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 F₂α. 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: this loss of sensitivity occurred to a greater extent in ALL than in CLL lymphocytes. GC activity was greater in normal T than B cells (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.

CONCISE REPORT

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

By Ugo Carpentieri, Jose J. Minguell, and Frank H. Gardner

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 centrifugation 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 7.4 (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).

Cytochrome c

Animal 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. 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 7.4 (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).

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Cytochrome c
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 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.

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 cells\(^3\) 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.\(^20\)\(^\text{-}^22\) 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 growth\(^23\)\(^\text{-}^25\) and in cells stimulated with chemical carcinogens\(^26\) 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.\(^3\)\(^\text{-}^9\) In our study, however, GC in leukemic lymphocytes is also less sensitive to agents such as NaN\(_3\) 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.\(^1\)\(^\text{-}^3\) Our study indicates that AC activity is decreased also in acute leukemia cells. This finding has been reported in other tissues with abnormal growth\(^29\) and in virus-transformed cells\(^30\) but not in acute leukemia lymphocytes. It agrees with the low level of c-AMP demonstrated in these cases\(^31\) 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.

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