Effect of a Thymic Factor on T Lymphocytes in B Cell Chronic Lymphocytic Leukemia: In Vitro and In Vivo Studies

By F. Lauria, D. Raspadori, and S. Tura

Abnormalities of T lymphocytes in B cell chronic lymphocytic leukemia (B-CLL) have been extensively documented by several immunologic investigations. Following recent studies pointing to the favorable effect of TP-1, a partially purified extract of calf thymus, on the T cell-mediated immunity of several diseases, including Hodgkin's disease, we have used monoclonal antibodies and the enriched T lymphocyte populations of 16 untreated B-CLL patients to evaluate the proportion of T cell subsets before and after the administration of TP-1. In addition, the proliferative response to phytohemagglutinin (PHA) and the helper function in a pokeweed mitogen (PWM) system were assessed. In ten cases, the effect of TP-1 was also studied in vitro by evaluating the same parameters before and after incubation of B-CLL T lymphocytes with the drug. The study demonstrated that in vivo administration of TP-1 increases significantly (P < .001) the proportion of the defective helper/inducer T cell population (OKT4-positive cells) in B-CLL, leading to a near-normal OKT4/OKT8 ratio. Furthermore, the improved phenotype profile was accompanied by an increased proliferative response to PHA and, in particular, by a significant increase (P < .01) of T helper capacity; this increase was, however, insufficient to enable the normalization of the serum immunoglobulin levels. The in vitro incubation of B-CLL T lymphocytes did not succeed in producing significant modifications in distribution and function.

MATERIALS AND METHODS

Heparinized peripheral blood samples were collected from 16 B-CLL patients and 15 normal controls. The patients, ten males and six females, were all untreated; they were aged 50 to 80 years, and according to Rai et al., six were in stage 0, five in stage I, four in stage II, and one in stage III.

TP-1 was prepared as described by Bergesi and Falchetti at the Istituto Farmacologico Serono, Rome. Briefly, calf thymuses were minced and extracted with ammonium acetate. The extract was heated to 70°C, filtered, and precipitated with ammonium sulfate. The precipitate was dissolved in water and subjected to ultrafiltration on Amicon PM-10 membrane. The filtrate was desalted on Sephades C-25 and gel-filtered on Sephadex C-50. The fractions used showed two characteristic bands with Rf 0.22 and 0.42 on polyacrylamide gel electrophoresis at pH 8.6. Controls for contaminating pyrogenic substances were performed by inoculating rabbits.
TP-1 was pyrogen free and the endotoxins were always less than 1 ng/mg of the TP-1 in all the samples used for in vitro and in vivo studies. To obtain enriched T cells, the lymphocyte surface expression of T helper/inducer cells (OKT4), and T suppressor/cytotoxic cells (OKT8) was assessed by counting at least 200 cells. The enriched T cell fractions were incubated with 50 ng/mL TP-1 for 15 minutes, and the percentage of positive cells was calculated as a function of the total number of cells recovered per well.

In Vivo Studies

In ten patients, PBL and enriched T lymphocytes (4 x 10⁶/mL) were suspended in RPMI medium containing TP-1 at a final concentration of 50 ng/mL. Previous experiments have shown this to be the ideal concentration in vitro. The cells were incubated one hour and 24 hours for T surface markers, whereas for the culture period. The tests were then performed as described above.

The rank sum test on log-transformed values was used for statistical analysis. The pretreatment values were compared to the posttreatment values. The relative helper T cell function was tested by measuring the serum immunoglobulin level after labeling with a fluorescein-conjugated goat anti-mouse antibody and counting at least 200 cells.

Table 1 illustrates the mean values of T cells (E rosette- and OKT3-positive cells), T cell subsets, and OKT4/OKT8 ratios, and values of functional studies before and after incubation with TP-1. The helper activity of enriched T lymphocytes was assessed by enumerating, on the peripheral blood lymphocytes (PBL), the E rosette-positive cells with sheep red blood cells (SRBC). In addition, the nonadherent cells were allowed to rosette a second time with SRBC after labeling with a fluorescein-conjugated goat anti-mouse antibody and counting at least 200 cells.
EFFECT OF TP-1 ON B-CLL T LYMPHOCYTES

Table 2. Clinical Characteristics, T Cell Distribution, and Functional Studies Before and After In Vivo Treatment With TP-1 in the 16 Patients With B-CLL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex and Age</th>
<th>Stage</th>
<th>WBC x 10³/L</th>
<th>Percent E Rosettes*</th>
<th>Percent OKT3†</th>
<th>Percent OKT4‡</th>
<th>OKT4/OKT8 Ratio</th>
<th>PHA (cpm)</th>
<th>PWM</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.F.</td>
<td>M 58 0</td>
<td>B</td>
<td>11.7 9.8</td>
<td>23 24 86 91</td>
<td>38 81</td>
<td>26 24</td>
<td>1.46 3.37</td>
<td>26,591</td>
<td>36,961</td>
</tr>
<tr>
<td>C.A.</td>
<td>M 64 I</td>
<td>A</td>
<td>39.1 50.9</td>
<td>11 9 93 89</td>
<td>42 64</td>
<td>47 41</td>
<td>0.89 1.56</td>
<td>4,149</td>
<td>33,852</td>
</tr>
<tr>
<td>Z.A.</td>
<td>F 73 0</td>
<td>B</td>
<td>11.2 13.7</td>
<td>38 32 87 95</td>
<td>29 68</td>
<td>48 45</td>
<td>0.60 1.61</td>
<td>30,101</td>
<td>64,918</td>
</tr>
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<td>Z.G.</td>
<td>M 65 0</td>
<td>B</td>
<td>49.3 48.6</td>
<td>16 15 77 87</td>
<td>28 58</td>
<td>24 30</td>
<td>1.16 1.93</td>
<td>4,627</td>
<td>28,595</td>
</tr>
<tr>
<td>R.A.</td>
<td>F 55 0</td>
<td>A</td>
<td>18.0 14.8</td>
<td>14 16 83 86</td>
<td>16 50</td>
<td>40 31</td>
<td>0.40 1.61</td>
<td>6,974</td>
<td>32,908</td>
</tr>
<tr>
<td>B.L.</td>
<td>M 67 I</td>
<td>B</td>
<td>17.2 16.5</td>
<td>11 13 92 88</td>
<td>42 38</td>
<td>41 39</td>
<td>1.02 0.90</td>
<td>11,028</td>
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</tr>
<tr>
<td>Z.F.</td>
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<td>B</td>
<td>16.5 17.0</td>
<td>28 27 85 80</td>
<td>39 35</td>
<td>42 22</td>
<td>0.92 1.59</td>
<td>17,313</td>
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</tr>
<tr>
<td>N.G.</td>
<td>F 73 I</td>
<td>A</td>
<td>19.1 20.7</td>
<td>21 23 84 90</td>
<td>50 46</td>
<td>43 35</td>
<td>1.16 1.31</td>
<td>14,090</td>
<td>36,941</td>
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<tr>
<td>M.T.</td>
<td>M 61 0</td>
<td>A</td>
<td>10.8 16.3</td>
<td>26 30 89 87</td>
<td>35 56</td>
<td>40 32</td>
<td>0.87 2.06</td>
<td>10,004</td>
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<td>N.A.</td>
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<td>B</td>
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<td>9 14 83 88</td>
<td>24 43</td>
<td>32 35</td>
<td>0.75 1.22</td>
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</tr>
<tr>
<td>T.W.</td>
<td>F 54 I</td>
<td>A</td>
<td>35.7 38.4</td>
<td>18 17 89 94</td>
<td>26 42</td>
<td>30 36</td>
<td>0.86 1.16</td>
<td>6,425</td>
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<tr>
<td>G.A.</td>
<td>M 71 II</td>
<td>B</td>
<td>48.1 44.3</td>
<td>21 23 92 88</td>
<td>27 30</td>
<td>28 30</td>
<td>0.84 1.58</td>
<td>13,121</td>
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<td>R.C.</td>
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<td>B</td>
<td>32.3 30.1</td>
<td>16 21 93 95</td>
<td>40 56</td>
<td>40 28</td>
<td>1.00 2.00</td>
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<td>12,907</td>
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<td>B.F.</td>
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<td>A</td>
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<td>41 32</td>
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<tr>
<td>M.G.</td>
<td>F 67 II</td>
<td>A</td>
<td>44.1 51.4</td>
<td>26 21 89 91</td>
<td>38 64</td>
<td>45 40</td>
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<tr>
<td>F.C.</td>
<td>M 72 II</td>
<td>A</td>
<td>63.4 47.9</td>
<td>19 23 88 88</td>
<td>36 58</td>
<td>38 26</td>
<td>0.95 2.23</td>
<td>4,149</td>
<td>33,852</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>35.7 35.5</td>
<td>19 20 87 89</td>
<td>34 55</td>
<td>37 32</td>
<td>0.91 1.70</td>
<td>13,504</td>
<td>27,818</td>
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</table>

B, before; A, after administration of TP-1.
*PBL fraction.
†T-enriched fraction.
‡No. of CyIg-positive cells recovered/well x 10³.

rosette formation and by OKT3 MoAb on the unfractionated lymphocytes was 19% and 20%, respectively. (OKT3 data are not shown in the Table 2.) After enrichment of T lymphocytes (mean value, 87% of OKT3-positive cells), the distribution of T cell subsets before the administration of TP-1 showed a significantly reduced (P < .001) proportion of OKT4-positive cells (helper/inducer) and a significant increase (P < .01) of OKT8-positive cells (suppressor/cytotoxic) compared with normal T lymphocytes. Due mainly to a reduction of OKT4-positive cells, the mean OKT4/OKT8 ratio was significantly reduced (P < .001). In summing up percentages of OKT4 and OKT8 in six patients studied before the administration of TP-1, the results were lower than the OKT3 percentage. Following in vivo treatment with TP-1, the proportion of E rosettes and OKT3-positive cells assessed on the mononuclear fraction appeared consistently unmodified. However, the proportion of OKT4-positive cells assessed on the enriched T-cell fraction rose from 34% to 55% (P < .001), while that of OKT8-positive cells appeared to be only moderately reduced compared with pretreatment values (Table 2).

The response of T lymphocytes to PHA was significantly reduced in all patients studied compared with normal controls (P < .001). After TP-1 administration, an increase (P < .01) in the response to PHA was observed in 10/14 cases, but only in three patients was a complete normalization reached (Fig 1). Despite treatment with TP-1, the overall mean PHA stimulation index was still significantly depressed compared with normal controls (P < .001) (Table 2).

The effect of TP-1 treatment on the helper capacity was assessed on isolated T cells of 16 cases of B-CLL patients and on ten controls cocultured with normal purified B cell in the presence of PWM. As illustrated in Table 2 and Fig 2, B-CLL T lymphocytes showed a constant reduction in helper activity in all cases studied. In 13/16 cases, the administration of TP-1 produced an enhancement of the T helper/inducer capacity. Despite this improvement, however, we were unable to detect any increase in the level of immunoglobulins. In no patient did the administration of TP-1 produce side effects.

**DISCUSSION**

Thymic hormones have been shown in both animal and human models to exert an essential role in the differentiation and maturation of thymocytes into functionally mature T lymphocytes, both in periphery and in secondary lymphoid organs. Moreover, an omeostatic activity of thymic hormones has been demonstrated in peripheral T cells, leading to a better in
vitro response to subliminal antigenic signals. These experimental findings were later confirmed by the beneficial effect of thymic hormones, and of TP-1 in particular, in human pathologic conditions, such as immune defects, recurrent viral infections, etc. More recently, TP-1 has been investigated in vitro and in vivo in patients with Hodgkin’s disease, and an improvement of the immunologic competence, such as an increase in the proportion of E rosette-forming cells and in the response to PHA, has been achieved. In our study, we did not record any increase in the proportion of E rosette-forming cells, but the administration of TP-1 did increase significantly the proportion of OKT4-positive cells (helper/inducer) in B-CLL patients, leading to a near-normal OKT4/OKT8 ratio. Furthermore, the improved phenotypic profile was accompanied by an increased proliferative response to PHA (P < .01) and by an improved helper function in a PWM-stimulated assay (P < .01). The level of serum immunoglobulins, however, was not affected by the administration of TP-1, and no effect was observed on the T cell distribution and function when B-CLL T lymphocytes were incubated in vitro with TP-1. The fact that a correction of T cell subset imbalance was obtained only when giving TP-1 in vivo (no effect was observed in vitro) suggests that the drug may play an important role in activating or restoring T cell physiologic maturation pathways through the thymic compartment and/or in T-dependent areas of peripheral lymphoid organs. However, it is ineffective on peripheral mature T cells. Interestingly, in six cases, a relatively high proportion of T lymphocytes that were characterized by the absence of both antigens recognized by the OKT4 and OKT8 MoAbs, previously demonstrated mainly in patients with advanced disease, was recorded; this suggests a maturation impairment that may be restored by the thymic hormone. Thus, it appears that TP-1 does not produce any increase in the total number of T cells, as suggested by the unmodified number of E rosette-forming cells, but does produce a maturation effect on the OKT4 cell compartment.

Although the exact implications of T cell abnormalities in B-CLL are yet to be fully understood, they may, nonetheless, contribute to the gradual accumulation of immature B cells, which occurs as the disease progresses, and especially, to the immunodeficiency that frequently accompanies this disease. The reduced antibody production in B-CLL may in fact relate to the reduced proportion of OKT4-positive cells associated with an increase of OKT8-positive cells, which is complicated also by an intrinsic defect within the T helper cell population. It is intriguing that TP-1 appears to affect not only the percentage of OKT4-positive cells, but also the functional behavior of these cells. In this respect, although the exact mechanism of the action of TP-1 on the T-cell compartment of B-CLL needs to be clarified, the possibility that it may act by promoting an increase of T-soluble factors (eg, interleukin 2) should be taken into consideration. More studies are warranted in order to assess whether the positive effect on the T cell compartment may be prolonged by a “maintenance” regimen, as preliminary data suggest that the effect of TP-1 may decline rapidly within 1 month after ceasing treatment. This study will clarify whether “continuous” treatment is capable of influencing the level of serum immunoglobulins in B-CLL and whether it may tangibly affect the course of the disease, particularly in patients with more advanced and complicated disease.

REFERENCES


Fig 1. PHA response of B-CLL T lymphocytes before and after administration of TP-1 in 14 patients.

Fig 2. Helper/inducer capacity of B-CLL T lymphocytes assessed in a PWM-stimulated system before and after therapy with TP-1.
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