Decreased 5′-Nucleotidase Activity in a T Lymphocyte Subpopulation From Patients With B Cell Chronic Lymphocytic Leukemia

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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 5′N could be detected only in the T cells from patients whose B cells had activity. The specific activity of 5′N was lower in normal T4 cells than in the T8 cells of patients with CLL. The T8 cells, however, had a significantly lower 5′N activity than normal T8 cells.

Recent studies have determined the 5′-nucleotidase (5′N) activity in T4 and T8 subpopulations of normal lymphocytes. Decreased levels correlated with an increase in suppressor cell function. The enzyme may be a useful marker in disorders of antibody synthesis associated with imbalances of regulatory T cells, such as congenital agammaglobulinemia or common variable immunodeficiency.

Hypogammaglobulinemia is a common finding in patients with chronic lymphocytic leukemia (CLL). The immunodeficiency may be related to the neoplastic monoclonal B cell proliferation in this disorder. In two thirds of patients with CLL, the B cells lack 5′N activity (5′N−) as well as immunoreactive protein. In the remainder, the cells have a normal or even supranormal activity (5′N+). Although B cell proliferation is considered the hallmark of this disease, an increase in the absolute number of T cells is generally found that may contribute to the immunodeficiency. In addition, a variety of functional and biochemical differences between normal and CLL T cells has been described. This includes the lactate dehydrogenase (LDH) isoenzyme pattern, actin content, and helper/suppressor functions. Relatively little is known about 5′N in CLL T lymphocytes. To date, the activity has been studied only in the unfractionated T lymphocytes of these patients. In view of the suggestion that this enzyme may serve as a marker for immunoregulatory T lymphocytes, we decided to determine the 5′N in the T cell subsets, as defined by the monoclonal antibodies OKT4 and OKT8, from patients with B-CLL. These studies show that while there was no difference between 5′N levels of T4 cells from normal subjects and patients with CLL, the T8 cells in CLL patients had a marked decrease in activity.

**MATERIALS AND METHODS**

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

Subpopulations were enriched by sheep erythrocyte rosetting techniques using trypsinized, antibody-coated erythrocytes in the presence of complement (E,C5) for B cells and neuraminidase-treated erythrocytes (Eα) 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 lysis technique using the monoclonal antibodies, OKT4 and OKT8. The percentage of helper and suppressor cells was determined by the enzyme-linked immunosorbent assay using monoclonal antibodies and a peroxidase-linked anti-mouse IgG. Viability, measured by trypan blue 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 AM32P, 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 CLL 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 CLL lymphocytes are shown in Fig 1.

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* T4 and T8 cells are those T lymphocytes that react with the monoclonal antibodies OKT4 and OKT8, respectively.
from the blood of a single patient. The shaded areas correspond to the range of 5'N activity of their normal counterparts. The preparation of OKT4-positive and OKT8-positive cells was 42.3% ± 7.3% and 40.7% ± 14.5% in the T-enriched cells, 70.3% ± 3.1% and 41.1% ± 3.5% in the T4-enriched cells, and 20.4% ± 8.1% and 69.5% ± 7.0% in the T8-enriched cells. The viabilities in the T-, B-, and null-enriched cells were greater than 90%. The T4-enriched cells had 86.4% ± 9.0% viable cells, while the T8-enriched cells were 82.9% ± 9.1% viable. The average recovery of the T4 cell preparation was 45.3% ± 12.3% and that of the T8 cell fraction was 55.3% ± 14.3%.

Following E7AC 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 E7 rosette formation over that in unfractionated preparations. After E7AC and E7 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 1.49 ± 0.37 and that of the CLL T cells was 1.19 ± 0.50, which is in agreement with previously published values.

As reported before, the enzyme activity of the normal B cells was significantly higher \((P = .003)\) than that of normal T cells. In agreement with recent studies, the 5'N activity of normal T8 cells was three times that of the T4 cells. Normal null cells had the lowest 5'N levels.

5'N could not be detected in the T cells from CLL patients whose B cells lacked activity (Fig 1, patient 0). In contrast, T cells from patients whose B cells were 5'N+ had activity and, hence, were analyzed in this study. These T cells had a relatively narrow range of 5'N activity (0.15 to 0.36 \(\mu\)m/h/mg), which was unrelated to the level in the B cells from the same donors (Fig 1) \((r = .01)\). Although the mean specific activity was lower than that of normal T cells, this difference was not statistically significant \((P = .068)\).

When the 5'N of the individual subsets from 5'N+ T cell preparations was determined, the specific activity of the CLL T4 cells (0.17 ± 0.02 \(\mu\)m/h/mg) was similar to that of their normal counterparts (0.13 ± 0.08 \(\mu\)m/h/mg) \((P = .245)\). In contrast, the T8 cells from leukemic patients had a lower enzyme level (0.17 ± 0.02 \(\mu\)m/h/mg) than those of the normal T8 cells (0.41 ± 0.11 \(\mu\)m/h/mg). Although the number of patients was small, this difference was highly significant \((P = .003)\), and there was no overlap between the activities of normal T8 cells and those of CLL.

The 5'N activity of CLL null cells varied (Fig 1). In three of four cases, the enzyme level was higher than 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 (Fig 1, ■) 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, 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\(^2\) 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.

REFERENCES


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