Increased Cyclic Nucleotide Phosphodiesterase Activity Associated with Proliferation and Cancer in Human and Murine Lymphoid Cells

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SUMMARY

Guanosine cyclic 3':5'-monophosphate phosphodiesterase activity of murine leukemic cells (L1210) was 400 times that of human peripheral blood lymphocytes and 15 times that of murine splenocytes. Adenosine cyclic 3':5'-monophosphate (cyclic AMP) phosphodiesterase activity of L1210 cells was 320 times that of human peripheral blood lymphocytes and 24 times that of normal murine lymphocytes. The total cyclic AMP and guanosine cyclic 3':5'-monophosphate phosphodiesterase activities of rapidly growing cultured human lymphoid cells (normal and leukemic lymphoblastoid cell lines) were also markedly elevated when compared to quiescent peripheral blood lymphocytes. Human peripheral blood lymphocytes stimulated by phytohemagglutinin showed a 10-fold increase in total enzyme activity.

Leukemic and normal murine cells contain a low-affinity cyclic AMP phosphodiesterase as normally observed in most mammalian tissues. In contrast, kinetic analyses of cyclic AMP phosphodiesterase activities of human B and T lymphoblastoid cells were similar to those of peripheral blood lymphocytes in that no evidence of a low-affinity enzyme was found. All the lymphoid cells tested showed a 3 to 4 S enzyme form by linear sucrose gradient fractionation. Proliferating and quiescent human cells also contain a higher-molecular-weight cyclic AMP-specific form (5.9 S), while the murine cells contain a 7.0 S higher-molecular-weight form. Both the 5.9 and 7.0 S forms show anomalous kinetic behavior. These results are discussed with respect to the biochemical nature of cyclic nucleotide phosphodiesterases and the role of this enzyme system in the proliferation of lymphoid cells.

INTRODUCTION

Evidence from a number of different experimental cell systems suggests that cyclic nucleotide phosphodiesterases play an important role in regulating cellular levels of cyclic nucleotides that may be important factors in controlling cell growth. Alterations in catalytic activities, substrate affinities, and physical forms of cyclic nucleotide phosphodiesterases accompany changes in the growth state of cultured cells (11, 13, 21). The changes noted in cyclic nucleotide phosphodiesterase activities appear to correlate with changes in the steady-state levels of cyclic nucleotides (12). In addition, activity differences have been detected between normal cells and their malignant counterparts (5, 10, 11, 16), and changes in affinity for cyclic AMP and maximum velocities have been reported in lymphocytes from patients with squamous cell carcinoma of the head and neck (2).

Our previous studies have shown that cyclic nucleotide phosphodiesterases of HPBL have kinetic and physical properties that are distinct from those of other mammalian tissues (18). In addition, we have suggested that this enzyme plays an important role in the process of lymphocyte proliferation. In this report we have compared the physical and kinetic properties of cyclic AMP and cyclic GMP phosphodiesterases from quiescent and proliferating, normal and malignant, human and murine lymphoid cells. We demonstrate that cyclic AMP and cyclic GMP phosphodiesterases are increased by mitogenic stimulation of resting lymphocytes. A preliminary report of this work was published previously (4).

MATERIALS AND METHODS

Isolation and Purification of HPBL. HPBL were purified as described previously (18) from 60 to 120 ml of normal donor blood. Fibrin and platelets were removed by swirling with glass beads, and lymphocytes were separated by Ficoll-Hypaque gradient centrifugation after addition of 220 mM CaCl₂:160 mM MgSO₄ and incubation for 30 min at 37° with tetracarbonyl iron (50 mg/ml) to minimize monocyte contamination. The isolated lymphocytes were washed twice with MEM (10 min), and any remaining erythrocytes were lysed with 1 to 3 treatments of hypotonic buffer (280 mM NaCl:30 mM Tris-Cl, pH 7.2) at 37° for 10 min. After 2 additional washes in MEM, the cell preparations contained >98% lymphocytes and 0 to 2% monocytes as determined by light microscopic analysis.

Isolation and Purification of DBA/2 Murine Splenic Lym-
phocytes. Two-month-old male DBA/2 mice obtained from Timco Laboratories, Houston, Texas, were killed by cervical dislocation. The spleens were removed and homogenized by hand in 5 ml of MEM medium in a ground-glass homogenizer. Freed cells were then diluted into 60 ml of MEM medium containing 10% heat-inactivated (56°, 30 min) fetal calf serum and purified by carbonyl iron treatment, Ficoll-Hypaque gradient centrifugation, and erythrocyte lysis with hypotonic Tris-NH₄Cl buffer, as described for HPBL. Murine lymphocyte-purity was >99% under light-microscopic examination.

Source and Maintenance of Lymphoid Cell Lines. Three human lymphoblastoid cell lines expressing B-cell characteristics, CRG, JC, and BEQ, were established in this laboratory from normal donors, according to the procedure of Hersh et al. (6). Once established, these cell lines were maintained in suspension in Falcon 75-cm² flasks by continuous passage (3 times weekly) in RPMI Medium 1640 supplemented with 20% heat-inactivated fetal calf serum. This cell line usually possesses Epstein-Barr virus and surface immunoglobulins for IgG, IgA, and IgM as determined with fluorescent antimmunoglobulins (8). These lines were checked for surface immunoglobulin and were found to be positive. They also induced vigorous blastogenic responses when used as stimulator cells in 1-way mixed-lymphocyte reactions.

The human lymphoblastoid T-cell line, CEM, was derived from a child with lymphocytic leukemia and kindly supplied by Dr. Dean Mann and Dr. S. Mayassi, National Cancer Institute, Bethesda, Md. This cell line was maintained by continuous passage (50% split, 3 times weekly) in RPMI Medium 1640 supplemented with 8% heat-inactivated fetal calf serum. The final cell preparation was brought to 0.33 x 10⁹ lymphocytes/ml in RPMI Medium 1640 supplemented by 10% autologous serum, penicillin (100 units/ml), streptomycin (100 µg/ml), and medium were replaced by fresh medium) in RPMI Medium 1640 supplemented with 10% heat-inactivated fetal calf serum. This cell line did not induce a blastogenic response when used as a stimulator cell in a 1-way mixed-lymphocyte reaction. All cell lines were checked at monthly intervals for the presence of Mycoplasma and remained free of this contamination.

Stimulation of HPBL by PHA. PHA was used to stimulate HPBL obtained from normal human donors and purified as indicated for HPBL except that the tetracarbonyl iron incubation was omitted. Resulting cell purity was 80 to 90% lymphocytes, 7 to 20% monocytes, and 0 to 3% granulocytes. The final cell preparation was brought to 0.33 x 10⁶ lymphocytes/ml in RPMI Medium 1640 supplemented with 10% autologous serum, penicillin (100 units/ml), streptomycin (100 µg/ml), and glutamine (0.4 µmole/ml). Cultures (3 ml) were incubated in 16- x 125-mm glass culture tubes; PHA-M (330 µg/ml) was added to some cultures. Incubation was at 37° in a humidified atmosphere of 5% CO₂ in air.

Measurement of [³H]Thymidine Incorporation. Five µCi of [³H]thymidine (specific activity, 1.9 Ci/m mole) were added to 10⁶ lymphocytes in 16- x 125-mm glass tubes 3 hr prior to termination of incubation. Incubation was terminated by cooling cells to 4°. Cells were washed with 0.9% NaCl solution (4°), treated with 5% trichloroacetic acid (30 min, 4°), washed 1 additional time with trichloroacetic acid, and washed with methanol. Acid-precipitable material was dissolved in 0.5 ml of Soluene (30 min, 60°), and radioactivity was measured in 10 ml of scintillation fluid.

Assay of Cyclic Nucleotide Phosphodiesterase Activity. Cyclic nucleotide phosphodiesterase activity was measured by a modification of a 2-step radioisotope procedure of Thompson and Appleman (17). Unless otherwise indicated, lymphoid cell pellets were suspended in 40 mM Tris-Cl (pH 8.0):5 mM 2-mercaptoethanol and sonically disrupted for 10 sec at a setting of 50 (80 watts) using the microprobe of a Biosonic IV sonicator. Microscopic examination showed 100% cell breakage.

Reaction mixtures (0.4 ml) contained 40 mM Tris-Cl (pH 8.0), 10 mM MgCl₂, 5 mM 2-mercaptopethanol, cyclic [³H]AMP or cyclic [³H]GMP (~100,000 cpm), cyclic AMP or cyclic GMP as indicated, and 0 to 200 µl (0 to 2 x 10⁶ cells) of cell sonicate. Reactions were incubated at 30°, usually for 10, 20, and 30 min, and terminated by boiling for 1 min. Snake (Ophiophagus hannah) venom (0.5 mg/ml) was added (100 µl) to the chilled samples and incubated for 10 min at 30°. One ml of methanol was added to each sample, and [³H]-nucleosides were then separated from unreacted cyclic nucleotides with Dowex 1-X8 resin. One ml of an aqueous 1:4 resin slurry was added to a 5.75-inch Pasteur pipet column supported on a Lucite plate that covered a 19- x 15- x 4-cm box capable of holding a vacuum. The reaction mixture was applied to the column, and a vacuum was applied to elute the contents into scintillation vials previously placed in the box below the columns. Eight ml of Aquasol were added to the vials, and radioactivity was determined with fluorescent antiimmunoglobins (8). These procedures less than 5 to 15% of the reaction products bind nonspecifically to the resin. In addition, a sensitivity 3-fold greater than that of previously published assays is obtained.

Cyclic AMP Extraction and Immunoassay. Cell pellets (1 to 5 x 10⁶ cells) were extracted with 0.4 n perchloric acid, sonically disrupted (5 sec, setting of 50, Biosonic IV sonicator), and neutralized with 2 M Tris-Cl (pH 7.6). One ml of a 30% solution of neutral alumina in water was added to each sample. The samples were mixed and centrifuged at 1000 x g for 5 min. The supernatants were applied to a column of Dowex 1-X8 anion-exchange resin (200 to 400 mesh) prepared from 2 ml of a 1:4 aqueous slurry in a Pasteur pipet. The columns were washed with 5 ml of water and 5 ml of 0.001 n HCl in 50% methanol. Cyclic AMP was eluted with 6 ml of 0.01 n HCl in 50% methanol, lyophilized, dissolved in 0.5 ml of water, and assayed by radioimmunoassay according to the method of Steiner et al. (15) as modified by Thompson and Williams (19).

Sucrose Gradient Density Centrifugation. Linear 5 to 20% sucrose gradients (5 ml) in 40 mM Tris-Cl (pH 8.0):5 mM 2-mercaptoethanol:10 mM MgCl₂ were prepared in polyallomer centrifuge tubes. Cell pellets containing 30 to 50 x 10⁶ cells were sonically disrupted in 0.2 ml of the above buffer and layered on the gradients. Bovine serum albumin (3 mg) in the same buffer was layered on a separate gradient and used as a molecular weight standard (4.3 S). Gradients were centrifuged at 192,000 x gmax in a SW 50.1 rotor for 15 to 16 hr (4°) and collected as 8 drops/fraction after the bottom of the tube was pierced.

Protein was determined either by the method of Lowry et al. (9) or by the fluorescent method of Udenfriend et al. (20), with bovine serum albumin as standard.
Materials. Cyclic $[^{3}H]$AMP (specific activity, 16.3 or 23 Ci/mmol), cyclic $[^{3}H]$GMP (specific activity, 4.1 Ci/mmol), and $[^{3}H]$thymidine (1.9 Ci/mmol) were purchased from Schwarz/Mann, Orangeburg, N. Y., and New England Nuclear, Boston, Mass. Cyclic $[^{3}H]$AMP and cyclic $[^{3}H]$GMP were purified by Dowex 1-X8 (200 to 400 mesh) anion-exchange chromatography and stored at $-20^\circ$ in acidic 50% ethanol. The following materials were purchased as indicated: snake (O. hannah) venom, cyclic AMP, cyclic GMP, RNase and Pronase, Sigma Chemical Co., St. Louis, Mo.; 2,7-diamo-10-ethyl-9-phenylphenanthridium bromide (ethidium bromide), Calbiochem, Los Angeles, Calif.; Ficoll, Pharmacia Fine Chemicals, Inc., Piscataway, N. J.; Dowex 1-X8 (200 to 400 mesh), BioRad Laboratories, Richmond, Calif.; Hypaque-M (90%) Winthrop Laboratories, Dallas, Texas; fluorescein-conjugated goat anti-human immunoglobulin, Meloy Laboratories, Springfield, Va.; enzyme grade sucrose, Merck and Co., Rahway, N. J.; RPMI Medium 1640, MEM, L-glutamine, penicillin:streptomycin solution, Grand Island Biological, Grand Island, N. Y.; carbonyl iron powder (Grade SS), GAF Corporation, Texas City, Texas; glass beads (3 mm in diameter), Scientific Products, McGraw Park, Ill.; PHA-M, Difco Laboratories, Inc., Detroit, Mich.; and 4-phenylspiro[furan-2(3H),1-phthalonitrile3,3'-dione (Fluram), Roche Laboratories, Nutley, N. J. All other chemicals were of commercial analytical grade quality.

RESULTS

Cyclic Nucleotide Phosphodiesterase Activities of Lymphoid Cells. The specific activities of cyclic AMP and cyclic GMP phosphodiesterases in lymphoid cells of different origins were measured with the use of 0.025 and 50 $\mu$M cyclic AMP and 1 $\mu$M cyclic GMP substrate concentrations. The activities of both cyclic AMP and cyclic GMP phosphodiesterases in HPBL were the lowest of any of the cells tested (Table 1). Previously, Thompson et al. (18) found undetectable cyclic GMP phosphodiesterase activity in HPBL. However, the more sensitive assay procedure used in this study allowed the determination of the entire reaction product formed and enabled detection of a very low level of cyclic GMP phosphodiesterase activity in HPBL.

The cyclic AMP and cyclic GMP phosphodiesterase activities per $10^6$ cells of all of the human lymphoid cell types tested were severalfold higher than in HPBL (Table 1). The specific activities of these enzymes were not as markedly different since the cultured cells contained 100 $\mu$g of protein per $10^6$ cells and HPBL contained 40 $\mu$g of protein per $10^6$ cells. The T-cell line (CEM) had total and specific cyclic AMP phosphodiesterase activity 2- to 4-fold higher than the activities of the B-cell lines (CRG, JC, BEQ). The cyclic GMP phosphodiesterase activities of these cultured cells showed no clear differences between T- and B-lines.

The cyclic nucleotide phosphodiesterase activities of the B lymphoblastoid cell lines (CRG, JC, BEQ) were determined with the use of nearly confluent cultures (densities near $2 \times 10^6$ cells/ml) at which time they form ball-like aggregates in suspension. The CEM line was harvested at the same density but did not aggregate. Since the levels of phosphodiesterase activity can vary during various stages of cell growth in other cultured lines (11), we tested the possibility that the activities shown in Table 1 were not representative for the lymphoblastoid cells. Lymphoblastoid cell line CRG was grown to near confluence (1.5 x $10^6$ cells/ml) and diluted to 0.5 x $10^6$ cells/ml (or in other experiments to 0.25 x $10^6$ cells/ml). Little change in total activity or affinity for cyclic AMP was observed upon dilution, and the specific activity remained constant during growth from zero time to 96 hr in culture (data not shown).

Cyclic AMP phosphodiesterase activity of murine DBA/2 isolated splenic lymphocytes with 50 $\mu$M cyclic AMP substrate was 1.7 to 4.5 times that of the human lymphoblastoid cells and 13 times that of HPBL (Table 1). However, when measured at 0.025 $\mu$M cyclic AMP, the murine activity was lower. This difference is the result of the lower affinity of the DBA/2 cyclic AMP phosphodiesterase (see below). Cyclic GMP phosphodiesterase activity of the DBA/2 splenic lymphocytes was severalfold higher than cyclic GMP phosphodiesterase activity in all of the human lymphoid cell types. Both cyclic AMP and cyclic GMP phosphodiesterase activities in the murine leukemic cell lines, L1210, were markedly higher than those observed in any of the other cell types examined. The activities were 10- to 20-fold higher than those measured in normal DBA/2 splenic lymphocytes and more than 400-fold higher than some of the human lymphoid cells. Hait and Weiss (5) also found 20-fold differences in phosphodiesterase activities between L1210 and DBA/2 lymphoid cells.

Kinetic Analysis of Cyclic Nucleotide Phosphodiesterases of Various Lymphoid Cells. The kinetic behavior of cyclic AMP phosphodiesterase activity in lymphoid cells was analyzed with the use of substrate concentrations.

### Table 1

<table>
<thead>
<tr>
<th>Activity</th>
<th>Cyclic AMP</th>
<th>Cyclic GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPBL</td>
<td>0.09</td>
<td>2.2</td>
</tr>
<tr>
<td>Human lymphoblastoid cell lines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B lines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRG</td>
<td>0.25</td>
<td>6.6</td>
</tr>
<tr>
<td>JC</td>
<td>0.28</td>
<td>9.8</td>
</tr>
<tr>
<td>BEQ</td>
<td>0.26</td>
<td>9.8</td>
</tr>
<tr>
<td>T line</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEM</td>
<td>0.99</td>
<td>18</td>
</tr>
<tr>
<td>Normal murine splenic lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murine L1210 leukemic cell line</td>
<td>0.75</td>
<td>707</td>
</tr>
</tbody>
</table>

All cells were harvested or isolated as indicated in "Materials and Methods." After 2 washes in MEM, the recentlyrifuged (1500 rpm/10 min) cells were suspended as $10 \times 10^6$ cells per 200 $\mu$l of 40 mM Tris-Cl (pH 8.0):5 mM 2-mercaptoethanol sonically disrupted cells, and diluted to 10$^6$ cells/100 $\mu$l for activity analyses as described in "Materials and Methods." Activities are the means of 1 to 8 separate cell populations for each. Where multiple cell populations were analyzed, the standard error was no greater than 15% of the mean.
ranging from 0.04 to 50 μM cyclic AMP (Charts 1 and 2). Lineweaver-Burk plots ranging from substrate concentrations of 0.04 to 2 μM were nonlinear in all of the lymphoid cells tested (Chart 1). Extrapolation of the linear portion of the plots indicated the presence of a high-affinity form of cyclic AMP phosphodiesterase in all the cells. The human lymphoid cells have apparent affinities for cyclic AMP of 0.1 to 0.4 μM and the murine cells have affinities of 0.8 μM. The nonlinear kinetics are much more evident in the murine cells and cultured human cell lines than in HPBL. We have previously reported that HPBL show apparent Michaelis-Menten kinetic behavior (18). However, these initial studies were limited to activity analyses below 2 μM, and we could not clearly document the nonlinearity of the plots without the more sensitive assay used in the present study.

Kinetic analyses of lymphoid cell cyclic AMP phosphodiesterase activity at cyclic AMP substrate concentrations from 1 to 50 μM cyclic AMP are shown separately in Chart 2 for graphic clarity. In agreement with our earlier findings (18), these data indicate that none of the human lymphoid lines shows evidence of a low-affinity enzyme typically observed in most mammalian tissues (1). However, both murine cell types do contain the low-affinity enzyme form (apparent Kₐ = 36 and 70 μM). Maximum velocity calculations confirm the very high activity of the murine L1210 cells relative to the other cell types measured at nonsaturating substrate concentrations (Table 1).

With the exception of isolated HPBL, cyclic GMP phosphodiesterase activities in the lymphoid cells showed typical Michaelis-Menten kinetic behavior when tested from 0.5 to 50 μM cyclic GMP (Chart 3). The affinities for cyclic GMP were 10- to 60-fold higher in the human lymphoid cell types than in normal murine splenic lymphocytes or the L1210 leukemic cell line.

Physical Analysis of Cyclic Nucleotide Phosphodiesterases in Lymphoid Cells. The observed anomalous kinetic behavior of these cyclic nucleotide phosphodiesterases could be the result of negative cooperative interactions on 1 enzyme, the presence of separate enzyme forms differing in affinity for substrate, or both. To distinguish between these possibilities, we separated the enzyme forms by linear 5 to 20% sucrose gradient centrifugation (Chart 4) and determined the kinetic behavior of the forms (Chart 5). The major portion of cyclic AMP phosphodiesterase activity in 3 human lymphoid cells tested, HPBL, lymphoblastoid B-cells (CRG), and lymphoblastoid T-cell line (CEM), sedimented near 5.5 to 6 S; each had a minor peak of activity between 3 and 4 S. In contrast, the majority of the murine cell activities sedimented near 7 S, although activities were evident in the 3 to 4 S region of the gradients as well. The recovery of all activities was 50 to 75% for each cell type.

The sedimentation coefficients of the 2 cyclic AMP phosphodiesterase activities suggest that these peaks might...
Kₐₘ, ~6 µM). Lineweaver-Burk analysis from 2 to 50 µM cyclic AMP showed the L1210 7 S enzyme to have anomalous kinetic behavior with an extrapolated apparent Kₐₘ of 80 µM (data not shown; available upon request). The CRG 5.9 S form also showed deviation from linearity with a lower extrapolated apparent Kₐₘ of 1.3 µM.

Analysis of cyclic GMP phosphodiesterase activity by linear sucrose gradient fractionation revealed differences in the sedimentation constant of the enzyme among the different cell types (Chart 4). Both murine cell types demonstrated a single peak of activity sedimenting at 7 S that was coincident with the heavier form of cyclic AMP phosphodiesterase. In contrast, HPBL showed 2 peaks of cyclic GMP phosphodiesterase activity, a heavy form at about 6.5 S and a lighter form at 3 to 4 S. The human lymphoblastoid B-cell line showed a major peak of cyclic GMP phosphodiesterase also at 6.5 S and contained a shoulder of activity at about 4 to 5 S. Cyclic GMP phosphodiesterase in the human T-lymphoblastoid cells sedimented mainly at 3.6 S (Chart 4).

Cyclic AMP Levels in Lymphoid Cells. Total cyclic AMP content was measured in isolated, purified HPBL and in human and murine lymphoblastoid cell lines (Table 2). Variation among individuals was observed when cyclic AMP content was measured in isolated HPBL, which made it represent polymeric forms of the same enzyme. This possibility was examined with L1210 cells and HPBL as the sources of enzyme activity. L1210 enzyme from cells homogenized in buffer containing 5 mM reduced glutathione and centrifuged in linear sucrose gradients (5 to 20%) containing reduced glutathione (5 mM) showed activity profiles (4 and 7 S forms) identical with those of L1210 cells homogenized and centrifuged in sucrose and buffer containing oxidized glutathione (5 mM). Different amounts of homogenate protein placed on the gradient (1 to 5 mg) produced no difference in the proportion of high-molecular-weight to lower-molecular-weight activity seen in the gradient profile. Unlike the changes previously shown in HPBL (18), frozen and freshly homogenized L1210 cells showed similar activity profiles. Previously, we described an apparent "aging" change where HPBL cell homogenates stored at 4° for 24 hr showed only the 3.5 S enzyme form, when at zero time both 3 to 4 S and 5.5 to 6 S forms were observed (18); effects of aging were not found in L1210 cells.

Lineweaver-Burk plots from 0.04 to 2 µM cyclic AMP showed that the low-molecular-weight (3 to 4 S) forms have apparent Kₐₘ's of 1 to 2 µM for both the human and murine cells. The 5.9 S and 7 S forms showed distinct kinetic differences (Chart 5). When analyzed from 0.04 to 2 µM cyclic AMP, the 5.9 S form of the human CRG line had a much higher substrate affinity (apparent Kₐₘ, ~0.2 µM) than did the 7 S form of the murine L1210 line (apparent
Cyclic AMP levels in lymphoid cells were isolated and purified as described in "Materials and Methods." Cell lines were grown to a density of approximately 10^6 cells/ml. All cells were extracted and assayed for cyclic AMP as described in "Materials and Methods." Cyclic AMP (pmoles/10^6 cells) HPBL (9) 1.6 ± 0.6 Human lymphoblastoid cell lines B lines CRG (8) 1.0 ± 0.2 BEQ (9) 0.9 ± 0.2 T line CEM (9) 0.4 ± 0.06 Murine L1210 leukemic cell line (9) 0.3 ± 0.08

PHA Stimulation of HPBL. When HPBL were stimulated to undergo mitogenesis by PHA, increases of as much as 20-fold in both cyclic AMP and cyclic GMP phosphodiesterase activity were found (Chart 6). Monahan et al. (10) reported that PHA stimulates total cyclic AMP phosphodiesterase specific activity by 2-fold in HPBL after 2 days in culture. The 72-hr PHA-stimulated HPBL enzymes showed kinetics similar to those of resting lymphocytes. Linear sucrose gradient fractionation of the PHA-activated enzymes showed an increase in all cyclic AMP and cyclic GMP phosphodiesterase forms (data not shown).

DISCUSSION

These results demonstrate that cyclic nucleotide phosphodiesterases of human lymphoid cells differ markedly from those of murine origin. Both the long-term human lymphoblastoid cell lines and normal peripheral blood lymphocytes were stimulated to undergo mitogenesis by PHA, increases of as much as 20-fold in both cyclic AMP and cyclic GMP phosphodiesterase activity were found (Chart 6). Monahan et al. (10) reported that PHA stimulates total cyclic AMP phosphodiesterase specific activity by 2-fold in HPBL after 2 days in culture. The 72-hr PHA-stimulated HPBL enzymes showed kinetics similar to those of resting lymphocytes. Linear sucrose gradient fractionation of the PHA-activated enzymes showed an increase in all cyclic AMP and cyclic GMP phosphodiesterase forms (data not shown).
phocytes do not appear to have a low-affinity cyclic AMP phosphodiesterase, which is in agreement with our earlier studies (18). The absence of the low-affinity system has been confirmed by both physical and kinetic data. This enzyme is, however, evident in the murine leukemic cell line as well as in normal murine splenic lymphocytes. Coincidently, the murine cells have been shown to have a higher-molecular-weight enzyme form than that of the human lymphoid cells. As discussed previously (18) other investigators have reported the presence of a low-affinity enzyme in human lymphocytes, but in none of these studies were techniques used that adequately removed platelets from their preparations; therefore possible cell contamination could influence their results.

In previous studies of peripheral blood lymphocyte cyclic AMP phosphodiesterase, we have speculated that the 3 to 4 S form, which has a high affinity for cyclic AMP and displays typical Michaelis-Menten kinetic behavior, may represent a basic subunit of cyclic nucleotide phosphodiesterase. This study further supports this hypothesis and suggests that negative cooperativity and low affinity for cyclic AMP are a consequence of multiple arrangements of the lower-molecular-weight form. Both the human and murine lymphoid cells possess a high-affinity low molecular-weight (3.5 S) cyclic AMP phosphodiesterase form displaying Michaelis-Menten behavior. Moreover, the human lymphoblastoid 5.9 S enzyme form is twice the molecular weight of the 3.5 S form and the L1210 7 S enzyme form is 3 times the molecular weight of the 3.5 S form. In contrast to the lower-molecular-weight enzyme forms, the higher-molecular-weight enzyme forms in both the human and murine cells display anomalous kinetic behavior. However, the 5.9 S human enzyme form is distinctly different from the 7 S murine form. The 5.9 S form has markedly higher affinity for cyclic AMP than does the 7 S enzyme and is relatively specific for cyclic AMP, whereas the 7 S enzyme is not specific and hydrolyzes both cyclic AMP and cyclic GMP.

If the low-affinity cyclic AMP phosphodiesterase results from multiple subunit aggregation as we have hypothesized, then the absence of low-affinity enzyme in human lymphoid cells suggests that either multiple aggregation is prevented in this cell type or this cell type lacks a factor responsible for promoting multiple subunit interaction. Alternatively, Russell and Pastan (14) have proposed that the low-affinity cyclic AMP phosphodiesterase in mammalian cells is genetically distinct from the higher-affinity enzyme. The absence of the low-affinity enzyme in human lymphoid cells would suggest, therefore, that either the gene for this enzyme is not expressed in this cell or it lacks a regulatory factor necessary for the expression of this gene product(s). Our data do not clearly distinguish between aggregation and genetic mechanisms.

Measurement of cyclic AMP levels indicated that a correlation exists between cyclic AMP content and high-affinity cyclic AMP phosphodiesterase activity in cultured lymphoid cell lines. A clear extension of this correlation to results on isolated HPBL was complicated by variation in lymphocyte cyclic AMP content among different individuals. Variation in cyclic AMP content from individual to individual was confirmed in a separate study by Dr. Uri Lewinski of this laboratory. In 26 separate determinations of cyclic AMP content in isolated peripheral blood lymphocyte from 9 breast cancer patients, the average cyclic AMP content was 1.25 ± 0.23 pmoles/10^6 cells, but the range was from 0.29 to 5.6 pmoles/10^6 cells.

Distinct differences in kinetic and physical properties of cyclic nucleotide phosphodiesterases between human and murine lymphoid cells suggest that studies of cyclic nucleotide metabolism in murine lymphoid cells may not be adequate model systems for human lymphoid cells. In this regard, it seems pertinent that murine splenic cells, but not HPBL, can be activated by exogenous addition of cyclic GMP and analogs of cyclic GMP (22-25). In contrast, the results in this report do indicate that cyclic AMP phosphodiesterases in human lymphoblastoid cell lines are kinetically and physically similar to those in HPBL. Hence, these cell lines may prove to be useful models for the examination of hormonal and mitogenic effects on cyclic AMP metabolism in isolated human lymphoid cells.

The murine L1210 and human lymphoblastoid cell lines are rapidly proliferating cells when compared to lymphocytes isolated from peripheral blood or mouse spleen. Coincidently, these rapidly growing cells have markedly enhanced cyclic nucleotide phosphodiesterase activity as compared to their quiescent counterparts. When the quiescent human lymphocytes were stimulated by PHA, we also found marked increases in cyclic AMP and cyclic GMP phosphodiesterase activities. These findings suggest that increased cyclic nucleotide phosphodiesterase activity is associated with proliferation and cancer in human and murine lymphoid cells.

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