Human Lymphocytes: 5′-Nucleotidase-Positive and -Negative Subpopulations

ROBERT SILBER, MARYROSE CONKLYN, GEORGE GRUSKY, and DOROTHEA ZUCKER-FRANKLIN

From the Department of Medicine, New York University School of Medicine, New York 10016

ABSTRACT The enzyme, 5′-nucleotidase (5′N) (E.C.-3.1.3.5) is present in lymphocytes isolated from the blood of normal subjects. This activity is markedly decreased or not detectable in the cells from three-quarters of patients with chronic lymphocytic leukemia (CLL), while supranormal levels are found in less than 10% of the cases. To determine whether the decreased 5′N value in CLL represents a lower activity per cell or fewer enzyme-containing cells than in the normal, conditions were established for the histochemical measurement of 5′N in human lymphocytes. It was found that the cells isolated from the blood of normal subjects or patients with CLL consist of 5′N-positive and 5′N-negative subpopulations. Normal subjects who had high 5′N specific activity were shown to have a greater percentage of 5′N-positive cells than individuals with low 5′N activity. Patients with CLL who had no activity by standard chemical assay had no 5′N-positive cells, while the exceptional patient with CLL with a higher than normal specific activity showed an increased percentage of 5′N-positive cells. It is suggested that the selective proliferation of 5′N-positive and 5′N-negative populations may account for the heterogeneity of 5′N in CLL.

INTRODUCTION

The enzyme 5′-nucleotidase (5′N)[1] is present in many cell types, usually bound to membranes (1, 2). Heterogeneity for this activity has been demonstrated in chronic lymphocytic leukemia (CLL) (3, 4). While the lymphocytes isolated from blood of 75% of patients with this disorder have markedly decreased or absent activity, rare normal or even supranormal 5′N levels are found in the cells from other patients (5). In lymphocytes from all normal subjects studied to date, 5′N is readily detected, but the range of specific activity (0.1–0.9 μmol/h per mg) is wider than that found in this laboratory for most of the other lymphocyte enzymes (5).

Serial 5′N determinations on lymphocytes isolated over a 3½-yr period from normal subjects and patients with CLL revealed that the level for a given subject is relatively stable. The results shown in Table I indicate that 5′N in lymphocytes from a subject in the high normal range (R. T.) and low normal range (J. S.) were significantly different from each other ($P < 0.001$), and a similar consistency was observed for 5′N activity-deficient (I. G., G. R.) or "supranormal" level CLL cells (W. G., N. S.) as well as in patients with subnormal levels.

The finding that individuals have consistently low or high 5′N specific activity can be interpreted in at least two ways: The level could reflect a homogeneous increase or decrease in the activity of all cells, or alternately, the preponderance of a subpopulation with a different level of the enzyme. Since these possibilities could not be investigated by the chemical determination of the activity in homogenates or suspended intact lymphocytes, the histochemical determination of 5′N (6, 7) was adapted to human lymphocytes.

METHODS

Lymphocytes were purified by centrifuging blood on a Hypaque-Ficoll gradient to eliminate erythrocytes and granulocytes. A glass wool column was used to remove platelets and monocytes. The final preparation contained greater than 95% lymphocytes, which were viable, responded to phytohemagglutinin, and had the normal T- and B-cell distribution previously reported from this laboratory (5, 8). These cells were washed in

Dr. Zucker-Franklin is a recipient of a Career Development Award 5-KO3-AI09572.

Received for publication 2 June 1975 and in revised form 31 July 1975.

[1] *Abbreviations used in this paper:* CLL, chronic lymphocytic leukemia; 5′N, 5′-nucleotidase.

TABLE I

Lymphocyte 5'-Nucleotidase Activity for Given Individuals Tested over Various Time Intervals

	1972	1973	1974	1975
Normal subjects				
S. R.	0.4	0.5, 0.7, 0.3, 0.3	0.3, 0.4, 0.3	0.3
J. S.	0.4	0.2	0.2, 0.2	0.1, 0.2
R. T.		0.6, 0.5, 0.8, 0.8	0.6, 0.5, 1.0, 0.5, 0.6, 0.7	0.6, 0.8
J. M.	0.2	0.3, 0.2	0.4	
L. J.	0.4	0.5, 0.5	0.5	0.4
CLL				
G. R.		0.13, 0.09	0.08, 0.09, 0.1, 0.1	
I. G.	<0.02, <0.02	<0.02, 0.03	0.03	
W. G.			1.9, 2.1	1.8
N. S.			2.1, 1.6	1.7

Samples were obtained at intervals of 1–3 mo during the years indicated above. Assays were performed in duplicate as previously described. Specific activity is expressed as micromoles per hour per milligram protein.

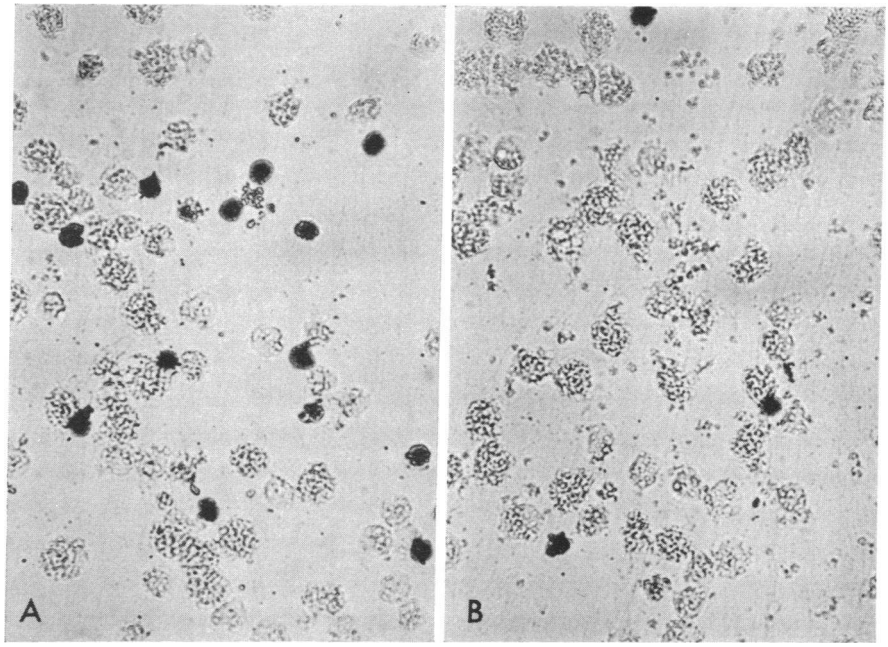

FIGURE 1 Histochemical assay of 5'N in lymphocytes isolated from the blood of normal subjects. Smears prepared from lymphocyte suspensions as described in the text were processed on the same day without fixation. Smears were incubated in Coplin jars in a mixture containing the following: 0.25 M Tris maleate buffer, pH 8.0, β-glycerophosphate, 0.02 M, Na K tartrate 0.01 M, Mg $(NO_3)_2$ 0.01 M, Pb $(NO_3)_2$ 0.002 M and 5'-AMP 0.003 M. In the blank, 2'3'-AMP (0.003 M) was substituted for the 5'-AMP. Usually controls of CLL lymphocytes in which no activity was detectable by the radioisotope assay were also included. At the end of the incubation period (usually 20 h) slides were rinsed in H_2O, immersed for 30 s in 2% ammonium sulfide, and rinsed again in H_2O. In some experiments (not shown) the slides were fixed in 0.4% glutaraldehyde for 10 min before the ammonium sulfide. This modication generally improved the cell morphology. To facilitate the counting of unstained (negative) cells, the slide was counter-stained for 1 min with 1% neutral red (C.I. no. 50040). All the cells shown are lymphocytes. Cells which had a black precipitate were scored as positive for 5'N activity. The percentage of positive cells was determined on coded slides by an observer who counted 500 cells. (A) Lymphocytes from a subject R. T., a high normal (× 1,200 magnification). (B) Lymphocytes from J. S., a low normal.

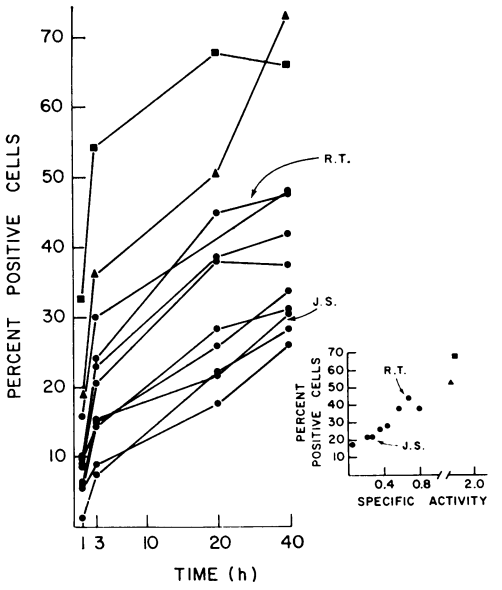

FIGURE 2 Histochemical determination of lymphocyte 5'N activity: effect of incubation time and relationship to chemical assay. In the experiment shown, cells were obtained from nine normal subjects and two patients with CLL. The effect of incubation time on the percentage of cells showing 5'N activity is shown in the figure: (●), normal subjects; (▲, ■), CLL. The inset shows the relationship of 5'N activity determined chemically to the percentage of cells showing 5'N activity after 20 h of incubation. A similar correlation exists for the other staining periods as well. In over 50 controls (using 2'3'-AMP or 5'N-negative CLL lymphocytes) the positive cells were below 2%. This experiment was performed three times with identical results.

0.25 M Tris maleate-5% bovine serum albumin, pH 8.0 and resuspended in this buffer in a final concentration of 2–4 × 10^7 cells/ml. Smears were prepared on glass slides from 10-μl aliquots of this suspension.

RESULTS AND DISCUSSION

The results shown in Fig. 1 indicate that normal lymphocytes are either positive for 5'N (black cells) or negative for this activity. Although no intermediate forms were seen in unfixed preparation, it is possible that a spectrum ranging from no activity to full activity may be present in individual cells which is not detected in the histochemical assay. Controls in which 2'3'-AMP was substituted for the usual substrate 5'-AMP, or lymphocytes from patients with CLL, in which no activity was detected by chemical assay, showed no positive cells. The controls were negative despite the presence of β-glycerophosphate in the assay mixture since lymphocytes lack alkaline phosphatase activity and tartrate was included as an inhibitor of acid phosphatase.

An experiment was performed to evaluate the relationship between lymphocyte 5'N as determined by the chemical assay (5) which measures this activity in a suspension of cells, and the percentage of cells positive for this enzyme determined histochemically. Slides prepared from lymphocytes with a wide range of 5'N activity were stained, and the percentage of positive cells after variable staining periods were determined. Fig. 2 shows the relation of these values to the chemical assay. After each staining period there was an excellent correlation between the percentage of positive cells and the value determined by chemical assay (for the 20-h period a positive correlation was found, $r = 0.94$ by the least-squares plot). This result indicates that the level of 5'N specific activity reflects primarily the predominance of the positive subpopulation. The figure includes data on the lymphocytes of two patients with CLL in whom the supranormal levels by the chemical assay were associated with a higher than normal percent of positive cells.

We have previously presented evidence that the level of this enzyme is unrelated to the B/T subpopulations (5). The effect of cell age on 5'N activity is unknown. Current studies indicate that the finding of "high" and "low" normal subjects occur in a familial pattern, suggesting a genetic control of activity.[2]

The finding of 5'N-positive and 5'N-negative subpopulations in normal lymphocytes allows for some speculation on the observed heterogeneity in CLL patients. Mutation (or selective proliferation) of a 5'N-negative lymphoid line could give rise to 5'N-negative CLL and conversely the rare supranormal CLL may originate from the 5'N-positive normal cell.

At least three explanations can be offered for the high incidence of 5' negativity in CLL: (*a*) the negative subpopulation may be more likely to proliferate or (*b*) rare 5'N-negative normal subjects may exist, constituting a population more likely to develop CLL; (*c*) the activity is lost in the course of leukemogenesis. The possibility that environmental factors may be responsible for the loss of 5'N activity in the majority of CLL cells must also be considered, particularly since profound fluctuations in this activity have recently been reported in macrophages in responses to exogenous stimulation (9). Further clinical and experimental studies are required to decide which if any of these hypotheses may account for the heterogeneity of 5'N in CLL. The studies reported above describe another marker for the increasingly complex pattern of lymphocyte subpopulations.

ACKNOWLEDGMENTS

This investigation was supported by U. S. Public Health Service Research grants CA-11655 and AM-012274, and an American Cancer Society grant CI-122.

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