Interleukin (IL)-10 and IL-6 Are Produced in Vivo by Non-Hodgkin’s Lymphoma Cells and Act as Cooperative Growth Factors

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ABSTRACT

The in vivo production of interleukin (IL)-10, IL-6, IL-2, and tumor necrosis factor (TNF)-α in tumor samples was investigated by immunohistochemistry in 54 non-Hodgkin’s lymphomas (NHLs). Respectively, 55, 89, 23, and 29% of tumor samples were found positive for IL-10, IL-6, IL-2, and TNF-α expression by immunohistochemistry. Using reverse transcription-PCR, the mRNA of IL-10 and IL-6 were detectable in all samples tested and in 90 and 34% of the samples for TNF-α and IL-2, respectively. In 13 patients, fresh tumor tissue was available for B NHL cell purification with Dynabeads. IL-2, IL-6, IL-2, and TNF-α were detectable in the supernatant of 38, 100, 0, and 23% of purified tumor cell preparations (PTCPs), respectively. All patients with detectable IL-10 in culture had increased serum IL-10. IL-6 production by tumor cells and serum IL-6 levels were also found to be highly correlated (P < 0.0001). This suggests that tumor cells are a major source of serum IL-10 and IL-6 in these patients. Exogenous IL-10, IL-6, IL-6, and TNF-α significantly enhanced the [3H]thymidine uptake in 13 of 13 (100%), 5 of 13 (38%), 9 of 13 (69%), and 1 of 20 (10%) PTCPs costimulated with anti-CD40, respectively, IL-2, IL-6, and TNF-α synergized with IL-10 in 54, 23, and 30% of PTCPs. The combination of IL-10, IL-2, and IL-6 induced the maximal level of proliferation in 12 (92%) of 13 PTCPs. CD40 ligand mRNA expression was also detectable in vivo using reverse transcription-PCR in 28 of the 29 (97%) tumor samples tested, including 11 of those tested for IL-10, IL-6, IL-2, and TNF-α expression. These results show that IL-10, IL-6, IL-2, and TNF-α are produced in NHL tumors and may cooperate in vivo to increase NHL cell proliferation.

INTRODUCTION

The network of cytokines exerts regulatory effects on the proliferation of normal lymphocytes, but their interaction in vivo on the proliferation of malignant lymphoma cells is poorly understood. In this study, the roles of IL-10, IL-6, IL-2, and TNF-α on the proliferation of NHL cells were investigated.

IL-10 is a pleiotropic cytokine produced by B and T lymphocytes as well as monocytes (1—4) that acts as a growth factor for normal activated human B lymphocytes, B lymphoma cell lines, and HIV-related NHL (5, 6). Besides its growth factor activity, IL-10 inhibits macrophage-dependent T-lymphocyte activation and proliferation (7). IL-10 also inhibits the expression of the MHC class II molecules on monocytes-macrophages and blocks cytokine production by monocytes, macrophages, natural killer cells, and Th1 and Th2 lymphocytes (4, 8—11). In vivo, the production of IL-10 in human tumors has been reported in HIV-related and non-related NHL, and serum IL-10 levels have been found increased in vivo in patients with NHL (12—15).

IL-6 is produced in vivo in several tumors, in particular in multiple myeloma and renal cell carcinoma (16, 17). IL-6 may also play an important role in NHL (18—23): (a) IL-6 is an autocrine growth factor for human B-cell lymphoma cell lines (19, 20); (b) IL-6 mRNA and protein expression are detectable in malignant lymphoma cells by IHC (21); and (c) serum IL-6 levels are increased in lymphoma patients and correlate to a poor prognosis in diffuse large cell NHL (22, 23). TNF and lymphotoxin α mRNA are detectable in fresh lymphoma samples (24), whereas TNF receptor p80 chain is expressed on NHL cells (26). Although TNF is an autocrine growth factor in chronic lymphocytic leukemia (27), the possible in vivo growth-promoting effect of TNF is not demonstrated in NHL. The role of IL-2 in the proliferation of NHL cells in vivo has been poorly investigated. Normal B cells and NHL cells express IL-2 receptors and IL-2 has been reported to induce the proliferation of NHL cell lines (28—30).

The objectives of this study were to investigate the production of IL-10, IL-6, TNF-α, and IL-2 in vivo in NHL tumor samples and to analyze their role on the proliferation of fresh tumor cells cultured in vitro. The results obtained indicate that IL-10 and IL-6 are produced in vivo in tumor samples, and that IL-10, IL-6, IL-2, and TNF-α additively stimulate the proliferation of NHL cells cultured in vitro, suggesting an autocrine/paracrine role for these cytokines in vivo in patients with NHL.

PATIENTS AND METHODS

Patients and Tumor Samples. The 54 patients analyzed were diagnosed and treated in the Centre Léon Bérard. All had non-HIV-related B or T NHL and no known cause of congenital or acquired immunosuppression. Tumor biopsies and serum were obtained prior to cytotoxic treatment and stored at −80°C and in liquid nitrogen, respectively. Table 1 shows the clinical characteristics of these patients. In those 54 patients, 13 tumor cell purification was performed in 13 B NHL tumor samples for in vitro proliferation and evaluation of cytokine production.

Sources of Cytokines and Reagents. Purified rhIL-10 was provided by Dr. F. Rouset (Schering-Plough, Dardilly, France) and was used at 100 ng/ml in proliferation assays; rhIL-2 (Eurocetus, Amsterdam, the Netherlands) was used at 100 units/ml; rhIL-6 (Sandoz, Basel, Switzerland) was used at 40 ng/ml; rhTNFa (Eurocetus) was used at 10 ng/ml. Anti-CD40 antibody (mAb 89) was provided by Dr. Fr. Rouset and was used at 0.5 μg/ml (31—35). Anti-IL-10 (mAb 85) was provided by Dr. J. Joab (Institut G. Roussy, Villejuif, France). The following antibodies were used: mAb 202 (R&D, Minneapolis, MN), mAb B-F6 (Innotest, Besançon, France), and mAb B-C7 (Innotest) directed against B-F6 (Innotest, Besançon, France), anti-CD3 (Dako SA, Trappes, France), FITC-conjugated anti-CD20 (Dako SA, Trappes, France), anti-CD19 (Dako SA, Trappes, France), anti-CD20 (Dako SA, Trappes, France), and anti-CD20 (Dako SA, Trappes, France). The following antibodies were used: mAb 202 (R&D, Minneapolis, MN), mAb B-F6 (Innotest, Besançon, France), and mAb B-C7 (Innotest) directed against B-F6 (Innotest, Besançon, France), anti-CD3 (Dako SA, Trappes, France), anti-CD19 (Dako SA, Trappes, France), and anti-CD19 (Dako SA, Trappes, France). The following antibodies were used: mAb 202 (R&D, Minneapolis, MN), mAb B-F6 (Innotest, Besançon, France), and mAb B-C7 (Innotest) directed against B-F6 (Innotest, Besançon, France), anti-CD3 (Dako SA, Trappes, France), anti-CD19 (Dako SA, Trappes, France), and anti-CD19 (Dako SA, Trappes, France).
sections or cytotoxicrefrigured smears) were fixed for 10 min with acetone at 4°C, incubated for 60 min with the first antibody, and then for 30 min with an alkaline phosphatase-conjugated rabbit antirabbit immunoglobulin, followed by a 30-min incubation with an alkaline phosphatase-conjugated swine antirabbit immunoglobulin (Dako SA) as the second antibody. The third step was an avidin-biotin complex (Dako SA). The same incubation time and revelation technique were used.

RT-PCR and Agarose Gel Electrophoresis. Total RNA was extracted from nitrogen frozen tissues by mechanic breaking with a polytron in RNA B solution (Bioprobe, Montreuil-sous-bois, France). RNA were extracted in chloroform, precipitated in isopropanol, resuspended in diethylpyrocarbonate water and quantified with a spectrophotometer (Beckman, Gagny, France). The cDNA template for RT-PCR was synthesized from RNA by Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc., Cergy-Pontoise, France).

PCR for IL-10 mRNA detection was performed as follows: preamplification at 95°C for 5 min; for specificity enhancement, a “hot start” was performed by maintaining the temperature at 69°C, with cycles 1 to 30 at 95°C for 30 s (strand separation), 58°C for 1 min (annealing), and 72°C for 2 min (primer extension). The reaction was then held at 72°C for 7 min. The primers for the IL-10 mRNA were 5'-AATGTCCTGAGTCACAGCTGACTG-3' (sense) and 5'-AATGGGGAATCTTTTTCACAAAGGGCTGGGT-3' (antisense). Two successive PCR 30 cycles were performed for IL-10 detection. The positive and negative controls were the cDNA of the BJAB cell line and the BL-41 cell line, respectively.

PCR for IL-2, IL-6, and TNF-α mRNA detection was performed with Clontech amplimer sets (Ozyme, Montigny-Le-Bretonneux, France) according to the manufacturer’s protocol. PCR of the cDNA was performed as follows: preamplification at 94°C for 4 min; cycles 1 to 35 at 94°C for 1 min (strand separation), 60°C for 45 s (annealing), and 72°C for 2 min (primer extension). The reaction was then held at 72°C for 7 min. Negative control was performed without cDNA adjuvant in the master reagent mix. Positive control PCR products were provided in the amplimer sets.

PCR for CD40 ligand mRNA detection was performed as follows: preamplification at 94°C for 4 min, with cycles 1 to 35 at 94°C for 1 min (strand separation), 60°C for 45 s (annealing), and 72°C for 3 min (primer extension). The reaction was then held at 72°C for 10 min. The primers for the CD40 ligand mRNA were 5'-TCACCTTGTCAGTTCAATCGG-3' and 5'-GTGAATGGATACCTGTTGCGAATGG-3'. The positive control was the cDNA of the L cell line transfected with CD40 ligand (provided by F. Rouset). Two negative controls were performed, without cDNA adjuvant in the master reagent mix, and with cDNA of the CDw32/FcyRII-transfected L cell line.

Primers designated to amplify an ubiquitously expressed gene β-actin were used to test the efficiency of cDNA synthesis. Negative control was performed without cDNA adjuvant in the master reagent mix. All reactions were performed with Perkin-Elmer DNA Thermal cycler model 480.

Amplified PCR products appeared as bands of the size indicated on the provided Product Analysis Certificate (for IL-6, IL-2, and TNF-α) when viewed under UV light. Amplified PCR fragments were 658, 305, 628, 444, and 149 bp for IL-10, IL-2, IL-6, and CD40 ligand, respectively.

Tumor Cell Preparation and Purification. B lymphoma cells were extracted from tumor tissues after mechanic or enzymatic disaggregation (DNase, collagenase, and hyaluronidase). Adherent cells were removed after overnight incubation in petri dishes. The proportion of B and T cells in tumor tissues after disaggregation was determined using a FITC-conjugated anti-CD20 (anti-Leu 16 FITC) and a phycocerythrin-conjugated anti-CD3 (anti-Leu 4 PE). Cells were incubated 30 min with the first antibodies and then washed in PBS with sodium azide. Cells were resuspended in PBS with sodium azide and analyzed by flow cytometry with a FACScan analyzer (Becton Dickinson). An indirect technique was used to separate T cells from B cells, with Dynabeads M450 coated with goat antiserum IgG in a ratio of 20:1 beads to total cells (10^6 cells/ml). Total cells at 2 × 10^6 cells/ml were pretreated with a primary antibody specific for surface antigens on target T lymphoma cells. Primaries antibodies were an anti-CD2, CD5, and CD7 pool provided by Dr. A. Bernard (INSERM U343, Nice, France) and used at 20 µg/ml. Magnetic separation was used to remove free beads and T cells coated with beads. The proportion of NHL B cells was controlled by the FITC-conjugated anti-CD20 antibody detection. A minimum of 80% purity was achieved for 12 of 13 tumor samples. To determine the clonality of the B-cell population, matched fluorescent antibodies were used: rabbit antihuman light chains κ/FITC and λ/PE. Ninety-four % of cells expressed a single κ or λ chain in 11 of 13 tumor cell
Cytokines in Non-Hodgkin's Lymphoma

Preparations. This percentage was not modified after a 96-h culture in the presence of cytokines.

Cytokine Detection in the Supernatant. Cell supernatants were collected after a 96-h culture of purified NHL B cells. Samples were tested for IL-10 using a two-sided sandwich IL-10 ELISA assay. The assay was purchased from Innotest and recognizes specifically human IL-10. Serum IL-10 determinations were performed according to the kit procedure. IL-2 and IL-6 were determined using ELISA tests from Immunotech. TNF-α levels were measured using the Genzyme ELISA kit (Cambridge, MA).

Proliferation Assays. Purified B NHL cells (10^6 lymphoma B cells) were cultured in 96-well U-shaped microtiter plates and stimulated for proliferation in the presence of 10^8 irradiated (7500 rad) CDw32 L cells and anti-CD40 antibody (mAB 89). The CDw32/FcγRII-transfected Ltk (-) cell line (CDw32 L cells) was kindly provided by Dr. F. Rousset. All reagents tested for their stimulatory or inhibitory activity were added at the beginning of the culture. Cells were pulsed after different culture times (24, 48, 72, and 96 h) with 1 μCi/well [3H]thymidine 25 Ci/mmol (Amersham, Les Ulis, France) for 22 h. cpm incorporation was measured by tritium detection using standard liquid scintillation counting techniques on a beta counter (Packard, Rungis, France). Every experiment was performed in triplicates. Each result was confirmed by three independent experiments.

Statistical Analysis. Results of thymidine incorporation (expressed in cpm) in proliferation assays were compared using double-sided Student’s t test. The Fisher Exact Test was used to compare the proportion of samples with detectable cytokine levels in the different subgroups.

RESULTS

Immunohistochemical and RT-PCR Detection of Cytokine in Tumor Samples. Thirty (55%) of the 54 tumor samples were found positive for IL-10 expression by IHC in tumor cells and/or macrophages (Fig. 1, A and B). IL-10 was detected in tumor cells in 9 of 30 (30%) tumor samples, in macrophages in 3 of 30 (10%), and in both in 9 of 30 (30%; Table 1). In 9 (30%) patients, the nature of cells staining positively for IL-10 was not determined. Forty-eight (89%) of the 54 tumor samples were found positive for IL-6 expression by IHC, in both tumor cells and macrophages (Fig. 1, C and D). Fifteen of 52 (29%) samples were positive for TNF-α expression, mostly in macrophages. Eleven of 47 samples (23%) were weakly positive for IL-2 in nontumoral cells. IL-10 and IL-6 detection was observed in all NHL subtypes. However, IL-10 expression was more frequent in intermediate or high-grade NHL as compared to low-grade NHL (68% versus 29%; P = 0.01). TNF-α was detected in 40% of the 38 intermediate or high-grade NHLs but in none (0%) of the 16 low-grade NHLs studied (P = 0.01). Cytokine expression by IHC was not found correlated to the T/B phenotype. In four patients, the pattern of sequential biopsies were performed at the time of diagnosis and relapse. IL-10 and IL-6 IHC expression was consistent in different samples collected in the same patients at different times of tumor progression (Table 2).

The correlations between cytokine expression were then evaluated. Fourteen of the 15 tumor samples with TNF-α detected by IHC were also positive for IL-10 expression (P = 0.0001). The presence of TNF-α was thus observed almost exclusively in patients with IL-10-positive tumors in IHC. The expressions of IL-2 and TNF-α were also found marginally correlated (P = 0.05). The expressions of IL-6, IL-10, and IL-2 were not correlated.

Thirty-one of the frozen tumor tissues were analyzed by RT-PCR
for IL-10 mRNA expression and 29 for TNF-α, IL-6, and IL-2 mRNA expression. All 31 tumors expressed IL-10 mRNA, as evaluated by RT-PCR, of which 19 (61%) were positive in IHC. IL-6 mRNA was detected by RT-PCR in the 29 (100%) samples, which were all positive for IL-6 expression in IHC. Twenty-six of 29 (90%) tumors had detectable TNF-α mRNA by RT-PCR, of which 8 (31%) had a positive immunostaining for TNF-α. Ten of 29 (34%) tumor tissues expressed IL-2 mRNA as evaluated by RT-PCR, of which none was positive in IHC.

**Comparison of Cytokine Production in Situ, in Culture, and in Serum.** In 13 of these 54 patients, fresh tumor tissue was available for B NHL cell purification. PTCPs were cultured and tested for cytokine production in supernatant. Cytokine expression in tumor tissues, in the supernatant, and in the serum of the same patient was compared (Table 3).

IL-10 was detectable in the supernatant of four of five PTCP cultures obtained from IL-10-positive tumors in IHC as compared to one of eight IL-10-negative tumors in IHC (P = 0.03; Table 3). The four tumors with detectable IL-10 in supernatant had detectable serum IL-10. However, among the nine patients with detectable serum IL-10, only four had IL-10-positive tumors by IHC, and five PTCPs produced IL-10 in the supernatant (Table 3). This suggests that tumor cells are an important but not exclusive source of circulating IL-10 in these patients.

IL-6 was detected by IHC in the 13 tumor samples (Table 3) and in the supernatant of the 13 (100%) PTCPs. All 10 (100%) patients with increased serum IL-6 had detectable IL-6 in tumors by IHC. IL-6 concentration in serum and in the supernatant were highly correlated (r² = 0.82, P < 10⁻⁴), indicating that tumor cells are the major source of circulating IL-6 in these patients.

IL-2 was detectable in none of the supernatants of tumor cell cultures but was found increased in the serum of 7 (70%) of the 10 patients tested. Only one (8%) of these patients had detectable IL-2 in tumor samples by IHC (Table 3). TNF-α was detected in the supernatant of 3 of these 13 PTCPs in both positive and negative samples as evaluated by IHC. Among the three patients with detectable serum TNF-α, two had undetectable TNF-α in tumor samples, and only one had detectable TNF-α in the supernatant of the PTCP supernatant (Table 3).

**Modulation of Purified NHL B-Cell Proliferation by Cytokines.** All 13 PTCPs had a very low level of spontaneous proliferation when cultured either alone or in the presence of IL-6, IL-10, and IL-2 (data not shown). The stimulatory activity of cytokines was, therefore, tested in the presence of an additional costimulation, i.e., irradiated CDw32 L cells in the presence of an anti-CD40 mAb89. In these conditions, maximal [³H]thymidine uptake was obtained after 96 h of culture (Fig. 2). To assess the relevance of this system to the in vivo situation, the expression of the mRNA of CD40 ligand was investigated by RT-PCR in 29 primary tumors (Fig. 3). CD40 ligand mRNA was detectable in 28 of the 29 samples tested, including the 11 tumors from which PTCPs were obtained and which were tested.

Anti-CD40-activated, NHL-purified tumor B-cell preparations were cultured in the presence of IL-10, IL-2, IL-6, and TNF-α, either alone or in combination. IL-10, IL-6, and IL-2 enhanced [³H]thymidine uptake in 13 (100%), 5 (38%), and 9 (69%) of 13 PTCPs, respectively (Table 4; Fig. 2). TNF-α stimulated NHL B cell proliferation in only 2 of 10 (20%) PTCPs (data not shown). IL-10 induced the highest increase of [³H]thymidine uptake as compared to IL-2 and IL-6 (Fig. 2).

The interaction of cytokines was then investigated (Table 4; Fig. 2). IL-10 and IL-2 exerted a significantly additive effect on [³H]thymidine uptake in 7 of the 13 (54%) PTCPs. IL-6 and IL-10 exerted an additive effect in 3 of the 13 (23%) PTCPs. TNF-α and IL-6 had an additive effect on [³H]thymidine uptake in 3 of 10 (30%) PTCPs (data not shown). In 12 of 13 patients, the combination of IL-10, IL-6, and IL-2 induced the highest level of B-cell proliferation (Table 4).

**DISCUSSION**

The objectives of this study were to investigate the presence of IL-10, IL-6, IL-2, and TNF-α in NHL tumor samples, their correlation to cytokine serum levels in the same patients, and the growth-promoting effect of these cytokines on the proliferation of fresh B NHL cells. IL-10 is produced by a broad spectrum of normal cells including B and T lymphocytes, monocytes (1-4), Epstein-Barr virus-transformed B cells (31), and malignant cells in HIV-related NHLs (1-4, 12, 32, 33). An overproduction of IL-10 in vivo in patients with NHL has been described in several reports (13-15). The results here further indicate that all NHL tumors of patients with no known cause of immunosuppression express IL-10 mRNA, as detected by RT-PCR, and that a majority (61%) produce detectable amounts of

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**Table 2 Cytokine expression in sequential biopsies**

<table>
<thead>
<tr>
<th>Patients</th>
<th>IL-10</th>
<th>IL-6</th>
<th>IL-2</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>GER initial diagnosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>First relapse</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Second relapse</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LOP initial diagnosis</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>First relapse</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CAT initial diagnosis</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>First relapse</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TAU initial diagnosis</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>First relapse</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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**Table 3 Comparison of cytokine expression in tumors, in supernatant of tumor cell cultures, and in serum from the same patients**

<table>
<thead>
<tr>
<th>Patients</th>
<th>IL-10</th>
<th>IL-6</th>
<th>IL-2</th>
<th>TNFα</th>
</tr>
</thead>
<tbody>
<tr>
<td>BON</td>
<td>31</td>
<td>&lt;5</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BES</td>
<td>299</td>
<td>10</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>DUR</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>ARM</td>
<td>22</td>
<td>18</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BER</td>
<td>79</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>GES</td>
<td>74</td>
<td>9</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>RAF</td>
<td>25</td>
<td>&lt;5</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MAR</td>
<td>ND</td>
<td>&lt;5</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ORI</td>
<td>52</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>VIS</td>
<td>18</td>
<td>7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>THI</td>
<td>F</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>LAR</td>
<td>G</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>LOW</td>
<td>48</td>
<td>34</td>
<td>500</td>
<td>+</td>
</tr>
</tbody>
</table>

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* N, normal for healthy subjects. IL-10: N, <5 pg/ml; IL-2: N, <5 pg/ml; IL-6: N, <8 pg/ml; TNF: N, <5 pg/ml. *S, serum. Sup, supernatant; ND, not determined.

* Patients for whom tumoral cells were purified by an indirect technique with beads. <, 0% of cells in tumor sample; +/-, <1%; +, 1-5%; ++, 5-50%; +++, >50%.
IL-10 protein in their tumors. IL-10 was detectable in tumor cells and/or in macrophages in these patients. Four of the five PTCPs obtained from IL-10-positive samples also produced IL-10 in vitro in the supernatant, and all patients whose PTCPs produced IL-10 in supernatant had increased serum IL-10. However, four patients with increased serum IL-10 had undetectable IL-10 production, both in tumor and in the culture supernatant. Although this could be due to a low sensitivity of IHC, these results suggest that NHL tumors cells and infiltrating cells are an important but not the exclusive source of circulating IL-10 in patients with NHL.

IL-6 mRNA and protein expression was found detectable in a majority of patients in both tumor and/or nontumor cells. Lymphoma cell lines, anaplastic large cell lymphomas, Lennert lymphomas, HIV-related NHLs, in particular of the immunoblastic type, and Hodgkin's disease have been reported to produce IL-6 (19–21, 34–39). In addition, increased serum IL-6 levels have been reported in patients with lymphomas (22, 40). The results presented here further indicate that purified tumor cells are capable to produce IL-6 in supernatants in vitro and that IL-6 production by tumor cells in vitro is highly correlated to serum IL-6 levels in these patients. These results strongly suggest that NHL tumors are the major source of IL-6 in vivo.

TNF-α mRNA expression has been reported previously in 34% of patients.

Table 4 Additive effects of IL-10, IL-6, and IL-2 on thymidine incorporation of PTCPs costimulated with an anti-CD40 antibody

<table>
<thead>
<tr>
<th>Patients</th>
<th>[3H]Thymidine mean cpm (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[+1]</td>
</tr>
<tr>
<td>BON A</td>
<td>373 (27)</td>
</tr>
<tr>
<td>BES B</td>
<td>1007 (39)</td>
</tr>
<tr>
<td>DUR B</td>
<td>1184 (173)</td>
</tr>
<tr>
<td>ARM B</td>
<td>1850 (271)</td>
</tr>
<tr>
<td>BER B</td>
<td>311 (60)</td>
</tr>
<tr>
<td>GES B</td>
<td>194 (6)</td>
</tr>
<tr>
<td>RAF C</td>
<td>2890 (125)</td>
</tr>
<tr>
<td>MAR C</td>
<td>352 (40)</td>
</tr>
<tr>
<td>ORI E</td>
<td>333 (21)</td>
</tr>
<tr>
<td>VIS F</td>
<td>2327 (75)</td>
</tr>
<tr>
<td>THI F</td>
<td>2289 (114)</td>
</tr>
<tr>
<td>LAR G</td>
<td>10352 (472)</td>
</tr>
<tr>
<td>LOP G</td>
<td>2300 (38)</td>
</tr>
</tbody>
</table>

* Significantly greater than control (P < 0.05 Student's t test).
* Significantly greater than IL-10 alone (P < 0.05 Student's t test).
TNF-α-positive tumor samples were also positive for IL-10 expression in cell culture. IL-2 significantly stimulated NHL B cell proliferation in vitro. Importantly, IL-10, IL-6, and IL-2 additively stimulated the proliferation of PTCPs, indicating a possible cooperative mitogenic effect of these cytokines in vivo in patients with NHL.

Serum IL-10, IL-6, and TNF-α have been reported to correlate to survival in patients with active NHL (13, 22, 23, 41). The data presented here indicate that these observations could result, at least in part, from their growth-promoting activities in vivo on tumor cells. Modulation of cytokine production by tumor cells may represent a potential therapeutic option in non-HIV-related NHL, as already reported for HIV-related NHL (12).

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5504


Interleukin (IL)-10 and IL-6 Are Produced in Vivo by Non-Hodgkin's Lymphoma Cells and Act as Cooperative Growth Factors


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