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

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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 membrane antigen and proliferative response to mitogen. The appearance of activation antigens (transferrin, HLA-DR, and interleukin 2 [IL 2] receptor) was normal in CLL T cells post-mitogen exposure. Despite the normal presentation of IL 2 receptor on CLL T cell membrane, there was decreased production of IL 2 by CLL patients (v controls) (39.6 ± 10.2 cells per milliliter v 64.6 ± 11.0 cells per milliliter). Finally, we were able partially, but not fully, to reconstitute CLL T proliferative response to mitogen by adding purified exogenous IL 2. These findings suggest that CLL 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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chronic lymphocytic leukemia (CLL), typically a monoclonal B cell malignancy, is frequently accompanied by various peripheral blood T cell abnormalities. These include T cell lymphocytosis, low T4-T8 ratios with inadequate helper activity for B cell proliferation and immunoglobulin synthesis, and depressed to absent natural killer activity and autologous mixed lymphocyte reactivity. In addition, variably decreased responses to mitogen-induced (phytohemagglutinin [PHA] or concanavalin A (Con A)) proliferation have been reported by several investigators. We have recently described a subgroup of CLL patients in whom only a minority of erythrocyte rosette positive (ER+) peripheral blood T cells reacted with monoclonal antibodies to T3, a 20- to 25-kd membrane antigen normally present on mature T cells and medullary thymocytes. In contrast to other CLL patients whose T3-ER+ ratios were more normal (≥0.6), T cells from this CLL subgroup exhibited markedly defective responses to PHA and Con A. The T3 antigen is now known to be required for normal T cell activation. Consequently, we have attempted to better define the mechanisms of abnormal T cell responsiveness to lectins in this group of CLL patients by enzymatically exposing additional membrane T3, studying the appearance of membrane activation antigens, measuring interleukin 2 (IL 2) production, and enhancing proliferative responses with exogenous normal IL 2.

Materials and Methods

Eleven B-CLL patients and 11 normal donors, matched for age and sex, were studied. CLL patients were staged according to the method of Rai: stages 0 to 1, five patients; stage 2, four patients, and stage 4, two patients. No patient had received chemotherapy within four weeks of study.

Preparation of ER+ cells. T cells were isolated by virtue of their ability to rosette with sheep erythrocyte (SRBC). Peripheral blood mononuclear cells were prepared from heparinized venous blood by ficoll-hypaque centrifugation, resuspended in RPMI 1640 containing 10% fetal bovine serum (FBS), and depleted of adherent cells by incubation in sterile plastic Petri dishes for one hour. Nonadherent cells were incubated with SRBC pretreated with aET (2-aminoethylisothiouronium hydrobromide), and the ER+ cells were pelleted by centrifugation through ficoll-hypaque. ER+ lymphocytes were freed of SRBC by brief hypotonic lysis in distilled water. This procedure gave a purity 98% or greater for ER+ lymphocytes (measured by rerosetting with SRBC-AET). Treatment of ER+ lymphocytes with neuraminidase. ER+ cells (2 x 10^7/mL) suspended in PBS were incubated with Vibrio cholerae neuraminidase (50 U/mL) (Behring Diagnostics, Somerville, NJ) at 37°C for 30 minutes with shaking. The cells were then washed repeatedly with PBS to remove excess enzyme and resuspended in RPMI-10% FBS.

Detection of T cell membrane antigens. The following monoclonal antibodies were used to detect surface membrane antigens of ER+ lymphocytes: OKT3, TAC (anti-IL 2 receptor), OKT9 (anti-transferrin receptor), and 5/12 (anti-IA). (Monoclonal antibodies OKT3 and OKT9 were purchased from Ortho Laboratories, Raritan, NJ; TAC was generously provided by Dr Thomas Waldmann, National Institutes of Health; 5/12 was a generous gift of Dr Soldano Ferrone, New York Medical College, New York.) ER+ cells (1 x 10^6/mL) in RPMI-10% FBS were incubated (4°C, 30 minutes) with 20 to 50 µL of an appropriate dilution of monoclonal antibody. The cells were then washed twice with RPMI-10% FBS and incubated for an additional hour with 100 µL of a 1:40 dilution of fluorescence-activated mouse F(ab) anti-immunoglobulin (Dako, Burlingame, Calif). After thorough washing, the cells were examined with a Zeiss fluorescence microscope equipped with epifluorescence filters.

PHA-induced mitogenesis. ER+ cells (2 x 10^3) isolated from controls or CLL patients were mixed in microtitre wells (Linbro, Hamden, Conn) with 1 µg/mL purified PHA (PHA-A, Burroughs Wellcome, Triangle Park, NC). After 72 hours of incubation (37°C, 5% CO2), 2 µCi of tritiated thymidine ([3H]Tdr) (New England Nuclear, Boston) was added. After an additional 24-hour incubation, cells were harvested and radioactivity measured by liquid scintillation spectrometry. Results were calculated and expressed as mean ± standard deviation of the mean (SEM). In some studies, exogenous IL 2 was added simultaneously with PHA. The IL 2 was derived from normal, PHA-stimulated lymphocytes (see below), or recombinant IL 2 (generously provided by Cetus Corporation, Emeryville, Calif) was used. IL 2 concentrations ranging from 0.1% to 20% were used.

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IL 2 production and quantitation. Lymphocytes (10^7/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., using the CTLL-20, IL 2-dependent cell line. 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 × 10^4 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. 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. 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-
IL-2 production was somewhat lower in ER lymphocytes with <60% T3 reactive cells, but the difference was not statistically significant.

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 ratios. 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.

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 and 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, i.e., 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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