Defective T Cell Responsiveness in Chronic Lymphocytic Leukemia: Analysis of Activation Events

By Neil E. Kay and Manuel E. Kaplan

Chronic lymphocytic leukemia (CLL) is a B cell disorder in which major T cell proliferative defects are present. We investigated the nature of this deficit by studying several parameters known to be crucial in normal T cell proliferative response to mitogen. Purified peripheral blood T cells from B-CLL patients were analyzed for the presence of T3 antigen. We observed that CLI T cells have a direct correlation between levels of T3 membrane antigen and proliferative response to mitogen. The appearance of activation antigens (transferrin, HLA-DR, and interleukin 2 [IL 2] receptor) was normal in CLI T cells post-mitogen exposure. Despite the normal presentation of IL 2 receptor on CLI T cell membrane, there was decreased production of IL 2 by CLL patients (v controls) \(39.6 \pm 10.2 \text{ cells per milliliter} \times 64.6 \pm 11.0 \text{ cells per milliliter}\). Finally, we were able partially, but not fully, to reconstitute CLI T proliferative response to mitogen by adding purified exogenous IL 2. These findings suggest that CLI T cells have multiple defects that may impact on their proliferative potential. Further insight into these deficits may result in strategies that will facilitate immunologic restoration in T cells of these patients.

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Fig 1. Relationship between T cell proliferation and T3 membrane antigen. The T3-ER ratios of CLL () (n = 17) or control (●) (n = 6) subject lymphocytes are plotted vs the [3H]Tdr incorporation of the same lymphocytes PHA-induced [3H]Tdr incorporation.

IL 2 production and quantitation. Lymphocytes (10⁷/mL, control and CLL) in RPMI–10% FBS were incubated with 1 µg/mL PHA for 24 hours (37 °C, 5% CO₂). PHA–lymphocyte-conditioned media (PHA-LCM) supernatants were harvested and stored at −70 °C until being assayed for IL 2 activities. IL 2 measurements were performed by a modification of the method of Gillis et al.¹⁷ using the CTLL-20, IL 2-dependent cell line.¹⁸ CTLL-20 cells were maintained in RPMI–5% human serum and were fed every other day with 10% to 20% of a murine IL 2 source (spleen LCM derived from MLC). CTLL-20 cells were washed twice to remove residual IL 2, distributed into microtiter wells (0.5 to 1.0 x 10⁶ CTLL per well), and supplemented with various dilutions of normal or CLL PHA–LCM. Dilutions of a standard human IL 2 source were invariably included. After 24-hour incubation, the cells were pulsed for six hours with 2 µCi [3H]Tdr. A log probit plot of the standard IL 2 source was used to quantify IL 2 activities of the unknown PHA–LCM.

RESULTS

Relationship between CLL T cell proliferation and membrane T3 antigen. We have previously reported that CLL T cell populations containing low T3-ER⁺ ratios exhibit subnormal mitogenic responses to PHA.¹² The present studies confirm our previous findings and demonstrate a high degree of correlation between T3-ER⁺ ratios and levels of [3H]Tdr incorporation (Fig 1). Ratios ≥0.6 were invariably associated with more normal proliferative responses. Patients with ratios ≤0.3 (n = 5) had significantly less proliferation than patients with ratios ≥0.6 (P < 0.5; n = 10).

In an effort to augment the defective responses, we treated CLL cell populations having low T3-ER⁺ ratios with neuraminidase, which, as we have previously shown, enhances their T3 antigen expression.¹² Although neuraminidase treatment markedly increased the percentages of T3-positive cells from 38% to 78%, no significant improvement in PHA-induced [3H]Tdr incorporation resulted.

Appearance of membrane activation antigens in CLL and control T cells. Figure 2 summarizes the kinetics of appearance of various activation antigens after exposure of CLL and control T cells to PHA. During the first 48 hours, T9, TAC, and IA increased at similar rates in patient and control cells; thereafter, T9 and TAC gradually declined. In contrast, IA expression of control, but not of CLL, cells continued to increase between 48 and 72 hours. The rate and degree of expression of activation antigens bore no relationship to T3-ER⁺ ratios or to the magnitude of [3H]Tdr incorporation. The standard error of the mean for all points plotted in Fig 2 ranged from 4% to 10%.

IL 2 production by CLL and control T cells. CLL T cells were found to elaborate approximately 55% of control IL 2 activities in response to PHA stimulation. Table 1 summarizes the relationship of CLL ER⁺ lymphocytes to their membrane T3 presentation and PHA-induced IL 2 production and [3H]Tdr incorporation (Table 1). In general, the lower the T3-ER⁺ ratio, the lower the thymidine incorpora-
the percentage of ER cells that are T3 reactive. Controls. CLI patients were subdivided into three groups based on cyto
tes pre- and post-exposure to 10% IL 2 are plotted for CLI and cytes with <60% T3 reactive cells, but the difference was not cause after PHA stimulation CLL T cells developed various statistically significant.

2 production was somewhat lower in ER lympho-

tocytes 30% to 60% reactive with OKT3; and III, ER lymphocytes >60% reactive with OKT3.

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Enhancement of mitogenesis by exogenous IL 2. Because after PHA stimulation CLL T cells developed various activation antigens normally but produced diminished amounts of IL 2, we attempted to improve their mitogenic responsiveness by supplementing them with exogenous IL 2 (Fig 3). Addition of 10% IL 2 improved somewhat, but did not normalize, the proliferative responses of CLL T cells to PHA. The greatest degrees of improvement were seen in CLL patients exhibiting low T3-ER cell populations. Addition of higher concentrations of IL 2, or repetitive supplementation with IL 2 over the 72-hour incubation period, failed to further enhance T cell proliferative responses.

Table 1. CLL ER Lymphocytes in Relationship to Their T3 Membrane Antigen, IL2 Production, and PHA-induced [3H]Tdr Incorporation

<table>
<thead>
<tr>
<th>Group</th>
<th>T3 Positive (%)</th>
<th>IL 2 Production (μ/mL)</th>
<th>[3H]Tdr (cpm/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (0%-30%, n = 4)</td>
<td>13.5 ± 2.9</td>
<td>31.5 ± 27.8</td>
<td>2.9 ± 1.8 x 10⁻²</td>
</tr>
<tr>
<td>II (30%-60%, n = 4)</td>
<td>40.8 ± 1.0</td>
<td>32.8 ± 26.5</td>
<td>18.5 ± 1.4 x 10⁻³</td>
</tr>
<tr>
<td>III (&gt;60%, n = 6)</td>
<td>87.5 ± 3.6</td>
<td>45.8 ± 11.7</td>
<td>59.3 ± 6.8 x 10⁻³</td>
</tr>
<tr>
<td>Control (n = 11)</td>
<td>92.5 ± 2.6</td>
<td>64.6 ± 11.0</td>
<td>109.8 ± 15.8 x 10⁻³</td>
</tr>
</tbody>
</table>

CLL ER lymphocyte groups were arbitrarily divided into three groups: I, ER lymphocytes that were 0% to 30% reactive with OKT3; II, ER lymphocytes 30% to 60% reactive with OKT3; and III, ER lymphocytes >60% reactive with OKT3.

DISCUSSION

Activation of normal T cells may be arbitrarily conceptualized as occurring in three sequential stages: (1) engagement of Ti, the T cell antigen receptor intimately associated with T3, (2) development of multiple membrane activation antigens, including T9 (transferrin receptor), TAC (the receptor for IL 2); the T cell growth factor, and IA, (3) synthesis and elaboration of IL 2, which combines with TAC to trigger mitogenesis. In an effort to better understand the mechanisms of the defective proliferative responses of CLL T cells to PHA and other immunologic stimuli, we have examined each of the stages for possible abnormalities. We have previously reported that the peripheral blood of CLL patients showing exceedingly poor responses to PHA contain high percentages of ER lymphocytes that poorly express T3 membrane antigen. T3 is known to be required for normal T cell proliferative responses and development of cytotoxic effector cell functions. The studies described confirm our previous findings and emphasize the close relationship between T3 expression and mitogenic responsiveness (Fig 1). Although treatment of these low T3-ER cell populations with neuraminidase significantly improved their T3 expression, no parallel enhancement of mitogenic responsiveness occurred. The T3 antigen comprises several membrane glycoproteins containing different numbers of sialic acid residues. Because neuraminidase treatment exposes additional T3 antigenic sites without enhancing mitogenic responsiveness, additional yet undetected defects may be present within the CLL T3 complex. Alternatively, other abnormalities may be responsible for defective cell proliferation.

To investigate other possible abnormalities, we studied the appearance of various membrane activation antigens induced by PHA stimulation of CLL T cells. Surprisingly, the rates of appearance of T9, TAC, and IA were entirely normal, unrelated to the degree of T3 expression or to the mitogenic responsiveness of the individual cell populations. Although we detected significantly diminished IL 2 activity in PHA-CLL T cell conditioned media, no relationship was observed between the degree of abnormality in IL 2 elaboration, T3-ER ratios, and mitogenic responsiveness. Moreover, only minimal improvement in the proliferative responses of these cells to PHA could be induced by addition of exogenous IL 2, despite the presence of apparently normal numbers of IL 2 receptors. These findings suggest that receptor binding of IL 2 may be defective or that even more distal defects in the mitogenic sequence may be present, ie, in the generation of the recently described inducible cytoplasmic factor that initiates DNA replication.
Thus it appears that CLL T cells exhibit multiple abnormalities that may relate to their diminished proliferative potential. Continued exploration of the molecular nature of these abnormalities should yield insights that may permit immunologic reconstitution of patients with this disorder.

REFERENCES
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