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 (46%) 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 prognostic 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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Serum Interleukin-10 in Non-Hodgkin's Lymphoma: A Prognostic Factor
By Jean-Yves Blay, Nicolas Burdin, Françoise Roussel, Gilbert Lenoir, Pierre Biron, Thierry Philip, Jacques Banchereau, and Marie C. Favrot

INTERLEUKIN-10 (IL-10) was first characterized as a T-cell-derived cytokine able to block interferon gamma production by TH1 helper cell clones and as a B-cell-derived thymocyte growth factor. The cloning and sequencing of the murine and human IL-10 genes revealed an extensive homology with the BCRF1 gene, an open reading frame of the Epstein-Barr virus (EBV). The product of the BCRF1 gene, viral IL-10, exhibits partial IL-10 activity in vitro and could play an important role during EBV infection. IL-10 exerts multiple biologic effects on hematopoietic cell lineages, including monocytes, T and B lymphocytes. It is a growth and differentiation factor for B cells in humans and a growth factor for T lymphocytes in the mouse model. Besides IL-10 exerts immunosuppressive properties: It inhibits antigen-specific T-cell activation and blocks cytokine production by monocytes and macrophages in both human and mouse models.

HIV-related lymphoma cells have been shown to express IL-10 mRNA constitutively and to produce IL-10, which acts as an autocrine growth factor in several cell lines. We have investigated the possible involvement of IL-10 in the physiopathology of non-HIV-related non-Hodgkin's lymphoma (NHL) in vivo in humans. Using an enzyme-linked immunosorbent assay (ELISA) recognizing both human and viral IL-10, we measured the serum concentrations of IL-10 in 153 patients with a fully documented clinicopathologic history of NHL treated between 1979 and 1991 in our institute.

PATIENTS, MATERIALS, AND METHODS

Clinical and Pathologic Characteristics of the Patients
The 153 patients presented in this study have a fully documented history of NHL and were treated between April 1979 and June 1991 in the Departments of Pediatrics and Medicine at the Centre Leon Berard. Serum samples were collected, aliquoted, and stored at −80°C. Sera were collected at initial diagnosis in 86 patients, in first complete remission (CR) after induction chemotherapy in 29 patients, in first partial remission (PR) after chemotherapy in 23 patients, and at the first relapse in 15 patients. In 10 patients, serum samples were collected sequentially at an active phase of the disease (diagnostic and relapse) in PR (n = 1) or CR (n = 9). Histologic classification of the lymphomas is given according to the Working Formulation. Among the 86 patients at diagnosis, 16 had low-grade lymphomas, 38 had intermediate-grade lymphomas, and 32 had high-grade lymphomas. Among the 15 relapsing patients, 12 had intermediate-grade lymphomas and 3 had low-grade lymphomas. Clinical stage is given according to the Ann Arbor staging system.

The mean and median ages of the 86 patients tested at diagnosis were respectively 48 and 46, with a range from 3 to 89, and with 13 patients being under 18 years old. Children were treated with the COPAD regimen (cyclophosphamide, oncovin, prednisone, adriamycin) before 1981 and with the LMB regimens (Lymphomes Malins B, Société Française D'Oncologie Pédiatrique) thereafter. Adults were treated with CHOP-derived regimens (cyclophosphamide, adriamycin, oncovin, prednisone) before 1984 and the m/MBACOD regimens (methotrexate, bleomycin, adriamycin, cyclophosphamide, oncovin, dexamethasone) between 1984 and 1987. After 1987, adults up to age 35 received the LMB program and adults over age 35 were included in the LNH87 program (French Multicenter Randomized Trial for Non-Hodgkin's Lymphoma).

EBV and Human Immunodeficiency Virus (HIV) Serology

EBV serology was performed in the 86 patients analyzed at initial diagnosis of lymphoma. None of these patients tested at diagnosis had a characteristic EBV serology associated with African Burkitt's lymphoma. Eleven patients were seronegative for EBV. All 153 patients were HIV negative as evaluated by serodiagnostic.

IL-10 Enzyme-Linked Immunosorbent Assay (ELISA)

Samples were kept at −80°C and thawed immediately before the determination of IL-10 level. No correlation was ob-
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 $-80^\circ$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 intraassay 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 133 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 $X^2$ 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 ($P < 10^{-6}$), 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 have an independent effect on survival in a multivariate model.
of lymphoma progression; this accounts for the fact that patients with detectable serum IL-10 at initial diagnosis in patients with intermediate or high-grade lymphoma at diagnosis. 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. 

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°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. 

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.

Table 1. Histology, Immunophenotype, and Clinical Stage of NHL in Patients at Diagnosis

<table>
<thead>
<tr>
<th>Working Formulation*</th>
<th>Number (%) of Patients With Detectable IL-10 (Total 39/86 [45%])</th>
<th>Range of Serum IL-10 Levels (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4 &lt;100-1,457</td>
<td>2 (50)</td>
</tr>
<tr>
<td>B</td>
<td>6 &lt;100-854</td>
<td>3 (60)</td>
</tr>
<tr>
<td>C</td>
<td>6 &lt;100-116</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Low grade (total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1 322</td>
<td>1 (100)</td>
</tr>
<tr>
<td>E</td>
<td>5 &lt;100-808</td>
<td>2 (40)</td>
</tr>
<tr>
<td>F</td>
<td>8 &lt;100-1,362</td>
<td>4 (50)</td>
</tr>
<tr>
<td>G</td>
<td>24 &lt;100-2,134</td>
<td>12 (50)</td>
</tr>
<tr>
<td>H</td>
<td>9 &lt;100-892</td>
<td>2 (40)</td>
</tr>
<tr>
<td>I</td>
<td>3 &lt;100-792</td>
<td>1 (33)</td>
</tr>
<tr>
<td>J</td>
<td>20 &lt;100-1,508</td>
<td>8 (40)</td>
</tr>
<tr>
<td>Intermediate/high grade (total)</td>
<td></td>
<td>32 (46)</td>
</tr>
</tbody>
</table>

Clinical stage (Ann Arbor) of the 86 patients studied at diagnosis:

<table>
<thead>
<tr>
<th></th>
<th>Number (%)</th>
<th>Range of Serum IL-10 Levels (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10 &lt;100-1,984</td>
<td>4 (44)</td>
</tr>
<tr>
<td>II</td>
<td>15 &lt;100-892</td>
<td>9 (60)</td>
</tr>
<tr>
<td>III</td>
<td>25 &lt;100-1,016</td>
<td>11 (44)</td>
</tr>
<tr>
<td>IV</td>
<td>34 &lt;100-1,457</td>
<td>14 (41)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 &lt;100-2,134</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Immunophenotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>10 &lt;100-892</td>
<td>4 (40)</td>
</tr>
<tr>
<td>B</td>
<td>48 &lt;100-1,855</td>
<td>18 (39)</td>
</tr>
<tr>
<td>Not determined</td>
<td>28 &lt;100-2,134</td>
<td>17 (80)</td>
</tr>
</tbody>
</table>

* A, small lymphocytic; B, follicular small cleaved; C, follicular mixed; D, follicular large; E, diffuse small cleaved; F, diffuse mixed; G, diffuse large; H, immunoblastic; I, lymphoblastic; J, small non cleaved.
that reflect tumor mass (LDH, clinical stage, number of extranodal sites) or the patient’s condition (age, performance status). Serum IL-10 was detectable in a significant proportion of patients with low-grade lymphoma. However, this subgroup was too small to study the prognosis value of IL-10.

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-
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.

ACKNOWLEDGMENT

We express our gratitude to Dr J. Abrams (DNAX, Palo Alto, CA) for the kind gift of the 9D7 and the 12G8 MoAbs and to Drs F. Chauvin and C. Lasset for their helpful comments on statistical analysis.

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