ABSTRACT

The altered gene expression seen in cancer could relate to differences in nonhistone chromatin proteins between normal and malignant tumor cells. Phenol-soluble nonhistone chromatin proteins were isolated from human normal and leukemic (chronic lymphocytic leukemia) B-cells, as well as long-term cultured human B-lymphocyte cell lines. High-resolution two-dimensional electrophoretic maps identified a group of three nuclear proteins with a molecular weight of 45,000 to 50,000 and an isoelectric range of 4.5 to 4.7, which were associated only with the human leukemic B-cells. Leukemic B-cells and cultured B-cell lines also expressed a variant form of nuclear actin and tubulin.

INTRODUCTION

NHCP\(^{1}\) have been studied extensively, and it is becoming readily apparent that these molecules play an integral role in the structure and function of the eukaryotic genome (for recent reviews, see Refs. 14, 31, 32, 56, and 60). Chromatin proteins stimulate RNA synthesis, induce gene transcription, and are usually associated with active, differentiated, and neoplastic tissues derived from numerous sources (12, 18, 33, 41, 45, 64). Unlike the histones, NHCP are heterogeneous and possess both species and tissue specificity (19, 28, 40, 48, 64). It has been suggested that differences in NHCP derived from normal or tumor cells may be related to altered gene expression (11, 13, 15, 47, 73, 74).

Studies (8, 11, 23, 29, 37, 53–55) of nuclear proteins derived from human leukemic, lymphoma, PHA, concanavalin A activated lymphocytes have shown distinct differences between the normal lymphocytes and their activated or neoplastic counterparts. Polycyclamide gel electrophoretic patterns of NHCP derived from normal lymphocytes were compared with those of various human leukemias, lymphomas, and PHA-activated lymphocytes (69, 70). Each lymphocyte type exhibited a characteristic gel pattern. Qualitative and quantitative changes in the high-molecular-weight protein species were associated with both PHA-stimulated normal lymphocytes and with high-grade neoplastic cells when compared to normal and more "quiescent" leukemic cells (e.g., CLL cells). Seeber et al. (52) recently used one-dimensional SDS gel electrophoresis to compare the phenol-soluble subgroup of chromatin proteins extracted from fresh normal and leukemic human leukocytes. A decrease in phosphorylation and synthesis of these proteins was associated with increased cell differentiation. Wu et al. (72), using 2-dimensional gel electrophoresis (44), also found that some higher-molecular-weight (\(M_\text{r} > 40,000\)) NHCP common to human hematological tumor cells were altered or absent in normal human lymphocytes.

It was of interest, therefore, to take advantage of the high resolution and sensitivity of the O’Farrell (44) technique of 2-dimensional gel electrophoresis to study freshly obtained human normal and untreated leukemic B-cells, as well as transformed cultured B-lymphocytes, to detect aberrations and/or markers common to one or each of these cell types. A group of 3 proteins with a molecular weight of 45,000 to 50,000 and a pI range of 4.5 to 4.7 appeared to characterize certain leukemic cell types. Furthermore, both actin and tubulin were found to be different in the leukemic lymphocytes when compared with the electrophoretic patterns obtained from normal B- or T-cells or transformed cultured human B-lymphocytes.

MATERIALS AND METHODS

Isolation of Peripheral Blood Leukocytes. Buffy coats from acid citrate dextran-treated blood from normal individuals were obtained from the Canadian Red Cross, Calgary, Alberta, Canada. Heparinized peripheral blood from leukemic patients was obtained from the Tom Baker Cancer Centre and the hematology clinics of Calgary hospitals.

Mononuclear cells were isolated by centrifugation over Ficoll-metrizoate according to a procedure supplied by the manufacturer (Lyphophrep; Gallard Schlesinger Chemical Manufacturing Corp.). The mononuclear fractions obtained from patients with CLL that contained greater than 95% leukemic blasts were used directly without further purification.

The T-lymphocytes in the mononuclear fraction obtained from normal buffy coats were isolated by erythrocyte rosette formation with sheep erythrocytes at 4\(^{\circ}\), followed by a second Ficoll/metrizoate centrifugation. Sedimented rosettes were treated with 0.83% Tris/NaCl buffer to lyse the erythrocytes, and the resultant T-lymphocyte-enriched population was stored at –70\(^{\circ}\). The remaining nonsedimented cell population was then depleted of monocytes and macrophages by incubation for 30 min at 37\(^{\circ}\) with a preparation of iron particles (lymphocyte-separating reagent; Technicon, Tarrytown, NY). Following an additional centrifugation over Ficoll/metrizoate, the interface cells (containing greater than 90% lymphocytes with surface immunoglobulin) were stored at –70\(^{\circ}\) for 24 hr.

Cell Lines and Cell Culture. The human B-lymphoblastoid cell lines T5-1 and 6.1.6, established from the peripheral blood of a patient with infectious mononucleosis, were obtained from Dr. D. Pious, Seattle Medical School, Seattle, WA. The B-lymphoblastoid cell lines, NC-37 and Daudi, and the T-cell line, Jurkat, were obtained from Dr. J. Gordon, McGill University, Montreal, Quebec, Canada. The cell lines were maintained without agitation in RPMI-1640 medium supplemented with 15% fetal calf serum and 1.0 mM L-glutamine.

Preparation of Acidic Nuclear Proteins. All procedures were carried out at 4\(^{\circ}\), and all solutions contained 100 mM PMSF.

Freshly isolated cells were washed 3 times and resuspended in 0.25 M sucrose-3 mM CaCl\(_2\)-10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4 (Buffer A). Plasma membranes were disrupted by the addition of 1% Triton X-100. Following centrifugation, the crude nuclear pellet was purified through 2.2 M sucrose-3 mM CaCl\(_2\)-10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4. Integrity of nu-

1 Supported by the Medical Research Council of Canada.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: NHCP, nonhistone chromatin proteins; PHA, phytohemagglutinin; SDS, sodium dodecyl sulfate; CLL, chronic lymphocytic leukemia; PMSF, phenylmethylsulfonyl fluoride.

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clear membranes was ascertained by phase-contrast microscopy. Purified nuclei were washed twice in Buffer A and then again in Buffer A containing 1% Triton X-100. Phenol-soluble nuclear proteins were then extracted according to a modification of the procedure reported by Teng et al. (64). Dialysis buffers all contained 0.02 M glycine and 100 µM PMSF. The final dialysis (in 9 M urea and 5% mercaptoethanol) was performed at room temperature for 12 hr (64).

In Vitro Labeling of Proteins. The ε-amino groups on the lysine residues of the phenol-soluble proteins were labeled by reductive methylation with formaldehyde and sodium borohydride (34). Equivalent numbers of 1 x 10⁶ leukemic cells, 1 x 10⁷ normal B-cells, and 5 x 10⁸ 6.1.6 B-cell line cells, were labeled. Labeled proteins were dialyzed against 9 M urea-0.02 M glycine-5% mercaptoethanol and then concentrated using a Millipore ultrafiltration unit with a molecular weight cut-off of 10,000.

In Vivo Labeling of Cells with [35S]Methionine. Subconfluent 6.1.6 cells were suspended at 1.25 x 10⁶ cells/ml in 20 ml of methionine-free RPMI-1640 medium (Grand Island Biological Co.) and labeled with 5 µCi of L-[35S]methionine/ml (1100 mCi/mmol) (New England Nuclear) for 24 hr at 37°C. Cells were then washed in cold (4°C) RPMI-1640 medium and pelleted, and phenol-soluble nuclear proteins were obtained as above.

Gel Electrophoresis. Two-dimensional gel electrophoresis was carried out according to the method of O'Farrell (44) with the following modifications. Triton X-100 was used instead of Nonidet P-40, and 4% amphotolites of pH ranges 4 to 6, 6 to 8, and 5 to 7 were used in the ratio of 4.5:4.5:1.0. The proteins were focused in the first dimension in the presence of 9.5 M urea for 6800 V hr. Subsequently, the gels were equilibrated for 10 min in SDS sample buffer and then applied to 10% polyacrylamide discontinuous SDS slab gels. Second-dimension electrophoresis was performed for 6 hr at 22.5 ma/gel. Bovine serum albumin, human transferrin, ovalbumin, and soybean trypsin inhibitor were used as protein standards of known mass and isoelectric point in the 4 to 8 pH range. The pH range of the isoelectric focussing gels was determined by the method of Rickwood et al. (48). Following electrophoresis, slab gels were placed into 50% ethanol:10% acetic acid for 1 hr, stained for 18 hr in 0.25% Coomassie brilliant blue in methanol:acetic acid:water (5:1:5), and destained in 10% isopropl alcohol:10% acetic acid. Gels were then processed for fluorography according to the method of Bonner and Laskey (4). The gels were placed on Whatman No. 3 filter paper, sealed with Saran wrap, and dried under vacuum, and the ³⁵S-labeled proteins were detected by autoradiography using Kodak XRP-Royal X-omat film at -70°C. Approximately 325 µg (1 x 10⁶ cpmp) of the 6.1.6 B-cell line protein were analyzed by 2-dimensional gel electrophoresis. Approximately 90 µg (100 to 350 x 10⁴ cpmpm) and 70 µg (100 x 10⁵ cpmp) of protein from the leukemic patients and the normal B-cell, respectively, were also analyzed.

Densitometry Reading of Autoradiographs. The polypeptide spots labeled in Figs. 1 to 7 have been designated as Spots 1, 2, and 3 going from the spot of slowest to most rapid electrophoretic mobility (see best in Fig. 3). Densitometry scanning of these spots was performed by R. P. Tracey (66). Spot density ratios for each of the 3 spots, compared to the actin molecules from the same gel, were determined and tabulated for the normal B-cell and 3 representative leukemic patients' samples.

RESULTS

The patients with CLL were diagnosed on the basis of a lymphocytosis of 10,000/cu mm with predominantly mature small lymphocytes and a consistent clinical picture. In most instances, the leukemic cells were over 70% of the peripheral blood leukocytes. Patients were mostly untreated or on, at most, 10 mg of Prednisone daily at the time of study.

Surface markers of peripheral blood mononuclear cells from these patients were studied serially over 1 to 2 years. All were B-cell leukemias, expressing surface membrane immunoglobulin and DR antigens and forming no rosettes with sheep erythrocytes. Monocyte contamination was extremely low (<3%), since phagocytic cells were removed by carbonyl iron.

Comparison of gels was carried out by superimposition of gel autoradiographs from each patient, using the position of major protein spots as reference points, in addition to molecular weight and isoelectric point determinations. Only reproducible, well-defined spots were compared. Data obtained from 4 human cultured long-term B-cell lines, normal human B-cells, T-cells, and 7 patients with CLL are reviewed in Table 1. Two-dimensional gels are presented for one transformed B-cell line, 6.1.6 (Fig. 2, A and B), normal human B-cells (Fig. 1, A and B), and each of 5 leukemic patients in Figs. 3 to 7. Densitometry scanning of the spots at molecular weights of 45,000 to 50,000, compared to their respective actin polypeptides (Table 2), was performed on the autoradiographs of the normal B-cell and 3 representative patient samples.

Table 1

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Proteins Common to All Cell Types

Actin. The group of 5 to 6 protein spots at a molecular weight of 43,000, with pls ranging from 5.1 to 5.6 (Fig. 1, A and B), was tentatively identified as normal nuclear B-cell actin and was present in varying amounts in all cell types examined (Figs. 1 to 7; Table 1). As tentatively identified in the normal B-cell, actin consisted of at least 5 to 6 prominent forms and was labeled A', α, β, γ, δ, and ε, going from the acidic to more basic polypeptides (Fig. 1B). Similarly, normal T-cell actin consisted of 6 major components (Table 1). A similar pattern was seen in the B-cell line when the proteins were labeled in vitro with sodium borohydride (Fig. 2) or in vivo with [³⁵S]methionine (Fig. 2B). Four major actin polypeptides were detected by Coomassie blue staining (Fig. 2A). Actin derived from CLL cells was variably expressed, usually consisting of only 4 to 5 visible subunits. While β actin was the predominant species in normal B-cells, other species appeared to be more prominent in leukemic cells (Figs. 3, 6, and 7). Furthermore, actin expression varied from

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patient to patient (Figs. 5 to 7). While Patients B (Fig. 3) and S (Fig. 7) each exhibited 4 spots of about equal intensity, Patients M (Fig. 5), H (Fig. 6), and R (Fig. 4, A and B) showed an unequal distribution of labeling intensity. Patient R showed an increased number of actin subunits compared with all the other patients examined (Table 1). The number and relative intensity of the actin components appeared unchanged in Patient R, who was studied on 2 separate occasions (Fig. 4 A and B).

There was insufficient protein in the leukemic gels to be detected by Coomassie blue staining.

Tubulin. The $\alpha$ and $\beta$ subunits of tubulin (Fig. 2; Table 1) were present in all the B-cell lines at a molecular weight of 50,000 to 53,000, and a $\pi$ of 5.0 to 5.1. Both the $\alpha$ tubulin doublet and the slightly more acidic and slightly lower-molecular-weight $\beta$ subunit are seen best in Fig. 2. The $\alpha$ and $\beta$ subunits are present in about a 1:1 ratio. Normal B-cells showed only trace amounts of $\alpha$ and $\beta$ tubulin in approximately a 1:1 ratio (Fig. 1A). In contrast, $\alpha$ tubulin was the predominant subunit in all leukemic maps (Figs. 3 to 7; Table 1), and $\beta$ tubulin was expressed in only minimal amounts. Tubulin appeared to be more prominent (compared to actin) in all leukemic and B-cell lines than in normal B- or T lymphocytes.

Proteins Found Only in Certain Cell Types

A series of 3 protein spots at molecular weights of 45,000 to 50,000 and a $\pi$ of 4.5 to 4.7 appeared only in the leukemic cells examined (indicated by 45 in Figs. 3 to 7 and designated as 1, 2, and 3 in Fig. 3) and not in any of the normal B- or T-cells or B-cell lines examined (Figs. 1 and 2; Table 1).

A group of 3 proteins at molecular weights of 56,000 to 60,000 and a $\pi$ of 5.1 to 5.5, situated just above the tubulin polypeptides (seen best in Fig. 3, labeled 56), appeared to be common to most CLL patients. However, in December (Fig. 4B) and June (Fig. 4A) in Patient R, the most basic and lower-molecular-weight protein of this group (indicated in Fig. 3 by the third arrow situated slightly below and just to the right of the 2 arrows below 56) was present only in minimal amounts. The normal B-lymphocyte also lacked the more basic lower-molecular-weight protein of this group (Fig. 1A).

Densitometry Scanning

Ratios of the $M$, 45,000 to 50,000 ($\pi$s of 4.5 to 4.7) spot densities relative to their respective actins were determined on 3 representative leukemic patient autoradiograms and the normal B-cell autoradiogram (Table 2). The spots at a molecular weight of 45,000 (labeled 45 in Figs. 1A and 3 to 7) were designated as Spots 1, 2, and 3, going from the uppermost to lower arrow (see Fig. 3). The density of spots 1, 2, and 3 in the normal B-lymphocyte represented only trace (1 to 5%) amounts of these proteins compared to the ratios of these proteins in the 3 patients' samples. Spots 1, 2, and 3 appeared in an approximate 2:1:1 ratio, respectively, in the 3 patients studied.

DISCUSSION

Published studies (52, 69, 70, 72, 74) have indicated that normal lymphocytes exhibit distinctive electrophoretic profiles of nonhistone chromatin proteins compared with their activated or neoplastic counterparts. Using high-resolution 2-dimensional SDS:polyacrylamide gel electrophoresis, coupled with high-specific-activity [3H]borohydride labeling of phenol-soluble nuclear proteins, we have also identified several distinct proteins which may differentiate normal and transformed from neoplastic lymphocyte types.

The extraction of phenol-soluble acidic nuclear proteins was first described by Teng et al. (64). In our study, satisfactory resolution of phenol-soluble nuclear proteins was obtained within the molecular weight range of 20,000 to 150,000 and over a pH range of 4.0 and 7.0.

Introduction of a tritium label into protein by reductive methylation was first described by Means and Feeny in 1968 (43). The specificity and limitations of this methodology have been reviewed (3, 26, 71). This technique was chosen to label the phenol-soluble nuclear proteins primarily because it does not alter the immunological, biological, or chemical characteristics of most proteins (17, 61), and it can be performed in the presence of 9 M urea. There is no need to culture the fresh lymphocytes as the nuclear proteins may be extracted and labeled immediately following leukocyte isolation. Therefore, any postsynthetic modifications or alterations of the nuclear proteins would be accomplished prior to the introduction of the tritium label.

Reproducibility of the in vitro sodium borohydride protein-labeling technique and its effects upon the 2-dimensional gel patterns of the phenol-soluble nuclear protein were verified as follows. The Coomassie blue-stained 2-dimensional gel pattern of unlabeled NHCP obtained from the B-cell line, 6.1.6 (Fig. 2A), was compared with the patterns obtained following either in vitro [3H]labeling (Fig. 2) or in vivo [35S]methionine labeling (Fig. 2B). The major protein polypeptides of actin and tubulin were resolved in a similar manner irrespective of the label or staining technique. No significant changes in molecular weight or charge were introduced by the in vitro labeling technique. The minimal quantitative and qualitative changes are probably due to the differing sensitivities of staining versus radiolabeling of proteins. Some higher-molecular-weight proteins ($M$ > 55,000) were not evident in the [35S]methionine gel. These may have been proteins that were present in the sample that was labeled in vitro but were not synthesized during the [35S]methionine-labeling period. It is also possible that numerous proteins undergo postsynthetic modifications (1, 18, 31), which may render them more or less susceptible to reductive [3H]methylation. Comparison of the 2-dimensional gel patterns from the same patient studied on 2 separate occasions (Fig. 4, A and B) show only minor qualitative differences, probably a result of a slight overlap of the gel in Fig. 4B. The patient-to-patient variation (Figs. 3 to 7) is minimal and, as noted in other similar electrophoretic studies of leukemia cells (69, 70, 74), may be related to a differential distribution of
the cells throughout the cell cycle, which has been associated with changes in nuclear protein profiles (42, 59). Protein extraction and electrophoretic methods vary greatly among laboratories, and pH measurements are relatively inaccurate in the presence of 9 M urea (67), so that direct comparison of protein maps is difficult. However, characteristic molecular weights and isoelectric points can be assigned to common proteins.

The polypeptides tentatively identified as actin and tubulin were resolved consistently and were used as reference markers to compare gels of varying patterns. There is strong evidence to support the contention that these marker proteins are in fact actin and tubulin. (a) The molecular weights and isoelectric points for actin obtained by Coomassie blue staining, L-[35S]methionine label, and reductive 3H methylation agree with published values (5, 6, 27, 35, 36). (b) Resolution of the α tubulin doublet and the β tubulin subunit in the B-cell line is especially convincing in view of the work by others (6, 35, 36, 50), who have shown similar electrophoretic resolution of these polypeptides. (c) The relative proportions of tubulin seen here in normal B- and T-cells and transformed and leukemic cells are in agreement with published reports (6, 35, 36, 50).

(d) These proteins appear to be present in the appropriate concentrations relative to other nuclear proteins, as reported by Douvas (24) and others (5, 27, 35, 36) for actin and by Rubin (50) for tubulin.

Actin is known to be a major protein of the nucleus (7, 10, 11, 25, 67) and the only abundant cellular protein at that molecular weight. The 3 major forms of actin we observed in normal B- (Fig. 1B) and T-cells are tentatively labeled as muscle (α) and nonmuscle (β and δ) actins. β and δ actin are found in most if not all eukaryotic cells (see review by Pollard and Weihing, 1974, Ref. 46). α actin is the more acidic subunit of actin and is found only in the cytoplasm of muscle cells (5, 36) and in the nucleus of nonmuscle cells (27), where it may be involved in the contractile function of chromatin condensation. In addition to the above subunits, we resolved several other actin polypeptides. Two of these may correspond to the 2 unstable actin polypeptides, δ and ε reported (25) to be located on the basic end of the electrophoretic map of nerve cell actin. Others (5, 35, 36) have detected an additional A' actin polypeptide on the acidic end of the actin polypeptide (labeled A' in Fig. 1B). The heterogeneity of the actin polypeptides as seen in 2-dimensional gels here and elsewhere may be due to variations in primary structure or to secondary modification. In view of these complexities and of the limitations of electrophoretic resolution, we have made a tentative identification of actin subunits in our maps based on charge characteristics and on the relative intensity of radioactive labeling.

The presence of 4 or more subunits of actin in the B-lymphoblastoid cell lines (Fig. 2, 24 and B; Table 1) as determined by 3 different labeling techniques compares with the finding of Laevitt et al. (36), who reported that additional novel forms of β and δ actin are synthesized by transformed T-leukemic cells (Molt-4), and of Garrels and Gibson (27), who reported 5 different forms of actin in a rat B-35 nerve cell line. The B-cell leukemias also expressed a variant form of actin. The β subunit was not always the most abundant, and in some patients (e.g., Fig. 4), there were increased numbers of actin subunits, much like those seen in the B-cell line. In one patient (Fig. 5), there was an unexpected loss of some subunits. Despite precautions (use of 100 μM PMSF) taken to inhibit proteolysis, increased degradation of the actin polypeptides in this sample cannot be ruled out. Liebes et al. (38) observed differences between myosin Mg²⁺ ATPase activation by CLL and normal B-lymphocyte cytoplasmic actin. They failed to detect electrophoretic and tryptic peptide differences between the normal and leukemic actins but suggest their methodologies may not have been sensitive enough to resolve a possible substitution of nonpolar amino acids into the CLL actin. The amount of actin relative to total phenol-soluble nuclear proteins, as determined by visual comparison of radioiodel intensity in the normal B-cell, was much greater than that in the transformed B-cell line and only slightly greater than that in the leukemic lymphocytes. This finding is in general agreement with others, who also found a reduced percentage of actin in leukemic T-cells compared to normal T-cells (36) and in CLL lymphocytes compared to normal B-lymphocytes (58). This may be relevant to the report of Douvas et al. (25), who observed that a higher concentration of nuclear actin exists in resting rather than proliferating cells. It would appear, therefore, that the leukemic process is associated with synthesis of novel forms or altered proportions of the standard actin subunits. Laevitt et al. (36) speculate that unknown genetic events or mutations which alter actin expression may contribute to the process of malignant transformation.

Tubulin is a M, 52,000 protein with isoelectric points ranging from 4.9 to 5.2 (9, 50). Cytoplasmic tubulin contains at least one α and a β subunit (35, 50). The observed electrophoretic pattern of normal B-cell nuclear tubulin (Fig. 1B) appears similar to that published by others for cytoplasmic tubulin (6, 35) and cell surface tubulin (2, 50), suggesting the 3 are alike. As reported by others (35, 50) for normal B- and T-lymphocytes, we observed only negligible amounts of tubulin in both normal B- and T-cells. These same investigators (2, 50) found a greater abundance of α and β tubulin in transformed T-cells compared to normal T-cells, not unlike that noted in this study, where tubulin was more evident in transformed B-cells and B-leukemic cells, when compared to normal B- or T-cells. It has been suggested (35, 36) that changes in tubulin expression may reflect altered growth rate rather than differences between normal and neoplastic cells per se. However, this argument is inconsistent with our observations that 2 different types of resting B-cells (leukemic and normal) express divergent forms of tubulin subunits, while the rapidly growing B-cell lines express both forms. A recent report by Bravo and Celis (5) indicates that the expression of α and β tubulin varies dramatically during the cell cycle in rapidly dividing cultured cells. Therefore, altered tubulin expression may be a cell cycle-related phenomenon or could be related to stage of cellular differentiation.

A group of 3 acidic proteins (M, 45,000 to 49,000; pl 4.5 to 4.7) common to all CLL cells (Figs. 3 to 7) was not apparent in the 2-dimensional maps of normal B- or T-lymphocytes or 4 B-cell lines. The greater intensity of radioactive label in the most acidic M, 43,000 protein in the leukemic cells is striking. This finding was verified by comparative densitometry scanning of several relevant gels. Visual and/or densitometry data (Table 2) indicated that these proteins were not present in significant amounts in the normal B-lymphocyte. There is not enough evidence to ascertain whether these proteins (M, 45,000 to 49,000; pl 4.5 to 4.7) seen in our study are related to the M, 49,000 protein found by Schmidt et al. (51) in Novikoff hepatoma cells or the M, 47,000 protein hepatic nuclear kinase (65). Nuclear...
proteins with Mr, ~50,000 subunits have also been observed in several other normal, transformed, and malignant tumor cell systems (16, 20, 22, 24, 39, 51, 53, 54, 62, 63, 72, 73). However, the proteins discussed in most of these studies may not be related to the 3 proteins observed in our study. Either the distribution of these proteins in normal or tumor tissue types (22, 24, 39, 53, 54, 62), their molecular weights (16, 63), or their pIs (16, 20) were different enough from those seen in our study to suggest that they are not the same proteins.

This new group of proteins may be represented in the study by Seeber et al. (52) of one-dimensional electrophoretic profiles of phenol-soluble nuclear proteins obtained from patients with CLL. Close study of photographs of the gels shows evidence of protein in the molecular weight range of 45,000 to 50,000, which was absent in normal B-cells, granulocytes, and acute lymphoblastic leukemia cells. Furthermore, distribution of 32P radioactivity in this study (52) indicated that perhaps these proteins are phosphorylated. Using 2-dimensional gel electrophoresis, Wu et al. (72) may have resolved this group of nuclear proteins (Mr, 45,000 to 49,000; pI 4.5 to 4.7) which were present in purified nuclei of cultured fetal cells IMR-90, WI-38, and Hela cells and not evident in normal B-lymphocytes, liver cells, acute myeloblastic leukemia cells, or lymphoma cells. However, since methods of protein extraction and electrophoresis differ between our study and these 2 reports, it is difficult to be certain that these proteins all have the same identity.

The proteins (Mr, 45,000 to 49,000; pI 4.5 to 4.7) seen in our study were probably not artifically generated during sample preparation or electrophoresis. Precautions were taken to avoid proteolytic degradation (100 μM PMSF) and carbamylation (57) (use of 0.02 M glycine) of protein. Deamidation in the presence of base (49) causes a shift of about 0.1 pH unit in the acidic direction which is similar to the approximate 0.08 to 0.1 pH unit difference among Spots 1, 2, and 3. However, deamidation, carbamylation, and carboxymethylation probably do not account for the molecular weight differences (27).

Modifications of nuclear proteins may play a regulatory role in determining the content, function, and electrophoretic profiles of leukemic (70) and other cell types (1, 21, 30). Metabolism, e.g., posttranslational modifications, of nuclear proteins varies between normal and neoplastic cells (2). It is possible that the presence of these 3 proteins (with molecular weights of 45,000 to 49,000) may reflect the altered metabolism of CLL cells. Posttranslational modifications, such as methylation (46, 68), phosphorylation, and acetylation (68), alter the charge and molecular weight of proteins. As the proteins become modified, they migrate more to the acid end of the gel and show concomitant increases in molecular weight. However, as the molecular weight increases, the pH of Spots 3, 2, and 1 becomes more alkaline. It is possible that such protein modifications might render certain proteins more or less susceptible to 3H-reductive methylation and result in unequal methylation of the ε-lysine amino groups, producing different electrophoretic patterns.

Nuclear actin represents about 6% of the total nonhistone protein fraction by weight (10, 25). Densitometry scanning of Spots 1, 2, and 3 (Table 2) indicated that, relative to actin, these proteins represented between 0.67 and 1.4% of the total nonhistone protein fraction. The molecular weight differences, the pIs, and the concentrations (relative to actin) of these 3 proteins suggest that these polypeptides are separate gene products. Clearly, tryptic peptide and amino acid analysis of these proteins following further purification is necessary in order to reveal the nature of their cellular uniqueness, structure, and possibly functional differences.

Takami and Busch (63) and Wu et al. (72) have identified a protein with a molecular weight of 56,000 and a pI of 5.8 that is absent from normal liver cells and lymphocytes but present in tumors and fetal cells. We have also tentatively identified this protein as the lower and more basic part of a group of proteins present in some leukemias (seen best in Fig. 3 as the group of 3 spots above tubulin). It is present in transformed B-cell lines but absent in normal B-cells. It is of interest that this protein showed minimal expression at one time in Patient R (Fig. 4A) and was reexpressed at a later date (Fig. 4B). Its presence in the immature rapidly growing transformed B-cell lines (Fig. 2), coupled with its presence and variable expression in the CLL cells (Figs. 3 to 7), is consistent with its suggested role as an oncotelial or growth related protein (72).

High-resolution 2-dimensional gel electrophoresis has provided a technique suitable for analysis of a complex group of lymphocyte phenol-soluble nuclear proteins. This method was extended to detect proteins labeled in vitro by reductive 3H methylation. A group of 3 proteins (Mr, 45,000 to 50,000; pI 4.5 to 4.7) was found only in CLL cells and not in normal B- or T-lymphocytes or transformed B-cell lines. The increased sensitivity of the above techniques heightens the prospect of finding and correlating nuclear proteins with specific cellular functions in differentiation and neoplasia.

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Fig. 1. Autoradiograph of 2-dimensional gel electrophoretic separation of phenol-soluble NHCP from human normal B-lymphocytes. Fig. 1A is a complete gel. A, actin; Tα and Tβ, α and β subunits of tubulin; 45, relative location of M, 45,000 to 50,000, pI 4.5 to 4.7, proteins seen only in leukemic maps but not normal B-cells; 56, M, 56,000 to 60,000 proteins, pI 5.1 to 5.5, present in normal B-cells as a dyad only. Fig. 1B, magnified portion to show details of nuclear actin spots. Subunits are labeled as A', α, β', β, γ, δ, and ε.
Fig. 2. Autoradiograph of 2-dimensional gel electrophoretic separation of phenol-soluble NHCP from long-term, cultured, human B-lymphoblastoid cell line 6.1.6 (Fig. 2A). A, actin; Tα and Tβ, tubulin subunits; 45, relative location of M, 45,000 to 50,000, pI 4.5 to 4.7, seen only in leukemic cells. Fig. 2B is a photograph of a Coomassie blue-stained 2-dimensional gel of phenol-soluble NHCP from the same human B-lymphoblastoid cell line 6.1.6. A, actin; α and β, tubulin subunits. Fig. 2C is an autoradiograph of 2-dimensional gel electrophoretic separation of in vivo [35S]methionine-labeled phenol-soluble NHCP from the cell line 6.1.6. A, actin; α and β, tubulin subunits.
Fig. 3. Autoradiograph of 2-dimensional gel electrophoretic separation of phenol-soluble NHCP from leukemic cells from CLL Patient B. A, actin; α and β, subunits of tubulin; 45, M, 45,000 to 50,000 proteins, pH 4.5 to 4.7, seen only in leukemic cells. These spots are designated as 1, 2, and 3, going from the top to the bottom of the autoradiograph. 56, M, 56,000 to 60,000 proteins, pH 5.1 to 5.5, present as a triad in leukemic cells (small arrows).

Fig. 4. Autoradiograph of 2-dimensional gel electrophoretic separation of phenol-soluble NHCP from leukemic cells of CLL Patient R. Fig. 4A is from June 1980; Fig. 4B is from December 1980. A, actin; α and β, subunits of tubulin; 45, M, 45,000 to 50,000 proteins, pH 4.5 to 4.7, seen only in leukemic cells.
Fig. 5. Autoradiograph of 2-dimensional gel electrophoretic separation of phenol-soluble NHCP from leukemic cells of CLL Patient M. A, actin; α and β, subunits of tubulin; 45, M, 45,000 to 50,000 proteins, pl 4.5 to 4.7, seen only in leukemic cells.

Fig. 6. Autoradiograph of 2-dimensional gel electrophoretic separation of phenol-soluble NHCP from leukemic cells of CLL Patient H. A, actin; α and β, tubulin subunits; 45, location of M, 45,000 to 50,000 proteins, pl 4.5 to 4.7, seen only in leukemic cells.

Fig. 7. Autoradiograph of 2-dimensional gel electrophoretic separation of phenol-soluble NHCP from leukemic cells from CLL Patient S. A, actin; α and β, tubulin subunits; 45, location of M, 45,000 to 50,000 proteins, pl 4.5 to 4.7, seen only in leukemic cells.
Phenol-soluble Nonhistone Chromatin Proteins in Chronic Lymphocytic Leukemia

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