The Proliferative Response of B Cell Chronic Lymphocytic Leukemia to Interleukin 2: Functional Characterization of the Interleukin 2 Membrane Receptors

By Ivo Touw, Lambert Dorssers, and Bob Löwenberg

To determine the growth properties of B cell chronic lymphocytic leukemia (B CLL) and to identify possible abnormalities thereof, we examined the in vitro action of interleukin 2 (IL2) in four patients. Using radiolabeled IL2 and monoclonal antibodies reactive with IL2 membrane receptors we showed that CLL cells, after their activation in vitro, express IL2 receptors of a high- as well as a low-affinity type, exactly as has been reported for normal T and B blasts. In three of the four reported cases, CLL proliferation (measured with [3H]-thymidine incorporation) depended on the addition of phytohemagglutinin (PHA) to activate the cells and IL2 (optimal concentration, 10 to 100 U IL2/mL). In contrast, the cells of the fourth case of CLL (CLL-4) proliferated in an autonomous fashion, i.e., without a need for PHA and IL2 in culture. Specific blocking of the IL2-binding sites with anti-IL2 receptor monoclonal antibodies almost completely inhibited the proliferation of these cells, which indicated that functional IL2 receptors were required for the autonomous proliferation. The demonstration of low concentrations of IL2 activity in the culture medium conditioned by the cells suggests that endogenous IL2 had been responsible for the spontaneous [3H]-thymidine uptake by the CLL cells of patient 4. However, we were unable to extract IL2 mRNA from the cells (neither fresh nor after various in vitro incubations) in quantities detectable by Northern blot analysis that would prove that the CLL cells of patient 4 were actively synthesizing IL2 during culture. Thus, individual cases of B CLL are subject to variable growth regulation involving functional IL2 receptors on the cell surface: (1) after activation with PHA the cells respond to exogenous IL2 in a fashion similar to normal B lymphocytes, or (2) the cells are stimulated by endogenous IL2 (or an IL2-like activity) and do not require activation with PHA.

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INTERLEUKIN 2 (IL2) is one of the polypeptide hormones controlling the proliferation and differentiation of B cells via surface membrane receptors. Studies in our laboratory have focused on the role of IL2 in the proliferation of leukemic B cells, one of the purposes being to analyze whether the leukemic cells express abnormal responsiveness to the hormone. Using a colony culture technique, we have shown that in acute lymphoblastic leukemia (ALL) of B cell origin, i.e., common ALL and pre-B ALL, IL2 stimulates proliferation, although only when additional factors derived from leukocyte feeder cells are present in culture as well. The nature of the latter factors has not been established yet. In B cell chronic lymphocytic leukemia (B CLL), IL2 as a single stimulus induces in vitro colony formation provided that the CLL colony-forming cells are activated with the phorbol ester 12-0-tetradeacanoyl phorbol-13-acetate (TPA) or the lectin phytohemagglutinin (PHA). Proliferation of CLL cells stimulated by IL2 and TPA has also been reported in [3H]-thymidine ([3H]-Tdr) incorporation assays.

In the present study we characterize the function of the IL2 membrane receptors of B CLL cells by using a radiolabeled IL2 preparation and further analyze IL2 responsiveness in microculture. We demonstrate that in vitro activated CLL cells express two classes of IL2 receptors, one with a high affinity and one with a low affinity for IL2, a property that these cells share with normal activated T and B blasts. Results from the microculture experiments show that CLL cells of three patients require IL2 and activation by PHA for proliferation. On the other hand, in a fourth case, CLL cells proliferate without supplementation of IL2 and PHA to the cultures. The results of additional experiments suggest that the "spontaneous" proliferation of these CLL cells is in fact not IL2 independent but controlled by endogenous IL2.

MATERIALS AND METHODS

Leukemic cells. CLL cells were isolated by Ficoll-Isopaque (Nyegaard, AS, Oslo) separation from the peripheral blood of patients who had not received cytoreductive therapy for at least 12 weeks. All patients had given informed consent. T lymphocytes were removed by rosetting with 2-aminoethylisothiouronium bromide (AET)-treated sheep erythrocytes followed by a second Ficoll-Isoaque separation.

The T cell-depleted leukemic cell fractions contained 97% to 100% small lymphocytes and always less than 0.5% E rosette-forming cells. Peripheral blood leucocyte counts and immunotypes are listed in Table 1.

Cell cultures. Proliferation of CLL cells was measured by [3H]-Tdr uptake. Cells (1 x 10^6) were cultured in triplicate in round-bottom microtiter wells (Greiner, Alphen a/d Rijn, The Netherlands) in 100 μL culture medium. This culture medium consisted of Dulbecco’s modified Eagle’s minimal essential medium, heat-inactivated fetal calf serum (6.7% vol/vol), heat-inactivated horse serum (6.7% vol/vol), trypticase soy broth (6.7% vol/vol) supplemented with dialyzed bovine serum albumin (0.75% wt/vol), egg lecithin (3 x 10^-5 mol/L), Na2SeO3 (10^-7 mol/L), iron-saturated human transferrin (7.7 x 10^-4 mol/L), and B-mercaptoethanol (10^-4 mol/L) as modified from Gilbert and Iscove. Additions to the cultures included 0.1% vol/vol PHA (reagent grade, Wellcome Reagents, Dartford, England), pure recombinant IL2 with a specific activity of 1.9 x 10^6 U/mg protein or approximately

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3 × 10^13 U/mol (Biogen SA, Geneva), and monoclonal antibodies (MoAb) reactive with IL2 membrane receptors (see the like-named section). Cultures were performed in triplicate. Sixteen hours before harvesting the cells (Tittertrack cell harvester 550; Flow Laboratories, Irvine, Scotland), 0.1 μCi of 3H-TdR (Amersham Corp, UK; specific activity, 2 Ci/mmole) was added to each microwell. Radioactivity was measured by liquid scintillation counting (Beckman LS 3800, Beckman Instruments, Fullerton, CA).

Suspension cultures of CLL cells, normal peripheral blood mononuclear cells, and Jurkat cells were performed in test tubes (1) to assess IL2 activities elaborated in the culture medium (2) to extract RNA from the in vitro stimulated cells, and (3) to determine binding of radiolabeled IL2 to in vitro stimulated cells. Cells (2 × 10^6/ml culture medium) were incubated either with PHA (0.1% vol/vol) plus TPA (10 ng/ml), PHA alone, or without additions. After various incubation periods (ranging from four hours to seven days) followed by centrifugation, the culture supernatants were collected, filter sterilized (0.22-μm filter, Millipore, Bedford, MA) and stored at -80°C. For radiolabeled IL2-binding experiments, the cells were incubated for one hour in fresh culture medium and washed five times with ice-cold α-medium supplemented with 1% wt/vol bovine serum albumin (BSA).

IL2-producing Jurkat cells (a gift from Dr L.A. Aarden, Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam) were cultured in RPMI medium supplemented with 10% FCS (Hyclone, Logan, UT) and 1% wt/vol bovine serum albumin (BSA) to remove endogeneous IL2. After 18 hours of suspension culture in the presence of 0.1% α-medium containing 1% wt/vol BSA to remove endogeneous IL2. Then, 10^6 cells in 50 μl α-BSA medium were plated in wells of medium-prewetted (α-BSA), millipore 96-well, 5.0-μm filter-bottom plates (Millipore). To determine the amount of specific binding, serial dilutions of pure, recombinant 125I-IL2 (Amersham, UK) ranging from 6.25 to 800 pmol/ml were added to the cells. The cells were incubated with the radiolabeled IL2 for one hour at 37°C. These conditions allow for optimal saturation of high- and low-affinity binding sites. To correct for nonspecific binding of the 125I-IL2, incubations were also performed in the presence of excess (ie, thousandfold concentrations) of nonlabeled recombinant IL2. All incubations were carried out in duplicate. At the end of the incubation, the culture supernatants were harvested by centrifugation, and 100 μl of the filter bottoms were washed twice with 100 μl ice-cold α-BSA medium. These washings were carried out rapidly (two to five seconds per wash) to prevent dissociation of specific IL2 binding. After drying (30 minutes at room temperature) the filter bottoms were punched out and collected in gamma counter vials. Radioactivity was counted in a 4/200 automatic gamma counter (Micromedic System, Horsham, PA). The data were analyzed by using the Ligand computer program of Munson and Rodbard.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Leukocyte Count (x 10^7/L)</th>
<th>Immunotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL-1</td>
<td>379</td>
<td>M</td>
</tr>
<tr>
<td>CLL-2</td>
<td>161</td>
<td>M + D</td>
</tr>
<tr>
<td>CLL-3</td>
<td>268</td>
<td>M + D, A, G</td>
</tr>
<tr>
<td>CLL-4</td>
<td>152</td>
<td>M + D</td>
</tr>
</tbody>
</table>

Table 1. Hematologic Data of CLL Patients

RESULTS

Expression of IL2 receptors on the CLL cells and binding of radiolabeled IL2. IL2 receptors, as assessed in indirect fluorescence with MoAb all2r and flow cytometry, were absent or weakly expressed on the fresh CLL cells. In all cases IL2 receptors were maximally expressed on the cells after 18 hours of suspension culture in the presence of 0.1%...
FUNCTIONAL IL2 RECEPTORS ON B CLL CELLS

Fig 1. Flow cytometric fluorescence (histogram) analysis of IL2 receptors on activated CLL cells (CLL – 3) and activated normal peripheral blood T lymphocytes (PBT). After 36 hours of in vitro incubation in the presence of 0.1% vol/vol PHA and washings, cells were stained with MoAb all2r and GAM/FITC. The dotted curve represents the fluorescence profile of the cells stained with GAM/FITC alone. Staining with a nonrelevant MoAb dotted curve represents the fluorescence profile of the cells

ings. cells were stained with MoAb all2r and GAM/FITC. The

0.1 % vol/vol PHA and wash-

vitro incubation in the presence of

normal peripheral blood T lymphocytes (PBT). After 36 hours of in

receptors on activated CLI cells (CII

revealed a fluorescence profile identical to that of the GAMIFITC

control.

PHA. Flow cytometric histogram analysis indicated that IL2 receptor densities on the in vitro activated CLL cells were lower than on PHA-activated normal T lymphocytes (Fig 1). A binding assay using 125I-IL2 was performed to determine the affinity and numbers of IL2 receptors on the activated CLL cells. For comparison, IL2 receptors on activated T lymphocytes were also studied. Specific binding of the radiolabeled IL2 was measured at concentrations ranging from 6.25 to 800 pmol/L. Scatchard plot analysis (Fig 2) indicated that CLL cells expressed two classes of receptors similar to the T cells, ie, one with an affinity for IL2 in the picomolar range and one with an affinity in the nanomolar range. The data of these experiments are listed in Table 2.

Proliferative response of CLL cells to IL2. DNA synthesis by CLL cells in culture was estimated by 3H-TdR incorporation at various time points for 11 days. Cells were cultured under different stimulatory conditions, ie, in the presence of PHA (0.1% vol/vol) and IL2 (50 U/mL), PHA alone, IL2 alone, and without these additions (Fig 3). In CLL cases 1, 2, and 3, PHA and IL2 were required for induction of proliferation. Omission of one or both of these components resulted in significantly less or no incorporation of 3H-TdR. 3H-TdR uptake was significantly reduced, ie, by 45% to 77% when anti-IL2 receptor MoAb interfering with the IL2 binding had been added to the cultures (at their initiation). This confirmed that stimulation occurred through IL2–IL2 receptor interaction.

In contrast, 3H-TdR incorporation by the cells of CLL-4 was completely independent of the addition of PHA and IL2 to the microcultures.

To ensure that the proliferating cells of the four patients were indeed the leukemic cells (and not, eg, residual normal T lymphocytes) immunologic analysis was performed. From this it appeared that on day 7 of culture the cells expressed the clonal phenotypes characteristic of the neoplasm (Table 1). The cultured cells contained less than 1% T cells, as assessed by E rosetting and MoAb anti-T3 labeling in indirect immunofluorescence. Thus, T cells did not significantly contaminate the in vitro growth of the CLL cells. Leukemic cell growth was also confirmed by cyogenetic analysis. On day 7 of culture of the cells of CLL-1, 13 of 15 of the spread metaphases expressed abnormal karyotypes

Table 2. Binding of Radiolabeled IL2

<table>
<thead>
<tr>
<th>Cell Type*</th>
<th>Mean Number of Receptors per Cell Kd (pmol/L)</th>
<th>Low-Affinity Binding†</th>
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<tbody>
<tr>
<td></td>
<td>Mean Number of Receptors per Cell Kd (pmol/L)</td>
<td>Mean Number of Receptors per Cell</td>
</tr>
<tr>
<td>CLL-1</td>
<td>34</td>
<td>90</td>
</tr>
<tr>
<td>CLL-2</td>
<td>7.5</td>
<td>64</td>
</tr>
<tr>
<td>CLL-3</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>CLL-4</td>
<td>1.2</td>
<td>11</td>
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<tr>
<td>Normal T lymphocytes‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor 1</td>
<td>10.4</td>
<td>171</td>
</tr>
<tr>
<td>Donor 2</td>
<td>19</td>
<td>154</td>
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Abbreviation: ND, not determined.

*Cells had been cultured for 72 hours in the presence of 0.1 % vol/vol PHA.
†Estimated using the Ligand computer program of Munson and Rodbard.29
‡Isolated from normal peripheral blood.

Fig 2. Specific binding of 125I-IL2 to activated normal periph-

eral T lymphocytes and activated CLL cells (patient 3). T cells and

CLL cells had been incubated in the presence of 0.1% vol/vol PHA

for 36 hours. The data are expressed as Scatchard plots; the

insets show the relationships between the concentrations of free

and specifically bound 125I-IL2.

Fig 3. 3H-TdR incorporation by CLL cells under different

stimulatory conditions in microculture. (A - A), PHA (0.1% vol/

vol) + 50 μL IL2 (50 U/mL); (■ - ■), PHA alone; (△ - △), IL2 alone;

and (○ - ○), no additions. Left panel shows the data of the

experiments with CLL – 1. Comparable results were obtained

with the cells of CLL – 2 an i CLL – 3. In the right panel, the data

of 3H-TdR uptake of CLL – 4 are plotted. The cells were cultured

for 11 days.
including inv(X), 22p+, and +22. In CLL-4, 30 of 31 analyzed metaphases appeared abnormal (ie, expressing 14q+) after seven days of culture.

IL2 dependence was investigated by adding IL2 in increasing concentrations to the microcultures (Fig 4). In CLL-1 to -3, optimal proliferation occurred in the presence of 50 to 100 U IL2/mL. On the other hand, in CLL-4, maximal incorporation of ^3H-TdR was obtained in cultures without any addition of IL2. This could suggest that the cells of CLL-4 lacked IL2 dependence or that the cells produced IL2 and that proliferation was stimulated by endogenous IL2 (see the next paragraph). On several occasions we attempted to establish CLL cell lines by prolonged culturing and refeeding of the cultures but had negative results.

**Analysis of the "spontaneous" proliferation of CLL cells (case 4).** In CLL-1 to -3, proliferation depended on the presence of PHA and IL2. In contrast, in CLL-4 the rate of proliferation reached a maximum although no IL2 (and PHA) had been supplemented to the cultures (Figs 3 and 4). Epstein-Barr virus nuclear antigen was absent in these cells, which indicated that these cells had not been transformed by Epstein-Barr virus. We addressed the question as to whether spontaneous proliferation in CLL was truly IL2 independent or possibly mediated through endogenous IL2.

First, we examined the effect of four different MoAbs, reactive with IL2 receptor determinants, on the proliferation of the cells of CLL-4 (Table 3). The addition of MoAbs aiIL2r, anti-Tac, and 18E6.4, which compete with IL2 for receptor binding, completely inhibited ^3H-TdR incorporation. In addition, incubation of the cells with MoAb 36 A1.2, an anti-IL2 receptor antibody that does not block IL2 binding, did not exert an inhibitory effect on proliferation. These results indicated that the IL2 receptors (and more specifically the IL2-binding sites of these receptors) had a role in the proliferation even though the cells had not been exposed to exogenous IL2. We hypothesized that these receptors were activated by endogenously produced IL2. To test this possibility, IL2 activities elaborated by the CLL-4 cells in the culture medium were estimated. Media conditioned by CLL cells in suspension cultures were collected at various times (one to seven days) and assayed on a murine

![Fig 4. ^3H-TdR incorporation by CLL cells in relation to the concentration of IL2 added to the cultures. The cells were incubated with or without IL2 for ten days. Left section, CLL – 2 (cells were cultured in the presence of 0.1% vol/vol PHA); right section, CLL – 4 (cells were cultured in the absence of PHA).](image)

<table>
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<th>Table 3. Effect of Four Different Anti-IL2 Receptor MoAbs on the Spontaneous Proliferation of CLL Cells (CLL-4)</th>
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<tr>
<td>MoAb*</td>
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<tr>
<td></td>
</tr>
<tr>
<td>allIL2 r</td>
</tr>
<tr>
<td>anti-Tac</td>
</tr>
<tr>
<td>18E6.4</td>
</tr>
<tr>
<td>36 A1.2</td>
</tr>
</tbody>
</table>

IL2 (or PHA) was not added to the cultures.  
*Each of the MoAbs was added at saturating concentrations (as assessed in indirect immunofluorescence) to microcultures at the initiation of the cultures.  
†Assessed on day 8 of culture. Data are expressed as means ± SD of triplicate cultures. Figures in parentheses represent the percentages of control values.

IL2-dependent (CTLL) cell line. IL2 activities (ie, at concentrations varying from 0.15 to 1.5 U/mL) were indeed detectable in these CLL cell-conditioned media. By comparison, PHA- and TPA-activated Jurkat cells produced 50 to 100 U IL2/mL culture medium.

Finally, we applied Northern analysis by using an IL2 cDNA probe to test whether IL2 mRNA was present in the cells of CLL-4 (fresh and after various in vitro incubations), which would indicate that the cells were capable of synthesizing IL2. We were repeatedly unable to detect IL2 mRNA in the cells of CLL-4, even when relatively large quantities (ie, estimated at 2 to 5 µg) of purified, poly(A)+, mRNA preparations had been loaded onto the gels (Fig 5). At the same time, IL2 mRNAs from control cells, ie, PHA/TPA-activated Jurkat cells, as well as from PHA/TPA-activated normal peripheral blood T cells, were readily demonstrated (Fig 5). Control hybridization of the blot with actin cDNA indicated that the RNA extracted from the CLL cells had been intact. The inability to show IL2 mRNA in cell samples of CLL-4 could suggest that the endogenous IL2 in the

Fig 5. Autoradiographs of blotted poly(A) + RNA samples from CLL cells, Jurkat cells, and peripheral blood T lymphocytes. The blot was first hybridized with a radioactive IL2 cDNA probe (upper section). After removal of the IL2 cDNA by highly stringent washings, the blot was rehybridized with actin cDNA (lower section). Ribosomal RNA (28S and 18S) was run in a separate lane (stained with ethidium bromide) and used as a size marker. Lane 1, CLL – 4 after incubation (22 hours) in the presence of PHA and TPA; lane 2, CLL – 4 after incubation (six hours) with PHA/TPA; lane 3, CLL – 4 after incubation (22 hours) without the addition of PHA/TPA; lane 4, CLL – 1, after incubation (22 hours) with PHA/TPA; lane 5, peripheral blood T lymphocytes after incubation (22 hours) with PHA/TPA; lane 6, PHA/TPA-activated (six hours) Jurkat cells; lane 7, Jurkat cells, 1/10 the amount of lane 6.
culture media conditioned by these cells was not due to active production but represented an activity previously absorbed by the CLL cells from an exogenous source. On the other hand, it remains possible that the CLL cells did synthesize small quantities of IL2 but that the amount of IL2 mRNA remained below the detection level of the Northern blot analysis.

**DISCUSSION**

Recently it was shown that IL2 is a growth stimulator of B lymphocytes, and detailed information with respect to the responsiveness of B cells to IL2, their requirements of activation for the expression of membrane receptors for IL2, and the binding properties of the IL2 receptors has become available. Similar studies carried out with leukemic cells should reveal whether the response of the cells to growth and differentiation stimuli has been altered as a result of neoplastic transformation. The present experiments were undertaken to determine the properties of IL2 receptors and the IL2 responsiveness of leukemic B lymphocytes, ie, in four cases of B CLL, and to establish whether these features were different from those reported for normal B lymphocytes.

From radiolabeled IL2 binding assays it became apparent that activated CLL cells express two classes of IL2 receptors, ie, with a high (picomolar range) affinity and with a low (nanomolar range) affinity (Table 2). Comparable data have been reported for murine B cell blasts. Mean densities of IL2 receptors per cell on the mouse blast cells appeared to be considerably (about 200 times) higher than those on human B CLL cells. On the other hand, Muraguchi et al, without discriminating between high- and low-affinity binding sites, estimated the mean numbers of IL2 receptors per normal activated human B cell at 320 (with a mean Kd of 457 pmol/L). The low mean numbers of high-affinity IL2-binding sites per cell that we observed in CLL (ie, ranging from 11 to 90) could indicate that these functional receptors are not equally distributed over the total CLL cell population but concentrated on the IL2-responsive clonogenic fraction that comprises only a small minority (less than 0.1%) of the total CLL cell population. It should be emphasized that the cell suspensions had not been enriched for blast cells as was described by Robb et al. This may also explain why the mean numbers per cell of high-affinity IL2 receptors on normal activated T lymphocytes that we observed were ten to 20 times less than those reported by Robb et al for density gradient purified T blasts.

In three cases (CLL-1, -2, and -3), proliferation as measured by 3H-TdR incorporation was stimulated by IL2. The optimal IL2 concentration in these cases was 50 to 100 U/mL (Fig 4), ie, in the same order as reported for proliferation of normal B lymphocytes. For an optimal response to IL2 the cells of CLL-1, -2, and -3 also required activation by PHA. This lectin has been previously shown to activate CLL cells. In separate experiments not reported here, the phorbol ester TPA was used as the activation signal with comparable results.

In contrast to the earlier described response pattern of CLL-1, -2, and -3, the cells of CLL-4 proliferated independently on exogenous IL2 and did not require PHA as an activating factor (Figs 3 and 4). Nonetheless, after in vitro incubation the CLL-4 cells expressed functional IL2 receptors with a high affinity for IL2 (Table 2). Upon further analysis it became evident that spontaneous proliferation of the CLL-4 cells was most likely induced by endogenous IL2: specific blocking of IL2 receptors with monoclonal antibodies almost completely abrogated cell proliferation (Table 3). Moreover, concentrations of IL2 activity within the picomolar range were detected in the growth medium in which the cells from CLL-4 had been cultured. One could argue that this endogenous IL2 activity had been produced by activated contaminant T lymphocytes. For two reasons we consider this possibility unlikely: (a) In the fresh (T cell–depleted) material no T lymphocytes were detectable, and immunologic and cytogenetic analysis indicated that T lymphocytic cells had not expanded during culture either; (b) T lymphocytes require activation (eg, by the lectin PHA) or a phorbol ester for IL2 production, whereas spontaneous proliferation of the CLL-4 cells and elaboration of IL2 into the culture medium occurred in the absence of PHA. Therefore it is most plausible to assume that indeed the CLL cells (CLL-4) elaborated IL2 in the culture medium. The suggestion that normal and transformed B cells can produce cytokines, including IL2, was recently raised by several investigators. To investigate whether the cells of CLL-4 were able to synthesize IL2 we attempted to detect IL2 mRNA in the cells, with, however, negative results (Fig 5). Thus, if the cells really produced IL2, they formed IL2 mRNA in a quantity below the detection level of the Northern analysis. Typically, this could be the case when only a small portion of the CLL cells, eg, the proliferating subpopulation under the applied in vitro conditions, synthesized IL2. More sensitive detection methods, eg, S1 nuclease mapping or in situ hybridization techniques, will be needed to clarify this issue. Other explanations for the failure to detect IL2 mRNA are that the cells of CLL-4 produced an IL2-like activity with a protein structure discrepant from T cell–derived IL2 or that the CLL cells released IL2 that had previously been absorbed by the cells from external sources.

Taken together, the present experiments have established that the proliferation of CLL cells in vitro is regulated by IL2 through direct IL2–IL2 receptor interaction, similar to that of normal B lymphoblasts. In addition, the experimental data suggested that in some cases the CLL cells are capable of autocrine IL2-mediated stimulation. Autocrine growth may be the result of neoplastic transformation, and it has been suggested as a mechanism of maintaining tumor cell proliferation. On the other hand, it has become clear that normal cells may be controlled by autocrine mechanisms of growth as well. For example, T lymphocytes proliferate in response to endogenous IL2. It is unknown whether (subpopulations of) normal B cells may also be capable of autostimulation. Therefore, at the present time, it remains uncertain as to whether the growth characteristics of the cells of CLL-4 represent those of a normal B lymphocytic subset or whether they are typical of the transformed status of the neoplastic cells. Irrespective of this question, it is intriguing that primary tumor cells from individual patients that apparently descended from the same type of cell (ie, early B lympho-
cyte) may show markedly different in vitro growth characteristics.

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The proliferative response of B cell chronic lymphocytic leukemia to interleukin 2: functional characterization of the interleukin 2 membrane receptors

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