small cleaved; F, diffused mixed; G, diffuse large; H, immunoblastic; I, lymphoblastic; J, small noncleaved.

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Three questions are raised by this study: (1) the cellular origin of IL-10, (2) the role of EBV in these observations, and (3) the putative role of IL-10 in lymphomagenesis.

B lymphoma cell lines, EBV-transformed B cells, activated T cells, and macrophages have the capacity to produce IL-10 in vitro in humans. IL-10 could be produced by malignant cells as recently shown in HIV-associated NHL. However, the observation that serum IL-10 level is not correlated to tumor burden argues against this hypothesis. Furthermore, detectable IL-10 was found in all histologic or immunologic subtypes of NHL; a result that would lead to the conclusion that follicular and germinal B lymphocytes as well as T lymphocytes at different stages of differentiation are able to produce IL-10 after transformation. Alternatively, IL-10 may be produced by normal cells under deregulated stimulation by malignant cells.

The assay used in this study detects both human and viral IL-10. None of patients at diagnosis had the characteristic serologic pattern of EBV-associated NHL. IL-10 was detected in a similar proportion of EBV-seronegative and EBV-seropositive patients. This indicates that the presence of detectable IL-10 in these patients is not strictly associated with a previous EBV infection, as determined by serological criteria, in patients with NHL. However, additional studies are required to determine whether or not EBV is involved in these observations.

The biologic significance of the unfavorable prognostic value of IL-10 remains unclear. Although the presence of IL-10 in the serum of these patients with NHL could be an epiphenomenon, previous reports suggest at least two different mechanisms of action for this cytokine. IL-10 may be a growth factor for malignant B cells. Supporting this hypoth-
IL-10 was recently shown to be a growth factor for HIV-related lymphoma cell lines and to play a role in lymphomagenesis in a mouse model of lymphoma associated with a retroviral infection. In this model, the persistent activation of CD4+ T cells producing IL-10 is responsible for the chronic B-cell proliferation. An alternative interpretation could rely on the immunosuppressive properties of IL-10. Congenital or acquired T-cell immunodeficiency is a well-known predisposing condition for the development of lymphoma in humans. In vitro, IL-10 inhibits antigen-dependent T-cell proliferation by downregulating class II major histocompatibility complex expression on monocyte-macrophages and blocks cytokine production by macrophage in vitro. In human pathology, the presence of IL-10 mRNA in leprosy skin lesions correlates to the multibacillary form of leprosy, a condition associated with defective T-cell immunity. The high concentrations of IL-10 found in patients with NHL and possibly present in tumor sites could conceivably exert an inhibitory effect on macrophage and antigen-specific T-cell response at the tumor site and thus contribute to lymphoma progression in vivo.

In conclusion, these results indicate that serum IL-10 is increased in a subgroup of patients with NHL and correlates to a poor outcome in patients with intermediate- or high-grade NHL. The biologic mechanisms responsible for the presence and the prognostic value of serum IL-10 in these patients are under investigation.

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