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Cyclic Adenosine 3′:5′-Monophosphate-dependent and -independent Protein Kinases in Human Leukemic Cells

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ABSTRACT

The pattern of protein kinase activity in leukemic cells from patients with chronic myelocytic leukemia, acute myeloblastic leukemia, acute monocytic leukemia, chronic lymphocytic leukemia, and acute lymphoblastic leukemia was studied and compared with normal peripheral blood granulocytes and lymphocytes.

Our data showed that: (*a*) histone kinase activity was slightly lower in leukemic cells than in normal cells, whereas casein kinase activity was 2- to 3-fold higher in leukemic cells; (*b*) cyclic adenosine 3′:5′-monophosphate stimulated 1.4- to 1.6-fold histone kinase activity of both normal and leukemic cells, whereas it did not stimulate casein kinase activity; (*c*) the ratio of histone kinase activities to casein kinase activities correlated directly with the maturation of the white blood cells; and (*d*) histone and casein kinase activities of extracts from normal and leukemic cells behaved similarly on chromatography on phosphocellulose and casein/Sepharose 4B.

These results suggest that the increase in casein kinase activity is not due to the appearance of a new type of casein kinase but to an increase of the casein kinases 1 and 2 present in normal cells.

INTRODUCTION

Several reports have evidenced the role of cyclic nucleotides, mainly cyclic AMP,[3] on the control of cell proliferation and differentiation in both granulocytes (7, 24) and lymphocytes (3, 10). Therefore, cyclic AMP-dependent protein kinases, direct mediators of the cyclic AMP action, are presently the subject of studies on leukemic cells (6, 25).

It is known that leukocytes contain cyclic AMP-independent histone and casein kinases (15, 16, 18, 22), and the potential relevance of such enzymes to the understanding of cancer is suggested by the demonstration that the products of viral genes associated with transformation possess such enzymatic activity (23, 26). Thus, information about the pattern of cyclic AMP-independent protein kinases in leukemic cells seems of interest.

Previous works have shown higher specific activities of cytosol cyclic AMP-independent casein kinases in AML[3] cells compared to the normal myeloid cells (6) but lower casein kinase activity in tumor lymphocytes from CLL cells compared to normal human lymphocytes (25).

In this paper, we describe the quantification of cytosolic protein kinase activities in leukemic cells from both granulocytic and lymphocytic cell lines in comparison to normal human granulocytes and lymphocytes. All of the leukemic cells studied had slightly lower histone kinase activity and higher casein kinase activity than did normal cells. Nonetheless, the types of protein kinases in both normal and leukemic cells were similar, as judged by chromatography on phosphocellulose and casein/Sepharose.

MATERIALS AND METHODS

Chemicals. Casein was Hammarsten quality from Merck (Darmstadt, Germany). Casein coupling to Sepharose 4B was performed according to the method of Farron-Furstenthal and Lightholder (9). [γ-^{32}P]ATP was obtained from the Radiochemical Centre, Amersham, England; histone (type 2A), dextran, and cyclic AMP were from Sigma Chemical Co., St. Louis, Mo.; phosphocellulose (P-11) was from Whatman, Ltd., Maidstone, Kent, England; and Sepharose 4B from Pharmacia, Uppsala, Sweden. All other reagents were of analytical grade.

Blood Samples. Normal human blood and samples from blood of newly diagnosed (untreated) patients with leukemia were obtained by standard techniques. EDTA was used as anticoagulant.

The diagnoses were established by standard criteria, including May-Grünwald-Giemsa and cytochemical staining of blood and marrow samples according to standard techniques. The leukemic samples used consisted of more than 90% blast cells in AML, AMOL, and ALL; greater than 95% leukemic B-lymphocytes in CLL; and more than 90% granulocytes in CML.

Leukocyte Purification. Polymorphonuclear leukocytes from normal donors were isolated as described by Esmann (8).

Normal lymphocytes were isolated as indicated by Ribas *et al.*[4]

The viability of both types of cells was more than 96% as determined by the standard trypan blue exclusion method.

Venous blood (20 to 30 ml) was withdrawn from patients affected by different leukemias, and leukocytes were separated from RBC by differential sedimentation (dextran was added when gravitational sedimentation was too slow). Platelets were removed by differential centrifugation at 400 × *g*. A nearly pure population of pathological cells was obtained at this stage, contaminated only by a few erythrocytes which were removed by hypotonic lysis.

Extracts. Extracts were prepared from isolated leukocytes by homogenization in 50 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA, with a glass homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 27,000 × *g* for 15 min. The resulting supernatant was used for the determination of protein kinase activity.

Chromatography on Phosphocellulose. Supernatants from 27,000 × *g* centrifugation were centrifuged for 90 min at 50,000 × *g*. The

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[3] The abbreviations used are: cyclic AMP, cyclic adenosine 3′:5′ monophosphate; AML, acute myeloblastic leukemia; CLL, chronic lymphocytic leukemia; AMOL, acute monoblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelocytic leukemia; PMN, normal peripheral blood granulocytes.

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Table 1
Cytosol protein kinase activities in myeloid cells

		Activity (units/mg protein)					
		Histone kinase			Casein kinase		
	No.[a]	−Cyclic AMP	+Cyclic AMP	±	−Cyclic AMP	+Cyclic AMP	±
PMN	25	0.24 ± 0.07[b]	0.34 ± 0.07	1.42	0.31 ± 0.08	0.25 ± 0.09	0.81
CML	8	0.15 ± 0.03	0.25 ± 0.02	1.66	0.41 ± 0.14	0.42 ± 0.16	1.09
CML/PMN		0.625	0.73	1.17	1.32	1.68	1.34
AML	4	0.18 ± 0.02	0.28 ± 0.05	1.55	0.58 ± 0.04	0.58 ± 0.04	1.00
AML/PMN		0.75	0.82	1.09	1.87	2.32	1.23

[a] Number of patients or healthy volunteers.
[b] Mean ± S.D. of samples studied.

high-speed supernatant was adjusted to 60% saturation with 100% saturated $(NH_4)_2SO_4$, and after gentle stirring for 20 min it was centrifuged at 25,000 × *g* for 20 min. The precipitate was resuspended in 50 mM Tris-HCl buffer, pH 7.5, containing 1 mM dithiothreitol, 5% (v/v) glycerol, and 0.1 mM phenylmethylsulfonyl fluoride (Buffer A) and extensively dialyzed against the same buffer.

Dialyzed material from the 60% $(NH_4)_2SO_4$ precipitation was applied to a phosphocellulose column (1 x 2 cm) equilibrated with Buffer A. The column was washed with Buffer A, followed by KCl in Buffer A as indicated in the charts, and finally a linear gradient of 0.3 to 1.5 M KCl in Buffer A. Fractions of 1 ml each were collected, and samples were taken for protein kinase assay.

Affinity Chromatography of Casein Kinases. Pooled fractions from phosphocellulose were extensively dialyzed against Buffer A and then applied to a casein-Sepharose 4B column (1 x 3 cm) equilibrated with the same buffer. The column was washed with 15 ml of Buffer A followed by a 20-ml linear gradient of 0 to 1 M KCl in the same buffer. Fractions (1 ml) were collected and assayed for casein kinase activity in the absence of cyclic AMP.

Determination of Protein Concentration. The concentrations of all of the proteins were determined by modification (2) of the method of Lowry *et al.* (20) using bovine serum albumin as standard.

Assay of Protein Kinase. Protein kinase activity was measured as described previously (13) using either casein or histone as substrate at a concentration of 4 mg/ml. When cyclic AMP was included in the assay, its concentration was 20 μM. One unit of protein kinase is defined as the amount catalyzing the transfer of 1 nmol of phosphate from [γ-^{32}P]ATP (100 to 200 cpm/pmol) to exogenous protein substrate per min at 30°.

RESULTS

Histone and Casein Kinase Activities in Normal and Leukemic Myeloid Cells. As shown in Table 1, the specific histone kinase activities of cytosolic extracts from myeloid cells are slightly lower in leukemic than in normal cells (CML/PMN ratio, 0.7; AML/PMN ratio, 0.8). The effect of cyclic AMP on histone kinase activity resulted in a similar stimulation in all of the cases (1.4- to 1.6-fold).

On the contrary, the specific casein kinase activity is clearly higher in leukemic cells than in normal cells (1.7- and 2.3-fold for CML and AML cells, respectively). No stimulatory effect of cyclic AMP on casein kinase activity was found either in normal cells or in the 2 types of leukemia studied.

Chromatography on phosphocellulose of cytosolic extracts from both CML (Chart 1) and AML cells (not shown) exhibited a pattern similar to that found in normal polymorphonuclear leukocytes. Most of the histone kinase activity was in the flowthrough fractions, and the remainder, which bound to the exchange agent, was eluted with 0.3 M KCl in Buffer A. The

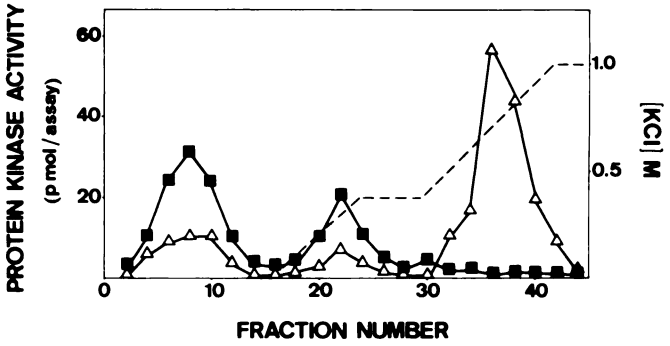

Chart 1. Chromatography on phosphocellulose of cytoplasmic protein kinases from CML leukocytes. Fractions (1 ml) were assayed for histone kinase activity in the presence of 20 μM cyclic AMP (■), casein kinase activity in the absence of cyclic AMP (△), and KCl concentration (– – –). Protein kinase activity is given as pmol of ^{32}P incorporated under standard assay conditions.

first peak of activity was stimulated by cyclic AMP, whereas the second was not. In fact, this second peak could be resolved into 2, one eluting at 0.2 M KCl and the other at 0.3 M KCl when a 0.2 M KCl wash was carried out before applying the 0.3 M KCl solution as observed previously (22).

Almost all of the casein kinase activity from leukemic cells was bound to phosphocellulose and eluted with 0.6 to 0.7 M KCl. This peak did not show histone kinase activity and was not stimulated by cyclic AMP.

Histone and Casein Kinases in Normal and Leukemic Peripheral Blood Lymphocytes. Specific histone kinase activities of cytosolic extracts from 4 samples of patients with CLL and 2 from patients with ALL were very similar to those of normal lymphocytes (CLL/lymphocyte ratio, of 0.9; ALL/lymphocyte ratio, 1.3). Cyclic AMP stimulated about 1.4-fold histone kinase activity of normal and leukemic lymphocytes (Table 2).

The specific casein kinase activity in CLL and ALL was 2- and 3-fold higher, respectively, than in normal lymphocytes. These casein kinase activities were not stimulated by cyclic AMP.

Chromatographic patterns found in leukemic lymphocytes were similar to those shown in myeloid cells. Thus, a histone kinase peak excluded from phosphocellulose and stimulated by cyclic AMP, a histone kinase peak eluted with 0.3 M KCl not activated by the nucleotide, and a casein kinase activity peak clearly separated from histone kinase activity, eluting at 0.6 to 0.7 M KCl, and not stimulated by cyclic AMP were observed.

Protein Kinases from Leukemic Cells and Cellular Maturity. The ratio of histone kinase activity to casein kinase activity in myeloid cell lines correlated clearly with maturation, as shown in Chart 2. This ratio is greater in normal PMN cells than

Table 2
Cytosol protein kinase activities in lymphoid cells

		Activity (units/mg protein)					
		Histone kinase			Casein kinase		
	No.[a]	−Cyclic AMP	+Cyclic AMP	±	−Cyclic AMP	+Cyclic AMP	±
Normal lymphocyte	25	0.24 ± 0.07[b]	0.34 ± 0.1	1.42	0.32 ± 0.09	0.28 ± 0.09	0.87
CLL	4	0.21 ± 0.10	0.28 ± 0.11	1.33	0.58 ± 0.17	0.59 ± 0.16	1.01
CLL/normal lymphocyte		0.875	0.82	0.93	1.82	2.11	1.16
ALL	3	0.32	0.45	1.40	0.88	0.82	0.93
ALL/normal lymphocyte		1.33	1.32	0.98	2.75	2.92	1.07

[a] Number of patients or healthy volunteers.
[b] Mean ± S.D. of samples studied.

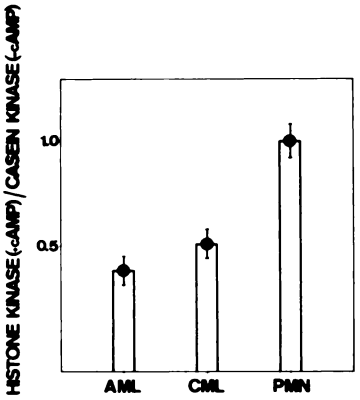

Chart 2. Histone kinase/casein kinase ratio in normal and leukemic granulocytic cells. Histone kinase activity was determined in the presence of 20 μM cyclic AMP, whereas casein kinase activity was measured in the absence of cyclic AMP. Results are mean (●) of 4 (AML), 8 (CML), or 25 (PMN) samples. Bars, S.D.

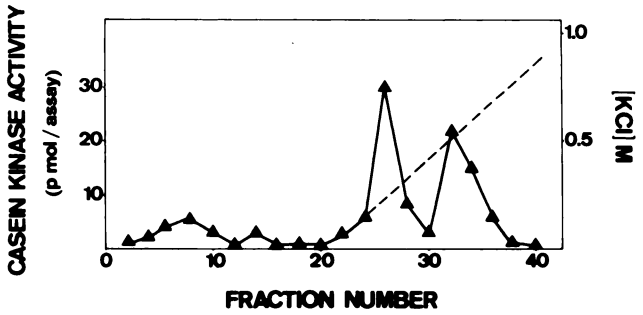

Chart 3. Resolution on casein/Sepharose 4B of casein kinases from leukemic cells. The pooled fractions from the phosphocellulose column were applied to a casein/Sepharose 4B column (0.8 x 3 cm). The column was washed with Buffer A and a linear gradient of 0 to 1 M KCl in the same buffer. Fractions (1 ml) were assayed for casein kinase activity in the absence of cyclic AMP (▲). - - - -, KCl concentration. Casein kinase activity is given as pmol of ^{32}P incorporated into casein under standard assay conditions.

that in leukemic cells from CML (nonblastic precursors) which, in turn, is greater than that in myeloid blast cells from AML (more than 95% of blasts).

Protein Kinases from Monocytic Leukemic Cells. The protein kinase content of another WBC line, the monocytic cells, was studied in a sample from an untreated patient with AMOL.

Chromatographic behavior on phosphocellulose of cytosolic extracts from AMOL cells showed peaks of histone kinase and casein kinase activities similar to those found in normal and leukemic myeloid and lymphoid cells. Three peaks of histone kinase activity were found, the flow-through and those eluting at 0.2 and 0.3 M KCl in Buffer A.

On the other hand, casein kinases were eluted from the column with 0.6 to 0.7 M KCl as fractions without histone kinase activity and not stimulated by cyclic AMP.

Casein Kinases 1 and 2 from Normal and Leukemic Cells. As described previously (22), casein kinases from leukocyte cytosol can be totally separated in 2 fractions either by gel filtration on Bio-Gel or by affinity chromatography on casein/Sepharose 4B.

Due to the low amount of cells available, the second method was used in order to evaluate the types of casein kinases present in leukemic cells.

In all of the leukemic cells studied, the casein kinase peak eluted from phosphocellulose was resolved in 2 fractions, which eluted at ionic strengths identical to those of the casein kinases 1 and 2 present in normal leukocytes. The elution profile of the enzymes from AMOL cells is shown in Chart 3 as typical.

DISCUSSION

The aim of this work was to study in parallel the cytosolic protein kinase pattern from normal and leukemic leukocytes in both myeloid and lymphoid cell lines.

In myeloid cells, the levels of histone kinase activity are slightly lower, and those of casein kinase activity are clearly higher in leukemic cells than those in normal PMN leukocytes. In all cases, only the histone kinase activity was stimulated by cyclic AMP, the effect being similar in leukemic and normal myeloid cells.

The chromatographic pattern on phosphocellulose of protein kinases from normal and leukemic myeloid cells was similar. This fact suggests that they contain the same general types of histone and casein kinases.

Similar results regarding the types of protein kinases and increased casein kinase activity in blast cells with respect to normal cells were reported by Elias *et al.* (6) when comparing normal and AML cells. However, these authors indicated that the cytosolic histone kinase activity in leukemic cells was slightly higher and less stimulated by cyclic AMP than in normal cells.

Our data from normal and leukemic lymphoid cells show no differences with respect to those described in myeloid cells. The cytosolic casein kinase but not the histone kinase activity is clearly higher in leukemic cells as compared to normal human lymphocytes, the chromatographic pattern of histone and casein kinases being similar in all cases.

It is important to note that, although the number of patients studied for each individual type of leukemia ranged from 3 to

8, the leukocytes from the 19 leukemic patients showed an elevated casein kinase activity, indicating that this could be considered a common characteristic of leukemic cells.

In contrast to our observations, Weber *et al.* (25) reported recently that CLL cells contain lower values of histone and casein kinase activities than do normal lymphocytes. On the contrary, higher levels of both histone and casein kinase activities were described in lymphosarcoma cells (21) compared with normal lymphocytes.

Previous reports have shown that cell proliferation is practically absent in peripheral blood lymphocytes and lymphocytes from patients with CLL (25). Furthermore, the protein kinase content in bone marrow samples, which included proliferative cells, was similar to that in normal peripheral blood granulocytes (6). Thus, it seems reasonable to consider that the difference between normal and leukemic peripheral leukocytes may relate to the state of differentiation rather than cell proliferation.

The ratio of histone kinase activity to casein kinase activity in myeloid cells increased with cellular maturation, mainly due to changes in the levels of casein kinase activity. This fact suggests (*a*) a possible role of casein kinases on cellular functions related to cell differentiation; and (*b*) that the differences on specific protein kinase activities between normal and leukemic cells are not due to modification on cytosolic protein content, since the specific histone kinase and casein kinase activities show no parallel variations.

Monocytic cells contain 2 cytosolic casein kinases independent of cyclic AMP which have the same chromatographic characteristic of casein kinases from lymphocytes and granulocytes. Furthermore, the chromatographic pattern on phosphocellulose of histone kinases was also similar in all of these types of cells. These data, obtained from a population with more than 90% of monocytic cells from a patient with AMOL, would indicate that monocytes, cells very difficult to isolate from normal blood, contain the same general types of protein kinases as the rest of WBC.

In summary, chromatographic behavior on phosphocellulose and casein/Sepharose 4B of cytosolic extracts shows that leukocytes, either myeloid, lymphoid, or monocytic cell lines, contain 2 cytosolic cyclic AMP-independent casein kinases. Our data suggest that the increased casein kinase activity in leukemic leukocytes with respect to normal is due only to the amount of casein kinases present but not to the presence of different types of enzymes in these cells.

The physiological role of the casein kinases is not well understood to date, although they phosphorylate key enzymes, such as glycogen synthase, besides casein and phosvitin (11, 12, 18). Moreover, the following data could be relevant to the understanding of the possible role of these kinases in transformed cells: (a) Polyamines are well known activators of casein kinases (1), and polycation metabolism (ornithine decarboxylase activity) is related to tumor growth and DNA content (14); (*b*) casein kinase 2 from rabbit muscle is identical to initiation Factor 2 kinase (5); (*c*) leukocyte nuclei contain analogous kinases (6), and phosphorylation of acidic nonhistone protein is an important mechanism of gene regulation (17, 19); and (*d*) the products of viral gene associated with transformation possess casein kinase activity (14).

All of these lines of evidence suggest that protein phosphorylation catalyzed by cyclic AMP-independent enzymes acting on acidic proteins could be a mechanism involved in malignant transformation.

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