Decreased CD45RA T Cells in B-Cell Chronic Lymphatic Leukemia Patients: Correlation With Disease Stage

By Shoshanna Peller and Suzanna Kaufman

T-cell subsets CD4, CD8 and suppressor-inducers (CD45RA) were determined in 20 patients with B-cell chronic lymphatic leukemia (B-CLL). The proportion of CD4 and CD45RA was decreased when compared with T cells from normal subjects. CD8 was markedly increased. The activity of concanavalin A-induced suppressor cells was not significantly different from that of normal controls and was negatively correlated to the percentage of CD4 of B-CLL patients. The selective loss of CD45RA cells was more prominent in patients in advanced Rai stages of the disease (III to IV) than in early stages (0 to II). Six patients of the advanced stages group suffered from autoimmune hemolytic anemia, whereas no patient in the early stages of disease showed an autoimmune phenomenon. Our results may indicate a mechanism of autoimmune in B-CLL similar to that of patients with autoimmune diseases.

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cells were washed four times with PBS and resuspended in RPMI 1640. Fifty micrograms of mitomycin C was added and the test tubes were incubated for 45 minutes at 37°C. Subsequently, the cells were washed four times with PBS and resuspended in RPMI 1640 at a concentration of $1 \times 10^6$/mL. Viability of cells exceeded 80%.

Suppressor cell activity was measured by the reduction of lymphocyte transformation of the responders with phytohemagglutinin (PHA) in the presence of Con A-induced suppressors compared with control lymphocytes incubated without Con A. Lymphocyte transformation was performed in microtiter plates. Each well contained $10^5$ responders, 10% autologous plasma, 10 μg/mL of PHA, and RPMI 1640 to a total volume of 100 μL. Con A-induced suppressor cells or control cells ($10^5$ cells in 100 μL) were added. After 72 hours of incubation, 0.5 μCi of $^{3}H$-thymidine (specific activity, 49 Ci/mmol; Nuclear Research Center, Negev, Israel) was added to each well for the last 4 hours. The plates were harvested and radioactivity (counts per minute) was counted in a beta counter. Percentage suppression was calculated as follows:

$$\% \text{ Suppression} = 1 - \frac{cpm \text{ With Con A-Induced Suppressors}}{cpm \text{ With Control Lymphocytes}} \times 100$$

Statistical analysis was performed by unpaired Student's t-test, the Pearson's correlation coefficient, and the Mann-Whitney test.

RESULTS

Table 2 summarizes T-cell markers and the percentage of suppression in both patients and control subjects. We have observed a significant decrease in the percentage of CD45RA cells in T cells from B-CLL patients compared with normal controls. The CD4 population in B-CLL was also significantly decreased, whereas the CD8 cells were increased. There was no significant difference between the percent suppression of patients' T cells and normal T cells, although there was a wider variability of results within the patients. Most patients had suppression within normal range, two had negative suppression meaning enhancement, and nine patients had suppressor cell activity greater than the upper range of normal cells.

Our analysis showed a negative significant correlation ($r = -.57, P < .015$) between the percentage of CD4 and suppressor cell activity in the patients, as can be seen in Fig 1.

For further analysis, the patients were divided into two groups: a group of 10 patients at early stages of the disease...
CD45RA Cells in B-CLL

T-cell subsets and suppressor cell activity in early and advanced stages of B-CLL patients and normal controls. (I) Patients at stages 0 to II; (II) patients at stages III to IV; (III) normal controls.

The results of each group were compared with those of normal controls and showed the same significant differences observed with the group of 20 B-CLL patients, a decrease in CD4 and CD45RA and an increase in CD8. There was no significant difference between the suppressor cell activity in each group and the activity of normal lymphocytes.

A negative correlation between the percent of CD4 and percent of suppression was also observed both in patients with early disease stages and patients with advanced stages ($r = -0.6, P < .05$).

When T-cell subsets and Con A-induced suppression were compared between the two groups of patients, no difference was observed in the percent of CD4, CD8, and suppressor cell activity. The percentage of CD45RA, on the other hand, was markedly reduced in patients at stages III to IV compared with those at stages 0 to II (31.4 ± 7.8 vs 38.5 ± 6, $P < .03$) (Fig 2).

The results of determination of T-cell subsets CD4 and CD45RA by flow cytometry are summarized in Table 3. The patients had a marked decrease in CD4 cells, CD45RA cells, and cells bearing both markers (CD45RA+CD4+) when compared with controls ($P < .05$, $P = .05$, and $P < .005$, respectively). We did not compare in this case patients in early and advanced stages of the disease because the numbers in each group were too small. The differences between the cell subsets in one B-CLL patient and one control subject by flow cytometry can be seen in Figs 3 and 4.

**DISCUSSION**

Our study showed a decreased percentage of CD4 lymphocytes in B-CLL patients and an increased percent-

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**Table 3. Immunophenotyping by Flow Cytometry of Lymphocytes From B-CLL Patients and Normal Controls**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical Stage</th>
<th>% CD4</th>
<th>% CD45RA</th>
<th>% CD45RA+CD4+*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>33</td>
<td>32</td>
<td>6</td>
</tr>
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<td>2</td>
<td>II</td>
<td>25</td>
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<td>11</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>43</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>III</td>
<td>48</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>III</td>
<td>46</td>
<td>20</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Normal controls†

| (n = 4) | 58.3 ± 4.5 | 37.3 ± 6.4 | 24 ± 3 |

*Counted as percentage of CD4+ cells.
†Results are mean ± SD.

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**Fig 2. Immunophenotyping by flow cytometry of normal T lymphocytes. (a) CD4+ cells, (b) CD45RA+ cells, and (c) CD45RA+CD4+ cells by double coloring.**

**Fig 3. Immunophenotyping by flow cytometry of normal T lymphocytes. (a) CD4+ cells, (b) CD45RA+ cells, and (c) CD45RA+CD4+ cells by double coloring.**

**Fig 4. Immunophenotyping by flow cytometry of T lymphocytes from a B-CLL patient. (a) CD4+ cells, (b) CD45RA+ cells, and (c) CD45RA+CD4+ cells by double coloring.**
age of CD8 cells. These results confirm previous reports on these T-cell subsets in B-CLL.

The percentage of CD45RA-positive T lymphocytes in the B-CLL patients we studied was markedly reduced. This decrease was more prominent in the advanced Rai stages. This result contradicts a previous report showing an increase in the percentage of CD45RA-positive CD3 lymphocytes in a group of B-CLL patients when compared with normal controls with no correlation to the stage of the disease. Our study determined CD45RA-positive T cells in 20 patients and counted by dual-color cytometry the CD45RA-positive CD4 cells in six B-CLL patients. In both cases we observed a decrease in the CD45RA population, which was a result of a decrease in CD4+CD45RA- cells. This finding agrees with the results of Totterman et al., who demonstrated a decrease in CD45RA+CD4+ cells with correlation to Rai staging.

CD45RA cells were found to generate in vitro Con A-induced suppressor cells. Therefore, we studied these suppressor cells in T cells from B-CLL patients. Our results show no significant difference between the suppressor cell activity of patients and normal controls. Most patients had suppressor activity within the normal range. Nine patients showed excessive suppression. Excessive suppression was found earlier when suppressor cell activity of B-CLL patients was studied on B-lymphocyte function.

Selective loss of CD45RA cells plays a role in the pathogenesis of autoimmune diseases. Unlike our results and the results of others on normal or excessive suppressor cell activity in B-CLL, patients with autoimmune diseases like SLE and idiopathic thrombocytopenic purpura (ITP) had impaired suppressor cell activity. When we looked for autoimmune phenomena in our patients we observed that five patients had a positive DAT hemolytic anemia, whereas one patient had autoimmune hemolytic anemia with a negative DAT. As can be seen in Table 1, all these patients were in an advanced Rai stage (III to IV), a group that also had a prominent decrease in CD45RA. A previous report by Hamblin et al. showed that autoimmune in CLL prevails in higher percentages in patients with advanced disease stages than in patients with early stages.

The excessive activity of suppressor cells observed by us may be explained by the presence of activated CD8 cells, as was recently reported. The presence of a certain percentage of activated suppressor cells in our B-CLL patients may also explain the negative correlation between the percentage of CD4 and the suppressor cell activity observed by us. Excessive suppression can also be explained if CD4 cells other than CD45RA act as suppressor-inducers in B-CLL. This explanation awaits further studies.

Except for the imbalance of the immunoregulatory T cells in B-CLL patients shown here, it has been recently reported that the expanded CD5-positive B cells of B-CLL patients can produce in vitro, after stimulation, autoantibodies. These autoantibodies could be demonstrated in sera from these patients, but only in very low levels.

Our results show a decrease in CD45RA cells in B-CLL patients progressing in the more advanced clinical stages of the disease. These results and the observation that 6 of 10 patients in the advanced stages had autoimmune phenomena may imply an autoimmune mechanism in B-CLL triggered by selective loss of CD45RA similar to that of autoimmune diseases.

The pathogenesis of autoimmunity in B-CLL may be more complex because of other impaired immune components like CD8 cells and dysfunction of B lymphocytes.

Sequential studies currently being performed on our patients may further enlighten the mechanism of onset of autoimmunity in B-CLL patients.

REFERENCES


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