**Decreased 5'-Nucleotidase Activity in a T Lymphocyte Subpopulation From Patients With B Cell Chronic Lymphocytic Leukemia**

By Robert Silber and Maryrose Conklyn

The activity of the ectoenzyme 5'-nucleotidase (5'N) was determined in the T lymphocyte subpopulations from patients with chronic lymphocytic leukemia (CLL). 5'N could be detected only in the T cells from patients whose B cells also had enzyme activity. The specific activity of CLI could be detected only in the T cells from patients whose B patients with chronic lymphocytic leukemia (CLI). 

The immunodeficiency may be related to the neoplasms of normal T4 cells, which was 0.13 ± 0.08 μm/h/mg. The CLI T8 cells, however, had a significantly lower 5'N activity (0.17 ± 0.02 μm/h/mg) than normal T8 cells (0.41 ± 0.11 μm/h/mg) (P = .003). Normal null cells had very low activity, while much higher levels were found in the null cells of CLI patients whose B cells had activity. These findings document a difference in activity of an enzyme between the T8 cell population of patients with CLI and that of normal subjects.

**MATERIALS AND METHODS**

Mononuclear cells (lymphocytes and monocytes) were isolated from the heparinized blood of normal subjects and untreated patients with CLI using the Ficoll-Hypaque gradient method. Monoclonal antibodies were removed by adhesion to plastic culture dishes. Purity, monitored by cell sizing using a Coulter (Hialeah, Fla.) Counter Model ZAU equipped with a channelyzer, was greater than 95%.

Subpopulations were enriched by sheep erythrocyte rosetting techniques using trypsinized, antibody-coated erythrocytes in the presence of complement (EAC) for B cells and neuraminidase-treated erythrocytes (Ea) for T cells. The erythrocytes were removed by NH₄Cl lysis. Purity of the B cell and T cell preparations was determined by rosetting assays.

The T cell subsets were separated into their T4 and T8 subpopulations by the antibody-complement lysing technique using monoclonal antibodies, OKT4 and OKT8. The percentage of helper and suppressor cells was determined by the enzyme-linked immunoabsorbent assay using monoclonal antibodies and a peroxidase-linked anti-mouse IgG. Viability, measured by erythrosin B dye exclusion, was greater than 90% in all but one experiment, where it was 76%. In some experiments, nonviable cells were removed by Ficoll-Hypaque sedimentation, but because 5'N activity is not affected by cell viability, dead cells were not removed routinely.

Lymphocyte 5'N activity was determined by the release of Pi from AMP, as previously described. Control assays contained 100 μmol/L α,β-methylene adenosine 5'-diphosphate, a specific inhibitor of 5'N. Protein was determined by the method of Lowry et al. Although 5'N is usually diminished in CLI B lymphocytes, most of the patients chosen for this study had detectable activity in their B cells as well as in their T cell subpopulations, and by necessity, represent a selected population.

**RESULTS AND DISCUSSION**

The 5'N activities of the T and B cell subsets of normal and CLI lymphocytes are shown in Fig 1.
Following E\textsubscript{T}AC rosette purification, there was an eightfold increase in the percentage of B cells in normal preparations, while the CLL T cell percentage was increased sevenfold by E\textsubscript{N} rosette formation over that in unfractionated preparations. After E\textsubscript{T}AC and E\textsubscript{N} rosette sedimentation, the cells remaining in the interphase contained about 75% null cells in either normal or CLL preparations. The T4 and T8 subsets were enriched to about 80% purity in normal and 70% in CLL lymphocytes. The T4/T8 ratio of normal T cells was three times that of the corresponding T cells, but lower than the 5’N levels of the B cells. Contamination by B cells was probably not sufficient to account for the values obtained for two reasons: (1) residual B cells contributed less than 25% of the total 5’N activity in these cells, and (2) in one patient the null cells actually had a slightly higher 5’N activity than the B cells. It therefore appears that the high 5’N activities in these preparations were indeed null cells. All of the above relationships were the same when enzyme activity was expressed per cell as well as in terms of protein concentration.

T lymphocytes from patients with B cell CLL manifest two anomalies in 5’N activity: (1) the absence or reduction of enzyme activity in T cells from patients whose B cells also lack the enzyme,\textsuperscript{11} and (2) a decrease in 5’N activity in the T8 subpopulation from B cell CLL patients who have 5’N+ lymphocytes. Thompson et al\textsuperscript{2} have shown the presence of a 5’N+ subpopulation in T8 cells. Conceivably, this could be expanded in CLL.

The absence of 5’N in the T cells of 5’N− B-CLL implies that the expression of 5’N may be determined in early precursor cells common to the T and B cell lineage. The 5’N− CLL lymphocytes may be derived from precursor cells in which the ability to express 5’N is lost or repressed. The 5’N+ CLL lymphocytes would then be derived from a subpopulation that retained 5’N expression. The heterogeneity of 5’N in this disorder could then be accounted for within the framework of this concept.

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R Silber and M Conklyn