Cyclin E Overexpression in Relapsed Adult Acute Lymphoblastic Leukemias of B-Cell Lineage

By Richard Scuderi, Karolina A. Palucka, Katja Pokrovskaja, Magnus Björkholm, Klas G. Wiman, and Pavel Pisa

Using Western blot analysis, we examined cyclin E and cyclin A protein levels in 19 patients with acute lymphoblastic leukemia (ALL) 15 B-ALL and four T-ALL). Whereas normal, nonproliferating peripheral blood mononuclear cells (PBMCs) expressed low levels of the 50-kD cyclin E, ALL blasts in the peripheral blood, although showing low-level or no proliferation as judged by FACS/cell-cycle analysis and cyclin A protein levels, expressed high levels of cyclin E, with a mean value similar to that of the proliferating Burkitt’s lymphomas cell line, Akata. The accumulation of a protein shown to shorten the G1 phase of the cell cycle, cyclin E, in growth-delayed leukemic blasts may reflect the malignant status of these cells. Before treatment, B-ALL cells expressed predominantly the 50-kD cyclin E. T-ALL samples displayed the 50-kD cyclin E protein and a smaller, approximately 43-kD cyclin E species. In paired B-ALL samples taken before treatment and at relapse, we found a significant overexpression of the 50-kD protein in relapsed samples (P < .006), plus the presence of up to four additional smaller–molecular-weight species of cyclin E, illustrating clear diagnosis versus relapse differences. Cyclin E expression in ALL blasts may correlate to the relative malignant status of the cells, with higher protein levels reflecting a more advanced stage of the disease and a greater potential to proliferate under permissive conditions.

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IN THE PAST DECADE, intensive treatment regimens and marked improvements in supportive care have resulted in an 80% complete remission rate in patients with acute lymphoblastic leukemia (ALL).

However, in contrast to children with ALL, most initially cured adult patients relapse in the first 3 years following treatment and die from a refractory disease. These patients are generally resistant even to high-dose conditioning therapy administered before allogeneic bone marrow transplantation. The biologic mechanisms behind the evolution of leukemic blasts from the time of diagnosis to the time of relapse are poorly understood. The immunophenotype, as well as the karyotype, of the leukemic blasts often remain unchanged at relapse.

Multiple drug resistance (mdr1), although the only drug-resistant phenotype associated with poor prognosis in ALL, does not explain the therapeutic failures at relapse. This indicates that other biologic mechanisms may be responsible for the evolution of leukemic blasts into more aggressive populations.

In an attempt to further characterize biologic traits of adult ALL blasts, we studied the cyclin E and cyclin A cell cycle–regulatory proteins. The mammalian cyclin E gene encodes a protein of approximately 50 kD discovered by its ability to restore entry into the S phase of the cell cycle in budding yeast (Saccharomyces cervisiae) strains devoid of G1 cyclins. Cyclin E mRNA and protein levels oscillate during the cell cycle, with a peak in late G1 near the S phase boundary followed by a decrease as cells proceed through S phase. Activated kinase complexes consisting of cyclin E and cyclin-dependent kinase 2 (cdk2) are capable of phosphorylating such substrates as the retinoblastoma protein (pRb), and appear to be important in progression through G1. Acceleration of the G1- to S-phase transition by overexpression of cyclin E was demonstrated in vitro in mammalian fibroblasts and HeLa cells, while blocking of cdk2 by antibodies inhibited the entry of mammalian cells into S phase. Aberrant expression of cyclin E is found in several tumor types and some leukemias.

The approximately 60-kD cyclin A protein appears later in the cell cycle than cyclin E, at the G1/S-phase transition, is capable of associating with and activating cdk2, and is required for progression through S phase. The correlation between cyclin A expression and S phase makes the protein a useful marker of cellular proliferation.

For our studies, samples from both B- and T-lineage ALL patients were used for comparison. In addition, patient samples taken from diagnosis and relapse were studied to observe cyclin E and A expression in the context of leukemia progression. The cyclin E protein was detected in all samples to varying degrees, with higher levels of expression found in relapsed samples versus samples from diagnosis. In addition, in our small group of patients, the only two survivors in disease-free remission at the date of study completion had the lowest cyclin E levels at the time of diagnosis.

MATERIALS AND METHODS

Primary leukemic cells. A total of 19 adult patients seen at our hematology unit with B- and T-ALL (15 and four patients, respectively) were included in this study. The mean age was 34 years and ranged from 16 to 79. In five B-ALL cases and one T-ALL case, paired samples from diagnosis and relapse were available. The diagnosis of leukemia was established according to the French-American-British classification based on the bone marrow morphology and cytochemistry and the immunophenotype. The median percentage of blasts in the bone marrow and/or peripheral blood for all samples was 80% (range, 40% to 98%) as determined by flow cytometry and/or morphology. Each sample was further enriched by Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient centrifugation before
experimental analysis (median % blasts after Ficoll-Paque, 83%; range, 50% to 99%). The percentage of leukemic blasts in patients studied both before treatment and at relapse is listed in Table 1. Peripheral blood was collected in most cases (one bone marrow sample was included: patient no. four relapse, 80% blasts in bone marrow). Mononuclear cells collected in heparinized glass tubes were separated by Ficoll-Paque gradient centrifugation, frozen by a programed freezer in 50% AB serum and 10% dimethyl sulfoxide (DMSO) at a density of 107 cells/mL, and stored in liquid nitrogen until used. Before protein analysis, frozen cells (1 mL) were thawed at 37°C in an equal volume of fetal calf serum (FCS) GIBCO BRL, penicillin (100 U/mL), and 10% heat-inactivated FCS for 3 days with or without 0.1% heat-inactivated FCS for 93 hours to obtain activated cells for FACScell-cycle analysis.

Mononuclear cells from a buffy coat were stimulated with PMA (0.3 ng/mL), phorbol-12-myristate 13-acetate (PMA) (4 ng/mL) and OKT3 (25 ng/mL) in RPMI 1640 medium and 10% heat-inactivated FCS for 3 days with or without 0.1% heat-inactivated FCS for up to 48 hours based on previous experimental procedures.22 B cells were cultured in Iscove’s modified medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% heat-inactivated FCS for 3 days with or without 0.1% heat-inactivated FCS, formalin-treated Staphylococcus aureus Cowan 1 (SAC) and 10 U recombinant interleukin-2. A Ficoll-Paque mononuclear fraction of cells (PBMCs) from a buffy coat were stimulated with PMA (4 ng/mL) and OKT3 (25 ng/mL) in RPMI 1640 medium and 10% FCS for 93 hours to obtain activated cells for FACS/cell-cycle analysis.

**Immunophenotyping.** Leukemic blasts and PBMCs were evaluated by multiparameter flow cytometry (FACScan; Becton Dickinson, San Jose, CA) and direct three-color immunofluorescence staining using the following monoclonal antibodies: CD2, CD3, CD14, CD20, and CD34 HLA-DR (Becton Dickinson); CD10, terminal deoxynucleotidyl transferase (Dakopatts, Glostrup, Denmark); and CD19 TRI (Caltag, Emeryville, CA). Normal B and T lymphocytes were isolated (in separate tubes) from PBMCs by magnetic beads coated with monoclonal antibodies specific for CD20, CD2 and CD2 (Dynal, Oslo, Norway), respectively, according to manufacturer’s instructions. T lymphocytes were incubated at a density of 2 × 105/mL in RPMI 1640 medium with 10% heat-inactivated FCS, l-glutamine (1 mmol/L), penicillin (100 U/mL), streptomycin (100 μg/mL), phytomagglutinin (1 μg/mL), phorbol-12-myristate 13-acetate (PMA) 0.3 ng/mL, and calcium ionophore A23187 (0.12 μg/mL) in 37°C and 5% CO2 for up to 48 hours based on previous experimental procedures.22  B cells were cultured in Iscove’s modified medium supplemented with penicillin (100 U/mL), streptomycin (100 U/mL), and 10% heat-inactivated FCS for 3 days with or without 0.1% heat-inactivated, formalin-treated *Staphylococcus aureus* Cowan 1 (SAC) and 10 U recombinant interleukin-2. A Ficoll-Paque mononuclear fraction of cells (PBMCs) from a buffy coat were stimulated with PMA (4 ng/mL) and OKT3 (25 ng/mL) in RPMI 1640 medium and 10% FCS for 93 hours to obtain activated cells for FACS/cell-cycle analysis.

**Cell lines.** The Burkitt’s lymphoma cell line, Akata,33 was grown under standard conditions in RPMI 1640 medium (GIBCO BRL) with 10% heat-inactivated FCS, and harvested during an exponential phase of growth.

**In vitro stimulation.** Normal B and T lymphocytes were isolated (in separate tubes) from PBMCs by magnetic beads coated with monoclonal antibodies specific for CD20, CD2 and CD2 (Dynal, Oslo, Norway), respectively, according to manufacturer’s instructions. T lymphocytes were incubated at a density of 2 × 105/mL in RPMI 1640 medium with 10% heat-inactivated FCS, l-glutamine (1 mmol/L), penicillin (100 U/mL), streptomycin (100 μg/mL), phytomagglutinin (1 μg/mL), phorbol-12-myristate 13-acetate (PMA) 0.3 ng/mL, and calcium ionophore A23187 (0.12 μg/mL) in 37°C and 5% CO2 for up to 48 hours based on previous experimental procedures.22

### Table 1. Characteristics and Disease History of Relapsed Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Morphology/Immunophenotype</th>
<th>Stage</th>
<th>Cytogenetics</th>
<th>Phenotype</th>
<th>% Blasts*</th>
<th>Remission Duration (mo)</th>
<th>Chemotherapy Prior to Relapse</th>
<th>S-G2/M Phase (% of total cells)</th>
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<td>1</td>
<td>45</td>
<td>L2 Null ALL</td>
<td>D</td>
<td>46,XY</td>
<td>CD19*; 10'; DR+</td>
<td>88</td>
<td>Dnr, Cy, Vcr, Asp, VP16, AraC, Amsa</td>
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<td>46,XY</td>
<td>CD19*; 10'; 34'; DR+</td>
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<td>5</td>
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<tr>
<td>3</td>
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<td>D</td>
<td>NA</td>
<td>CD19*; 15'; 10'; 34'</td>
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<td>5</td>
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</tr>
<tr>
<td>4</td>
<td>25</td>
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<td>CD19*; 10'; 34'; DR+</td>
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<td>Dox, Cy, Vcr, VP16</td>
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</tr>
<tr>
<td>5</td>
<td>30</td>
<td>Common ALL</td>
<td>D</td>
<td>NA</td>
<td>CD19*; 10'; Tdt+</td>
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<tr>
<td>16</td>
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<td>D</td>
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<td></td>
<td>84</td>
<td>16</td>
<td>ND</td>
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</table>

**Abbreviations:** D, diagnosis; R, relapse; NA, not available. Amsa, amssercine; AraC, cytarabine; Cy, cyclophosphamide; Dnr, daunorubicin; Dox, doxorubicin; Mtx, methotrexate; Mxn, mitoxantrone; Vcr, vincristine; VP16, etoposide; Tdt, terminal deoxytransferase; ND, not determined.

* Morphologic evaluation of blasts in peripheral blood and bone marrow.

† Determined by propidium iodide staining and flow cytometry.
DNA staining was evaluated by fluorescence 2 intensity in the linear scale using FACSscan. Nonactivated and PMA/OKT3-activated PBMCs and the Burkitt’s lymphoma cell line, Akata, were used as controls.

**Western analysis.** Cell pellets were lysed in ice-cold immunoprecipitation buffer (1% Triton X-100, 0.1 mol/L Tris hydrochloride (pH 8.0), 0.15 mol/L NaCl, 5 mmol/L EDTA (pH 8.0), and 1 mol/L phenylmethylsulfonyl fluoride), followed immediately by the addition of an equal volume of modified 2X Laemmli buffer (155 mmol/L Tris hydrochloride (pH 6.8), 4% sodium dodecyl sulfide (SDS), and 25% glycerol). After 10 minutes of boiling, cell lysates were centrifuged for 10 minutes at 12,000 rpm. supernatant was pipetted to a new tube, and total protein concentration was determined by comparison to bovine albumin standards using the DC Protein Assay Kit (Bio-Rad, Hercules, CA). Thirty-six micrograms of protein from each lysate was boiled with 1/5 vol DB solution (3 parts 1 mol/L dithiothreitol and 2 parts 0.1% bromophenol blue in ethanol) for 3 to 5 minutes, separated by 10% SDS-polyacrylamide gel electrophoresis, and electroblotted onto nitrocellulose membranes (0.45 μm; Bio-Rad). Transfer efficiency was confirmed by Ponceau-S staining. Membranes were blocked in blocking solution (5% nonfat milk and PBS-T [PBS with 0.05% Tween-20]) for 1 hour. Mouse antihuman cyclin E monoclonal antibody (Pharmingen, San Diego, CA) diluted 1:400 or mouse antihuman cyclin A monoclonal antibody (Santa Cruz, Santa Cruz, CA) diluted 1:100 in blocking solution were used for antigen detection. Secondary horseradish peroxidase-conjugated antimouse IgG antibodies (Amersham, Amersham Place, UK) were diluted 1:7,000. Antibody-protein interactions were detected using the ECL system (Amersham).

**Densitometry.** Scanning and densitometry of ECL-exposed films was performed using equipment from Bio-Rad (Molecular Analyst, version 2.0). The mean absorbance (A) multiplied by the area of each signal (A × area) was used to compare samples. Only the 50-kD cyclin E species was included in scanning and densitometry analysis. Absorbance values from different gels were normalized with respect to the Akata cell line, which was included on every blot and given the arbitrary A × area value of 45 U.

**Statistical analysis.** The nonparametric Wilcoxon test was used for analysis of densitometric values from diagnosis versus relapse samples. Simple linear regression was used to compare levels of cyclin E at the time of diagnosis with overall patient survival.

**RESULTS**

**FACS/cell-cycle analysis.** Propidium iodide staining of cellular DNA followed by FACS analysis (Fig 1) revealed a characteristic noncycling (G0/G1) fluorescence peak in leukemic blast cell samples at diagnosis and relapse. A similar pattern was observed in resting PBMCs. PBMCs stimulated for 93 hours with PMA and OKT3, as well as exponentially growing Akata cells, on the other hand, showed a fluorescence pattern characteristic of proliferating cells, with a significant fraction of cells in S and G2/M phases. The percentage of cells in the S and G2/M phases of the cell cycle is shown for most of the samples from diagnosis and relapse (Table 1). Patients no. 1 and 2 showed a slight increase in the percent of S- and G2/M-phase cells at relapse versus diagnosis, whereas patients no. 3 and 5 showed no clear differences. The S- and G2/M-phase fraction for the Akata cell line was 31%.

**Cyclin E protein expression.** Cyclin E protein levels were examined by Western blot in extracts of leukemic blast cells from patients with B- and T-ALL and compared with Akata, normal nonproliferating cells (PBMCs) and normal peripheral blood T and B cells (Fig 2A to C, upper panels). The Akata cell line had moderate cyclin E expression with a predominant 50-kD species and a less intense band just below it. PBMCs expressed cyclin E at a low level. Similarly, purified B cells (CD 19 >90%) had only low amounts of the 50-kD cyclin E. However, activated B cells showed a clear increase in the expression of cyclin E after 3 days of SAC stimulation in culture. Relative intensities of cyclin E protein expression in Akata cells and in stimulated and unstimulated peripheral blood cells as determined by densitometry are shown in Fig 3A.

Dilution experiments were performed to confirm that the expression of cyclin E observed in leukemic blast cells was a true reflection of the blasts and was not influenced by the small percentage of other cells in the peripheral blood (Fig 4) that are also present in the PBMC fraction after Ficoll-Paque isolation, ie, mainly lymphocytes, and to a small degree, erythrocytes and granulocytes.

Blasts from 15 B-ALL patients at diagnosis were examined and expressed varying levels of the 50-kD cyclin E as shown by Western blot (Fig 2A and C) and as quantified by densitometry (range, 9 to 131, A × area units; median, 42; mean, 50.1). The level of expression ranged from low, comparable to that of PBMCs or unstimulated B cells (patients no. 3, 6, and 13 to 15), to levels similar to Akata cells (patients no. 2, 5, and 12), to levels approaching and increasing above the level of SAC-stimulated B cells (patients no. 1, 4, 7, 8, 9, 10, and 11), as determined by densitometric values.

Isolated T cells (CD 2 >90%) from a healthy donor expressed low amounts of cyclin E (Fig 2B). However, after 48 hours of in vitro stimulation, the level of the 50-kD cyclin E protein increased along with the appearance of a smaller band just below it and one additional band at approximately 43 kD. T-ALL samples (patients no. 16 to 19) expressed varying amounts of cyclin E protein (range, 33 to 94 U; median; 43; mean, 57.4), and some samples (patients no. 16D and 17, 80 and 94 U, respectively) had levels of cyclin E similar to 48-hour mitogen-stimulated T cells (88 U). The remaining T-ALL samples had slightly lower cyclin E levels, but all values were greater than for both PBMCs and unstimulated T cells. A 43-kD species of cyclin E was observed in all T-ALL samples and stimulated T cells, but was not present in PBMCs or unstimulated T cells.

**Cyclin E overexpression in relapsed B-ALLs.** Comparison of B-ALL cells from five patients before treatment and at relapse revealed a consistent pattern of cyclin E overexpression among relapsed samples. Overexpression of the common 50-kD species and expression of between two and four additional faster migrating forms of the cyclin E protein were found in all relapsed B-ALL samples, compared with paired-patient samples from diagnosis (Fig 2C).

The apparent overexpression of cyclin E in relapsed B-ALL samples, as visualized on Western blots, was confirmed by densitometric analysis. In all five samples tested, there was a statistically significant (P < .006) increase in 50-kD cyclin E expression in relapsed samples (Fig 3B).
Fig 1. Histograms of propidium iodide-stained cellular DNA. DNA-staining fluorescence intensity on a linear scale (x axis) and cell number (y axis) are shown. Note the difference in DNA fluorescence intensity between nondividing samples (B to D and F) that show a G0/G1 peak and proliferating samples (A and E) that show cells in all phases of the cell cycle, including S and G2/M phases. B and C, patient no. 2, diagnosis and relapse; F, patient no. 19, relapse.

Fig 2. (A to C) Cyclin E and cyclin A protein expression as detected by Western blot with equal amounts of total protein lysates (36 µg) in each lane. Burkitt's lymphoma cell line, Akata, in exponential growth phase, normal in vitro stimulated with PHA, PMA, and calcium ionophore for 48 hours, CD2⁺ T cells, and 72-hour SAC-stimulated CD19⁺ B cells were included as controls of proliferating cells. Numbers represent individual patient samples obtained at time of diagnosis. In cases where samples from time of diagnosis and relapse were available, these are labeled D and R, respectively.
**Diagnosis and Relapse**

**Fig 3.** 50-kD cyclin E relative signal intensities as measured by scanning and densitometry of ECL-exposed Western blots. Values were normalized with respect to the signal intensity of the Akata cell line included on every blot (chosen value, 45 U). (A) Burkitt's lymphoma cell line, Akata, and normal PBMCs both stimulated and unstimulated. (B) B-ALL samples (patients no. 1 to 5) at relapse had significantly higher signal intensities of the 50-kD cyclin E paired samples from diagnosis ($P < .006$). The faster-migrating cyclin E isoforms (<50 kD) were not included in these values.

**Fig 4.** Dilution analyses of cyclin E expression by Western blot. In each lane, 36 µg total protein was loaded. Lane 1, normal PBMCs; lane 2, cell lysate from patient no. 16D; lanes 3 and 4, sample 16D diluted with normal PBMCs 3:1 and 1:1. The same dilution series was performed on patient sample no. 1R and is shown in lanes 5 to 7.

**Fig 5.** Distribution of cyclin E protein (50 kD) levels at diagnosis and resultant patient status ($n = 15$; 12 B- and 3 T-ALL). Four patients were excluded from this analysis due to incomplete data (patients no. 11 to 13 and 19). Most patients died from the disease (x). Three patients were alive at the time this study was completed. Two patients with low cyclin E levels were long-term survivors (C; patient no. 6, 44 months; patient no. 14, 69 months). One survivor with high cyclin E expression recently relapsed after 14 months' remission (○).

**Cyclin E expression and patient survival.** Although statistical significance was not obtained in our small patient group, the only two disease-free survivors in remission from B-ALL at the time this study was completed had the lowest levels of cyclin E at the time of diagnosis (Fig 5).

**Cyclin A expression.** The 60-kD cyclin A protein was undetectable by Western blot in isolated B cells from the peripheral blood of healthy donors, and was expressed at a low level in enriched T cells (Fig 2A and B). Expression of cyclin A was increased in peripheral blood B and T cells after mitogenic stimulation with SAC (B cells) or PMA, PHA, and calcium ionophore (T cells) in vitro (Fig 2A and B, respectively). Most of the blast cell extracts from ALL patients showed little or no expression of cyclin A (Fig 2A to C). The highest cyclin A expression was found in the proliferating Burkitt's lymphoma cell line, Akata. Cyclin A protein was detected in two B-ALL patients at relapse (patients no. 4 and 5), while the same patients showed no cyclin A at diagnosis. In samples from the other three patients studied before treatment and at relapse, levels of cyclin A either were undetectable (patients no. 1 and 2) or showed a slight increase at relapse (patient no. 3). T-ALL patient samples expressed little (patients no. 16R and 17) or no cyclin A (patients no. 16D, 18, and 19R).

**DISCUSSION**

T- and B-ALL peripheral blood blast cells expressed cyclin E protein at levels as high as the exponentially ex-
panding cell line, Akata. Noteworthy is that most ALL blast samples appeared to have few or no cells in the S or G2/M phases of the cell cycle despite having relatively high levels of cyclin E protein. This strong expression of a G1-promot-
ing cyclin without any clear proliferation may be a reflection of the loss of normal growth characteristics in ALL blasts. We conclude that cyclin E protein levels in peripheral blood ALL blasts reflect the malignant nature of these cells, with higher levels corresponding to a more advanced stage of the disease.

In comparing T-ALL and B-ALL at diagnosis, we ob-
served cell-lineage differences in the expression patterns of cyclin E. Both T- and B-ALL blast samples had relatively high cyclin E protein expression combined with low prolifer-
ation rates in the peripheral blood. Despite these similarities, the samples differed in the expression of a 43-kD cyclin E species that was consistently present in T-ALL, but only present in two B-ALL (patients no. 2 and 4). In T-ALL, the 43-kD species was present at an intensity similar to that of the 50-kD cyclin E species in three of four patients (patients no. 16 to 18). Interestingly, a 43-kD splicing variant of hu-
man cyclin E was described that has lost its ability to com-
plex with cdk2.34 Although a function for the 43-kD species is unknown, this alternatively spliced cyclin E transcript may be preferentially expressed in T-lineage ALLs as compared with B-ALLs.

Samples from five B-ALL patients were available for com-
parison of diagnosis and relapse. In these samples, in-
creased expression of the 50-kD cyclin E (P < .006), plus the presence of various cyclin E isoforms, consistently ac-
 companied progression of the disease from diagnosis to re-
lapse. Similar findings were described in solid tumors in which the expression of cyclin E isoforms was predomi-
nantly observed in malignant tissues rather than normal sur-
rrounding tissue.33 These isoforms may represent splicing variants that maintain normal cyclin E function or have distinc-
t functions. In addition to the 43-kD splicing variant,34 others have recently described three classes of cyclin E tran-
scripts that code for two distinct proteins designated E-L (52 kD) and E-S (50 kD), both of which can shorten the G1 phase in vitro.35 Alternatively, the isoforms may indicate posttranslational modifications of the protein, eg, phosphory-
lation, which could cause increased cyclin E levels by stabi-
lizing the protein. Although the nature of the cyclin E iso-
forms remains unclear, B-ALLs at relapse displayed additional accumulation of cyclin E proteins, and these may be an indication of the malignant progression of B-ALL blasts from diagnosis to relapse.

Cyclin A was used as a marker of blast proliferation.36 In a majority of the tested samples, cyclin A expression was low or undetectable, consistent with the low-proliferation findings from FACS/cell-cycle analysis. However, in two patients, there was some discrepancy in these two param-
ters, ie, increased levels of cyclin A were observed without a significant increase in the proliferating fraction. This finding suggests that, at least in some cases, accumulation of cyclin A can occur in ALL blasts without further progression of these cells through the cell cycle, much like the situation with cyclin E. Nevertheless, because cyclin A was detected in only a fraction of the samples, cyclin E is a more practical parameter to study, since it was detected in all samples.

Of interest is the apparent contradiction between the high levels of cyclin E protein and the low or nonproliferative behavior of leukemic blasts in the peripheral blood. Consid-
ering the role of cyclin E as an essential and rate-limiting protein involved in the G1- to S-phase transition of the cell cycle,13-18 one would expect to find a greater number of dividing cells in a population expressing high levels of cyclin E. However, several lines of evidence suggest that the slow or noncycling status of peripheral blood blasts is not due to an inherent property of the cells, but to their localization in the bloodstream. Cell density and/or availability of growth factors in the peripheral blood have been suggested as possible reasons for the low proliferation rates observed in pa-
tients.35 Mauer et al36 described the presence of both a prolif-
erating fraction of large ALL blasts in the bone marrow and a smaller nonproliferating pool of blasts in the peripheral blood. Additional studies showed that the smaller nonprolifer-
erating blasts could home to the proliferating pool and re-
sume cell division spontaneously37 or after chemother-
aphy.38,39 Furthermore, ALL blasts from the peripheral blood of some patients are able to home and aggressively prolifer-
ate in the lymph nodes, bone marrow, spleen, and thymus when intravenously injected into severe combined immuno-
deficiency disease (SCID) mice.40,41 These examples demon-
strate how cyclin E may reflect the malignant status of ALL blasts—not by directly influencing their growth in the pe-
ripheral blood, but by revealing their potential to proliferate when conditions change. In this manner, those samples with higher expression of cyclin E would be expected to have a proliferation advantage in more permissive conditions due to the role of cyclin E in driving cell cycle progression.

Studies by our group on the growth of human ALL blasts in SCID mice revealed differences in engraftment rates, with relapsed B-ALL samples having a more aggressive behavior in mice as compared with samples from diagnosis.42 Over-
expression of cyclin E, by virtue of its role in driving the G1-
to S-phase transition of the cell cycle, may play a role in the increased engraftment of relapsed ALL blasts in SCID mice by providing a growth advantage.

In addition to growth differences in SCID mice, biologic differences between blasts from diagnosis and at relapse are manifest in the behavior of leukemia in patients. The progression of ALL from diagnosis to relapse is often ac-
 companied by an increased resistance to chemotherapy,43 with most patients failing to achieve disease-free survival after relapse.43 However, the evolution of the disease at the cellular level is not well understood. Commonly studied pa-
rameters such as immunophenotype and karyotype are often un-
changed in the two populations,4 and therefore do not provide any additional information. Clearly, new parameters that allow for rapid assessment of biologic differences in leukemic blast cells would be helpful for learning more about the nature of leukemic blast cells. In this study, cyclin E was easily detected in patient samples by Western blot and proved to be a useful parameter for illustrating clear differ-
ences between B-ALL blasts from diagnosis and relapse. Cyclin E levels from patients at diagnosis were compared with the patients' clinical course. No significant correlations were observed with respect to cyclin E levels and time to remission or length of remission. However, it was interesting that the only two disease-free survivors in our patient group had the lowest levels of cyclin E protein at diagnosis. More studies should be performed on a larger number of samples and patient outcomes closely monitored to evaluate whether cyclin E expression levels have value in diagnostics. If cyclin E proves successful in assessing the grade of proliferation potential or malignancy in ALL blasts, it may be a useful parameter for assigning patients to different risk groups at diagnosis and applying aggressive chemotherapeutic regimens where necessary.

ACKNOWLEDGMENT

We thank the Clinical Pharmacology laboratory at Karolinska Hospital for assistance in scanning and densitometry, Gunilla Hansson for helpful information on patient histories, and J. Avila-Carino, who kindly provided interleukin-2 and SAC.

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