T-Cell Activation and Subset Patterns Are Altered in B-CLL and Correlate With the Stage of the Disease

By Thomas H. Tötterman, Mats Carlsson, Bengt Simonsson, Mats Bengtsson, and Kenneth Nilsson

Two-color FACS analysis was used to study activated and "functional" T and natural killer (NK) cell subsets of circulating lymphocytes in 23 patients with B-type chronic lymphocytic leukemia (B-CLL) and in 30 healthy subjects. As compared with controls, B-CLL patients had increased absolute numbers of phenotypically activated, HLA-DR+ CD4+ and CD8+ cells and T suppressor/effector (CD11b+CD8+) cells. When patients in Rai stages II through IV (n = 11) were compared with cases in Rai stages 0 through I (n = 12), the former group of patients had higher numbers of activated CD4+ and CD8+ T cells and decreased levels of suppressor/effector T cells. The absolute numbers of T suppressor/inducer (CD45RCD4) cells were elevated in patients with stage 0 through I disease but within normal range in stage II through IV leukemia. We further showed that the absolute numbers of NK-like (CD16+) cells and their activated counterparts (DṚCD16̣) are elevated in B-CLL patients as compared with healthy subjects. The comparison of relative T and NK subsets in the blood of patients and controls showed that the proportions of CD4+, CD8+, and CD16+ cells expressing the activation marker HLA-DR were increased in B-CLL. Furthermore, the percentage of T-suppressor/inducer (CD45R+) cells within the CD4+ population was decreased in the patients. The proportion of T-suppressor/effector (CD11b+) cells within the CD8+ subset was reduced in subjects with stage II-IV disease only. When stimulated in vitro with the T-cell--dependent inducer TPA, B-CLL cells from patients in Rai stages II through IV secreted larger amounts of IgM as compared with cells from stage 0 through I patients. A positive correlation was observed between the degree of phenotypic activation of CD4+ T-helper cells and their functional capacity to augment IgM secretion by autologous B-CLL cells. Our findings indicate a tumor cell-directed regulatory role of T cells (and possibly NK cells as well) in B-CLL. Furthermore, monitoring of phenotypically activated and functional T-cell subsets may be helpful in the prediction of disease progression and timing of therapy in B-CLL.

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MATERIALS AND METHODS

Patients and Controls

Twenty-three consecutive patients fulfilling the histopathologic, 28 clinical, and immunologic criteria for B-lymphocytic CLL were studied. None of the patients had detectable serum monoclonal Ig bands in standard immune electrophoresis. None of the patients had received cytoreductive therapy for 3 months before study. The patients were classified according to the prognostic staging system of Rai et al., 29 which is based on a clinical estimation of the tumor load (0 through IV). Furthermore, disease activity (active or stable CLL) was estimated according to the guidelines of Levin et al., 30 and is based on signs of an expanding tumor cell mass. Patients with active CLL had a significant increase (30% to 50%) in circulating lymphocytes with or without a parallel decrease in hemoglobin (Hb) or platelet levels during the preceding 3 months.

Absolute circulating lymphocytes were determined using an electronic blood cell counter (Technicon H1, Technicon Instruments, Tarrytown, NY). S-LDH and S-TK were determined with technics obtained from circulating lymphocytes to correlate with both Rai stage and clinical activity of CLL. 31

Absolute circulating lymphocytes were determined using an electronic blood cell counter (Technicon H1, Technicon Instruments, Tarrytown, NY). S-LDH and S-TK were determined with techniques described previously. 31,32 The levels of S-TK and S-LDH were earlier shown to correlate with both Rai stage and clinical activity of B-CLL. 33 Patient lymphocyte marker data were compared with data on circulating lymphocytes obtained from 30 healthy blood donors (20 men, 10 women, mean age 39 years, range 24-72 years).

Cells

Twenty milliliters heparinized venous blood was collected by standard aseptic techniques. Mononuclear cells (MNCs) were isolated on routine density gradients (Ficoll-Hypaque, Pharmacia, Uppsala, Sweden).

Enrichment of Non-B Lymphocytes

To enable accurate quantification of small populations of double antibody-binding cells in two-color FACS analysis (described below), T and NK cells were negatively enriched before being stained. Magnetic polystyrene immunobeads (Dynabeads, Dynal, Oslo) precoated with IgM-type anti-CD19 pan-B cell MoAb (AB-1, Dynal) were added to 100 x 10^6 MNCs in phosphate-buffered saline (PBS) with 10% fetal calf serum (FCS, GIBCO Europe, Glasgow, Scotland) at a bead/cell ratio of 2:1. The mixture was gently mixed for ten minutes at +4°C on a rocker platform. Rosette-forming B cells were removed with a cobalt-samarium magnet. The procedure was repeated once or twice. With this protocol, T and NK cells were enriched by a factor of 2 to 6. In control experiments using cells from ten patients, we compared the proportions and phenotypic activation (HLA-DR expression) of T-subset cells and NK cells in unfractored lymphocytes and in the corresponding cell populations negatively enriched for T and NK cells. No significant differences were observed (data not shown).

Staining of Cells

For immunofluorescence staining, MNCs and T/NK cell-enriched populations were washed and suspended in PBS-2% FCS-0.1% sodium azide; 1 x 10^6 cells/50µL were incubated for ten minutes at room temperature with combinations of fluorescein (FITC, green)- and phycoerythrin (PE, red)-labeled MoAbs, washed twice, and mounted in PBS-2% FCS-1% paraformaldehyde (BDH, Poole, UK) for FACS analysis. The MoAbs used to define T- and NK-cell subsets and activated cells are shown in Table 1. Isotype-specific FITC and PE-labeled MoAbs were used as negative controls. All MoAbs were obtained from Becton Dickinson, Mountain View, CA.

FACS Analysis

Fluorescent cells were analyzed on a FACStar (Becton Dickinson) flow cytometer/sorter equipped with a 5-W Argon ion laser run at 0.5 W and emitting at 488 nm. Green fluorescence (FITC) was collected through a 530/30 filter, and red fluorescence (PE) was collected through a 585/42 filter. For each combination of MoAbs, 10,000 viable lymphocytes were gated and analyzed for red-green fluorescence. In stainings of CD4+ and CD8+ cells with Leu-3 and Leu-2, respectively, only brightly fluorescent cells were gated to avoid possible contribution of dim CD4+ monocytes and dim CD8+ NK-like cells. 34 Data were processed with a Hewlett-Packard 217 computer (Fort Collins, CO) and Consort 30 (Becton Dickinson) software.

IgM Secretion

MNCs were suspended in RPMI 1640 medium (Flow Laboratories, Ayrrshire, Scotland) supplemented with 100 IU/mL penicillin, 100 µg/mL streptomycin, 50 µg/mL gentamycin, 2 mmol/L glutamine, and 10% of a selected batch of newborn calf serum (GIBCO). Cells (1 x 10^6/mL) were thereafter cultured in triplicate 0.2-mL cultures in 96-well tissue culture plates (Costar, Cambridge, England). The functional differentiation of B-CLL cells was determined by measuring the IgM secretion in response to TPA 1.6 x 10^-7 mol/L (Sigma). 35 TPA-driven IgM secretion was earlier shown to be strictly dependent of accessory T cells. 36 We also measured IgM secretion of B-CLL cells in an accessory cell-independent induction system. 37 B-CLL cells were activated with 0.1% formalinized Staphylococcus Cowan A particles (SAC, a gift from Dr A-C Rydén, Pharmacia Diagnostics AB, Uppsala, Sweden) for two days followed by addition of rIL-2 (100 U/mL; Amgen, Amersham, England) plus 25% (vol/vol) of B-cell stimulatory factor-containing supernatant (BSF-MP6). BSF-MP6 is produced by a T-T hybridoma cultured serum-free and was earlier shown to have B-cell growth and differentiation-promoting activity distinct from IL-1 alpha, IL-1 beta, IL-2, IL-4, IL-5, IL-6, interferons, colony-stimulating factors (CSFs), and tumor necrosis factor beta (TNF beta). 38,39 The IgM levels in five-day culture supernatants were determined by enzyme-linked immunosorbent assay (ELISA) as described previously. 40

Statistics

Differences in marker-carrying cells were analyzed with the Mann-Whitney U test for nonparametric data. The significance of correlation coefficients (r) was tested with Student’s t test.

Table 1. MoAbs Used to Define T and NK Cells

<table>
<thead>
<tr>
<th>MoAb</th>
<th>CD*</th>
<th>Cell Population Defined</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu-4</td>
<td>3</td>
<td>All T cells</td>
<td>31</td>
</tr>
<tr>
<td>Leu-3</td>
<td>4</td>
<td>T helper/inducer (Th1) cells</td>
<td>32</td>
</tr>
<tr>
<td>Leu-2</td>
<td>8</td>
<td>T suppressor/cytotoxic (Ts) cells</td>
<td>33</td>
</tr>
<tr>
<td>Leu-18</td>
<td>46R</td>
<td>T suppressor/inducer (TaI) cells</td>
<td>34</td>
</tr>
<tr>
<td>Leu-15</td>
<td>11b</td>
<td>T suppressor/effectector (Ts) cells</td>
<td>35</td>
</tr>
<tr>
<td>Leu-11</td>
<td>16</td>
<td>NK-like effectector (NK) cells</td>
<td>36</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>NS</td>
<td>Activation marker on CD4+, CD8+, and CD16+ cells</td>
<td>37, 38</td>
</tr>
<tr>
<td>Anti-IL-2R</td>
<td>25</td>
<td>Activation marker on CD4+, CD8+, and CD16+ cells</td>
<td>38, 39</td>
</tr>
</tbody>
</table>

*CD, cluster of differentiation; NS, CD number not specified at present.
Ethical Considerations

The present investigations were part of routine diagnostics at the University Hospital. Informed consent was obtained in every case.

RESULTS

According to clinical criteria, the patients could be divided into two groups of roughly similar size that differed with respect to Rai stage and disease activity (Table 2). Serum levels of LDH and TK were significantly higher among patients with progressive CLL and Rai stages II through IV, confirming results of earlier studies. The absolute numbers of circulating total lymphocytes and T cells (CD3+) were not significantly different between the groups. Detailed results are presented with respect to Rai stage. Very similar results were obtained, however, when the patients were classified according to disease activity (Table 2).

Circulating T- and NK-Cell Subsets

B-CLL patients as compared with controls. The absolute circulating numbers of activation and "functional" subset marker-carrying T- and NK-like cells in patients and controls are shown in Table 3. Since total lymphocytes, T cells (CD3+), and NK (CD16+) cells were higher in the patients, a significant increase was also observed in every T- and NK-cell subset analyzed. The CD4/CD8 ratio was decreased in progressive B-CLL as compared with controls (Table 3).

The relative numbers of CD4+ (T helper/inducer), CD8+ (T suppressor/cytotoxic), and CD16+ (NK-like) cells among lymphocytes were very low in B-CLL due to the expansion of monoclonal B cells. The proportions of activation and functional subset marker-carrying cells within the CD4+, CD8+, and CD16+ populations in B-CLL were altered as compared with normal. Thus, when all 23 B-CLL patients were compared with 30 controls, the percentage of phenotypically activated, DR+ cells of CD4+ cells was higher (mean + SEM 14 + 3 v 2 + 0.3, P < .001), as was the proportion of DR+ cells within the CD8+ population (21 + 6 v 5 + 0.6, P < .001). Receptors for IL-2 (CD25) were undetectable on T cells in B-CLL and in healthy subjects. The percentage of phenotypic T suppressor/inducer (CD45R+) cells within the CD4+ subset was decreased in B-CLL (21 + 5 v 54 + 3; P < .01), whereas the proportion

Table 2. Clinical and Laboratory Findings in the 23 B-CLL Patients Related to Rai Stage and Disease Activity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rai Stage</th>
<th>Disease Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–I (n = 12)</td>
<td>II–IV (n = 11)</td>
</tr>
<tr>
<td>Mean age, sex (M/F)</td>
<td>75; 8/4</td>
<td>66; 7/4</td>
</tr>
<tr>
<td>Total lymphocytes, f mean (range)</td>
<td>70 (6–209)</td>
<td>102 (9–196)</td>
</tr>
<tr>
<td>T cells (CD3+), mean (range)</td>
<td>5.3 (0.9–8.5)</td>
<td>7.4 (1.4–26)</td>
</tr>
<tr>
<td>S-LDH‡</td>
<td>5.9 (1.8–7.5)</td>
<td>8.1 (4.5–13)</td>
</tr>
<tr>
<td>S-TK‡</td>
<td>4.9 (1.6–24)</td>
<td>13.6 (3–39)</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.
*P-value for difference, Mann-Whitney U test.
†Circulating cells/µL x 10⁵.
‡Serum lactate dehydrogenase (µkat/l, upper normal limit = 6.7).
§Serum thymidine kinase (U/µl, upper normal limit = 5.0).

Table 3. Circulating Absolute Numbers* of T- and NK-cell Subsets in 23 B-CLL Patients and 30 Healthy Subjects

<table>
<thead>
<tr>
<th>Cell Subset</th>
<th>B-CLL Patients</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Rai Stage 0–I (n = 12)</td>
<td>Rai Stage II–IV (n = 11)</td>
</tr>
<tr>
<td>Total lymphocytes</td>
<td>70 + 16</td>
<td>102 + 24</td>
</tr>
<tr>
<td>CD4/CD8 ratio</td>
<td>1.03 + 0.35</td>
<td>0.82 + 0.35</td>
</tr>
<tr>
<td>CD4+</td>
<td>3,300 + 400</td>
<td>3,730 + 950</td>
</tr>
<tr>
<td>DR+CD4+</td>
<td>240 + 20</td>
<td>810 + 32</td>
</tr>
<tr>
<td>CD25+CD4+</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>CD8+</td>
<td>3,200 + 252</td>
<td>4,500 + 280</td>
</tr>
<tr>
<td>DR+CD8+</td>
<td>520 + 80</td>
<td>1,330 + 175</td>
</tr>
<tr>
<td>CD25+CD8+</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>CD45R+CD4+</td>
<td>910 + 180</td>
<td>445 + 80</td>
</tr>
<tr>
<td>CD11b+CD8+</td>
<td>460 + 90</td>
<td>200 + 59</td>
</tr>
<tr>
<td>CD16+</td>
<td>1,250 + 560</td>
<td>1,650 + 750</td>
</tr>
<tr>
<td>DR+CD16+</td>
<td>185 + 50</td>
<td>240 + 45</td>
</tr>
</tbody>
</table>

*Cells per microliter whole blood, mean ± SEM.
†P-value for difference between stage 0–I v stage II–IV B-CLL, Mann-Whitney U test.
‡P-value for difference between stage 0–I B-CLL v controls.
§P-value for difference between stage II–IV B-CLL v controls.
| Cells/µL x 10⁵. |
of T suppressor/effector (CD11b⁺) cells within the CD8⁺ subset was unaltered (11 + 3 v 12 + 1.2, NS). The proportion of phenotypically activated, DR⁺ cells within the NK (CD16⁺) cell population was increased in B-CLL (14 + 4 v 3 + 0.3; P < .001).

**Early v late Rai stage disease.** When 12 B-CLL patients in Rai stages 0 through I were compared with 11 patients in stages II through IV, several differences were observed with respect to T and NK cell markers (Table 3). The absolute numbers of phenotypically activated, DR⁺CD4⁺ and DR⁺CD8⁺ T-subset cells were significantly higher (by a factor of 3) in cases with advanced leukemia. This was not the result of differences in absolute numbers of CD4⁺ or CD8⁺ cells (Table 3), but instead was due to larger proportions of activation marker-carrying cells within each subset in cases with advanced disease (Fig 1). The numbers of circulating CD4⁺ cells with suppressor/inducer (CD45R⁺) phenotype were lower in patients with Rai stages II through IV (Table 3). Again, this was due to a decreased percentage of CD45R⁺ cells within the CD4⁺ subset (Fig 1). Similarly, the circulating absolute and relative numbers of T-suppressor/effectortype (CD11b⁺CD8⁺) cells were lower in advanced B-CLL as compared with early-stage disease, although this difference was of marginal significance (Table 3 and Fig 1).

**Functional Studies**

The in vitro accessory functions of patient T cells were studied by testing their capacity to regulate IgM secretion of autologous B-CLL cells. In these experiments, cells from nine cases in Rai stages II through IV were compared with cells from 12 patients in Rai stages 0 through I. Two different protocols were used, ie, a T-cell-dependent (TPA) and a T-cell-independent (SAC + IL-2 + BSF-MP6) induction system. Figure 2 shows that spontaneous IgM secretion by PB B-CLL cells was minimal. B-CLL cells cultured for five days with TPA secreted significant amounts of IgM, and cells from patients with Rai stages II through IV exhibited a significantly higher secretory response than cells from cases with stage 0 through I disease (means + SEM 1750 ± 300 v 400 ± 140 ng/mL, P < .01). A significant positive correlation (r = .72, P < .02) was obtained between the percentage of DR⁺ cells within the CD4⁺ subset and the IgM secretory response to TPA in vitro.

When the T-cell-independent induction protocol (SAC + IL-2 + BSF-MP6) was used to trigger B-CLL cells from the same patients, the amounts of IgM secreted were considerably lower. No differences were observed in the IgM secretory response with regard to the Rai stage using this protocol.
induction protocol (110 + 79 + 320 + 210 ng/mL; P = .46) (Fig 2).

DISCUSSION

The present study has confirmed the earlier findings of absolute T lymphocytosis and a relative increase in T-suppressor/cytotoxic (CD8\(^+\)) cells in B-CLL.\(^{5-10}\) We extended the phenotypic characterization of B-CLL T cells and showed that B-CLL patients, compared with healthy subjects, have increased absolute numbers of phenotypically activated DR-positive CD4\(^+\) and CD8\(^+\) cells and T suppressor/effector (CD11b\(^+\)CD8\(^+\)) cells in the blood. Among the patients with B-CLL, the numbers of circulating T suppressor inducer (CD45R \(\times\) CD4\(^+\)) cells were elevated in early Rai stages (0 through I) but were normal in more advanced (Rai stage II through IV) disease. We further observed that circulating numbers of NK-like (CD16\(^+\)) cells and their activated counterparts (DR \(\times\) CD16\(^+\)) are elevated in B-CLL as compared with healthy subjects.

The present analysis of relative T and NK subsets in the blood of B-CLL patients and controls showed that the proportions of HLA-DR expressing CD4\(^+\) and CD8\(^+\) T cells and CD16\(^+\) NK-like cells were increased. The percentage of suppressor/inducer (CD45R \(\times\) CD4\(^+\)) cells among CD4\(^+\) cells is decreased in B-CLL, whereas T suppressor/effector (CD11b\(^+\)) cells within the CD8\(^+\) subset are normal or slightly decreased (advanced disease).

CD4\(^+\) T-helperlike cells, when in contact with antigen-presenting accessory cells or mitogen, may undergo activation as evidenced by induction of surface HLA-DR and IL-2 receptors, followed by IL-2–dependent proliferation. Recent studies showed that naive CD4\(^+\) cells unprimed by antigen express the CD45R epitope (detected by MoAbs such as Leu-18 and 2H4) of the leucocyte common antigen (T200) and have the capacity to induce antigen-specific or non-specific T suppressor (CD11b\(^+\)CD8\(^+\)) cells.\(^{35,43}\) On activation with antigen and reversion to memory cells, CD4\(^+\) cells lose CD45R and gain expression of other epitopes of the LCA detected by CDw29 (4B4) and UCHL1 MoAbs.\(^{34,46,47}\) The CD45R \(\times\) CDw29 \(\times\) CD4\(^+\) cells are of pure helper type with respect to B-cell proliferation, Ig secretion, and killer cell (CD11b\(^+\)CD8\(^+\)) generation. Recently, patients with active multiple sclerosis were shown to lose the T-suppressor/inducer population selectively.\(^{48}\) We have observed similar changes in patients with rejecting kidney allografts\(^{49}\) and in autoimmune conditions such as Graves’s disease.\(^{50}\)

Our findings of in vivo-activated CD4\(^+\) cells with a parallel decrease of the suppressor/inducer (CD45R \(\times\) CD4\(^+\)) phenotype in B-CLL are compatible with a chronic antigenic stimulation and accumulation of memory helper/inducer CD4\(^+\) cells. The nature of the triggering antigens in B-CLL are not known, but one likely source is the autologous leukemic cells. This view is strengthened by two observations in the present study. First, the increase in activated CD4\(^+\) cells and decrease in suppressor/inducer cells was significantly more pronounced in cases with advanced disease having a progression in their lymphocyte counts. Second, the functional experiments showed that the T-helper cell-depen-

dent\(^{55-27}\) TPA-driven IgM secretion of autologous B-CLL cells was substantially higher in cases with advanced disease, and there was a positive correlation between CD4\(^+\) cell activation and the IgM response. In the T-cell–independent assay using SAC activation followed by rIL-2 plus BSF-MP6,\(^{52}\) no differences in IgM secretion were noted, indicating that changes in T but not tumor B cells accounted for the exaggerated IgM response. Therefore it is conceivable that an immunologic regulatory interplay between T cells and B-CLL cells may occur in vivo as well. Activated CD8\(^+\) and CD4\(^+\) cells were also recently described in multiple myeloma and monoclonal gammopathies of undetermined significance. In panning experiments, patient’s DR \(\times\) CD8\(^+\) cells bound specifically to Petri dishes coated with the idiotypic serum M-protein.\(^{51}\) In the present study, patients with progressive disease had higher levels of activated T cells and lower levels of suppressor/inducer cells as compared with stable disease. These results indicate that the T-cell activation and functional subset patterns fluctuate in individual B-CLL patients, although prospective follow-up studies are needed to substantiate this.

We further observed that absolute numbers of circulating T cells with suppressor/effector (CD11b\(^+\)CD8\(^+\)) phenotype are increased in B-CLL. This is the result of a substantial elevation in CD8\(^+\) cells, whereas the percentage of CD11b\(^+\) cells within the CD8\(^+\) subset is normal or slightly reduced in active B-CLL. The large proportion of DR \(\times\) CD8\(^+\) cells within the CD8\(^+\) subset indicates that suppressor/effector cells are activated. Thus, these findings provide a phenotypic basis for earlier functional studies indicating excessive suppressor T-cell function in B-CLL.\(^{12,13,15}\) In accordance with these results, the study of Massaia et al\(^{52}\) recently demonstrated that activated CD8\(^+\) cells in progressive multiple myeloma have the suppressor/effector (CD11b\(^+\)) phenotype.

The present results indicate that absolute circulating numbers of CD16\(^+\) NK-like cells and activated (DR \(\times\) CD16\(^+\)) NK cells are elevated in B-CLL. Early studies of NK-like cells indicated that Fcy-receptor \(\times\) E rosetting cells, which include CD8\(^+\) T cells and some NK cells, were elevated in CML.\(^{67}\) Velardi et al\(^{53}\) reported increased absolute numbers of lymphocytes binding the Leu-7 MoAb, whereas the percentage of E rosetting cells binding Leu-11 (CD16) MoAb was subnormal in another study.\(^{54}\) The NK-cell cytotoxic function was earlier reported to be decreased in B-CLL,\(^{20,22}\) but could be corrected by treatment with IL-2 or IFN. Whether NK cells are important in suppressing the tumor clone in B-CLL is unclear. The sensitivity of B-CLL cells to lysis by NK cells may fluctuate, however. We observed earlier that patients’ tumor cells are more susceptible to lysis by allogeneic NK cells during periods of progressive disease.\(^{55}\) In the present study, we found no disease activity-related differences in numbers or activation stage of NK-like cells. Recent studies showed that NK cell clones have the capacity to activate autologous B cells and secrete several lymphokines with B-cell growth and differentiation promoting properties.\(^{56}\) We observed that B-CLL cells from patients with higher Rai stages had an exaggerated IgM response to TPA. Since this was correlated with changes in phenotypic T but not NK cell activation, it is
less likely that NK cells regulate B-CLL cells, at least under in vitro conditions.

We have observed increased numbers of activated T cells, activated NK cells, and T-suppressor cells in B-CLL, with a relative reduction in the T-suppressor/inducer phenotype. Several of these changes were more exaggerated in patients with advanced Rai stages as compared with early stages.

Thus, monitoring of T-cell activation and T-cell functional subsets may prove useful in determining disease activity and timing of therapy in B-CLL. Furthermore, the degree of phenotypic CD4+ T-cell activation was positively correlated with functional help to autologous B-CLL cells in vitro. This indicates that the tumor cells of B-CLL may be under the regulatory control of T cells in vivo.

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