Abnormal T-Cell Subpopulation Function in CLL: Excessive Suppressor (Tγ) and Deficient Helper (Tμ) Activity With Respect to B-Cell Proliferation

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T-cell function directly influences several B-cell functions. The effect of T-cell subgroups on B-cell function (DNA synthesis) was evaluated for controls and patients with B-cell type of CLL. Control and CLL intact T cells, T cells with receptors for IgG (Tγ), and T cells without Fc receptors at isolation (T non-γ) were admixed with control B cells. Two predominant differences between control and CLL T cells were observed. First, CLL Tγ cells were excessively effective at suppressing B-cell DNA synthesis, and secondly, control T non-γ cells were more efficient than CLL T non-γ at promoting control B-cell DNA synthesis. While it is unclear whether the qualitative and quantitative T-cell abnormalities are part of the CLL disease process, it is possible that excessive Tγ cell numbers and function may reflect an appropriate immune response to a malignant B-cell clone.

CHRONIC lymphocytic leukemia (CLL) is predominantly characterized by proliferation and/or accumulation of clonal B lymphocytes. Although T lymphocytes are not believed to be abnormal in patients with CLL, recent evidence suggests that T-cell subpopulations may be altered. We and others have described a marked imbalance in T-cell subsets, with increases in proportions of Tγ cells and a decrease in T cells with Fc receptors for IgM, in addition, Faguet has described excessive T-cell suppressor activity in these patients. Human Tγ and Tμ cells are believed to have suppressor and helper property, respectively, for B-cell function in a T-cell-dependent system. We have evaluated the functional levels of suppressor and helper activity of Tγ and Tμ cells in CLL patients. The in vitro effects observed may be related to B-cell proliferation in CLL.

MATERIALS AND METHODS

Patient Selection

CLL patients (n = 6) were all stages I–III as determined by Rai staging method. The control population (n = 8) consisted of healthy donors aged 35–65 yr. None of the patients had received chemotherapy or had received a staging method. The control population (n = 6) were all stages II–III as determined by Rai staging method. The control population (n = 8) consisted of healthy donors aged 35–65 yr. None of the patients had received chemotherapy or had received a staging method.

Mononuclear Isolation

Peripheral blood mononuclear cells (Mn) were obtained from controls or patients by centrifuging heparinized whole blood on Ficoll-Hypaque gradients. These cells were then depleted of adherent cells by incubating the Mn cells in plastic 75-mm tissue culture flasks (Falcon, Oxnard, Calif.) at 37°C for 45 min. This procedure routinely results in less than 1.5% macrophages being present in subsequent cell fractions as determined by latex ingestion and nonspecific esterase. The nonadherent cells were harvested, and then were rosetted with AET-sensitized sheep erythrocytes (SRBC) at 4°C for 2 hr. Two successive Ficoll-Hypaque gradients separated the rosetted T cells from the nonrosetted B and null cells (T-cell purity was 98%–99%). To further purify B cells, the nonrosetted B cells were allowed to rosette a second time with AET-sensitized SRBC, followed by another gradient. SRBC were removed from the rosetted cells by hypotonic lysis with distilled water.

T cells were then separated into subpopulations enriched for Tγ or depleted of T cells bearing receptors for IgG (T non-γ). This was accomplished by rosetting the purified T cells with ox red blood cells IgG sensitized as previously described. Rosetted cells were then separated by centrifugation through a third Ficoll-Hypaque gradient. The nonrosetted cells (T non-γ) were isolated from the interface, and a subpopulation of cells with IgG Fc receptors were obtained in the pellet. Ox red blood cells (ORB C) were then removed by hypotonic lysis. The purity of Tγ-enriched cells, as determined by rosetting with ORBC-IgG, is routinely 96%–98% with this technique. T non-γ were utilized in place of positively selected Tμ cells because of the fragility of ORBC-IgM rosettes and the high percentage of Tμ cells formed in the isolated T non-γ population with in vitro culture. In studies of lymphocytes from normal individuals, the T non-γ cells function as helper cells and Tγ as suppressor cells in this assay system (submitted for publication).

The percentages of Tγ, T non-γ, T, and B cells were identified by techniques previously reported.

T-Cell Functional Assay

The ability of T-cell subgroups, Tγ and T non-γ from patients and controls, to alter B-cell DNA synthesis was studied in the following manner: To assess T non-γ effect on control B cells, B cells at a concentration of 5 × 10^6 were mixed with 5 × 10^6 to 2 × 10^7 T non-γ cells. T non-γ cells were irradiated with 3000 rad prior to addition of these cells in mixed culture. To assess Tγ effect on B-cell DNA synthesis, it was necessary to add irradiated T non-γ cells (2 × 10^7) to syngeneic B cells (5 × 10^5), since B cells alone with pokeweed mitogen (PWM) do not incorporate significant 3H-thymidine. Then patients or control Tγ cells were added at concentrations of 5 × 10^6 to 2 × 10^7 cells/ml to this cell mixture. Cultures in a final volume of 0.2 ml/well were stimulated with 1:100 dilution of pokeweed mitogen (20 µg/ml, Gibco, Grand Island, N.Y.) and cultures incubated for 4 days at 37°C in 5% CO2. The wells were then pulsed with 3H-thymidine (New England Nuclear, Boston, Massachusetts).
Mass.) and incubated for another 24 hr. Cells were then harvested on a MASH 11 cell harvester in H2O and counted in a Packard Scintillation Spectrometer. Results are reported as percent inhibition of enhancement between various controls and coculture cell population. Controls include each individual cell population and each mixture without mitogen and were processed with the mitogen-stimulated cultures in all experiments. The use of a 4-day incubation period and irradiated T non-γ cells resulted in minimal 3H-thymidine uptake for isolated T-cell subgroups of T-B-cell mixtures without mitogen. The range of dpm noted for mixtures of T-B cells without mitogen was from 55 ± 22 to 117 ± 31 (mean ± SEM). The raw counts of T-B-cell mixtures without mitogen were always subtracted from the same mixture with mitogen.

RESULTS

T-Cell Subpopulations

Figure 1 illustrates the levels of total T cells, Tγ, and Tμ cells in CLL patients versus controls. The total number of T cells in the CLL group was higher than for controls. The number of Tγ cells are significantly higher than controls, p < 0.01. Tμ cells in CLL were markedly reduced as compared to controls (p < 0.01), confirming our previous observations.4

T-Cell Subgroup Function

T-cell suppressor function. Equivalent concentrations of Tγ cells from controls and patients were analyzed for their ability to modulate DNA synthesis of a mixture of control B cell plus irradiated control T cell. CLL Tγ cells at all concentrations tested were more effective than control Tγ cells at suppressing B-cell 3H-thymidine incorporation (Fig. 2). In addition, both control and CLL Tγ cells increased suppression as cell concentrations increased, although the control Tγ cell effect plateaued at a cell concentration (2 × 10^9) where CLL Tγ suppression was still increasing. This effect was not related to cell death, since serial sampling of cell mixtures for cell count and trypan blue exclusion were not significantly different from the initiation of culture.

T-cell helper function. As indicated in Materials and Methods, irradiated T non-γ cells from controls and patients were utilized to assess T-cell helper function. All control mixtures utilized allogeneic
rather than autologous T non-γ cells. Equivalent concentrations of T non-γ cells from controls were consistently much more effective than CLL T non-γ at enhancing control B-cell 3H-thymidine incorporation (Fig. 3). Indeed, CLL T non-γ cells were poorly effective at all cell concentrations in promoting B-cell 3H-thymidine incorporation; at cell concentrations of $1 \times 10^5$, CLL T non-γ led to increases of about 500 dpm compared to 2300–3000 dpm for control T non-γ (Fig. 3).

DISCUSSION

There is increasing evidence that in certain diseases T cells are not qualitatively or quantitatively normal despite the fact that they do not constitute the predominant or malignant cell type. While earlier investigations of CLL suggested that T cells were not easily distinguishable from control T cells, recent delineation of T-cell subgroups has permitted more detailed T-cell analysis. Indeed, our findings indicate a multiplicity of functional abnormalities in CLL T-cell subgroups; excessive Tγ suppressor activity and a markedly deficient T-cell helper effect. The T-cell abnormalities reported here extend and confirm two recent investigations of CLL patients that observed either deficient T-cell helper or increased T-cell suppressor function.

Since T cells are abnormal in CLL in both subpopulation distribution and their functional influence on B-cell DNA and Ig synthesis, the fundamental question appears to be whether the abnormality reflects an appropriate response to the B-cell proliferation or is it part of the initial disease process. If the T-cell abnormalities were integrally involved in the disease, one might expect to see the T-cell dysfunction early in the course of CLL. Our preliminary studies indicate that in stage 0–1 CLL, T-cell defects of qualitative or quantitative nature are more difficult to detect than in advanced CLL.

An alternative hypothesis, which is perhaps more tenable, is that T-cell dysfunction reflects a response by the immune system to an increasing B-cell malignant clone. T cells are effective in cytotoxic responses, including cell-mediated lympholysis, natural killer activity, and antibody-dependent cytotoxicity. Indeed, the Tγ subgroup has a more obvious role in those functions as noted in prior in vitro studies. Thus, the increase in Tγ cell effector portion of the immune system may be related to the immune response to a malignant B-cell clone. Since both cell types are easily obtainable from peripheral blood of these patients, this hypothesis is testable and may lead to a better definition or understanding of the T-cell–B-cell interaction in this malignancy.

REFERENCES

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