CONCISE REPORT

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

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Adenylate cyclase (AC) and guanylate cyclase (GC) activities were studied in normal B-enriched and T-enriched lymphocytes, in lymphocytes of children with acute lymphocytic leukemia (ALL), and in lymphocytes of adults with chronic lymphocytic leukemia (CLL). AC activity was greater in normal B than T lymphocytes (215 pmole/min/mg protein versus 80 pmole in the membrane-enriched fraction) and in both increased greatly after stimulation with isoproterenol and more so with prostaglandins E and F2a. In leukemic lymphocytes, AC showed depressed activity (20 pmole in ALL cells and 55 pmole in CLL cells) and was less sensitive to hormonal stimulation.

In normal and leukemic lymphocytes, AC activity was lower in normal T than B cells (in membrane-enriched fraction: 10.2 pmole versus 5.3 pmole). It increased little with isoproterenol and prostaglandins stimulation, and much more with sodium azide and dehydroascorbic acid stimulation. GC activity was increased in both types of leukemic lymphocytes (23 pmole for ALL cells and 18 pmole for CLL cells) and was insensitive to stimulation. Possible derangement of cyclase and cyclic nucleotide regulation in leukemic cells is suggested.

Information on the activity of adenylate cyclase (AC) and guanylate cyclase (GC) in normal and leukemic lymphocytes is sparse,4-6 as are data on the effect of modulating substances on these enzymes.4,8,9 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.9,9 GC, which catalyzes the formation of c-GMP from GTP in the presence of Mn2+, is present in independently regulated soluble and particulate fractions.5,10 A role for the cyclic nucleotides these two enzymes produce has been suggested in metabolism and division of normal and abnormal lymphocytes.6,11-13

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 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)44 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 centrifugation on cold Ficoll-Hypaque.13 Identification was performed by E-rosette and EAC-rosette technique.16,17 The cells were frozen-thawed twice and Teflon-homogenized in 10 mM Tris-HCl buffer pH 7.4 (fraction I). They were centrifuged 5 min at 120 g and the pellet resuspended in the same buffer (fraction II). The supernatant was recentrifuged 60 min at 100,000 g and two more fractions were obtained: supernatant or fraction III (considered to contain mainly soluble enzymes), and fraction IV (membrane-enriched pellet, considered to contain particulate enzyme).

Cyclase assays were performed as described, with slight variations.4,8,9 The mixture for AC assay 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), PGE1, and PGF2α were added separately at a final concentration of 10 μM. In other cases sodium azide or dehydroascorbic acid (DHA) was used at final concentrations of 1 mM and 5 mM, respectively. Five micrograms of catalase or prostaglandin F2α (PGF2α) were added also at a final concentration of 10 μM. 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
divisions 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
findings of this study and, to best of our knowledge,
stimulators in leukemic cells are the main
GC activity in normal B enriched
lymphocytes. This increase, however, combined with the
lower activity of AC in these cells may suggest that
circulating T cells are more actively cycling or function-
ing than circulating B cells. 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. Since increase of
c-GMP has been proposed as an initial short message
preceding cell division, both findings may be due to
the larger number of cells in mitosis without neces-
sarily being a characteristic of the leukemic popula-
tion. 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 growth
and in cells stimulated with chemical carcinogens
and Laetrile; it regressed with the use of anticancer
agents. Deregulation of GC activity in tumoral cells is
suggested.

A limited sensitivity of GC to isoproterenol and
prostaglandins in normal and leukemic cells is well
known. 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 character-
istic of the different cell populations or may be second-
dary 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.

Higher AC activity in normal B than in normal T
lymphocytes, its increase with isoproterenol and pros-
taglandins stimulation, depression of AC activity in
CLL lymphocytes with insensitivity to autocoids, and
a possible AC defect have been described. Study
indicates that AC activity is decreased also in acute
leukemia cells. This finding has been reported in other
tissues with abnormal growth and in virus-trans-
formed cells not in acute leukemia lymphocytes.
It agrees with the low level of c-AMP demonstrated in
these cases and the importance of this nucleotide in
the mechanism of cell division. Cyclic AMP is in fact
low in normal dividing cells and many tumoral tissues
as well. Insensitivity to stimulation in ALL cells
similar to that present in CLL cells also is demonstrat-
ated here and may suggest AC modification in both
types of lymphocytes.

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