**REVIEW ARTICLE**

**Advances in the Biology and Treatment of B-Cell Chronic Lymphocytic Leukemia**

By Susan O'Brien, Auro del Giglio, and Michael Keating

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in the western hemisphere. Diagnosis and staging of CLL are usually straightforward, but predicting an individual patient's prognosis is still a challenge. Cytogenetic abnormalities provide important prognostic information in CLL and may show its molecular heterogeneity. A search for oncogene abnormalities continues, although no consistent defects have been identified. New agents such as fludarabine produce high complete remission rates and have generated interest in earlier treatment as a first step in a potential cure. Fludarabine also makes autologous bone marrow transplant feasible as a consolidation therapy. Immunologic abnormalities and minimal residual disease persist in most patients in remission. Combining fludarabine with other active agents and devising effective postremission strategies may change the natural history of CLL.

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**BIOLOGY AND MOLECULAR ASPECTS**

**Cytogenetic and molecular studies.** Chromosomal abnormalities occur in 56% to 65% of patients with CLL who have sufficient metaphases for analysis. An extra chromosome 12 and a breakpoint involving the long arm of chromosomes 14 (14q+) or 13 (13q+) are frequent abnormalities present in 18%, 13%, and 10% of patients, respectively. Structural aberrations involving the long arm of chromosome 11 occur in 3% of patients. An abnormal karyotype in CLL portends a worse prognosis. Specific chromosomal abnormalities and multiple abnormalities are associated with worse prognoses. Among patients having only a single chromosomal abnormality, those with trisomy 12 have a worse prognosis. When all abnormalities (single and multiple) are considered, patients with abnormal chromosome 14 have the worst survival rate and those with 13q+ abnormalities have the best, although all survival rates are shorter than those seen in patients with a normal karyotype. The median survival rate in the latter group is more than 15 years. Patients with a low percentage of abnormal metaphases have a longer survival rate than those with 100% abnormal metaphases.

The rate of clonal evolution in CLL is variable. In a longitudinal follow-up study of 95 patients, minor changes in karyotype were noted in 15% and 15% showed clonal evolution in only one sample. In another study, karyotypic evolution was seen in 22 of 53 patients (42%), 12 (55%) of whom had disease progression, whereas progression was seen in only of 5 of 31 (16%) with stable karyotypes (P < .01). Abnormalities occurring during clonal evolution were different from the common baseline abnormalities; trisomy 12 was not observed as a secondary abnormality. Southern blot analyses of Ig gene rearrangements showed a multiband pattern in 22 of 38 patients (58%). Loss of the JH germline band or a Cu multiband pattern has been associated with advanced stage disease.

The molecular mechanisms underlying chromosome 12 abnormalities in CLL are unknown. Involvement of the ras gene has been postulated because of its location, but no ras gene mutations were found in 93 patients studied. Probes for chromosome 12, the most common cytogenetic abnormality in CLL, will facilitate rapid screening for trisomy 12 by fluorescence in situ hybridization (FISH). This approach is sensitive, specific, and evaluates cells in interphase. It has been also used to detect minimal residual disease after fludarabine chemotherapy.

The 14q+ abnormality involves a breakpoint at 14q32 at the site of the Ig heavy chain gene. Translocations such as t(11;14)(q13;q32) may juxtapose critical genes to the Ig heavy chain gene. Candidate genes located on 11q13 include BCL-1, CD20, int-2, hst, and prad-1. Rechavi et al reported no BCL-1 or BCL-2 rearrangements in 38 cases of B-CLL. Adachi et al noted a 10% incidence (3 of 32) of BCL-2 gene translocation to Ig λ or κ light chain genes. The 14q+ abnormality is frequent in prolymphocytic leukemia. Translocation of the BCL-2 gene is infrequent in CLL, but expression of the gene at the RNA and protein level is increased. Schena et al found BCL-2 mRNA expression in 11 of 11 cases of B-CLL clones, whereas no expression was seen in normal CD5+ B cells. Mariano et al found expression of BCL-2 mRNA in 20 of 20 patients with B-CLL; in 16 of these 20 patients, the level of expression was higher than that of normal peripheral blood mononuclear cells. Increased expression of BCL-2 protein [compared with a t(14;18)-bearing lymphoma cell line] was found in 14 of 20 cases of CLL (70%); these levels varied from 1.7 to 25 times the levels seen in normal peripheral blood lymphocytes.

From the Department of Hematology, University of Texas M.D. Anderson Cancer Center, Houston, TX; and Sao Paulo University Medical School, Sao Paulo, Brazil. Submitted January 25, 1994; accepted October 13, 1994. Address reprint requests to Susan O'Brien, MD, Hematology/Leukemia, Box 61, UT M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030. © 1995 by The American Society of Hematology. 3006-4971/95/8502-0031$3.00/0
this hypomethylation did not correlate with the relative levels of BCL-2 protein. In the above studies, no rearrangements of the BCL-2 gene were found, suggesting that alternate mechanisms are responsible for enhanced protein overexpression. Overexpression of BCL-2 leads to inhibition of apoptosis and prolonged survival of hematopoietic cells. Thus, the increased protein levels seen in B-CLL cells may be a crucial part of their malignant phenotype. Hanada et al studied this phenomenon in 3 cases of B-CLL with low, intermediate, or high levels of BCL-2 protein. After 72 hours of incubation, the viability of cells with low BCL-2 protein expression was ~30%, versus 60% to 80% of cells with higher BCL-2 levels. A morphologic picture consistent with apoptosis was noted in the cells with low protein level.

The retinoblastoma (Rb) gene is located on the long arm of chromosome 13. By Southern blot analysis, abnormalities of Rb are infrequent in CLL, having been reported in 1 of 88 patients in two studies. Abnormalities of other oncogenes and tumor-suppressor genes have been found in occasional patients with CLL; however, no characteristic pattern of abnormal gene expression has emerged. Gaidano et al reported mutations of p53 in 3 of 7 patients with B-CLL and a translocation involving chromosome 13. A study using in situ hybridization to interphase nuclei demonstrated Rb gene deletion in 11 of 35 cases (31%) of B-CLL; only 4 (11%) had abnormalities of chromosome 13 by conventional cytogenetic analysis.

Rb deletions in CLL are usually hemizygous. Recently, somatic-cell hybrids have been used to pinpoint the 13q breakpoint in CLL. Two groups have identified a high frequency of deletions of D13S25, a distinct locus separated from Rb by 530 kb. This deletion occurred with higher frequency than the loss of Rb; in 5 of 9 cases the deletion was homozygous, suggesting the presence of a new tumor-suppressor gene. Abnormalities in the expression of the Rb gene, including decreased RNA message and both underexpression and overexpression of the protein when compared with normal peripheral blood, have been reported.

Abnormalities of other oncogenes and tumor-suppressor genes have been found in occasional patients with CLL; however, no characteristic pattern of abnormal gene expression has emerged. Gaidano et al reported mutations of p53 in 6 of 40 (15%) cases of CLL and in 3 of 7 patients (43%) with Richter’s transformation. El Rouby et al found a similar incidence of p53 mutations in CLL (8 of 53 [15%]). Twenty-seven of twenty-nine patients (93%) without p53 abnormalities responded to treatment, versus only 1 of 7 patients (14%) with p53 abnormalities.

Abts et al described expression of the proto-oncogenes lck and c-fgr in lymphocytes from 21 of 21 patients (100%) with CLL, but found no such expression in normal purified B cells. c-fgr and lck belong to the src gene family, which encodes tyrosine kinase proteins; their abnormal regulation may be relevant in the pathogenesis of this disorder.

Rearrangement of T-cell receptor genes have been occasionally reported in B-CLL. Merup et al found T-cell receptor β gene rearrangement in 6 of 100 patients with B-CLL; 3 of the 6 patients had deletion of the long arm of chromosome 6. An additional patient had this abnormality in one metaphase: 6q deletions were found in only 4 of the remaining 94 patients.

**Immunophenotype.** Geisler et al have analyzed the immunophenotype of 540 newly diagnosed patients with CLL. All cases showed CD19 and/or CD20 (pan-B-cell antigens). CD5, an antigen present on mature T cells, was present in 93%. Using a cut-off of 30%, positivity for CD21, CD22, and CD23 was noted in 65%, 86%, and 71% of patients. The presence of CD23 was strongly correlated with survival, with CD23+ cases having a significantly shorter survival rate. Correspondingly, increased levels of serum-soluble CD23 confer a shorter survival rate in CLL patients. In contrast, Newman et al reported that patients with CLL and CD23 positivity had a higher Rai stage and a higher white blood cell count, suggesting more aggressive disease, but follow-up was too short to evaluate survival. CD23 regulation may have a regulatory role in B-cell proliferation, and studying the mechanism of its abnormal expression is of interest. Myelomonocytic antigens are frequently expressed in CLL and have been associated with interleukin-1 (IL-1) production, a diffuse pattern of bone marrow infiltration, high levels of C-FOS oncogene mRNA, and CD5 negativity.

**TREATMENT**

The diagnosis of CLL is not consonant with the decision to treat. Older age, a potentially indolent course, and accepted incurability of the disease using conventional therapies make observation without treatment a tenable decision. Updated results from the French group CLL80 protocol corroborate this approach. Among stage A patients randomized to observation versus daily chlorambucil, the 5-year survival rates were 82% versus 75% (P = .21). A delay in disease progression in the treatment group was counterbalanced by a shorter survival once progression occurred and by a higher incidence of epithelial cancers. Therefore, treatment for Binet stage A or Rai stage 0 disease is indicated only when progression or disease-related symptoms supervene.

**Response criteria.** The National Cancer Institute (NCI) and the IWCLL proposed uniform guidelines for response criteria (Table 1). A subset of patients with complete response (CR) may have residual lymphoid nodules, which are sometimes seen in normal marrow. Disappearance of the malignant clone by immunophenotypic parameters and gene rearrangement studies is not a requirement to define CR. Therefore, patients achieving CR may be heterogeneous, including hematologic and clonal CRs. Furthermore, the impact of achieving a clonal CR on the natural history of this disease is not clear.

**Conventional treatment.** The most commonly used chemotherapeutic regimen in CLL is the combination of chlorambucil (CLB) and prednisone. The evidence supporting the use of the combination instead of either drug alone is based on two small, randomized studies in which no significant difference in survival was evident for the different treatment groups. Responses with this combination ranged from 38% to 87%. This wide variation in response is a result of...
several factors, including differences in response criteria and in drug dose schedules. The issue of dose intensity for chlorambucil is evident in the trial conducted by Jaksic et al. In their study, 181 patients with CLL were randomized to treatment with continuous daily CLB (15 mg daily to CR or toxicity) or with weekly dose CLB (75 mg every week for 6 weeks) plus prednisone. The total dose of chlorambucil was six times higher with the daily CLB regimen, which resulted in a CR rate of 70% compared with 31% with the weekly schedule. Survival of patients randomized to the high-dose regimen was also significantly superior.

Several studies have investigated other drug combinations. COP (cyclophosphamide, vincristine, and prednisone) was associated with a response rate range of 44% to 48%, an outcome diversity accounted for by different patient characteristics, response criteria, and drug dose schedules. In randomized trials, COP results were no better to those obtained with CLB and prednisone. The addition of adriamycin to the COP regimen (CHOP) yielded superior survival results than COP alone and higher response rates than CLB plus prednisone. A recent update from the French randomized study of CHOP versus CLB plus prednisone for stage B CLL patients failed to show a significant survival difference between the two groups. When CHOP was compared with high-dose CLB, no significant differences in overall response rates were seen between regimens. Other combination chemotherapy regimens have included M2 (vincristine, BCNU, cyclophosphamide, melphalan, and prednisone), CMP (cyclophosphamide, melphalan, and prednisone), CAP (cyclophosphamide, adriamycin, and prednisone), and POACH (cyclophosphamide, adriamycin, cyclosporine, vincristine, and prednisone). Although these regimens induce higher CR rates, they are more toxic and are not clearly superior to CLB plus prednisone.

Resistance to CLB in CLL is poorly understood; alterations in DNA repair-synthesis activity and increased levels of sulfhydryl groups, especially GST, have been proposed. Resistance to other commonly used drugs in CLL (vinca alkaloids and anthracyclines) may be related to multidrug resistance (mdr) gene expression. Several groups have reported increased levels of mdr mRNA in a subset of patients with CLL. The expression of mdr-associated glycoprotein in some patients with CLL has been reported by several investigators. Pharmacologic intervention to ameliorate drug resistance mechanisms is of interest. Elevated levels of topoisoerase-I found in B-CLL lymphocytes suggest a role for the use of topoisoerase-I targeting agents such as topotecan or CPT-11 in the treatment of CLL.

Nucleoside analogues. Nucleoside analogues have shown significant anti-CLL efficacy. Especial promising is fludarabine, a fluorinated purine analogue. Fludarabine is rapidly dephosphorylated in the plasma, enters the cell by a carrier-mediated transport mechanism, and is then phosphorylated to F-ara-adenine triphosphate (F-ara-ATP) by deoxycytidine kinase. Because of its resistance to deamination by adenosine deaminase, F-ara-ATP accumulates in the cell, leading to suppression of DNA synthesis by inhibiting ribonucleotide reductase and DNA polymerase. The significant activity of fludarabine in lymphoid malignancies may be related to low levels of deoxynucleosides in lymphoid tissues and high deoxycytidine kinase activity.

Grever et al administered fludarabine to 26 previously treated patients with CLL at a dose of 20 mg/m^2 daily for 5 days. Fourteen patients (54%) had clinical improvement and toxicity was limited to myelosuppression. More than 300 patients have received fludarabine-based chemotherapy at MD Anderson Cancer Center since 1985. Keating et al administered fludarabine intravenously at a dose of 25 to 30 mg/m^2 for 5 days every 3 to 4 weeks to 68 previously treated patients. Nine patients (13%) achieved a CR and 30 (44%) achieved a partial response (PR), with most of the responses occurring within 3 months of treatment initiation. In this initial study, PR included responses in which the only evidence of disease after treatment was the persistence of lymphoid aggregates in the bone marrow. This was referred to as “nodular PR,” but is recently called “nodular CR,” creating some confusion about the rates of response reported in different series and in subsequent trials. Patients with nodular CR belong to the CR category by IWCLL and NCI criteria. Thus, CR rates in this study of previously treated

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Table 1. Response Criteria in CLL

<table>
<thead>
<tr>
<th>Site</th>
<th>CR Criteria</th>
<th>PR Criteria</th>
</tr>
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<tbody>
<tr>
<td>Physical exam</td>
<td>Decrease of lymphadenopathy, hepatosplenomegaly,</td>
<td>NA</td>
</tr>
<tr>
<td>Nodes</td>
<td>Normalization of CBC</td>
<td>NA</td>
</tr>
<tr>
<td>Liver/spleen symptoms</td>
<td>Neutrophils ≥1,500/μL or 60% improvement from</td>
<td>NA</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>Platelets ≥100,000/μL or 50% improvement from</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin ≥11.0 g/dL or 50% improvement from</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Lymphocytes ≥4,000/μL or 60% decrease</td>
<td>NA</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Normalization of bone marrow</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Nodular or focal lymphoid infiltrates are</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>compatible with CR</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Resolution of lymphadenopathy, hepatosplenomegaly</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>and constitutional symptoms.</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Change from stage C to A or B, or from stage</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>B to A</td>
<td>NA</td>
</tr>
<tr>
<td>Stable disease</td>
<td>No change in the stage of the disease</td>
<td>NA</td>
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</tbody>
</table>

Abbreviations: PMN, polymorphonuclear cells; NA, not applicable.
patients approached 30% by IWCLL/NCI guidelines. This led to a subsequent study with the same fludarabine regimen in 33 previously untreated patients. In this group, the CR and PR rates were 33% and 45%; considering patients who achieved a nodular CR, the CR rate increased to 72%. To enhance the single-agent activity of this drug, prednisone was added at a dose of 30 mg/m² orally for 5 days in combination with fludarabine administered at the same dose and schedule. Response rates did not improve and 14 episodes of Listeria monocytogenes sepsis or pneumocystis carinii pneumonia (PCP) were observed among 269 patients (5%) treated. This occurrence is less than 1% of all courses administered, but opportunistic infections had not been observed with fludarabine alone.

Toxicities observed with fludarabine include myelosuppression and infections. Complications are more frequent among nonresponding patients. Nausea, vomiting, stomatitis, diarrhea, and neurotoxicity, mainly mild peripheral neuropathy, occur in less than 5% of patients. Fludarabine does not cause alopecia. Encouraging results with fludarabine in CLL have been reported by other investigators, although shorter-exposure schedules may yield lower response rates.

Puccio et al used fludarabine 20 mg/m² bolus followed by 30 mg/m² daily for 48 hours as a continuous infusion. Response rates were inferior, but the total dose (80 mg/m²) and exposure duration were roughly half that of the daily for 5 days schedule (150 mg/m²). Based on the slow elimination of FaraATP from CLL lymphocytes, fludarabine was administered at 30 mg/m² weekly to 48 previously treated patients with CLL. The dose was 80% of that administered on a daily for 5 days schedule, but the overall response rate was only 29%. A 3-day schedule of fludarabine (30 mg/m² daily) in 80 patients with relapsed or refractory CLL produced a response rate of 46%, which is not significantly different from that reported with the 5-day schedule (unpublished data), but the incidence of CR was lower and detection of residual disease (by flow cytometry) was more frequent with the shorter course therapy.

Fludarabine has been approved by the Food and Drug Administration for use in refractory CLL and is now in phase III trials being compared with CLB alone, CLB plus fludarabine, CAP, and CHOP (Table 2). Hiddemann et al presented preliminary data from a multicenter randomized trial comparing fludarabine with CAP (750 mg/m² cyclophosphamide, 56 mg/m² Adriamycin, and 40 mg/m² prednisone × 5) in patients with CLL. The response rate in 106 patients receiving fludarabine was 58%, versus 42% of 102 patients treated with CAP. The response rate in previously treated patients was 45% (21 of 53) with fludarabine and 26% (13 of 50) with CAP. Side-effects, including nausea, vomiting, and alopecia, were more frequent with CAP. Binet et al randomized 183 stage B and 79 stage C CLL patients to one of three regimens: 25 mg/m² fludarabine daily for 5 days, CAP as described above, or mini-CHOP (300 mg/m² cyclophosphamide and 40 mg/m² prednisone orally for 5 days with 1 mg/m² vincristine and 25 mg/m² doxorubicin intravenously on day 1). After 5 months, the response rate in stage B patients was 94% with fludarabine, 72% with CAP, and 75% with CHOP (P = .08). There was no significant difference in response rates at 6 months for stage C patients, but only 79 patients had been randomized.

Two other purine analogues have been investigated. The Cancer and Leukemia Group B (CALGB) treated CLL patients with pentostatin (2-deoxycoformycin [DCF]) at a dose of 4 mg/m² intravenously weekly for 3 weeks and then every 2 weeks. One third of the patients had no prior therapy and 69% were Binet stage C. Three percent achieved a CR and 23% achieved a PR. Similar results were reported by Ho et al. Similar to the results with fludarabine, the most common toxicities were infections (52%) and myelosuppression. Other significant toxicities included nausea and vomiting (31%), rash (20%), and stomatitis (8%).

2-Chlorodeoxyadenosine (2-CDA) was investigated in CLL at 0.2 to 0.5 mg/kg/day for 7 days as a continuous infusion and yielded a PR rate of 22% in 18 previously treated patients. An update of the study showed a 44% response rate (4% CR and 40% PR) in 90 patients with CLL using NCI criteria. Although the CR rate appears inferior to that of fludarabine, 82 of the 90 patients treated had Binet stage C disease and 8 of 8 patients with stage A or B disease responded. Documented infections were seen in 18% of the patients, including three episodes of PCP and one episode of Listeria. Thrombocytopenia to less than 50 × 10⁹/µL was noted in 25% of the patients.

Twenty previously untreated patients received 2-CDA as a continuous infusion. The overall response rate was 85%, with CR being seen in 25%. The median duration of response is 8+ months. Three patients developed opportunistic infections; all had received steroid therapy for thrombocytopenia. The number of untreated patients receiving CDA is too small for significant comparison and imbalances in unaccounted prognostic factors may exist. Randomized studies comparing fludarabine with 2-CDA in CLL are needed, as well as studies using different dose schedules of 2-CDA.

The three purine analogues share structural similarities, and significant cross-resistance might be expected. Occasional patients resistant to fludarabine have achieved a remission with 2-CDA. Juliusson et al treated four consecutive patients who had not achieved a PR with fludarabine and all responded to 2-CDA, with one patient achieving CR. Other groups have reported negative results in this setting. Delanroy et al saw no responses in three fludarabine refractory patients treated with 2-CDA. In a similarly treated group of

Table 2. Results of Randomized Trials Comparing Fludarabine to Combination Chemotherapy

<table>
<thead>
<tr>
<th>Patients</th>
<th>Overall Response Rate (%)</th>
</tr>
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<tbody>
<tr>
<td>Fludarabine v CAP</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>Fludarabine</td>
</tr>
<tr>
<td>Pretreated</td>
<td>208</td>
</tr>
<tr>
<td>Untreated</td>
<td>105</td>
</tr>
<tr>
<td>Binet stage B</td>
<td>163</td>
</tr>
<tr>
<td>Binet stage C</td>
<td>79</td>
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14 patients, Saven et al.\textsuperscript{100} saw no responses; treatment was complicated by severe thrombocytopenia in 3 patients and pancytopenia in 2 patients. We have treated 28 patients whose disease was refractory to fludarabine with 2-CDA; 2 patients (7%) responded by NCI criteria and 1 additional patient had antitumor activity manifested by decreasing blood and marrow lymphocytosis, but persistent thrombocytopenia.\textsuperscript{102} Sixty-five percent of courses were accompanied by febrile episodes or infections and 10 patients died within 60 days of starting therapy with 2-CDA. The use of tandem nucleoside analogues may result in cumulative myelosuppression and immunosuppression with significant sequelae.

**Biologic response modifiers.** α Interferon induces a low response rate in previously treated patients.\textsuperscript{105} When previously untreated stage A CLL patients received α interferon, about 50% achieved more than 50% reduction in their absolute lymphocyte counts.\textsuperscript{106-107} Investigation of α interferon for remission maintenance (as for lymphoma and myeloma) is of interest.\textsuperscript{108,109}

Trials of α interferon in combination with other drugs such as chlorambucil and in high-risk stage A patients to decrease the rate of disease progression are underway.\textsuperscript{110,111} The mechanism of action of α interferon in CLL is unknown. Downregulation of cytokines such as tumor necrosis factor (TNF) and activation of monocytes with elevation of macrophage colony-stimulating factor (M-CSF) levels have been proposed.\textsuperscript{112,113} Alternatively, the induction of leukocyte adhesion molecule-1 (LAM-1) on CLL cells by α interferon may alter the homing pattern of these cells and simply cause a compartment shift.\textsuperscript{114}

CLL B cells may produce mRNA for IL-1β, transforming growth factor-β (TGF-β), TNF-α, IL-6, IL-7, and IL-8.\textsuperscript{115-119} IL-1 and IL-6 activity has been detected in supernatants from cultured CLL cells. IL-4\textsuperscript{120,121} and TGF-β\textsuperscript{112,113} have antiproliferative effects on CLL cells in vitro. Foa et al.\textsuperscript{124} found circulating levels of TNF in the serum of 20 of 24 patients (83%) with CLL. This cellular release was higher in patients with Rai stage 0-1 disease than in patients with Rai stage II-III disease. Although in this study exogenous TNF had little proliferative effect on CLL cells, other investigators have found significant stimulation by TNF.\textsuperscript{125,126} which may be enhanced by activation of CLL cells.\textsuperscript{127,128}

Moreover, TNF induced the nuclear transcription factor NFκB and c-fos and c-jun mRNA in B-CLL cells.\textsuperscript{131} The presence of circulation TNF receptors may stabilize TNF and increase the availability of this cytokine.\textsuperscript{125,126,129} Inhibition of TNF activity in culture by specific monoclonal antibodies corrected abnormal in vitro hemopoiesis in 11 of 15 CLL patients.\textsuperscript{132} Thus, TNF may exert a regulatory role in CLL. The addition of recombinant human IL-6 decreased TNF-induced growth of CLL cells in vitro,\textsuperscript{126,130} in contrast to the growth stimulation effects of IL-6 on other B-cell neoplasms. When IL-6 levels were measured in the serum of 76 patients with CLL, detectable levels (>50 pg/mL) were found in 28%. Similar to the findings with TNF, the incidence of IL-6 detection was related to disease stage; 55% of Rai O patients had IL-6 in the serum, versus 20% of Rai IV patients.\textsuperscript{126}

Soluble forms of TAC-reactive IL-2 receptors are expressed in most patients with CLL and serum levels increase with advancing stage.\textsuperscript{135,136} Cell surface positivity is noted on the B-CLL cells in 50% of cases. Kay et al.\textsuperscript{137} investigated recombinant IL-2 at a dose of 2 × 10^6 U/m^2 over 2 hours 5 times per week in 12 patients with refractory or progressive CLL after chemotherapy; no partial or complete responses were observed, but 7 of 10 patients with evaluable disease had some evidence of tumor reduction and 3 had disease progression.\textsuperscript{137} Using an IL-2 dose of 1.5 to 3 × 10^6 U/m^2, Allison et al.\textsuperscript{138} noted decreases in peripheral lymphocyte counts in 5 patients with CLL, but rebound to pretreatment levels upon discontinuation of the infusion. Toxicity was significant at the higher dose level.\textsuperscript{138}

Hematopoietic colony-stimulating factors (CSF) such as granulocyte-CSF (G-CSF) and granulocyte-macrophage-CSF (GM-CSF) may ameliorate postchemotherapy or disease-induced neutropenia\textsuperscript{139} and allow administration of higher doses of chemotherapy. Low-dose GM-CSF was occasionally superior to the conventional dose in increasing neutrophil counts.\textsuperscript{140}

**Monoclonal antibodies and receptor-targeted immunotoxins.** Grossbard et al.\textsuperscript{141} recently reported results from a phase I study of a monoclonal antibody toxin-conjugate directed at a B-cell-specific antigen (CD19) in 25 patients with refractory B-cell malignancies (mostly non-Hodgkin's lymphomas). By blocking the nonspecific binding sites of ricin, a modified immunotoxin was engineered and then coupled to an anti-CD19 monoclonal antibody. One CR, two PRs, and 8 minimal responses were observed. Toxicity consisted of dose-dependent increases in transaminase levels, fever, hypoalbuminemia, and thrombocytopenia. The results obtained are encouraging and clinical testing of this compound in CLL is underway. The use of a hybrid molecule of diphtheria toxin\textsuperscript{142} or Pseudomonas toxin with the IL-2 molecule\textsuperscript{143} have also been reported in CLL. The search for more specific targets (ie, CD5 and CD23) and approaches tailored to circumvent the formation of human antibodies against the monoclonal antibodies (ie, humanization of antibodies)\textsuperscript{144} will probably extend the applications of this therapeutic modality, particularly in the treatment of minimal residual disease.

**Bone marrow transplantation (BMT).** Experience with allogeneic BMT in patients younger than 50 years of age is slowly accumulating.\textsuperscript{145,146} The combined international experience was summarized for 47 CLL patients who received allogeneic transplants between 1984 and 1992.\textsuperscript{147} Conditioning usually consisted of cyclophosphamide and total body irradiation (TBI); the most common graft-versus-host disease (GVHD) prophylaxis consisted of methotrexate and cyclosporin. The median age was 42 years (range, 21 to 58 years) and 56% of patients were Rai stage III-IV at the time of BMT. GVHD was a significant problem and the most common cause of death post-BMT; 38% of patients experienced acute GVHD >grade II and 47% had chronic GVHD (extensive in 17%). The projected leukemia-free survival was 40% ± 18% at 5 years, with stage of disease being the most important factor influencing survival.

Rabinowe et al.\textsuperscript{148} treated 8 patients with CLL with a T-cell–depleted allogeneic BMT using cytoxan and TBI for conditioning. Patients were treated to a minor disease state...
with chemotherapy, including fludarabine in six patients. Only one patient had severe acute GVHD and eventually died of PCP pneumonia. With a median follow-up of 11.7 months, six patients remain in CR and one patient has bone marrow involvement as the only site of residual disease.

The experience at MD Anderson is similar to that of Rabine et al.189 Eleven patients were treated with allogeneic or syngeneic BMT (1 patient) using T-cell depletion and the same preparative regimen. However, all patients were refractory or relapsing after a median of three regimens and nine patients were staged Rai III-IV. All patients had received fludarabine. Eight patients achieved CR and one achieved PR. No patient developed GVHD >grade 2; one patient developed chronic extensive GVHD. Ten patients are alive with a median follow-up of 10 months.149 In contrast to the European experience, the incidence of severe GVHD, with consequent early death, is lower in the American studies. All but two patients in the American studies received therapy with fludarabine, a potent immunosuppressive agent, whereas none of the European patients was treated with this drug. T-cell suppression, seen with fludarabine, may interfere with antigen presentation to the donor cells, resulting in a decrease in the incidence of GVHD. Because of age constraints and donor availability, only a minority of CLL patients can be considered for allogeneic BMT. Its application in CLL is investigational, and patients should be treated in the setting of clinical trials.

Autologous BMT may be an alternative investigational option for this older group of patients. Purged autologous marrow has been used as consolidation remission in low-grade lymphoma, an analogous disease entity. Patients rendered negative for bcl-2 rearrangement at the polymerase chain reaction level had prolonged disease-free survival.150 Rabine et al.189 used monoclonal antibody-purged autologous bone marrow to treat 12 patients with CLL, using cyclophosphamide and TBI as the preparative regimen. As did the allogeneic BMT patients, all 12 achieved a minimal disease state with chemotherapy before BMT. One patient died with pulmonary hemorrhage and all but one of the other patients remain in CR with a median follow-up of 5 months.149

Khoury et al.141 treated 11 patients with CLL with purged autologous BMT. All patients had relapsed after fludarabine therapy and the median number of prior regimens was three. There was one death from cytomegalovirus infection and one death from complications of liver biopsy; 3 patients remain in CR with a median follow-up of 10 months.149

SPECIAL PROBLEMS

Autoimmune complications. When present in CLL, autoantibodies are preferentially targeted against hematopoietic cells. The incidence of autoantibodies in CLL ranges from 10% to 75%; the incidence of a positive Coombs test increases significantly with disease stage.151

The approach for patients with immune thrombocytopenia or autoimmune hemolytic anemia and CLL includes an initial trial of steroids (prednisone, 60 to 100 mg/day) for 2 weeks. Seventy-five percent of patients will respond, and an attempt to taper the steroid dose slowly should follow. For steroid refractory patients, alternatives include a trial of intravenous Ig or a splenectomy.

Pure red blood cell aplasia characterized by severe anemia, reticulocytopenia, and a marrow with less than 0.5% mature erythroblasts may occur in CLL in less than 1% of patients. Cyclosporin has demonstrated activity in this setting, even in patients resistant to conventional immunosuppressive therapy.152 A case of bone marrow aplasia associated with CLL that responded to antithymocyte globulin has been reported.153

The mechanism of the autoimmune manifestations of CLL is not understood. Two groups have reported that fusion of CLL and myeloma cells yields monospecific and polyspecific autoantibodies.154,155 These polyspecific autoantibodies are of the IgM class and exhibit a restricted use of highly conserved Ig V genes.154,156 The preferential use of these highly conserved V genes suggests that a biased selection of germinal Ig V gene repertoire, rather than somatic mutation, is responsible for the appearance of autoantibodies in this disorder.156-158 Inghirami et al.159 has reported a naturally occurring CD5+ B-cell subset that resembles the autoantibody producing B-CLL cells. In both situations, there is restricted expression of Ig V genes that raises the possibility that B-CLL may arise from this normal B-cell subset engaged in autoantibody production.157-160

Alternative explanations for the CLL autoimmune phenomena have been proposed, including increased spontaneous secretion of IL-6 by lymphocytes of CLL patients with autoimmune disease and decreased T-suppressor inducer cells (CD45RA).161,162

Transformation. Richter’s transformation occurs in about 3% to 10% of CLL cases and represents a change to a large-cell lymphoma (LCL) histology. Robertson et al.163 reported on 39 patients with CLL who developed LCL. This represented 3% of the patients enrolled on a computer-based registry at MD Anderson between 1972 and 1992.163 LCL was found in patients in all Rai stages and in 10 patients in CR after fludarabine therapy. The incidence of transformation after fludarabine or 2-CDA therapy was not significantly increased. Symptoms at transformation included progressive lymphadenopathy and systemic symptoms in the majority of patients. Extranodal involvement and paraproteinemia were noted in 41% and 44%, respectively. The most frequent clinical feature was elevation of lactate dehydrogenase (LDH), with 82% of patients evincing a value that was more than twice normal. In contrast, only 8% of CLL patients presenting to MD Anderson (treated or untreated) had this level of LDH elevation. As noted in other studies, median survival was short at 5 months, although responders to chemotherapy lived longer than nonresponders.164

It is unclear whether the LCL cells represent clonal evolution from B-CLL or the appearance of a new and unrelated clone.165-169 The possibility that in some cases de novo LCL arises in a background of a CLL-induced immunodeficiency cannot be ruled out. In the study by Robertson et al.163 12 of 15 patients showed the same light chain isotype in both the CLL and LCL cells. However, a change may simply represent isotype switch. Five patients who had Southern blotting performed on both types of cells showed an identical
pattern of heavy- and light-chain rearrangement. The availability of molecular techniques to study clonality coupled with the recent description of an NZB mouse model for CLL\textsuperscript{170} in which a Richter-like transformation has been observed may elucidate this question. Prolymphocytic transformation\textsuperscript{171,172} is clinically different from de novo prolymphocytic leukemia (PLL). Patients with prolymphocytic transformation have an indolent progression of anemia, thrombocytopenia, and splenomegaly associated with an increase in the percentage of prolymphocytes to 30% or more of the leukemic cells. Unlike de novo PLL, the immunophenotype of the prolymphocytes in prolymphocytic transformation is similar to the classic B-CLL clones, suggesting that, in most instances, prolymphocytic transformation involves the original B-CLL clone.

CLL evolving into acute lymphocytic leukemia is a rare phenomenon, occurring in less than 1% of cases, and has not been fully characterized.\textsuperscript{173} Isolated cases of CLL transformation into small noncleaved cell lymphoma, lymphoblastic lymphoma, hairy cell leukemia, and Hodgkin’s disease have been reported.\textsuperscript{174-177}

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