Nonhistone Protein Antigen Profiles of Five Leukemic Cell Lines Reflect the Extent of Myeloid Differentiation

By A. Goldberger, G. Brewer, L. S. Hnilica, and R. C. Briggs

The human leukemic cell lines, K562, KG-1, and HL-60, and the blast subclones, KG-1a and HL-60 blast, were utilized to relate differences in nonhistone protein antigens to stages of myeloid cell differentiation. Chromatin proteins were separated on SDS-polyacrylamide gels, transferred electrophoretically to nitrocellulose sheets, and visualized by the peroxidase-antiperoxidase method of Sternberger. Screening with antisera raised against total and dehistonized chromatin and a nuclear extract from these cells revealed quantitative as well as qualitative differences between the cell lines. A decrease in antigen content seemed to parallel progressive stages of myeloid cell development. The results indicate that a number of chromosomal protein antigens are lost or modified during differentiation. An antigen(s) of approximately 55,000 molecular weight was found in HL-60 chromatin, but was not present in its less differentiated subclone or in the other lines representative of earlier stage cells. Upon the induction of HL-60 cells to mature to end stages with 4 M retinoic acid, a significant increase in the mol wt 55,000 activity was seen. This antigen was detected only with antisera against HL-60 total chromatin and granulocyte nuclei, and it was found only in normal mature granulocytes and in the later stage cells of the HL-60 culture. Thus, the antigen appears to be associated with a differentiated myeloid function.

The regulation of transcription is a fundamental concept in the study of differentiation. A program of specific and ordered gene expression leads from multipotent precursor cells to mature cells in which transcriptional activity is restricted to that required for the specialized function of the cell. Nonhistone chromatin proteins are among the factors thought to be involved in regulating gene transcription. An overall scheme could require many levels of interactions for determining specificity, such as those between proteins and DNA, those between different proteins, and those between proteins and higher orders of DNA structure. Each of these interactions would require components present in varying amounts, and some would therefore be easier to detect and characterize. Weintraub's group has elucidated such an involvement for high mobility group (HMG) proteins 14 and 17, which are present in chromatin in relatively large amounts and yet are implicated in the structure of actively transcribed genes. Thus, additional elements appear necessary for the occurrence of this unusual chromatin structure related to transcription (DNase I sensitivity) at certain locations in the genome. The variation in the appearance of these sites between cell types is central to the process of cellular differentiation. Corresponding changes in chromatin proteins may be observed if they are involved in these events, either as a cause or as a consequence.

Hematopoiesis represents one of the better characterized systems available for studies of differentiation. The establishment of several human leukemic cell lines blocked at different stages of myeloid development provides an in vitro model system for comparison of the stages of myelopoiesis. Fukuda et al., used K562, KG-1 and its blast subclone KG-1a, and HL-60 to analyze changes in cell surface structures associated with granulocyte differentiation.

The fact that nonhistone protein-DNA complexes can elicit specific antisera allows the use of sensitive immunochemical techniques to probe the nonhistone protein complement of cells. Nonhistone protein antigen changes were observed in neoplasia and during carcinogenesis, as well as in differentiation.

In the present study, we have used immunochemical staining of proteins transferred from SDS-polyacrylamide gels to nitrocellulose sheets to screen for differences in the nonhistone protein antigens of five leukemic blood cell lines: K562, KG-1a, KG-1, HL-60 blast, and HL-60. As each line represents a different stage of myeloid development, it was hoped that there would be a correlation between the stage of differentiation and the nuclear antigen profile. A decrease in antigen content seemed to parallel the progression of differentiation, and qualitative changes were detected that may be associated with mature granulocytic functions. The results showed that a number of nonhistone protein antigens were lost or modified, while others appeared during the course of myeloid differentiation.

From the Departments of Biochemistry and Pathology and the A. B. Hancock, Jr., Memorial Laboratory of the Vanderbilt University Cancer Center, Vanderbilt University, School of Medicine, Nashville, TN.

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Address reprint requests to Dr. Robert C. Briggs, Department of Biochemistry, Vanderbilt University, School of Medicine, Nashville, TN 37232.
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MATERIALS AND METHODS

Cells

All the cell lines used in these experiments were maintained in suspension in RPMI 1640 medium with 15% heat-inactivated horse serum (GIBCO, Grand Island, NY) and gentamycin (40 μg/ml). K562 cells were grown in media supplemented with 1 ml of sterile 1 N HCl as well as 4 mM L-glutamine and 2 mM sodium pyruvate (GIBCO). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO2 in air and were resuspended at 105/ml when cell densities reached approximately 106/ml. HL-60 cell line was provided by Dr. R. C. Gallo, and the HL-60 blast cells came from Dr. Pierre Major. KG-I and KG-1a cells were obtained from Dr. H. P. Koeffler. Normal human granulocytes were isolated from fresh citrate-treated whole blood. The human origin of all the cell lines was demonstrated through restriction endonuclease mapping of the β-globin system, hCG, and hGH genes, using cloned human cDNA probes. A karyotype analysis of Giemsa-trypsin banded chromosomes from the HL-60 BII line further documented its human origin.

Induction of Differentiation

HL-60 cells at 105/ml were treated with 4 μM retinoic acid (RA). Nitroblue tetrazolium (NBT) reduction was used to assess the extent of differentiation. Cells were sedimented, resuspended to a final concentration of 2 x 105/ml in PBS (10 mM phosphate-buffered saline, pH 7.2), and incubated for 20 min at 37°C with an equal volume of 0.2% NBT in PBS containing 200 ng tetradecanoylphorbol acetate/ml. After pelleting and suspending in 0.1 ml PBS, at least 300 cells on a wet mount were counted to determine the percentage of cells capable of reducing NBT. Morphological differentiation was followed on Wright-stained Cytospin slide preparations. Large-scale cultures were harvested on the fifth day of induction for chromatin preparation and were 75%-95% NBT-positive.

Chromatin Preparation

After sedimentation of cells from the culture medium, all subsequent operations were performed at 4°C in solutions containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF). Cells were washed twice in 0.25 M sucrose, 5 mM MgCl2, and then lysed hypotonically in 30 volumes of 10 mM Tris-HCl, pH 7.5, 1 mM MgCl2 (Tris/Mg). The nuclear suspension was gently homogenized and then mixed 1:1 with 2.2 M sucrose, 5 mM MgCl2. Nuclei were homogenized vigorously until they appeared to be free of contamination by phase-contrast microscopy. The nuclei were collected by centrifugation through a pad of clean 2.2 M sucrose, 5 mM MgCl2 at 100,000 g for 1 hr. This ensured that only clean nuclei were used for chromatin isolation, as nuclei that have cytoplasmic tags do not sediment through the dense pad and are trapped at the interface of the two sucrose solutions. The nuclei pellets were washed in 0.25 M sucrose, 10 mM Tris-HCl, pH 7.5, 0.5% Triton X100, and chromatin prepared according to Spelsberg et al. Briefly, the pellets were homogenized 3 times in 0.08 M NaCl, 0.02 M EDTA, pH 6.3, with the volume of solution decreasing by 1/3 for each successive wash. The extent of breakage of the nuclei was followed by phase microscopy. Chromatin was then resuspended in 1/100 SSC (0.14 M NaCl, 0.014 M sodium citrate, pH 7.0) and collected by centrifugation. Samples were suspended in deionized water, and DNA concentration was determined by OD260 at 5 μg/ml of DNA. Each sample was adjusted to 1 mg/ml as DNA. Isolated granulocytes were fixed in 5 volumes of 3.7% formaldehyde in acetic acid for 1 min. After dilution with 10 volumes of PBS, the fixed cells were washed in PBS until the supernatant cleared and were then processed through chromatin preparation, omitting the Triton wash.

Cell Fractionations

One-fourth of the suspension of clean nuclei in Tris/Mg was used for cell fractionation. The nuclei were counted on a hemocytometer and the suspension was then centrifuged at 2,000 g for 10 min. This was repeated and the pellets combined for the nuclear fraction (P1). Centrifugation of the supernatant at 10,000 g for 10 min sedimented the mitochondria (P2), and the membrane fraction (P3) was pelleted 100,000 g for 1 hr. The remaining supernatant was considered to be the cytosol.

The three pellets from each fractionation were resuspended in a volume of deionized water, as determined by the following formula. As the nuclei suspension had been counted, the approximate number of nuclei in P1 was known. Using the value 7 x 1012 g DNA/nucleus, the amount of DNA in P1 was calculated, with a correction of 1.5 for the highly aneuploid K562 cells. Each pellet was then resuspended in the volume of dH2O that made P1 about 1 mg/ml as DNA, which not only equalized the fractions to each other, but also to the corresponding fraction from the other cell lines.

Localization of Antigenically Reactive Chromatin Proteins

Chromatin proteins were separated on sodium dodecylsulfate polyacrylamide slab gels by the method of Laemmli, except that a 7.5% acrylamide separating gel was used. Samples of chromatin and pellets were digested with DNase I (Worthington, Freehold, NJ) for 1 hr at 37°C and solubilized in sample buffer with Pyronin Y as the tracking dye, while cytosol was solubilized directly. Twenty-five micrograms of each chromatin (or the equivalent amount of cell fractions) was loaded into each well. Gels were stained with Coomassie brilliant blue and destained for visualization.

Unstained gels were electrophoretically transferred to nitrocellulose sheets, as described by Towbin. Proteins bound to the nitrocellulose were stained with amido black or were detected immunocytochemically by the peroxidase-antiperoxidase technique (PAP) of Sternberger, as adapted in our laboratory.

Cytospin preparations of cells from 5-day control or retinoic-acid-treated cultures were fixed for 1 min with 3.7% formaldehyde in ethanol. Slides were washed in PBS following this and each subsequent treatment. The cells were permeabilized by incubation in 0.1% saponin in PBS for 30 min, and the slides were then incubated in antibody monospecific for 55K (see below) for 48 hr at 4°C in a sealed Coplin jar. The "double PAP" technique, involving repeating the treatment of the slides with goat anti-rabbit gamma globulin (diluted 1:40 in 3% BSA, 10% calf serum, PBS) (University of Texas System Cancer Center, Veterinary Resources Division) for 1 hr at room temperature followed by PAP (1:200 in the same solution) for 1 hr, was used to intensify the staining reaction. The slides were then immersed in 0.3 mg/ml 3,3'-diaminobenzidine tetrahydrochloride, 0.005% H2O2, 0.05 M Tris-HCl, pH 7.5, to visualize the reaction.

Antisera

Several preparations were used as antigens to elicit heterogeneous antisera. These included total chromatin, and chromatin dehistonized using the method of Spelsberg et al. Chromatin was suspended in 2.5 M NaCl, 5 M urea, 50 mM sodium succinate, pH 5.0, to a final concentration of 0.17 mg/ml (as DNA), stirred at 4°C for 2 hr, and centrifuged at 100,000 g for 48 hr to pellet the dehistonized chromatin. The chromatin fraction of K562 nuclei solubilized by DNase I digestion after treatment of the cells with 30 μM hemin.
for 1 day, as well as nuclei isolated from human neutrophilic
granulocytes fixed in formalin/acetone, also served as antigens.
New Zealand male white rabbits received from 0.1 to 1 mg of
antigen. For the first 2 injections, antigen was emulsified in an equal
volume of Freund's complete adjuvant, whereas the second two doses
were emulsified in incomplete adjuvant. Antigen was administered
at multiple intradermal sites as well as toe pads. On the fifth week,
100 μg of antigen mixed with saline was given intravenously.
The animals were bled 1 wk later, serum was collected from the blood
and stored at −20°C.

Monospecific antisera against the mol wt 55,000 antigen was
prepared by extensive absorption of the heterospecific antiserum
raised to fixed granulocyte nuclei with total chromatin from the
HL-60 blast line, which does not contain this antigen but does share
most antigens with HL-60. Chromatin used for the absorptions was
quantitated in the SSC suspension and aliquoted so that pellets with
known amounts of chromatin were obtained upon centrifugation.
These pellets could be resuspended directly into antiserum diluted
1:12.5 in 3% BSA, 10% calf serum in PBS to a final concentration of
1 mg/ml, without causing further dilution. The antiserum was
absorbed on ice for 2 hr, 4 hr, and overnight with sedimentation of
chromatin (3,100 g, 20 min) between each step, and absorption was
repeated overnight as necessary. Absorbed antiserum was tested on
immunotransfers of HL-60 chromatin prior to use without dilution
for immunocytochemistry.

RESULTS

Differences in Nonhistone Chromatin Antigens
Among K562, KG-1, and HL-60 Cell Lines

Immunohistochemical localization of nonhistone protein
antigens with heterogeneous antisera revealed differences between the cell lines (Fig. 1). HL-60 chromatin
appeared to have a reduced amount of antigenically reactive nonhistone proteins when compared with
K562 chromatin using antisera raised to K562 total chromatin (Fig. 1A). KG-1 was intermediate in antigen content, in that it retained some antigens in
common with K562, which were reduced in HL-60, whereas others were reduced in both KG-1 and HL-60 compared to K562. Changes due merely to variations in chromatin preparations were eliminated by running several different samples of chromatin from each cell line. It can be seen that the differences between the three cell lines were reproducible.

When an identical set of samples was transferred to nitrocellulose and stained for protein with amido black, the differences in total protein content between the cell lines were not as great as the changes in antigenically reactive proteins (Fig. 1B). HL-60 chromatin proteins overall had a slightly decreased intensity of staining, indicating that the level of chromosomal proteins present in this cell line relative to DNA is reduced compared to the other cell lines.

Screening of other sera raised against chromatins or nuclear fractions from the three cell lines gave similar results. When antisera to HL-60 total (Fig. 2A) and dehistonized (Fig. 2B) chromatin were used, more reactive proteins were localized in K562 and KG-1 chromatins than in HL-60, the homologous chromatin. This seems to indicate that many of the changes in nonhistone protein profiles among the cell lines were quantitative rather than qualitative in nature.

Localization with antiserum to fixed granulocyte nuclei gave the most definite qualitative differences. These cells are the normal, terminally differentiated counterparts of the myeloid leukemic cell lines. Figure 2C shows that several new antigens were detected in HL-60 chromatin that were not present in K562 or KG-1 chromatin, in particular, an intensely staining band with a molecular weight of about 55,000 (arrow) (55K). This same antigen was also detected by antisera

Fig. 1. Differences of nonhistone protein antigens
among K562, KG-1, and HL-60 cells. (A) Immunotransfer
staining reaction of antiserum raised against K562 total
chromatin (1:50) with total chromatin preparations
from K562 (lanes 1 and 2), KG-1 (lanes 3 and 4), and
HL-60 (lanes 5–7). (B) Amido black staining for protein
of an identical transfer. Each lane represents a different
preparation of chromatin and contains an equivalent
amount of chromatin as DNA.
A 1 2 3 4 5 6 7
B 1 2 3 4 5 6 7
C 1 2 3 4 5 6 7

Fig. 2. Detection of antigens by antisera to HL-60 chromatin and mature granulocyte nuclei. Immunotransfer staining reaction of antisera to (A) HL-60 total chromatin (1:25), (B) HL-60 dehistonized chromatin (1:25), and (C) fixed granulocyte nuclei (1:50). Chromatin preparations are K562 (lanes 1 and 2), KG-1 (lanes 3 and 4), and HL-60 (lanes 5–7).

to HL-60 total chromatin (Fig. 2A, arrow), but not by that raised against HL-60 dehistonized chromatin (Fig. 2B). A band at this mobility was not seen on gels of the chromatins stained with Coomassie blue, indicating that it is not a major protein in chromatin.

Cellular Distribution of Antigens

While the differences in antigen content between the cell lines could have reflected real changes in nuclear antigens, they could also have been the result of antigens shifting to other subcellular compartments. Therefore, K562, KG-1, and HL-60 cells were fractionated and the fractions analyzed by immunotransfer staining with the antisera used previously (Fig. 3). The examples shown here indicate that many nuclear proteins (or antigenic determinants) were not actually restricted to the nucleus, but were found throughout the cell. However, the general distribution of antigens seen in chromatin was retained in the other fractions, as K562 fractions had the most antigens and HL-60 fractions the least (Fig. 3, B and E). Thus, the total antigen content of HL-60 cells appeared to be reduced relative to K562 and KG-1, instead of the nuclear proteins moving to other areas of the cell.

The 55K antigen, which was detected in HL-60 chromatin with antisera to granulocyte nuclei (Fig. 2C) and to HL-60 total chromatin (Fig. 2A), appears in all fractions of HL-60 cells to some extent when stained with the antisera to HL-60 total chromatin (data not shown), although more of this antigen was present in the nuclear and chromatin fractions. The antiserum to granulocyte nuclei (Fig. 3F) revealed the presence of this antigen only in chromatin and in the nuclear pellet. The antigen was not localized in fractions of the other cell lines with either antiserum (Fig. 3C), further suggesting that the activities in this region are unique to granulocytes and HL-60 cells.

Comparison of the immunocytochemically stained transfers to one stained for total protein with amido black (Fig. 3, A and D) indicated that the distribution of proteins in the various cellular fractions reflected that of the chromatin. As with the chromatin, the differences in amounts of protein in any fraction between the three cell lines were not as great as the differences detected immunologically.

Nonhistone Antigen Profiles of Blast Subclones of KG-1 and HL-60

K562, KG-1, and HL-60 cells have previously been utilized as a model of myeloid differentiation to study other cellular aspects such as cell surface markers. However, the changes in nonhistone chromosomal proteins between the three cell lines could conceivably have been due to the genetic differences between the
Fig. 3. Cellular fractionations of K562 and HL-60 cells. (A–C) K562 cell fractions. (D–F) HL-60 cell fractions. (A and D) Amido black staining for protein. (B and E) Staining with antisera to K562 total chromatin (1:100). (C and F) Staining with antisera to fixed granulocyte nuclei. Lanes in all transfers represent (1) total chromatin, (2) nuclear fraction—P1, (3) mitochondria—P2, (4) microsomal fraction—P3 and (5) cytosol.

cells. Each line originated from cells of different leukemia patients, and the three vary in both chromosome number and chromosome abnormalities. To try to rule out this possibility, blast subclones of KG-13 and HL-604 were obtained.

Separation and localization of the nonhistone proteins of these two cell lines with the same antisera used previously gave results that seemed to support a relationship between the stage of myeloid development of the cells and their nonhistone antigen profile (Fig. 4A).
KG-1a was very similar to KG-1, although some antisera showed slight quantitative enhancements of a few antigens in KG-1a chromatin. However, HL-60 blast appeared to be the link between the early myeloid cells (K562, KG-1a, KG-1) and HL-60, which at the promyelocyte stage is the most mature. There were a number of quantitative differences between HL-60 and its blast subclone, including an increased staining intensity of antigens throughout the molecular weight range of the gels. Again, amido black staining of the proteins transferred to nitrocellulose sheets (Fig. 4B) or Coomassie blue staining of the SDS-polyacrylamide gels (Fig. 5) indicate much less variation in total protein than in antigenic content.

A significant qualitative change appeared when chromatin proteins from all five cell lines were visualized with the antiserum to granulocyte nuclei. The prominent 55K antigen, which was present only in HL-60 (Fig. 2, A and C), was not present in its blast subclone. Further studies using more than one preparation of HL-60 blast and HL-60 chromatin (Fig. 6, A and B) showed that this result was reproducible. The antiserum against granulocyte nuclei (Fig. 6B) also did not detect the two lower molecular weight antigens in HL-60 blast chromatin, which appeared along with the 55K antigen in HL-60 chromatin. Several high molecular weight antigens were enhanced in HL-60 blast relative to HL-60 chromatin. Only a faint shadow of the 55K antigen was seen in HL-60 blast chromatin with antiserum to HL-60 total chromatin (Fig. 6A). The blast HL-60 chromatin does not show evidence of the 55K antigen with the antiserum to granulocyte nuclei, suggesting the presence of more than one antigen or determinant in this region.

Effects of the Retinoic Acid Induction of HL-60 on Nuclear Antigens

Retinoic acid induction of HL-60 maturation down the granulocytic pathway was used to further correlate the antigenic differences seen between the cell lines to myeloid differentiation. Matched cultures of control and induced cells were harvested the same day, and the nuclei were processed to chromatin at the same time to avoid artifacts due to preparation. Two sets of such matched samples were studied to determine the reproducibility of the results. Localization of the nonhistone protein antigens of these samples on nitrocellulose sheets with several heterogeneous antisera, including that against K562 total chromatin, indicated only a few changes (data not shown). Some high molecular
weight antigens were reproducibly reduced in intensity in chromatin from RA-treated cells. An antigen with molecular weight of about 50,000 increased in intensity in the induced cells.

The staining intensity of the 55K antigen was enhanced by RA in the two different chromatin samples prepared as detected with both the antiserum against fixed granulocyte nuclei and that against HL-60 total chromatin (data not shown). In addition, the antigen has been found in all eight specimens of normal peripheral blood granulocytes examined (data not shown).

Immunocytochemical techniques were used to determine which cells in HL-60 cultures contained 55K and to establish its intracellular location in situ. Extensive absorption of the antiserum to fixed granulocyte nuclei with HL-60 blast chromatin yielded an antiserum monospecific for 55K. As the HL-60 blast cells share most antigens with HL-60 except 55K (Fig. 6B), this absorption removed all other activities. When the absorbed antiserum was tested on HL-60 chromatin, 55K was the major activity remaining (data not shown).

Nuclei of later stage cells in the control culture appeared to give a stronger immunocytochemical staining reaction (Fig. 7A, arrows). In retinoic-acid-treated cultures (Fig. 7B), nearly all the cells had a more intense reaction than cells in the control cultures, and the mature cells had the strongest reactions. Slides incubated without antibody showed no reaction (Fig. 7C). A nuclear localization of the reaction was always seen (Fig. 7, A and B), supporting the results of the cellular fractionations (Fig. 3F).

**DISCUSSION**

The results suggest a correlation between the nuclear antigen profile of these leukemic cell lines and the stage of myeloid development that each is believed to represent. Several groups have characterized these cell lines with respect to their stage of myeloid development. Most of the differences in nuclear antigens seem to be quantitative, as antisera raised against HL-60 chromatin detected more antigens in chroma-
Fig. 7. Immunocytochemical localization of 55K in HL-60 and RA-treated HL-60 cells. (A and C) Control HL-60 cells. (B) HL-60 cells treated for 5 days with 4 μM RA. Slides in A and B were treated with monospecific antiserum to 55K. (C) No antiserum. Control and RA-treated cultures were 10% and 95% mature, respectively, as measured by NBT reduction. Neither induced cells incubated without antiserum nor cell lines negative for 55K on immunotransfere incubated with monospecific antiserum to 55K showed staining.

tins isolated from the less mature cell lines than in the homologous HL-60 chromatin. The overall amount of nonhistone protein is generally a reflection of the proliferation and metabolic state of the cell, and nonproliferating cells normally have much less nonhistone protein than cells that are rapidly dividing or than those having an active metabolism. Because all five cell lines used in this study actively divide at about the same rate, the changes noted here probably did not reflect differences in the metabolic rates of the cells. The progressive decrease in protein and antigen composition is consistent with the very low level of nonhistone proteins, relative to DNA, in the mature granulocytes, as observed previously.9

The antigen staining patterns of the blast subclones of KG-1 and HL-60 support the observations based on K562, KG-1, and HL-60. That KG-1 and KG-1a showed few antigenic differences was not unexpected, as they represent closely related stages of development. KG-1 cultures are characteristic of the earliest stages of myeloid development, myeloblasts, and promyelocytes, so an undifferentiated blast form of these cells is not far removed from the parent cell line. In contrast, there is a more obvious difference between the undifferentiated HL-60 blast line and HL-60 promyelocytes, especially as 5%–10% of HL-60 cells can spontaneously differentiate in culture beyond the promyelocyte stage.19 Thus, it is not surprising that the differences in nonhistone antigens between the two lines were more noticeable. The HL-60 blast line seemed to bridge the gap between HL-60 and the early myeloid lines, in that some antisera stained HL-60 blast chromatin with a pattern very similar to the early lines (K562, KG-1a, KG-1), whereas other antisera revealed an antigen pattern more intermediate between the early lines and HL-60.

We have included K562 in this study as a multipotential precursor cell,20–22 although others have identified this line as an erythroid precursor.23–25 There is a precedent for this use of K562 in the present type of study.7

The cell fractionations indicated that the decrease in antigen content seen with HL-60 was not simply the result of increased translocation of proteins out of the nucleus, for the antigens lost from the chromatin and crude nuclear fractions were not regained in any other fraction of the cell. As a cell fractionation represents the total cellular contents, any question of quantitative recovery of antigens in chromatin can be resolved. For example, the 55K antigen, which was undetectable in K562 chromatin, was also not detected in any of the other K562 fractions, showing that its loss from the chromatin was not just due to differences in recovery.

It is apparent that nuclear antigens are not an exclusive class, and either share antigenic determinants with other cellular proteins or are present in all cellular compartments. The overall reduction of antigens in HL-60 may be linked to its more mature stage of development, as well as to the unique ability of 5%–10% of the cells to further differentiate spontaneously in culture. The differences in distribution of the 55K antigen in HL-60 cells detected by the antiserum to granulocyte nuclei or to HL-60 total chromatin support the idea of more than one antigenic determinant in this region, as was seen with HL-60 blast chromatin. The significance of the distributions of these determinants is unknown.

In contrast to the inverse relationship between the overall nuclear antigen profile and the myeloid maturity of these cell lines, the appearance of some specific antigenic differences may be linked to mature granulocytic functions. The 55K antigen was only detected by
antisera to granulocyte nuclei or HL-60 chromatin and was only very slightly represented in the undifferentiated subclone of HL-60. Eight specimens of normal granulocytes contained this activity in nuclear preparations. In addition, a monospecific antiserum to 55K detected the antigen in mainly the more mature cells of control HL-60 cultures, whereas almost all cells of RA-treated, differentiated cultures reacted strongly with this antiserum. These results support the contention that the nonhistone proteins reflect the differentiation state of the cell. The fact that antiseras to dehistonized HL-60 chromatin did not detect the 55K antigen seems to indicate that it may be a relatively basic protein or one loosely associated with chromatin and thus is removed by dehistonization. One possible interpretation might be that this antigen is associated with nuclear events related to mature granulocyte activities. This may be related to the observation of the spontaneous maturation of HL-60 in culture, as well as to the inducibility of maturation by DMSO26 and retinoic acid.10 Antiseras specific for nuclear antigens of late stage myeloid cells have previously been reported.27-29 However, there is no information on possible similarities between these antigens and those detected in this study.

That we were able to detect much larger differences in nuclear antigens between these cell lines than would have been supposed from their protein profiles is due to the sensitivity and specificity of the immunochromatographic method employed. The cause of these changes in immunologic reactivity is presently unknown, although protein modifications may be involved. Further studies will be directed at elucidating the role of these nonhistone protein antigens in myeloid cell differentiation.

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