Peripheral blood (PB) T cells from 56 patients with B-cell chronic lymphocytic leukemia (B-CLL) were analyzed by two- and three-color immunofluorescence (IF) to determine the expansion of distinct T-cell subsets and their relationship with the clinical and biological features of the disease. We detected the expansion of an unusual T-cell subpopulation expressing lower CD4 or CD8 levels (CD4\(^a\), CD8\(^a\)) than classic T cells (CD4\(^b\), CD8\(^b\)). This subpopulation also expressed low levels of the CD3/TCR\(\alpha/\beta\) complex and was CD19\(^-\)CD13\(^-\)CD14\(^-\). A phenotypic analysis probing the activation level of CD4\(^a\), CD8\(^a\), CD4\(^b\), and CD8\(^b\) cells showed that they comprised increased counts of HLA-DR\(^+\), CD11b\(^+\), CD45RA\(^+\), and CD45RO\(^+\) cells. Subset expansion ranged from 2.1- to 13.6-fold. Statistical analysis showed that the size of some of these subsets was correlated to intrinsic features of the tumor. First, CD4\(^+\)HLA-DR\(^+\) cell counts were higher in patients with stage A than those with stages B and C disease. Second, CD8\(^+\)HLA-DR\(^+\) cell counts were higher in patients in stable remission than in those at diagnosis. Third, CD4\(^+\)HLA-DR\(^+\), CD4\(^+\)CD45RO\(^+\), CD4\(^+\)CD45RA\(^+\), and CD4\(^+\)CD11b\(^+\) cell counts were higher in patients whose tumor cells expressed high levels of surface immunoglobulin (slg) than in those expressing low levels. The involvement of CD4\(^b\) and CD8\(^b\) cells in most of these correlations suggests that they may be tumor-reactive cells. Similar cells described in human and murine autoimmune disease have been shown to be autoreactive anergic cells, which may derive from nonclassic pathways of T-cell development.

**B-CELL CHRONIC** lymphocytic leukemia (B-CLL) is a lymphoproliferative disease characterized by clonal expansion of CD5\(^+\) B cells that accumulate in bone marrow and peripheral lymphoid tissues. It is associated with secondary immunodeficiency and an enhanced risk of infection. In vitro studies have shown that peripheral blood (PB) T cells from B-CLL patients respond poorly to mitogens and display defective helper activity, whereas suppressor activities may be either increased, normal, or decreased. Moreover, the total T-cell number is decreased, CD4 to CD8 and CD45RA to CD45RO ratios are decreased, and activation markers, such as HLA-DR, CD25, and CD11b, are highly expressed. It has been suggested that this T-cell disregulation plays a role in the hypogammaglobulinemia and increased incidence of autoimmunity in B-CLL. Patients with advanced forms have higher counts of HLA-DR\(^+\) T cells and lower counts of both CD45RA\(^-\) and CD8\(^-\)CD11b\(^-\) T cells than those with initial disease, and this T-cell pattern may be directly induced by the antitumor immune response. Studies on another B-cell lymphoproliferative disease, multiple myeloma, are in line with this view. These patients have high counts of activated CD4\(^+\) and CD8\(^+\) cells in PB and bone marrow, and expansion of activated CD8\(^+\) cells correlates with disease progression. Moreover, these cells are oligoclonal, express antiidiotypic determinants specific for the autologous M protein, and display antiplasma cell cytotoxic activity.

The aim of this work was to analyze the relationship between T-cell expansion and tumor cells in B-CLL. Our approach was twofold: first, to map the expansion of distinct T-cell subsets; second, to see whether their size was correlated to the clinical and biological features of the disease. We found that T-cell expansion strikingly involves cells expressing low CD4 or CD8 levels. These cells also express activation markers and their expansion correlates with the clinical status and neoplastic cell phenotype.

**MATERIALS AND METHODS**

Patients. Fifty-six patients with B-CLL, observed as outpatients over a 6-month period, were studied. Mean age was 62.6 years (range, 39.8 to 88.2). Diagnosis was based on a PB expansion of cells expressing the phenotype surface immunoglobulin (slg)\(^+\)CD5\(^-\)CD19\(^-\) (>5 \times 10^9 cells/L), and bone marrow lymphoid infiltration greater than 30%. Moreover, these cells appeared to be monoclonal according to the expression of the Ig\(\kappa\) and \(\lambda\) light chains as evaluated by two-color direct immunofluorescence (IF), using the anti-\(\kappa/\lambda\) Simultest (Becton Dickinson, Mountain View, CA). Patients were staged according to the method reported by Binet et al:\(^15\) 42 were stage A, 10 stage B, and four stage C. Blood samples were taken at a median of 43 months from diagnosis (range, 1 to 186 months). Twenty-eight patients (26 stage A, two stage B) were analyzed at diagnosis or shortly after, and had never received either chemotherapy or radiotherapy. Twenty-three (11 stage A, eight stage B, four stage C) were on treatment with alkylating agents (chlorambucil or cyclophosphamide), with or without prednisone. Five patients (all stage A) were off therapy in stable remission after treatment with chlorambucil. Stable remission was defined as a partial response (ie, a decrease of PB lymphocyte counts and palpable disease >50%, hemoglobin >11 g/dL, and platelet count >100 \times 10^9/L) lasting for at least 6 months from discontinuation of chemotherapy. Forty age-matched healthy blood donors were used as normal controls.

B-CLLs were classified as CD13\(^+\), CD14\(^+\), CD33\(^+\), CD25\(^+\), or CD38\(^+\) more than 30% of the tumor cells were stained by
CD4⁺ and CD8⁺ CELLS IN B-CLL

Fig 1. Staining pattern of CD4⁺ and CD8⁺ cells from PBMC of (A, B) B-CLL patients and (C) normal controls. A similar pattern was found using different staining protocols. (A) CD4 or CD8 were stained using biotin-conjugated OKT4 or OKT8 mAb + RED-613-conjugated avidin. Negative control was obtained using a biotin-conjugated isotype matched mAb + the second reagent. (B and C) CD4 or CD8 were stained by direct IF using PE-conjugated Leu-3 or Leu-2 mAb. Negative control was obtained using isotype-matched PE-conjugated mAb. Staining curve of CD4⁺ and CD8⁺ cells were obtained by setting a gate on positive cells, then 5,000 positive cells were acquired.

Incomplete phenotyping resulted in some totals being less than 56. The phenotype was stable when eight patients were analyzed more than once.

IF assays. The following mAbs were used: Leu-2 (CD8), Leu-3 (CD4), Leu-4 (CD3), Leu-15 (CD11b), Leu-M7 (CD13), Leu-M3 (CD14), Leu-M9 (CD33), TCRb (Becton Dickinson); 2H4 (CD45RA), My7 (CD13), My4 (CD14), My9 (CD33) (Coulter Clone, Luton, UK); UCHL1 (CD45RO), Dako-CD5, Dako-CD19, Dako-CD11b (Dako Immunoglobulins, Copenhagen, Denmark); ß-TCR (T-Cell Science, Cambridge, MA); OKT4 (CD4), OKT8 (CD8) (ATCC, Rockville, MD). PB mononuclear cells (PBMC) were prepared by gradient centrifugation (Lymphoprep, Nycomed, Oslo, Norway) of venous blood and stained by direct IF. Lymphocyte staining was analyzed with a cytofluorimeter (FACScan, Becton Dickinson) using a gate set on small nongranular cells. Staining was performed in the presence of protein A–purified mouse Ig (100 μg/mL) to saturate Fc receptors. When the distribution of CD4⁺ and CD8⁺ cell subsets was investigated by two-color IF, a gate was set on positive cells, 5,000 cells were acquired, and the expression of HLA-DR, CD11b, CD45RA, and CD45RO was then analyzed on cells expressing low and high levels of CD4 and CD8. The staining pattern of CD4⁺ and CD8⁺ cells is shown in Fig 1. Cells in the main bright staining peak were considered as high cells, whereas cells comprised between the negative control marker and this peak were considered low cells. CD4 and CD8 expression by high cells was relatively uniform and gave rise to a sharp monomodal staining peak. By contrast, low cell staining was heterogeneous, since these cells expressed different amounts of the coreceptors. The MRFI cut-off levels for high and low cells were always the same for a given staining protocol. When three-color IF was performed, CD4⁺ or CD8⁺ cells were stained with biotin-conjugated OKT4 and OKT8 mAb and RED613-conjugated avidin (Becton Dickinson). The expression of two of these markers was detected with the corresponding phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated mAb. Absolute cell counts were calculated by multiplying the percent size of each subset by the total lymphocyte count provided by an automatic blood-cell counter.

Statistical analysis. Differences between means were calculated with Student’s t test and the nonparametric Mann-Whitney test when two groups were compared. One- and two-way analysis of variance (ANOVA) was used when three groups were compared. Computa-
Table 1. PB Counts of Cells Expressing High and Low CD4 or CD8 Levels in B-CLL Patients at Diagnosis and in Therapy

<table>
<thead>
<tr>
<th>Cells</th>
<th>Controls (n = 40)</th>
<th>CLLD (n = 28)</th>
<th>CLLT (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4b</td>
<td>772 ± 265*</td>
<td>1,962 ± 1,035*</td>
<td>1,326 ± 969f</td>
</tr>
<tr>
<td>CD4b</td>
<td>80 ± 54</td>
<td>472 ± 454*</td>
<td>523 ± 470*</td>
</tr>
<tr>
<td>CD8b</td>
<td>274 ± 185</td>
<td>771 ± 546*</td>
<td>682 ± 650t</td>
</tr>
<tr>
<td>CD8b</td>
<td>135 ± 72</td>
<td>522 ± 494*</td>
<td>313 ± 275f</td>
</tr>
</tbody>
</table>

Results are expressed as cells/μL (mean ± SD), with the ratio between patient and control values given in parentheses.

Abbreviations: CLLD, patients at diagnosis; CLLT, patients in therapy. Significantly different from controls: * P < .001, t P < .01 by ANOVA.

RESULTS

Recent studies have defined a new T-cell population that either does not express CD4 or CD8 or expresses lower amounts than classic CD4b and CD8b cells.\(^\text{17-24}\) Since CD4b and CD8b cell counts are increased in B-CLL, we investigated the distribution of high and low cells in these subpopulations. We found that CD4b and CD8b cell counts were increased by 5.9- and 3.9-fold, respectively, whereas CD4b and CD8b cells were less strikingly expanded (2.6- and 2.8-fold) (Table 1). The staining pattern of CD4b and CD8b cells is shown in Fig 1 (see Materials and Methods). To rule out the possibility that these data were artifactual due to contaminant monocytes or neoplastic B cells, we analyzed the expression of B- (CD19), T- (CD3, TCRα/β, TCRγ/δ), and monocyte (CD13, CD14) cell markers in the low and high CD4b and CD8b cells by two-color IF. Table 2 and Fig 2 show that these subpopulations comprise very few B cells and monocytes: more than 95% CD4b, CD4b, and CD8b cells and more than 90% CD8b cells displayed the CD3+/TCRα/β phenotype; CD8b cells also comprised a substantial proportion (7% ± 7%) of TCRγ/δ+ cells. It is noteworthy that CD4b and CD8b cells consistently expressed lower CD3 and TCRα/β levels than their high counterparts (Fig 2). Student's t test for paired samples showed that the differences between the CD3 and TCRα/β MRFI values in the high and low subpopulations were statistically significant (P < .05, n = 5; data not shown).

T-cell expansion in B-CLL is associated with increased expression of HLA-DR, CD11b, and CD45R0, and decreased expression of CD45RA.\(^\text{4,10}\) Since HLA-DR, CD11b, and CD45R0 are regarded as activation markers, we investigated whether they detect the same cells. Three-color staining of PBMC from six patients showed that the overlap between HLA-DR and CD11b expression was minimal (Fig 3). HLA-DR+ cells comprised variable proportions of CD45RA+ and CD45R0+ cells, whereas CD11b+ cells were mostly CD45RA+ (Fig 3). Expression of CD45RA and CD45R0 was also mutually exclusive, as expected, and these subsets comprised variable numbers of CD11b+ and HLA-DR+ cells (data not shown). These data, therefore, showed that CD45RA, CD45R0, HLA-DR, and CD11b detect CD4b, CD4b, CD8b, and CD8b subsets that only partially overlap, and prompted us to investigate their distribution in a larger number of patients (Table 3). This analysis showed that all these subsets were expanded in patients at diagnosis (CLL). Treated patients (CLLT) displayed a similar T-cell pattern, except for CD4b/CD45RA+, CD8b/CD45RA+, CD8b/CD45RA+, CD8b/CD11b+, and CD8b/CD11b+ cells, whose counts were lower in CLLT than CLLD patients. It is noteworthy that subset expansion ranged from 2.1- to 13.6-fold, and therefore was not homogeneous.

These data show that chemotherapy affects T-cell expansion only weakly and its effect is mostly negative and only significant on a few subsets. Moreover, they suggest that the main force driving the T-cell expansion is the tumor cell itself.

To define the role played by the tumor better, we looked for correlations between the T-cell pattern and two clinical parameters that provide evidence of tumor burden and malignancy, and that may influence the antitumor immune response. The first parameter was clinical stage, which can be regarded as an index of tumor burden. We compared T-cell counts of patients with low (stage A) and high (stage B or C) tumor burden. Only treated patients were included in this analysis, since all stage C patients were treated. Stage A patients had more CD4bHLA-DR+ cells than stage B and C patients (Table 4), whereas the other counts were similar in the two groups. The second parameter was stable remission, which corresponds to reduced tumor burden and malignancy. Patients in stable remission (CLLT) had higher counts of CD8bHLA-DR+ cells than CLLD (Table 4).

Finally, the relationship between an intrinsic feature of neoplastic cells, namely their phenotype, and the T-cell pattern was assessed. Patients were grouped according to their expression of sIg, CD5, CD13, CD14, CD33, CD25, and CD38 (see Methods). These markers were chosen because they (1) are heterogeneously expressed by B-CLL cells, as found in the course of routine diagnostic analysis, (2) recognize molecules that may speculatively play a role in modulating T-cell response (see Discussion), and (3) identify groups of patients large enough to allow statistical analysis. This analysis showed that high sIg levels are associated with higher CD4bHLA-DR+, CD4bCD45RA+, CD4bCD45R0+, and CD4bCD11b+ cell counts (Table 4), whereas no difference was found between the other groups.

DISCUSSION

This work analyzes T-cell subset distribution in PB of B-CLL patients, and its correlation with disease status. We

Table 2. Phenotypic Analysis of CD4b, CD8b, CD4b, and CD6b Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>CD3</th>
<th>CD8</th>
<th>TCRα/β</th>
<th>TCRγ/δ</th>
<th>CD19</th>
<th>CD13</th>
<th>CD14</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4b</td>
<td>96 ± 6</td>
<td>94 ± 3</td>
<td>1 ± 1</td>
<td>3 ± 3</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
<td></td>
</tr>
<tr>
<td>CD8b</td>
<td>91 ± 6</td>
<td>86 ± 6</td>
<td>7 ± 7</td>
<td>5 ± 4</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
<td></td>
</tr>
<tr>
<td>CD4b</td>
<td>98 ± 3</td>
<td>97 ± 3</td>
<td>&lt;1</td>
<td>1 ± 1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>CD8b</td>
<td>99 ± 2</td>
<td>97 ± 2</td>
<td>&lt;1</td>
<td>1 ± 1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as the percent of positive cells (mean ± SD, n = 4 to 6).
found that T-cell expansion involves several subsets differentially affected by disease progression and neoplastic cell phenotype. The main finding was that B-CLL patients display high counts of CD4<sup>+</sup> and CD8<sup>+</sup> cells, whose expansion was proportionally higher than that of classic CD4<sup>+</sup> and CD8<sup>+</sup> cells. Since T-cell expansion requires T-cell activation, we analyzed the expression by low and high cells of CD45R0, CD45RA, CD11b, and HLA-DR, which discriminate the T-cell activation stages. CD45R0 and CD45RA discriminate two subsets with different activation features, which have been reported to comprise memory and naive cells, respectively, although this view has been questioned, and may in fact correspond to activated and resting lymphocytes.\textsuperscript{25-27} CD11b is expressed by T cells with granular morphology; CD8<sup>+</sup>CD11b<sup>+</sup> cells are abundant in normal PB, constitutively express the interleukin-2 receptor (IL-2R) $\beta$ chain and display lymphokine-activated killer cell activity;\textsuperscript{28-30} CD4<sup>+</sup>CD11b<sup>+</sup> cells are rare in normal PB, but abun-
Coexpression of HLA-DR, CD11b, CD45RO, and CD45RA in PB CD4\textsuperscript{+}, CD8\textsuperscript{+}, CD4\textsuperscript{hi}, and CD8\textsuperscript{hi} cells from B-CLL patients. Data were obtained by three-color IF. Fluorescence-activated cell-sorter (FACS) plots show expression of CD11b (FL1) and HLA-DR (FL2) in CD4\textsuperscript{+}, CD4\textsuperscript{hi}, CD8\textsuperscript{+}, and CD8\textsuperscript{hi} cells; numbers represent the proportion of cells contained in each quadrant.

Three-color IF was performed on PBMC using PE-HLA-DR, FITC-CD11b and biotin-conjugated CD4 or CD8 mAbs + RED613-avidin; a gate was set on CD4\textsuperscript{+}, CD4\textsuperscript{hi}, CD8\textsuperscript{+}, and CD8\textsuperscript{hi} cells and coexpression of CD11b and HLA-DR was analyzed. All of the plots were obtained from the same patient. Bar graphs show the proportion of cells expressing CD45RA, CD45RO, and CD11b or HLA-DR in the HLA-DR\textsuperscript{+} (left) or CD11b\textsuperscript{+} (right) subset of CD4\textsuperscript{+}, CD4\textsuperscript{hi}, CD8\textsuperscript{+}, and CD8\textsuperscript{hi} cells and represent the means ± SD of data obtained from four to six patients.
CD4\(^+\) and CD8\(^+\) CELLS IN B-CLL

CD8\(^+\)CD11b\(^+\) cell expansion, suggesting a greater susceptibility of these cells to chemotherapy toxicity. The difference between CLL\(^0\) and CLL\(^1\) was not due to the heterogeneous stage distribution, since (1) these subsets were not differently expanded in patients grouped by clinical stage, and (2) similar difference was found when the comparison was confined to stage A patients (data not shown).

A search for links between T-cell expansion and the clinical and biological features of B-CLL showed that the expansion consistently involved CD4\(^0\) and CD8\(^+\) cells. CD4\(^+\)HLA-DR\(^+\) cell counts were higher in stage A patients than in patients with advanced disease (stages B and C). This was not due to differences in the impact of chemotherapy, since (1) all of these patients were treated, (2) CD4\(^+\)HLA-DR\(^+\) cell counts were not significantly different in treated and untreated patients, and (3) no difference was found between patients receiving alkylating agents as opposed to alkylating agents plus prednisone or showing different responses to chemotherapy (data not shown). These data suggest that high tumor masses have a negative influence on CD4\(^+\)HLA-DR\(^+\) cells, which may be due to real inhibition of these cell's expansion or to their trapping in the lymphoid tissues. A negative effect of high tumor masses is also suggested by the finding that stable remission was associated with increased CD8\(^+\)HLA-DR\(^+\) cell counts.

Correlations were also sought between T-cell expansion and B-CLL phenotype, using markers that are heterogeneously expressed by B-CLL patients and could speculatively play a role in T-cell recognition of the tumor. CD1434 and CD3335 are monocyte cell markers and may have a role in cell-to-cell interactions. A similar role may be possessed by CD38,\(^36\) which is expressed by activated T and B cells, whereas CD13\(^37\) is expressed by cells of the myelomonocytic lineage, corresponds to the aminopeptidase N, and probably modulates the function of inflammatory peptides. CD25 is the IL-2R \(\alpha\) chain, and its expression on B-CLL might affect T-cell activation by consuming IL-2. Last, we considered the expression level of sIg, which may function as a tumor-specific antigen. We found that the expression of high levels of sIg by neoplastic cells was associated with higher CD8\(^+\)HLA-DR\(^+\), CD4\(^+\)CD45R0\(^+\), and CD4\(^+\)CD45RA\(^+\) cell counts. In this case, CD4\(^+\)CD11b\(^+\) cells were also expanded. Comparison of patients expressing low and high levels of CD5 was used as a control for this analysis and no difference was found. It is noteworthy that sIg expression was the only phenotypic feature of the neoplastic cell that significantly affected the T-cell pattern. This is in line with data obtained on multiple myeloma, which suggest that T-cell activation is partly sustained by an immune response against idiotypic determinants of the M protein.\(^11\)

This work, therefore, indicates that CD4\(^0\) and CD8\(^+\) cells are an important component of the T-cell expansion displayed by B-CLL patients. They are not a general feature of lymphoproliferative disease, since they are not expanded in multiple myeloma patients (P. Omede, personal observation). They resemble the double-negative (DN) T cells, which are expanded in human and murine autoimmune diseases, and either do not express CD4 or CD8 or express low amounts.\(^12\)\(^24\) Low counts of these cells are normally present in PB, but Dellabona et al reported that oligoclonal TCR\(\alpha/\)

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**Table 3. PB T-Cell Subset Counts in B-CLL Patients at Diagnosis and in Therapy**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Controls (n = 40)</th>
<th>CLL(^0) (n = 28)</th>
<th>CLL(^1) (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4(^+)CD11b(^+)</td>
<td>58 ± 49</td>
<td>292 ± 295*</td>
<td>277 ± 297*</td>
</tr>
<tr>
<td>CD4(^+)HLA-DR(^+)</td>
<td>82 ± 44</td>
<td>579 ± 356*</td>
<td>708 ± 585*</td>
</tr>
<tr>
<td>CD4(^+)CD45R0(^+)</td>
<td>302 ± 175</td>
<td>863 ± 395*</td>
<td>689 ± 650*</td>
</tr>
<tr>
<td>CD4(^+)CD45RA(^+)</td>
<td>356 ± 186</td>
<td>736 ± 748†</td>
<td>310 ± 461†</td>
</tr>
<tr>
<td>CD4(^+)CD11b(^+)</td>
<td>16 ± 14</td>
<td>169 ± 224†</td>
<td>195 ± 275†</td>
</tr>
<tr>
<td>CD4(^+)HLA-DR(^+)</td>
<td>20 ± 14</td>
<td>218 ± 261*</td>
<td>271 ± 261*</td>
</tr>
<tr>
<td>CD4(^+)CD45RA(^+)</td>
<td>29 ± 27</td>
<td>171 ± 266†</td>
<td>160 ± 215†</td>
</tr>
<tr>
<td>CD4(^+)CD11b(^+)</td>
<td>64 ± 60</td>
<td>325 ± 340*</td>
<td>262 ± 2941†</td>
</tr>
<tr>
<td>CD4(^+)HLA-DR(^+)</td>
<td>44 ± 34</td>
<td>397 ± 323*</td>
<td>569 ± 562*</td>
</tr>
<tr>
<td>CD4(^+)CD45R0(^+)</td>
<td>102 ± 84</td>
<td>261 ± 169*</td>
<td>379 ± 383*</td>
</tr>
<tr>
<td>CD4(^+)CD45RA(^+)</td>
<td>171 ± 133</td>
<td>510 ± 428*</td>
<td>303 ± 3155‡</td>
</tr>
<tr>
<td>CD8(^+)CD11b(^+)</td>
<td>103 ± 65</td>
<td>361 ± 293*</td>
<td>191 ± 1855‡</td>
</tr>
<tr>
<td>CD8(^+)HLA-DR(^+)</td>
<td>41 ± 44</td>
<td>209 ± 198*</td>
<td>192 ± 210*</td>
</tr>
<tr>
<td>CD8(^+)CD45R0(^+)</td>
<td>44 ± 40</td>
<td>140 ± 188‡</td>
<td>137 ± 122‡</td>
</tr>
<tr>
<td>CD8(^+)CD45RA(^+)</td>
<td>90 ± 46</td>
<td>381 ± 418*</td>
<td>178 ± 231‡</td>
</tr>
</tbody>
</table>

Results are expressed as cells/\(\mu\)L (mean ± SD), with the ratio between patient and control values given in parentheses.

Significantly different from controls: * \(P < .001\), † \(P < .01\), ‡ \(P < .05\) by ANOVA.

§ Significantly different from CLL\(^0\): \(P < .05\) by ANOVA.

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**Table 4. Patients With Different Clinical Status or Tumor Phenotype Display Different T-Cell Patterns**

<table>
<thead>
<tr>
<th>Cell Subset</th>
<th>Stage B + C (n = 12)</th>
<th>Stage A (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4(^+)HLA-DR(^+)</td>
<td>160 ± 211</td>
<td>354 ± 2691‡</td>
</tr>
<tr>
<td>CD8(^+)HLA-DR(^+)</td>
<td>209 ± 198</td>
<td>483 ± 2761‡</td>
</tr>
</tbody>
</table>

Results are expressed as cells/\(\mu\)L (mean ± SD).

\(* \ P < .001\), † \(P < .01\), ‡ \(P < .05\) using Student's t-test (a) or the Mann-Whitney nonparametric test (b).
cell lineage, not dependent on positive and negative selection.\textsuperscript{22,23} or belong to an unique thymus-derived T-cell lineage, not dependent on positive and negative selection.\textsuperscript{24} Moreover, von Boehmmer et al.\textsuperscript{25} reported that if T cells obtained from female mice transgenic for a TCR\textalpha/\beta specific for the male antigen HY are transferred into male nude mice, expressing the correct genetic background, the HY-specific cells rapidly expand, but, after a few days, downmodulate the CD3/TCR and CD8 molecules and become unresponsive to stimulation via the CD3/TCR. It is noteworthy that CD4\textsuperscript{b} and CD8\textsuperscript{b} cells from B-CLL patients display a downmodulation of CD4, CD8, and CD3/TCR that is quantitatively similar to that described by these investigators, suggesting that these cells, too, may be anergic. This may account for the weak mitogenic response of T cells from B-CLL patients to stimuli acting via the CD3/TCR (personal observation). Porcelli et al showed that some human TCR\textalpha/\beta\textsuperscript{a} DN clones recognize antigens presented by CD1, a major histocompatibility complex class I-like molecule expressed in association with \beta\textsubscript{2}-microglobulin.\textsuperscript{39,40} This is noteworthy, since it has been reported that subsets of normal CD5\textsuperscript{a} and CD5\textsuperscript{b} cells express CD1c, and that B-CLL cells may express CD1a and/or CD1c.\textsuperscript{41,42} Further studies are needed to define the specificity of CD4\textsuperscript{a} and CD8\textsuperscript{a} cells and to assess their relation with CD1 expression by B-CLL cells. However, this work suggests that they may be tumor-specific cells that proliferate in vivo in response to tumor antigens, but have poor effector functions. Cells of this kind are easy to detect and purify, and could be a good system for studying antitumor immunity and a useful tool in immunotherapy.

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Expansion of T cells expressing low CD4 or CD8 levels in B-cell chronic lymphocytic leukemia: correlation with disease status and neoplastic phenotype

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