Characterization of Human Leukemia and Burkitt Lymphoma Cells by Their Acidic Nuclear Protein Profiles

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SUMMARY

The content of the acidic nuclear proteins in various types of human leukemia cells, normal lymphocytes, and Burkitt lymphoma cells grown in tissue culture has been examined. Human leukemias could be distinguished from each other and from other tissues by the banding pattern of the acidic nuclear proteins observed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The banding profile of the nuclear protein extracts from leukemia cells obtained from peripheral blood indicated a lack of the higher-molecular-weight nuclear protein species that are present in metabolically active tissue or in dividing cell populations. A correlation could be made between the activity of the leukocyte cell types in nucleic acid and protein synthesis and the content of the higher-molecular-weight acidic nuclear proteins. In general, cells that were more actively engaged in nucleic acid and protein synthesis contained larger amounts of high-molecular-weight proteins. Moreover, when chronic lymphocytic leukemia cells were stimulated to divide in tissue culture by the addition of phytohemagglutinin, the banding profile of the acidic nuclear proteins obtained from these cells was converted to a profile that was very similar to that of cultured Burkitt lymphoma cells. This alteration in protein content occurred prior to the onset of DNA synthesis and at a time when cell viability was 80 to 90% of the original cultured cells. Thus, the events leading to the onset of DNA synthesis and cell division in a previously dormant population of cells were accompanied by a significant shift in the type of acidic nuclear proteins contained in the cells. These findings may relate to the control of gene readout and to the events involved in malignant transformation of mammalian cells.

INTRODUCTION

The acidic (nonhistone) nuclear proteins of various tissues have been characterized by SDS-polyacrylamide gel electrophoresis as a heterogeneous fraction with a tissue-specific distribution (15, 30). Approximately 25 to 30 distinct protein bands, many of them in the higher-molecular-weight regions of the gels, have been identified in the tissues studied. In addition, an increased synthesis of 1 or more of the proteins in this fraction has been shown to precede or accompany activation of the flow of genetic information in tissues exposed to agents capable of effecting enzyme induction. For example, elevated synthesis of specific ANP's has been reported to occur in rat liver after administration of hydrocortisone (21) or phenobarbital (19) and in uterus after estradiol treatment (28, 29). An increase in the synthesis of proteins in the nonhistone fraction has been shown to precede the stimulation of DNA synthesis produced in (a) mouse salivary glands exposed to isoproterenol (25), (b) cultured human diploid fibroblasts after fresh medium has been supplied to stationary phase cells (17), and (c) in liver after partial hepatectomy (16). This evidence suggests that the ANP's play an important role in the control of gene readout in mammalian cells.

In the present study, we have examined the electrophoretic patterns of the ANP fractions obtained from various types of human leukemia cells and have attempted to relate these patterns to gene activity. Additionally, we have studied the changes in the electrophoretic pattern of the ANP fractions that appear to precede or accompany the metabolic changes induced in normal and leukemic lymphocytes by PHA stimulation. The latter cells respond to PHA with an increased thymidine incorporation into DNA that is comparable in magnitude to that which occurs under similar conditions in lymphocytes from normal donors (1), although the time of maximal response is delayed in the leukemic cell cultures (1, 18).

MATERIALS AND METHODS

Donors. Fresh, heparinized peripheral blood (10 ml) was obtained from 7 patients with acute or chronic leukemia. In some instances, multiple samples from 1 patient were obtained. Table 1 indicates the times that blood was drawn and which patients had received chemotherapy. Differential cell counts and evaluation of the maturity of the various types of leukemic cells were performed by hematologists at the Simpson Memorial Institute of the University of Michigan. Fresh, heparinized peripheral blood (500 ml) was also obtained from normal donors.

Isolation of Leukocytes. Leukemic blood samples were aspirated into a 10-ml syringe, which was then mounted vertically in an infusion pump of the type used to administer drugs at a constant rate to experimental animals. The blood samples were allowed to sediment at 37° for 30 min. Following this interval, the leukocyte-rich plasma layer was
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Table 1
Clinical information on sources of human leukemia cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Chemotherapy</th>
<th>Date obtained</th>
<th>Cell count</th>
<th>Differential (%)</th>
<th>Progranulocytes and myelocytes</th>
<th>Metamyelocytes, bands, and segmented neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Other</th>
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<tr>
<td>1</td>
<td>CGL</td>
<td>Cytosine arabinoside, Thio-guanine</td>
<td>6/15, 6/16, 6/17</td>
<td>160,000, 151,000, 111,000</td>
<td>69</td>
<td>15</td>
<td>12</td>
<td>Not done</td>
<td>Not done</td>
<td>1</td>
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<tr>
<td>2</td>
<td>CLL (&quot;mature&quot; lymphocytes)</td>
<td>None</td>
<td>6/21</td>
<td>178,000</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>96</td>
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<td>3</td>
<td>CLL (lymphosarcoma type lymphocytes)</td>
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<td>0</td>
<td>7</td>
<td>91</td>
<td>2</td>
<td>0</td>
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<td>20</td>
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<td>Vincristine, prednisone (begun 8/3)</td>
<td>7/16, 8/31, 9/21</td>
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<td>29</td>
<td>59</td>
<td>2</td>
<td>0</td>
<td>1 eosinophil</td>
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slowly expelled by activating the pump. The remaining blood was allowed to sediment for an additional 30 to 60 min, and the plasma layer that formed was expelled in a similar fashion. The yield of leukocytes obtained in this manner averaged between 85 and 90%. Since the differential cell count showed greater than 90% homogeneity for 1 cell type in most cases shown in Table 1, no further enrichment for a particular cell type was attempted.

Blood samples (500 ml) obtained from normal donors were placed in an 800-ml beaker. Then, 3.5 g of desiccated carbonyl iron powder (General Aniline and Film Corporation, New York, N. Y.) were gently stirred into the blood, and the suspension was thoroughly mixed. The blood was transferred to a 1000-ml graduated cylinder, and the suspension was continuously mixed for 30 min at 37°C. The graduated cylinder was then placed upright, and the iron was allowed to settle to the bottom of the cylinder for 5 min. The suspension was poured off into a liter beaker, and 150 ml of a solution of 3% gelatin in heparinized Eagle’s minimal essential medium were slowly stirred into the blood. The mixture was then divided into 3 equal volumes and placed in 250-ml graduated cylinders. The blood was allowed to stand in these cylinders at room temperature for 60 min. During this time, the erythrocytes and most of the neutrophils (which had ingested iron) settled to the bottom of the cylinder. The lymphocyte-rich plasma was aspirated into 50- or 100-ml syringes, which were then mounted vertically in the infusion pumps described above. The blood was slowly (100 drops/min) expelled from the syringes and passed through a magnetic field into a collecting vessel. The magnetic field removed most of the remaining phagocytic cells (neutrophils and monocytes) and a part of the nonphagocytized iron that remained in suspension. The final suspension of leukocytes contained 90 to 99% lymphocytes. Total recovery of lymphocytes from whole blood by this method was 70 to 80%. Because of the length of time required to isolate lymphocytes from normal blood (3 hr), the blood was always handled under sterile conditions.

Cell pellets obtained by centrifugation of the leukocyte-rich plasma (1000 X g, 5 min, 0°C) were resuspended in 10 volumes of a refrigerated HEPES (Calbiochem, Los Angeles, Calif.) buffer-saline solution (pH 7.2) containing (mM) NaCl, 140; HEPES, 10; KCl, 5.4; dextrose, 5.6; and Na2HPO4, 0.70. The suspension was centrifuged (1000 X g, 5 min, 0°C), and the resulting cell pellet was either quickly frozen at -76°C or utilized directly in the nuclear isolation procedure.

**Culture of Leukemic Lymphocytes and Burkitt Lymphoma (P3J) Cells.** Leukemic lymphocytes were cultured in the presence of PHA under conditions similar to those described by Abell et al. (1). Briefly, the leukocyte-rich plasma was diluted with an equal volume of Eagle’s minimal essential medium containing 15 units of heparin per ml. The cells were collected by centrifugation (250 X g, 30 min, 22°C) and resuspended in a medium containing 50% modified McCoy’s 5A medium with glutamine and 50% Eagle’s basal medium with glutamine (Grand Island Biological Co., Grand Island, N. Y.). The culture medium was supplemented with 10% fetal
bovine serum, streptomycin (50 μg/ml), and penicillin (50 units/ml). The cell concentration of the medium was adjusted to about 1.8 × 10⁶ cells/ml, and PHA-P (Difco Laboratories, Detroit, Mich.) was added at the beginning of the culture period to a concentration of 8 μg/ml. Following the culture period, the cells were collected by centrifugation (1000 × g, 5 min, 0°), washed with ice-cold HEPES buffer-saline, recentrifuged, and frozen as a pellet at −76°.

Burkitt lymphoma (P₃J) cells were maintained in tissue culture in the medium described above (but without antibiotics or PHA). The doubling time of the culture was about 17 hr in the log phase of growth. For purposes of this study, the P₃J cells were collected in the same fashion as described for the leukemic lymphocytes and were either frozen or used fresh for our experiments.

**Isolation of Nuclei.** All procedures were carried out at 0°. Nuclei were isolated by a modification of the procedure described by Wray and Stubblefield (32). Briefly, the cell pellet obtained above was suspended in a buffer containing (mM) 2-methyl-3,4-pentanediol (Eastman Kodak Co., Rochester, N. Y.), 500; CaCl₂, 1.0; and piperazine-N,N'-bis(2-ethanesulfonic acid) monosodium monohydrate (Calbiochem), 0.1. The cells were allowed to stand in this buffer for 5 to 10 min. Shearing was effected with a loose and then a tight pestle in a Dounce homogenizer. The exact number of strokes required to liberate more than 90% of the nuclei varied considerably, so the shearing process was monitored continuously with a phase-contrast microscope. The cells that had been frozen were ruptured with considerably greater ease than were the fresh cells.

Following the shearing step, the crude nuclear suspension was immediately pelleted (1000 × g, 5 min) and washed twice with an isotonic buffer (pH 8.5) containing (mM) sucrose, 250; Tris-HCl, 10, MgCl₂, 5.0; and β-mercaptoethanol, 5.0. The washed nuclei were then suspended in 2.0 ml of 2.2 M sucrose containing 10 mM Tris-HCl (pH 8.5) and 1 mM MgCl₂. The nuclear suspension was layered over 3 to 4 ml of this same sucrose-buffer solution and centrifuged at 34,000 rpm for 60 min in a Spinco SW 50.1 rotor. After centrifugation, a diffuse band was observed approximately one-third of the way down from the top of the centrifuge tube. This band contained intact cells and nuclei contaminated with cytoplasmic tags. Purified nuclei were pelleted at the bottom of the tube. The purified nuclear pellet was washed twice in the isotonic sucrose buffer, and the effectiveness of the isolation procedure was evaluated by phase-contrast microscopy. The nuclei were intact and free of cytoplasmic contamination.

**Extraction of the Nuclear Proteins.** The purified nuclear pellet was suspended in a high-ionic-strength saline buffer (pH 8.5) containing (mM) NaCl, 1,000; Tris-HCl, 50; MgCl₂, 5.0; and β-mercaptoethanol, 5.0. The pellet was suspended by means of vigorous shearing with a tight pestle in a small Dounce homogenizer. The resulting viscous solution was further disrupted sonically with a Bronnwill Sonifier (probe intensity, 32%) for 15 sec and then stirred on ice for 3 hr. The solution was centrifuged at 30,000 × g for 30 min. The supernatant from this step was then dialyzed for 14 hr against a buffer (pH 8.5) containing (mM) NaCl, 140; Tris-HCl, 10, MgCl₂, 5.0; and β-mercaptoethanol, 5.0. The dialysate was centrifuged at 30,000 × g for 30 min. The supernatant contained the ANP fraction. The pellet contained precipitated nucleoprotein, primarily nucleohistone. In some experiments, this pellet was reextracted with 1.8 M NaCl in 0.1 N NaOH, or the nuclear pellet was first extracted with 0.25 N HCl to remove histone and then extracted with 1.8 M NaCl-0.1 N NaOH.

The ratio of nuclear proteins to DNA was determined for many of the cell types. The ratio of total nuclear protein to DNA averaged 3.1 (range, 2.2 to 3.7; n = 12). The ratio of 0.25 N HCl-extractable protein to DNA averaged 2.1 (range, 1.6 to 2.4; n = 9). The ratio of nuclear proteins remaining after HCl extraction to DNA averaged 0.91 (range, 0.53 to 1.4; n = 9). Our data did not permit us to make correlations of nuclear protein content with either the type of cell or with the ANP electrophoretic banding patterns.

**Thymidine Incorporation.** The incorporation of thymidine-methyl-³H into DNA was used to estimate DNA synthesis in the PHA-stimulated leukemic lymphocyte cultures. On consecutive days, duplicate 4-ml aliquots were withdrawn and placed in individual culture flasks. Thymidine-methyl-³H (specific activity, 20 Ci/mmole; New England Nuclear Corp., Boston, Mass.) was added to each flask to a final concentration of 7.5 μCi/ml. The cultures were removed from the incubator 6 hr later and placed on ice. The cell suspension was centrifuged, and the cells were washed in ice-cold HEPES buffer-saline and frozen at −76°. Prior to analysis, cold 10% perchloric acid was added to the cell pellets. After thawing, the pellets were thoroughly dispersed, washed by suspension on a Vortex mixer, and then collected by centrifugation (1000 × g, 7 min). This procedure was repeated 3 times. Following this, the pellets were suspended in 0.5 ml of cold 10% perchloric acid. The nucleic acids were hydrolyzed by heating the suspension to 80° for 20 min, and 0.2-ml aliquots were analyzed for radioactivity in 10 ml of a toluene solution containing Scintisol-GP (Isolab, Inc., Akron, Ohio), 183 ml, and 3.0 g of 2(4-tert-butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole per liter of solution.

**Electrophoresis.** SDS-polyacrylamide gel electrophoresis was performed as previously described (15, 20). The protein samples were dialyzed for 12 to 16 hr against a buffer containing 0.01 M sodium phosphate (pH 7.0), 0.1% SDS, and 0.1% β-mercaptoethanol. Electrophoresis was carried out with a running buffer of 0.1 M sodium phosphate (pH 7.2) containing 0.1% SDS at 5 ma per tube for 5 to 6 hr. Identical patterns were obtained in gels from the same protein samples, which were run at different times. Following electrophoresis, the gels were fixed and stained for 3 to 8 hr in a solution of 0.25% Coomassie brilliant blue in methanol:acetic acid:water (5:1:5). Destaining was accomplished by soaking the gels in a solution containing methanol:acetic acid:water (2:3:35).

**Viability.** Cell viability was estimated by means of erythrosin B dye exclusion (9).

**RESULTS**

**Content of ANP's in Human Leukemia Cells.** Examination of the nonhistone or ANP's (ANP fraction) from various human leukemias by SDS-polyacrylamide gel electrophoresis...
indicated a notable paucity of high-molecular-weight proteins and the majority of proteins in the lower-molecular-weight regions of the gels (Figs. 1 and 2). The banding patterns obtained from these largely nondividing populations of peripheral blood cells were quite different from the banding patterns obtained from rapidly dividing cultured Burkitt lymphoma cells, which contained a heterogeneous population of high-molecular-weight proteins and a comparatively small amount of lower-molecular-weight proteins (Fig. 3, Gel 5). Although all the gels obtained from human leukemic cells (not grown in culture) seemed to be deficient in the higher-molecular-weight proteins, the profile obtained from myeloid leukemias differed from that of the lymphoid leukemias in that some higher-molecular-weight protein bands were detectable in the myeloid cells (Fig. 2). In contrast, the extracts from lymphoid leukemia cells did not contain distinct high-molecular-weight proteins (Figs. 1 and 3). Even when large amounts of protein (e.g., 90 \( \mu \)g) from the lymphoid cells were placed on the gels, only faint bands appeared in the upper half of the gels.

When the gels from various types of lymphoid cells were compared, a correlation between the ANP banding pattern and the “maturity” of the cell type could be made. Proteins extracted from immature leukemic lymphocytes contained species that banded out near the middle of the gels (Fig. 1, Gel 3; Fig. 3, Gels 1 and 2). In contrast, more fully mature leukemic cells of the large lymphocyte type (Fig. 1, Gel 2) and lymphocytes from normal donors (Fig. 4, Gel 1) showed smaller amounts of these proteins.

The ANP banding patterns were reproducible for blood samples obtained from consecutive sampling from the same patient. For example, the ANP patterns obtained from blood drawn on 3 consecutive days from a patient with chronic myelocytic leukemia were identical to that illustrated in Fig. 1, Gel 1. Likewise, the banding pattern remained unchanged over an 8-week period for another patient, although his chemotherapeutic regimen had been changed in the interim between the samplings (Table 1; Fig. 3, Gels 1 and 2).

The nature of the low-molecular-weight proteins observed on the gels of extracts from leukemic cells is not clear. Two of the low-molecular-weight bands from the leukemic lymphocytes (Fig. 1, dotted arrows) showed \( R_F \) values similar to those of the 2 main bands of protein extractable from intact leukemia or Burkitt lymphoma cell nuclei with 0.25 N
Fig. 3. Electrophoretic separation of ANP from the leukemic lymphocytes of Patient 3 and from Burkitt lymphoma cells. Left to right: Gel 1, unstimulated cells, obtained on July 7; Gel 2, unstimulated cells, obtained on August 31; Gel 3, cells obtained on August 2, ANP extracted 148 hr after addition of PHA to the cell culture; Gel 4, cells obtained on September 21, ANP extracted 69 hr after addition of PHA; Gel 5, Burkitt lymphoma cells, ANP extracted from cells in the late-log phase of growth in suspension culture. Twenty-five µg of protein were layered on each gel.

HC1 (gels not shown). This suggests either that these bands represent histone contamination of the ANP extracts or that these are low-molecular-weight nonhistone proteins that are not present in significant quantities in other tissues that have been analyzed. However, these bands were also present in the ANP fraction (extracted with 1.8 M NaCl:0.1 M NaOH) obtained from leukemic lymphocyte nuclei after histones had first been removed by 2 extractions in 0.25 N HC1. Thus, it appears that these bands represent nonhistone proteins.

Content of ANP’s in PHA-stimulated Leukemic Lymphocytes. When leukemic lymphocytes were grown in tissue culture in the presence of PHA, incorporation of thymidine-3H into DNA was increased 100-fold by the 6th day after addition of PHA (Chart 1). At the same time, the nonhistone nucleoprotein profile, as determined by gel electrophoresis, changed from a pattern with bands mainly in the lower half of the gels to a pattern very similar to that obtained from Burkitt lymphoblasts (Fig. 3; Chart 2). A 2nd experiment showed that there was a progressive disappearance of low-molecular-weight proteins accompanied by a progressive appearance of high-molecular-weight proteins after PHA addition (Chart 3). This process had begun at least as early as 22 hr following the addition of PHA and before the onset of increased thymidine incorporation into DNA. The alteration of the banding pattern observed after PHA stimulation cannot be explained on the basis of the loss of cells that contained no high-molecular-weight nuclear proteins and the emergence of cells in the population of lymphocytes that contained high-molecular-weight ANP, since the altered

Chart 1. Incorporation of thymidine-methyl-3H into the DNA of lymphocytes (obtained from Patient 3) that were grown in tissue culture in the presence of PHA. •—•, cells were obtained on August 31; •—•, cells obtained on September 21. Conditions are described in "Materials and Methods." Actual cpm in the samples analyzed were in the range of 1,800 to 250,000.

Chart 2. Electrophoretic separation of ANP (25 µg) obtained from PHA-stimulated leukemic lymphocytes and from late log-phase Burkitt lymphoma cells. Chronic lymphocytic leukemia (CLL) lymphocytes were obtained from Patient 3 on August 2 and cultured with PHA for 148 hr. Gels were scanned at 550 nm in a Gilford Model 2400 recording spectrophotometer with a linear transport attachment. The absorbance tracings are superimposed for comparison.
HOURS AFTER PHA

Chart 3. Electrophoretic separation of nonhistone proteins obtained from leukemic lymphocytes stimulated by various periods of culture with PHA. Cells were obtained from Patient 3 on September 21 and cultured in 3 flasks with equal volumes of media and concentrations of cells. PHA was added and the cultures were terminated after 7, 22, or 69 hr. Twenty-five μg of ANP were layered on each gel. The gels were scanned as described under Chart 2. The absorbance tracings are superimposed for comparison.

banding pattern occurred well before cell division had begun and at a time when cell viability of the original cultured cells was still 80 to 90%. A similar shift in the content of ANP was observed when normal lymphocytes were stimulated in tissue culture by PHA (Fig. 4).

Experiments Utilizing Other Extraction Procedures. In the interpretation of the preceding data, 3 possibilities should be considered. First, it is possible that the differences in the banding patterns of the various cell types are not due to absolute differences in ANP content but rather are a reflection of variation in the extractability of the ANP from chromatin. Second, the banding patterns might reflect differences in proteolytic enzyme activity in the various cells. Third, the banding profiles might reflect actual variation in the content of ANP, which would imply that different species of ANP are synthesized by the various cell types. Experiments were performed to test the 1st 2 possibilities.

In order to determine whether a portion of the nonhistone protein fraction was not extracted by 1.0 M NaCl or was reassociated with chromatin during dialysis from 1.0 M to 0.14 M NaCl, the chromatin pellet obtained after dialysis in 0.14 M salt was reextracted with 1.8 M NaCl in 0.1 N NaOH. The protein extract from this step represents “residual” nuclear proteins, reassociated nonhistone proteins, and histones. Although the proteins extracted in this fashion from Burkitt lymphoblasts contained a considerable number of bands in the high-molecular-weight range, high-molecular-weight species were not observed in extracts from unstimulated leukemic lymphocytes (Fig. 5). This suggests that, in fact, the high-molecular-weight proteins are absent in these latter cells, since back extraction of the chromatin with 1.8 M NaCl-0.1 N NaOH should have dissociated the majority of the chromatin proteins from DNA (23). The fractions extracted in 1.8 M NaCl-0.1 N NaOH from PHA-stimulated lymphocytes also contained a distinct band in the upper third of the gel (Fig. 5, arrows). This band was observed at 69 hr, but not at 7 or 22 hr after the addition of PHA. This protein also appeared in the extracts from Burkitt lymphoma lymphoblasts (Fig. 5) but was only faintly present in the 1.0 M NaCl extracts following dialysis to 0.14 M NaCl (Fig. 3). It may represent a histone, since it showed an RF value similar to that of one of the major bands observed on electrophoresis of nuclear proteins extracted with 0.25 N HCl from leukemic lymphocytes.

In other experiments, nuclei were isolated in the presence of 0.005 M sodium bisulfite, which has been shown to inhibit substantially the proteolytic degradation of nuclear proteins (12, 13). The nuclei were then twice extracted with 0.25 N HCl, followed by 2 extractions with 1.8 M NaCl-0.1 N NaOH. All of these extractions were performed in the presence of 0.005 M sodium bisulfite. The samples from the NaCl-NaOH extracts were pooled and analyzed by SDS-polyacrylamide gel electrophoresis. When the banding patterns obtained from leukemic lymphocytes and Burkitt lymphoma cells were compared after isolation by this procedure, it was again seen that the ANP fraction from the leukemic lymphocytes contained mainly lower-molecular-weight proteins and lacked many of the higher-molecular-weight protein species that were present in the Burkitt lymphoblasts (Fig. 6). Thus, the observed differences in the banding patterns of the ANP from

Fig. 4. Electrophoretic separation of proteins extracted from normal lymphocytes. Left to right: Gel 1, ANP extracted from the peripheral blood lymphocytes of a normal donor; Gel 2, ANP extracted from normal lymphocytes cultured in the presence of PHA for 72 hr.
Fig. 5. Electrophoretic separation of proteins extracted by 1.8 M NaCl:0.1 N NaOH from the nucleoprotein pellet that was precipitated after dialysis of the 1.0 M NaCl nuclear extract to 0.14 M NaCl. Left to right: Gel 1, proteins extracted from unstimulated chronic lymphocytic leukemia cells (obtained from Patient 3 on August 31); Gel 2, proteins extracted from cells stimulated by 147 hr of culture with PHA (cells obtained from Patient 3 on August 31); Gel 3, proteins extracted from cells stimulated by 148 hr of culture with PHA (cells obtained from Patient 3 on August 2); Gel 4, proteins extracted from late-log phase Burkitt lymphoma cells. The protein bands marked by arrows were present after 69 hr of culture with PHA but not after 7 or 22 hr (gels not shown). Twenty-five μg of protein were layered on each gel. The dark-staining bands at the lower third of the gels represent histones.

2. The former cells appear to be more active than the latter in DNA, RNA, and protein synthesis, as estimated by the incorporation of the appropriate radioactively labeled precursors in vitro (6). The observations that the patterns from the myeloid leukemia cells differ from those of the lymphoid cells could perhaps be a diagnostic aid in cases of acute undifferentiated leukemias.

We have also shown that the banding pattern of the nonhistone nuclear protein fraction from leukemic lymphocytes undergoes a “metamorphosis” after PHA stimulation to a pattern similar to that observed in Burkitt lymphoma cells. Moreover, the alteration of the ANP profile precedes the initiation of DNA synthesis induced by PHA. Recently, a similar observation of alterations in ANP banding patterns on SDS-polyacrylamide gels has been made (7). It was reported that a loss of certain low-molecular-weight ANP and an appearance of high-molecular-weight ANP occur during the process of differentiation in the slime mold Physarum polycephalum.

For evaluation of the significance of these observations, the various cell types appear to be due to actual differences in ANP content.

DISCUSSION

We have observed distinct differences in ANP content between various types of human leukemia cells. Moreover, the content of nonhistone proteins of the human leukemia cells contrasts with that of Burkitt lymphoma cells (Figs. 1, 2, and 3) and a variety of other tissues (15, 30). Our data also indicate that the ANP profile from normal lymphocytes is similar to that obtained from chronic lymphocytic leukemia cells that are morphologically like mature lymphocytes. The cells of the myeloid leukemias in this study apparently contain a greater proportion of the high-molecular-weight proteins than do the unstimulated cells of the lymphoid leukemias (Fig.
several mechanism through which the nonhistone nuclear proteins have been suggested to be involved in the regulation of gene readout must be considered. It has been postulated that these proteins could derepress portions of the genome through an interaction with histones (28, 31). There is controversy over whether the addition of the nonhistone protein fraction must precede the association of histones with DNA in an in vitro RNA polymerase assay to be effective in reducing histone inhibition of RNA synthesis (22, 28, 31). Other authors (4), however, have suggested that specific phosphorylation of certain nonhistone protein species could increase the affinity of these species for histone to an extent sufficient to displace already associated histone from DNA. It has also been shown that nuclear nonhistone proteins may specifically bind to purified DNA to stimulate transcription in vitro (30), thus suggesting a positive initiator function for these proteins. This positive control function could also be activated by phosphorylation of the nonhistone nuclear proteins (20, 30). Kleinsmith et al. (5) have demonstrated that the increased RNA synthesis that occurs shortly after PHA introduction into cultures of normal lymphocytes is preceded by an early stimulation of phosphorylation and dephosphorylation of nuclear nonhistone proteins. It has also been reported that the ANP fraction contains multiple protein kinases that apparently can utilize species of the nonhistone nuclear proteins as substrates (27). Thus, the phosphokinase activity of this fraction offers another mechanism through which the regulation of gene transcription could be achieved.

In metabolically active tissue, it is possible that specific nonhistone proteins may be activated to perform regulatory functions by differential phosphorylation and dephosphorylation. However, it is also a probability that, in some cases, an increased synthesis of 1 or more of the nonhistone protein species must take place in order for gene activation to occur (16, 17, 19, 21, 25, 28, 29). Furthermore, synthesis of a rather large number of high-molecular-weight ANP's could be a necessary prerequisite to the activation of a large number of genes, such as might occur when a nondividing population of cells is transformed into a metabolically active, dividing population. Since it has been shown that the acetylation of histones precedes gene activation in many tissues (8, 26), including PHA-stimulated normal lymphocytes (2, 10), histone acetylation may be involved in derepression of the portions of the genome coding for synthesis of nonhistone proteins. The ANP may then be activated by phosphorylation to finely regulate gene readout. An increased synthesis of nonhistone proteins has been shown to accompany histone acetylation in regenerating rat liver (16).

In the preceding discussion, we have suggested that the high-molecular-weight nonhistone nuclear proteins are involved in the stimulation of gene readout. The identity and function of the low-molecular-weight protein species that are present in nonstimulated leukemic cells is as yet unknown. Possibly, these low-molecular-weight proteins are inhibitory to transcription and may become displaced from chromatin by the higher-molecular-weight proteins as gene activation occurs. In support of the idea that certain nonhistone proteins can limit gene readout, Spelsberg et al. (24) have shown that the nonhistone proteins remaining attached to DNA after histones are removed restrict RNA synthesis in an in vitro system by about 25% (compared to the activity of the system with pure DNA as template). It is also possible that the lower-molecular-weight proteins might represent precursors of the high-molecular-weight proteins. If this were the case, lower-molecular-weight ANP's labeled with a radioactive amino acid should appear as high-molecular-weight species after PHA stimulation. We are currently attempting to answer this question.

The possibility that certain of the ANP extracts contain some nuclear membrane proteins cannot be excluded at this point. However, the banding pattern obtained with rat liver nonhistone proteins extracted from nuclei in the same fashion as in the present study compared very well to that obtained with nonhistone proteins extracted from rat liver chromatin (unpublished observations). This suggests that a major portion of the proteins obtained from isolated nuclei are chromatin-associated proteins.

In addition to a possible role for ANP's in the regulation of gene readout, it is likely that this fraction contains enzymes which are involved in DNA and RNA synthesis. For example, we have found that the ANP fraction from Burkitt lymphoma cells contains RNA polymerase activity (unpublished observation). Thus, the new species of ANP which appear in PHA-stimulated lymphocytes prior to the onset of DNA synthesis may contain both "regulator" and "effector" proteins.

Finally, it would be appropriate to consider the relationship of the nonhistone proteins to the pathogenesis of neoplasia. The similarity in the banding pattern of the nonhistone nuclear protein fractions of the PHA-stimulated leukemic lymphocyte and the Burkitt lymphoma cell has already been pointed out. An implication of these findings is that alterations in the content of the ANP, perhaps similar to those alterations observed when a resting population of lymphocytes is transformed to a dividing population by PHA, may be involved in the uncontrolled proliferation of malignant cells. It has been suggested that the uncontrolled proliferation of the malignant cell may be related to a defect in the capacity of the malignant cell to differentiate (11, 14). Gavosto et al. (3) have postulated that acute leukemia arises because of the "development of new lines of blast cells which have lost control over differentiation so that the genetic information governing the synthesis of specific proteins is lost, inhibited, or suppressed." Since certain ANP's may also have a role in differentiation (7), it is possible that an inability to synthesize or utilize "differentiation-specific" ANP's may be part of the basic defect that gives rise to neoplasia. Thus, the process that triggers the transformation of precursor or stem cells into malignant cells may bring about the synthesis of ANP's that are involved in promoting the readout of genes for cell proliferation, but normal differentiation fails to occur because of the inability of the cell to make or utilize the ANP involved in promoting the readout of genes for differentiation.

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REFERENCES


Characterization of Human Leukemia and Burkitt Lymphoma Cells by Their Acidic Nuclear Protein Profiles

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