CONCISE REPORT

Adenylate Cyclase and Guanylate Cyclase Activity in Normal and Leukemic Human Lymphocytes

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Understanding the activity of adenylate cyclase (AC) and guanylate cyclase (GC) is crucial as these enzymes play a significant role in the regulation of cell function. AC, which catalyzes the conversion of ATP to c-AMP in the presence of GTP and Mg2+, is almost exclusively located in the cell membrane and is stimulated by hormones through a receptor–enzyme complex. GC, which catalyzes the formation of c-GMP from GTP in the presence of Mn2+, is present in independently regulated soluble and particulate fractions.

This article reports the results of a study on AC and GC activities in four populations of normal and leukemic human lymphocytes. AC activity was lower and GC activity higher in normal T lymphocytes than in normal B lymphocytes. In leukemic cells, AC activity was very low and GC activity very high. Increased AC and GC activity occurred after appropriate stimulation in normal lymphocytes, but was absent in leukemic cells.

MATERIALS AND METHODS

Lymphocytes were isolated (Ficoll-Hypaque gradient followed by a discontinuous sucrose gradient) from the blood of 10 normal donors (6 children and 4 adults), 11 children with untreated acute lymphocytic leukemia (ALL), and 9 adults with untreated chronic lymphocytic leukemia (CLL). B and T lymphocytes were separated by rosetting with sheep erythrocytes and refractionin on cold Ficoll-Hypaque. Identification was performed by E-rosette and EAC-rosette technique. The cells were frozen-thawed twice and Teflon-homogenized in 10 mM Tris-HCl buffer pH 7.5. The mixture for AC and GC contained (final concentration): 5 mM creatine phosphate, 50 U/ml creatine phosphokinase, 20 mM Tris-HCl buffer, pH 7.4, 5 mM MgCl2, 1 mM ATP, 0.1 mM c-AMP, 0.01 mM GTP, 1 mM 1-methyl-3-isobutylxanthine, 1 mM dithiothreitol, and 2-3 x 106 cpm [α-32P] ATP. In some cases D-L isoproterenol (with or without D-L propranolol) and prostaglandins were added separately at a final concentration of 10 μM. In other cases sodium azide or dehydroascorbic acid (DHA) were used at final concentrations of 1 mM and 5 mM, respectively. Micrograms of catalase were also present with sodium azide. In both assays some fractions were pretreated with cellular derivatives (Triton X-100 and Lubral P3).

The reaction was stopped after 10 min of incubation at 30°C. The isolation of the labeled products was performed by sequential chromatography over Dowex AG 50W-X4 and alumina. Incorporation of 32P was expressed in picomoles per minutes per milligram protein. Statistical study was performed by the two-tailed Student's t test.

RESULTS

B cells ranged 69%–77% and T cells 16%–19% in normal B-enriched lymphocytes, while in T-enriched...
lymphocytes T-cell percentage increased from 71%–75% to 89%–94%. In patients with ALL, 82%–91% of the cells were null lymphocytes, 4%–7% T cells, and the remainder B cells. In patients with CLL, 73%–88% were B cells and the remainder T (8%–12%) and null cells (4%–6%). From 95% to 98% of the isolated cells were viable; 91%–98% were lymphoid elements, and the rest monocytes and neutrophils. The average of cells broken in each case was 92% (range 85%–94%), with a yield of 40–45 μg of proteins/10⁶ cells.

In cyclase assays the recovery ranged 65%–80% for c-AMP and 58%–68% for c-GMP. Pretreatment with detergents reduced the activity of the different fractions to 22%–28% of pretreatment activity, whereas addition of a phosphodiesterase inhibitor increased it by 50%–60% (data not shown).

**Adenylate Cyclase**

In normal B-enriched lymphocytes, the lowest activity of AC was found in fraction III (supernatant) (10 pmole/min/mg protein) and the highest in fraction IV (membrane-enriched pellet) (215 pmole). The same pattern at a significantly lower level (p < 0.01) was found in T-enriched cells (2 pmole for fraction III and 85 pmole for fraction IV). The activity increased (40%–100%) after stimulation with L-isoproterenol and prostaglandins. Propranolol completely inhibited the L-isoproterenol effect (data not shown). AC activity in ALL lymphoid elements was low in all fractions, compared to the corresponding fractions of normal cells (p < 0.01) (20 pmole versus 215 pmole in fraction IV) and did not increase significantly with stimulation. In CLL lymphocytes, the basal values were lower than in normal B-enriched lymphocytes (55 pmole versus 215 pmole), higher than in ALL cells (55 pmole versus 20 pmole), and essentially no different from those of normal T-enriched lymphocytes (p < 0.01). Activity increase after stimulation was higher than in acute lymphocytic leukemia cells.

**Guanylate Cyclase**

In normal B-enriched lymphocytes the highest value was found in the membrane-enriched pellet (fraction IV) (5 pmole) and the second highest value in the supernatant (fraction III) (3 pmole). The same pattern at significantly higher levels was present in normal T-enriched lymphocytes (10 pmole in fraction IV and 8 pmole in fraction III). When normal B and T lymphocytes were stimulated with L-isoproterenol or prostaglandins, activity did not increase (data not shown). A significant increase (p < 0.01) occurred with dehydroascorbic acid and sodium azide (100%–320%).

In ALL lymphocytes, GC was significantly higher (p < 0.01) than in the corresponding fractions of normal B and T cells (22 pmole versus 5 pmole and 10 pmole in fraction IV). When the fractions were stimulated, no significant increase occurred either with isoproterenol and prostaglandins or NaN₃ and DHA. GC activity in CLL lymphocytes showed values higher than in normal B and T cells (16 pmole versus 5 pmole and 10 pmole in fraction IV) but lower than in ALL lymphocytes. Results of stimulation were not significantly different from those obtained with ALL lymphocytes.

Figure 1 summarizes the results for AC activity in fraction IV (membrane-enriched pellet) and for GC activity in fraction III (supernatant) and fraction IV.
of normal and leukemic cells. Values for the other fractions were lower with similar patterns and are not shown.

DISCUSSION

Higher GC and lower AC activity in normal T cells than in normal B cells, increased GC activity in leukemic lymphocytes, and decreased sensitivity of GC to stimulators in leukemic cells are the main findings of this study and, to best of our knowledge, have not been reported before. Our data also confirm reduced AC activity with limited sensitivity to hormonal stimulation in leukemic lymphocytes.¹,²

Our observation that normal T-enriched lymphocytes have more GC activity than normal B-enriched cells in both particulate and soluble fraction is difficult to explain. This increase, however, combined with the lower activity of AC in these cells³ may suggest that circulating T cells are more actively cycling or functioning than circulating B cells.¹ Which functions of these cells and which form of GC are involved remain to be seen.

GC activity is increased in ALL lymphocytes. These data correlate with the higher level of c-GMP found in these and other tumoral cells.²⁰-²² Since increase of c-GMP has been proposed as an initial short message preceding cell division,¹¹ both findings may be due to the larger number of cells in mitosis without necessarily being a characteristic of the leukemic population. A similar relationship is suggested for CLL lymphocytes, in which cyclase G activity and c-GMP are also increased, although to a lesser extent than in ALL lymphocytes. Increased GC activity also has been reported in other cells with abnormal growth²³-²⁵ and in cells stimulated with chemical carcinogens²⁶ and Laetrile;²⁷ it regressed with the use of anticancer agents.²⁸ Deregulation of GC activity in tumoral cells is suggested.²⁷,²⁸

A limited sensitivity of GC to isoproterenol and prostaglandins in normal and leukemic cells is well known.³⁻⁵ In our study, however, GC in leukemic lymphocytes is also less sensitive to agents such as NaN₃ and DHA, which clearly stimulate GC in normal lymphocytes. This finding may be a characteristic of the different cell populations or may be secondary to the use of a cell-free system. It has, in fact, been suggested that adequate GC regulation probably is obtained only in intact cells and indirectly through mediators.⁵

Higher AC activity in normal B than in normal T lymphocytes, its increase with isoproterenol and prostaglandins stimulation, depression of AC activity in CLL lymphocytes with insensitivity to autacoids, and a possible AC defect have been described.¹⁻³ Our study indicates that AC activity is decreased also in acute leukemia cells. This finding has been reported in other tissues with abnormal growth²⁹ and in virus-transformed cells³⁰ but not in acute leukemia lymphocytes. It agrees with the low level of c-AMP demonstrated in these cases³¹ and the importance of this nucleotide in the mechanism of cell division.³¹ Cyclic AMP is in fact low in normal dividing cells and many tumoral tissues as well.⁶,³² Insensitivity to stimulation in ALL cells similar to that present in CLL cells also is demonstrated here and may suggest AC modification in both types of lymphocytes.

REFERENCES

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