Journal of Cellular Biochemistry 37:21–36 (1988)
Altered Glycosylation in Tumor Cells 69–84

Analysis of Cell Surface Glycoprotein Changes Related to Hematopoietic Differentiation

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A high-resolution technique has been used to study differentiation-related and leukemia-associated glycoproteins. Cells are labeled with the membrane-impermeable probe sulfo-N-hydroxysuccinimidyl-biotin. Nonionic detergent extracts are subjected to affinity chromatography on a number of immobilized lectins and after polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) and western transfer, the biotin-labeled glycoproteins are visualized by using avidin-horseradish peroxidase and 4-chloronaphthol. With the aid of the lectins concanavalin A, *Dolichos biflouros* agglutinin, *Lens culinaris* hemagglutinin, peanut agglutinin, pokeweed mitogen, *Ricinus communus* agglutinin I, soybean agglutinin, *Ulex europeus* agglutinin I (UEA), and wheat germ agglutinin, each purifies different glycoprotein subsets from the same cell type. Mature cells of distinct hematopoietic lineages differ considerably in their cell surface glycoprotein patterns.

This technique was used to analyze the glycoproteins of human leukemia cells before and after the induction of differentiation. K562 cells differentiated along different lineages after treatment with phorbol 12-myristate 13-acetate, sodium butyrate, dimethyl sulfoxide, or hemin. Limited specific alterations were observed with a number of lectins when K562 erythroleukemia cells were induced to differentiate. Among these, a number of bands were identified that were either lost or appeared after induction of differentiation with all four agents. In contrast, the glycoproteins bound by UEA were drastically diminished after induction of differentiation, and the remaining UEA-bound glycoproteins bore little resemblance to those of the cells before treatment. This high-resolution technique may be useful as a general method for the examination of cell surface glycoprotein differences. Once specific glycoprotein alterations are detected, lectin affinity chromatography and SDS-PAGE allow purification of antigens for the production of monoclonal antibodies.

Key words: leukemia, hematopoiesis, lectins, western transfer, differentiation

A limited number of methods exist that allow analysis of cell surface proteins and glycoproteins. These include lactoperoxidase-catalyzed radioiodination followed by autoradiography [1], selective sodium periodate oxidation of sialic acid residues followed by reduction with Na $B(^3H)_4$ and fluorography [2], and oxidation of terminal galactose

Received June 3, 1987; revised and accepted September 17, 1987.

or N-acetyl-galactosamine residues using galactose oxidase with or without neuraminidase digestion followed by reduction with Na $B(^3H)_4$ and fluorography [3].

An alternative approach to studying cellular glycoproteins involves the use of lectins. Lectins have been used to affinity purify glycoproteins followed by analysis in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [4]. Analysis of glycoproteins in gels or on western transfers has been performed by using lectins labeled either directly or indirectly with radioisotopes followed by autoradiography or fluorography [5–10] or with enzymes followed by visualization with chromogenic substrates that yield insoluble products [11–15].

We have previously used the western enzyme-linked lectin assay to analyze differentiation-related glycoproteins of the human hematopoietic system [15]. We have now developed a technique that restricts this analysis to cell surface components and have used it to analyze normal and leukemic hematopoietic cells. The development has allowed us to identify cell surface glycoproteins on K562 erythroleukemia cells which are altered after treatments with agents that induce differentiation.

MATERIALS AND METHODS

Cell Isolation

Peripheral blood cells were obtained from platelet donors; leukemia cells were obtained from patients by cell pheresis; and bone marrow cells were obtained from hematologically normal donors, with consent in accordance with institutional and federal guidelines.

Normal elements of peripheral blood were isolated as outlined in Figure 1. Platelets were directly isolated by using pheresis, and the associated cellular fraction was further separated by forming a buffy coat, separating neutrophils on a Ficoll-Hypaque density gradient, and separating monocytes from lymphocytes by elutriation [17] and T cells from the remainder of the lymphocytes by rosetting with 2-aminoethylisothiouronium bromide hydrochloride (AET)-treated sheep erythrocytes [17]. Each fraction was judged to be greater than 90% pure by morphologic examination of Wright's-stained cells.

Promyelocytes, myelocytes, and metamyelocytes were isolated from the peripheral blood cells of a patient with chronic myelogenous leukemia (CML) in chronic phase by using discontinuous Percoll gradient interphases of 0/40, 40/50, and 50/60% Percoll, respectively. These cells were greater than 80% pure by morphologic examination. Immature myeloid blast cells were purified by a combination of centrifugal elutriation [16] and discontinuous Percoll gradients [17].

Induction of Differentiation

Continuous human leukemia cell lines and fresh leukemia cells were induced to differentiate in culture and were examined before and after differentiation. K562 erythroleukemia cells were induced to erythroid maturation with hemin, neutrophilic differentiation with butyrate, megakaryocytic maturation with dimethylsulfoxide (DMSO; Sigma Chemical Co., St. Louis, MO), and monocytic differentiation with phorbol 12-myristate 13-acetate (PMA; Sigma), essentially as described by Sutherland et al. [18]. K562 cells were grown in supplemented Dulbecco's modified essential medium containing 2% rabbit serum [19] and were treated with 1.25×10^{-5} M hemin (Sigma) for 3 days, 15 mM butyrate for 4 days, 1.2% DMSO for 3 days, or 10^{-9} M PMA for 1 day. HL-60 promyelocytic leukemia cells were cultured in RPMI 1640 medium con-

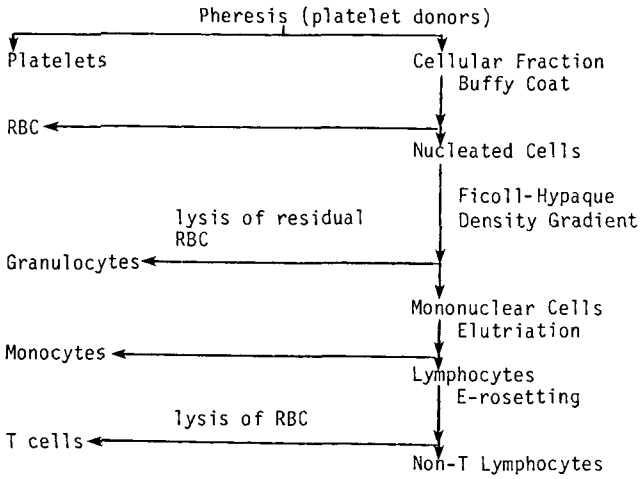

Fig. 1. Schematic diagram of cell isolation procedures. Formed elements of peripheral blood were isolated by pheresis to yield a platelet fraction and a cellular fraction. The cellular fraction was centrifuged to form a buffy coat and a pellet. After removal of the buffy coat, the pellet yielded purified erythrocytes. The buffy coat cells were fractionated on Ficoll-Hypaque density gradient to yield an interface fraction and a pellet. Treatment of the pellet cells with 0.83% NH_4Cl to lyse the residual erythrocytes afforded purified granulocytes. Centrifuged elutriation fractionated the mononuclear cells into a lymphocyte fraction and a purified monocyte fraction. The lymphocyte fraction was separated into T-enriched and T-depleted cells by rosette formation with AET-treated erythrocytes and separation of the rosettes on the gradient (Ficoll-Hypaque).

taining 10% fetal bovine serum (FBS) and were induced to granulocytic maturation [20] by using 1.2% DMSO for 5 days or to macrophage differentiation [21] by using 5×10^{-8} M PMA for 4 days.

Freshly isolated cells from patients with acute myelogenous leukemia (AML 1–5) or chronic myelogenous leukemia in blast crisis (BC-1) were treated with 1.6×10^{-9} M PMA for 20 hr or with 1.2% DMSO for 3 days in RPMI with 10% FBS.

Differentiation was analyzed by using morphology, cytochemistry, and phenotype analysis with monoclonal antibodies. Reduction of nitroblue tetrazolium (Sigma) as a measure of myeloid differentiation was assayed as described by Sutherland et al. [18]. Nonspecific esterase was assayed as a measure of monocytic differentiation using an α-naphthyl acetate esterase kit (Sigma), and neutrophilic granules were visualized with a Sudan black staining kit (Sigma). Hemoglobin was visualized by staining cytocentrifuged cells in 0.2% benzidine dihydrochloride in 10% acetic acid and 0.3% hydrogen peroxide for 10 min at room temperature.

For phenotype analysis, aliquots of 5×10^5 cells were stained with monoclonal antibodies L-243 (HLA-DR; American Type Culture Collection, Rockville, MD), My4, My7, My8, My9, Mol (Coulter Electronics, Hialeah, FL), and PM-81 (Le^x; from Dr. Ed Ball, Dartmouth Medical School), followed by fluorescein-isothiocyanate-goat anti-mouse immunoglobulin (FITC-GAM) and analysis by flow cytometry on a Coulter Epics C. CF-1-secreting hybridoma cells were obtained from Centocor, Inc. (Malvern, PA). We have found that CF-1 reacts broadly with freshly isolated acute leukemia cells and weakly with monocytes (Yong and Reading, manuscript in preparation).

Surface Labeling, Cell Extracts, and Lectin-Affinity Chromatography

Cells were washed three times in 150 mM NaCl, 5 mM sodium phosphate, pH 7.4, (phosphate-buffered saline-PBS), and labeled with 0.5 mM sulfo-N-hydroxysuccinimidylbiotin (S-NHS-B) at 3×10^7 cells/ml in PBS for 30 min on ice. The cells were washed twice in PBS and extracted in 0.5% Triton X-100, 0.5 M mannitol, 5 mM $CaCl_2$ 0.02% NaN_3, 100 μM phenylmethylsulfonyl fluoride (PMSF) at 10^8 cells/ml for 20 min on ice. The nuclei were removed by centrifugation for 3 min at 13,000g, and 100-μl aliquots were added to 100 μg of each lectin immobilized on agarose (Sigma). The immobilized lectins used and their sugar specificities are listed in Table 1. After incubation overnight at 4°C, the beads were washed three times with PBS, boiled in SDS sample buffer, electropheresed in 5–15% gradient slab gels, and transferred to nitrocellulose paper, as previously described [15]. Although preincubation with the specific hapten sugars inhibited binding of the glycoproteins to the immobilized lectins, sugar elution failed to quantitatively release the bound membrane glycoproteins, a finding that agrees with previous studies [22].

Staining Western Transfers

The western transfers were incubated in 1% bovine serum albumin (BSA), 1% polyvinylpyrrolidone (PVP) in PBS for 30 min at room temperature to block nonspecific protein binding. Avidin-horseradish peroxidase (avidin-HRP) was added at 1μg/ml for 30 min at room temperature. The sheets were washed 10 times with 0.05% Triton X-100 and incubated with hydrogen peroxide and 4-chloro-1-naphthol (4-CN) as previously described [15].

TABLE I. Lectins Used For Affinity Isolation of Cellular Glycoproteins and Their Carbohydrate Specificity

Lectin	Abbreviation	Specificity
Concanavalin A	ConA	M⟍ M$^{\beta}$— ⟋M
Dolichos biflorus agglutinin	DBA	A$^{\alpha}$— (anti-A^1) G$^{\alpha}$—
Lens culinaris hemagglutinin	LCH	M⟍ F ⟍M-N-N-ASN ⟋M
Lotus tetragonolobus agglutinin	LTA	F$^{\alpha}$— anti-O(H)
Peanut agglutinin	PNA	A- G$^{\beta}$⟋ (anti-T)
Pokeweed mitogen	PWM	Poly-N-acetyl-lactosamine
Ricinus communis agglutinin	RCA_1	G$^{\beta}$—
Soybean agglutinin	SBA	A$^{\alpha}$— G$^{\alpha}$—
Ulex europaeus agglutinin	UEA_1	F$^{\alpha}$— anti-O(H)
Wheat germ agglutinin	WGA	N$^{\beta}$ . . . N$^{\beta}$N-ASN and S-

M, mannose; A, N-acetylgalactosamine; G, galactose; N, N-acetylglucosamine; F, fucose; S, sialic acids; and the bonds indicate positions of linkage [27].

RESULTS

Induction of Differentiation

Differentiation of K562, HL-60, and five samples of freshly isolated leukemia cells was induced along the monocytic pathway by using PMA as previously observed [18]. Cells flattened, adhered to plastic, and displayed cytoplasmic vacuoles, ruffled membranes, and a macrophagelike appearance. K562, HL-60, and AML-1 cells were negative for nonspecific esterase (NSE) prior to treatment with PMA but became positive after treatment. K562 and AML-1 cells also became positive for nitro blue tetrazolium reduction after treatment with PMA. Inhibition of cell growth of K562 and AML-1 was apparent after 24 hr, but HL-60 was relatively resistant to growth inhibition by PMA. Nuclear maturation was evident in PMA treated HL-60 and AML-1 cells and to a lesser extent in K562 cells.

DMSO treatment induced granulocytic differentiation in HL-60 and AML-1 cells [20] but not in K562 cells. DMSO inhibited the growth of K562 cells, however, and resulted in extensive membrane blebbing. It has previously been reported that DMSO induces megakaryocytic differentiation in K562 cells with the appearance of 5′nucleotidase [18]. Nuclear maturation to the stage of metamyelocytes was evident in both HL-60 and AML-1 after DMSO treatment. Limited granulocytic differentiation was induced in K562 cells with sodium butyrate [18] as evidenced by sudan black neutrophilic granule formation. Hemin had a profound effect on K562 cells. Their proliferation ceased and their morphology changed to that of more mature erythroid cells, with the formation of rare orthochromatic normoblasts and a few erythrocytes. The cells had a positive benzidine reaction, and, in fact, the hemoglobin production was so marked that the cell pellets were red.

The surface phenotypes of the cells before and after treatment with differentiation-inducing agents were studied by flow cytometry with monoclonal antibodies. As seen in Figure 2, PMA induced the expression of monocytic markers My4, My8, Mo1, and CF-1. Treatment of AML-1 cells with DMSO increased the granulocyte marker PM-81 with little effect on the monocytic marker Mo1. Although these treatments had profound effects on several of the markers, the changes were not entirely restricted to the appropriate lineages (Table II). The monocytic markers Mo1, My4, and My8 were increased in HL-60, K562, and AML cells after almost all treatments, the exception being hemin treatment of K562 cells and DMSO treatment of AML-1 cells. The immature myeloid marker My9 increased in treated HL-60 and K562 cells, except for hemin treatment. The myeloid marker My7 was increased in most cases, the exception being hemin treatment of K562 cells and DMSO treatment of AML-1 cells. There were no major changes in HLA-DR expression with any of the treatments (data not shown). CF-1 was increased after PMA treatment of BC-1 and AML-1 cells (data not shown), and the intensity of staining was greatly increased on K562 cells after PMA treatment (Fig. 2).

Analysis of Cell Surface Glycoproteins

Sulfo-derivatives of biotin have been shown to be membrane-impermeable probes because of the negative charge of the sulfate group [23]. When erythrocytes were treated and analyzed directly, hemoglobin was not labeled, indicating that under the conditions used, internal proteins do not come into contact with the S-NHS-B (data not shown). Preincubation of the immobilized lectins with the appropriate sugar haptens blocked binding of the glycoproteins, and preincubation of the avidin-HRP with 2 mM biotin

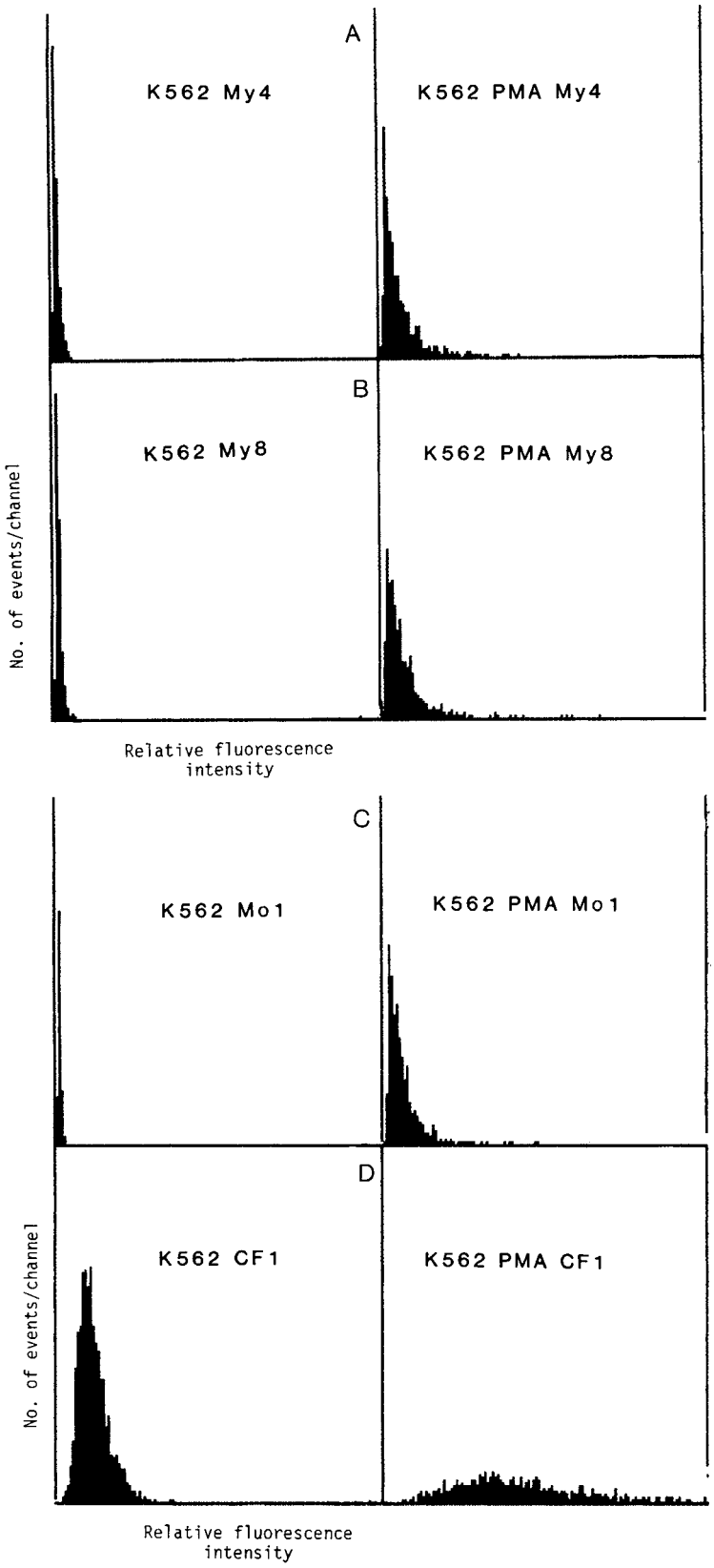
A
K562 My4
K562 PMA My4
B
K562 My8
K562 PMA My8
No. of events/channel
Relative fluorescence intensity
C
K562 Mo1
K562 PMA Mo1
D
K562 CF1
K562 PMA CF1
No. of events/channel
Relative fluorescence intensity

Figure 2

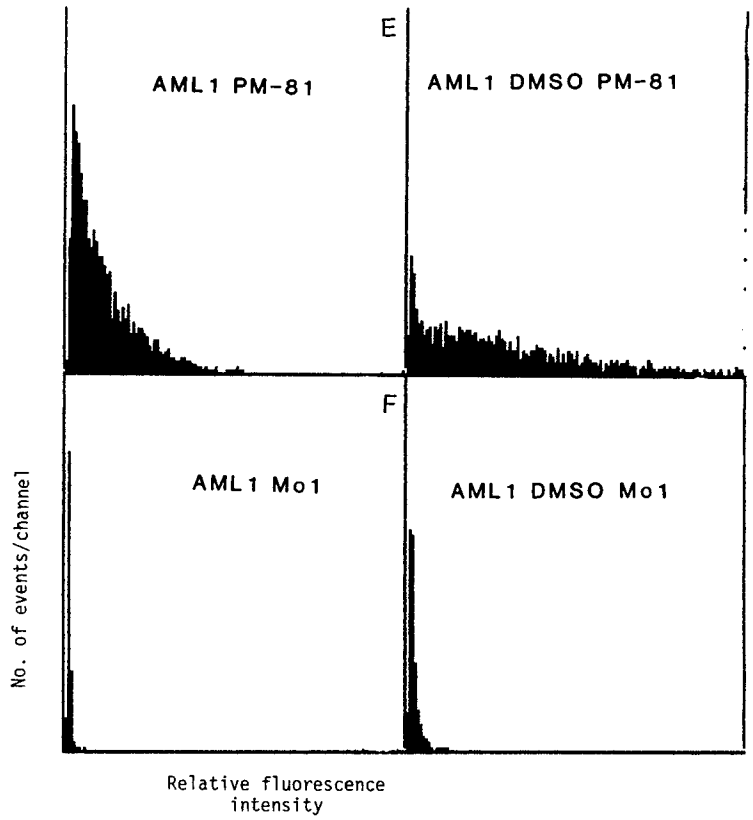

Fig. 2. Fluorescence histograms of K562 and AML-1 cells before and after induction of differentiation. **A:**K562 cells stained with My4 before and after treatment with PMA. **B:** K562 cells stained with My8 before and after treatment with PMA. **C:** K562 cells stained with Mol before and after treatment with PMA. **D:** K562 cells stained with CF-1 before and after treatment with PMA. **E:** AML-1 cells stained with PM-81 before and after DMSO treatment. **F:** AML-1 cells stained with Mo1 before and after DMSO treatment.

blocked staining (data not shown). The use of this cell surface probe allowed comparative analysis of glycoproteins from various hematopoietic cells types. In Figure 3, erythrocytes, promyelocytes, myelocytes, metamyelocytes, non-T lymphocytes, T lymphocytes, platelets, and monocytes were labeled with S-NHS-B, the cells were extracted with nonionic detergent, and the glycoproteins bound by DBA were isolated. These glycoproteins were separated by SDS-PAGE and transferred to nitrocellulose, and the transfers were stained with avidin-HRP and 4-CN to visualize only the surface glycoproteins bound by DBA. Each cell type displays different glycoproteins, indicating that they change as a function of differentiation. Although the T and non-T lymphocytes appear similar, there are characteristic differences such as the band indicated at "a" in lane 6, which is present in T lymphocytes but not non-T lymphocytes. The glycoproteins bound by DBA in maturing granulocytes show a dramatic decrease from promyelocytes to myelocytes to metamyelocytes, and, in fact, mature neutrophils did not show any glycoproteins (data not shown). Patterns of different glycoproteins associated with different hematopoietic elements were observed with all lectins tested, although different subsets

TABLE II. Surface Phenotype of Leukemia Cells Before and After Induction of Differentiation*

	Mo1	My4	My7	My8	My9	PM-81
HL-60	8% (25)	54% (23)	23% (19)	37% (23)	55% (26)	5% (2)
PMA	19% (47)	60% (58)	45% (46)	58% (56)	69% (61)	7% (37)
DMSO	32% (41)	86% (58)	54% (42)	75% (53)	83% (58)	11% (35)
K562	2% (9)	20% (11)	5% (10)	18% (11)	15% (10)	97% (73)
PMA	33% (45)	35% (47)	17% (34)	39% (48)	37% (40)	98% (208)
Butyrate	27% (19)	37% (20)	29% (17)	48% (25)	48% (18)	99% (215)
DMSO	23% (20)	43% (21)	33% (19)	42% (21)	38% (20)	81% (38)
Hemin	1% (14)	8% (15)	4% (14)	8% (15)	6% (14)	97% (150)
AML-1	24% (13)	9% (14)	35% (8)	23% (9)	91% (10)	83% (38)
PMA	49% (22)	38% (20)	91% (59)	50% (22)	61% (18)	79% (64)
DMSO	28% (25)	21% (33)	41% (22)	43% (20)	88% (23)	90% (94)
BC-1	7% (12)	2% (12)	10% (10)	2% (13)	3% (10)	68% (29)
PMA	13% (6)	5% (5)	56% (6)	4% (5)	3% (5)	60% (10)

*Numbers in Tables are % positive cells and mean channel of fluorescence intensity.

were observed with each lectin used. In Figure 4 for instance, extracts of S-NHS-B surface-labeled monocytes were affinity purified on various lectins, and the surface glycoproteins bound were visualized with avidin-HRP and 4-CN. Each lectin visualized different subsets of the monocyte surface glycoproteins. Major monocyte glycoproteins appeared at M_r 170 kDa, 145 kDa, 120 kDa, and 87 kDa, but there were as many as 30 individual bands visible in the WGA transfer, and the majority of these were distinct from the 21 bands visible in the Con A transfer.

K562 cells were surface labeled with biotin, and the glycoproteins were affinity purified on various lectins. The lanes from the individual gels were of differing length so that a direct photograph was not applicable for comparison, but the bands were analyzed for their molecular weight (MW) from a linear plot of log MW vs migration for the MW standards on each gel, and the bands were scored for intensity as heavy, medium, or faint. This information was compiled into Figure 5. RCA bound 11 surface glycoproteins; 33 bands were obtained with PWM, 43 with SBA, 22 with WGA, 46 with UEA, 45 with ConA, 52 with DBA, and 7 with PNA. It is apparent from Figure 5 that none of the lectins detected the same subset of surface glycoproteins in K562 cells.

After induction of differentiation with PMA, butyrate, DMSO, or hemin, the glycoprotein profiles from K562 cells bound by each lectin were changed. Two types of differences were observed: (1) alteration in the staining of specific glycoproteins and (2) alterations in the overall staining after purification on a given lectin. In general, a loss of RCA-bound glycoprotein was observed with induction of differentiation. When larger numbers of differentiated cells were processed in an attempt to visualize changes in the remaining RCA-bound glycoproteins, some specific alterations were observed. As seen in Table III, a decrease in the 120-kDa band was apparent after all four treatments, as were increases in the bands at 150 and 125 kDa. Numerous other minor changes were apparent in the RCA-bound surface glycoprotein profiles.

There was an increase in the WGA-bound surface glycoproteins after treatment with PMA, DMSO, and hemin, but not with butyrate. No WGA-bound glycoproteins were identified which showed a consistent increase or decrease after treatment with all

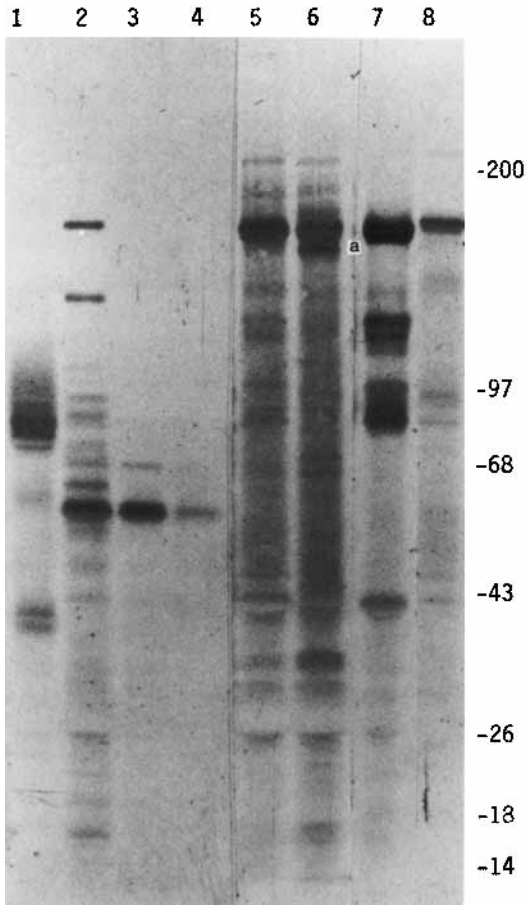

Fig. 3. Extracts of various cell types surface labeled with biotin, affinity purified on DBA, and stained with avidin peroxidase, hydrogen peroxide, and 4-chloronaphthol. **Lane 1**, purified erythrocytes. **Lane 2**, enriched promyelocytes. **Lane 3**, enriched myelocytes. **Lane 4**, enriched metamyelocytes. **Lane 5**, enriched B lymphocytes. **Lane 6**, purified T lymphocytes. **Lane 7**, purified platelets. **Lane 8**, purified monocytes. Standards are myosin heavy chain (M_r 200,000), phosphorylase B (M_r 97,400), bovine serum albumin (M_r 68,000), ovalbumin (M_r 43,000), α-chymotrypsinogen (M_r25,700), β-lactoglobulin (M_r 18,400), and lysozyme (M_r 14,300). A prominent band present in T but not non-T lymphocytes is indicated by the letter "a" in lane 6.

four agents. After treatment of K562 cells with various agents, there was an increase in the PWM-bound surface glycoproteins, which was the most obvious after PMA and hemin treatment. No consistent decreases were observed after treatment with all four agents, but there was an increase in the 18-kDa band in all four cases. There was a decrease in the DBA-bound K562 cell surface glycoproteins after treatment with butyrate and DMSO but not with PMA. After treatment, there was a decrease in the bands at 200, 180, 175, and 155 and an increase in a band at 39 kDa with all four agents. A number of smaller differences were also apparent. With Con A-bound glycoproteins, decreases were observed with all four treatments in bands at 180, 165, 150, 97, and 39 kDa. No bands were consistently increased.

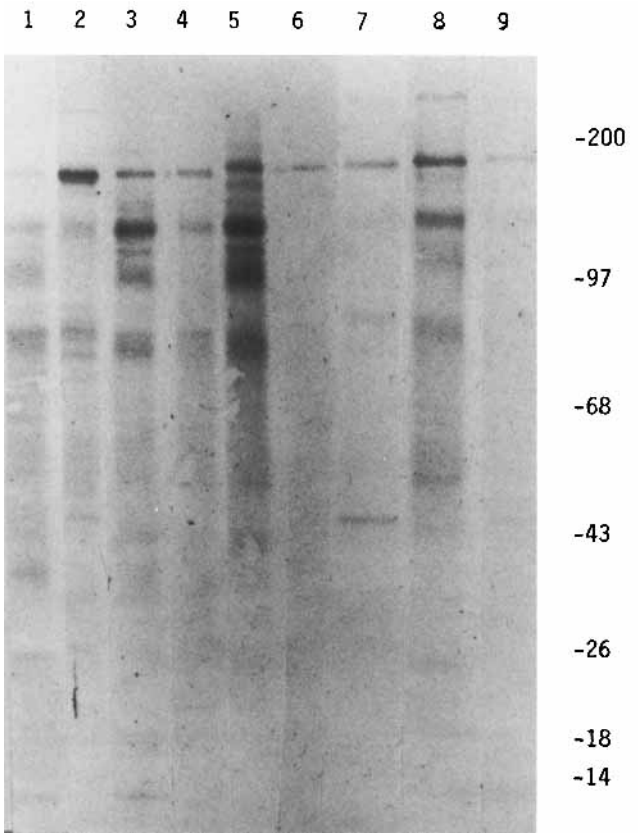

Fig. 4. Extracts of purified monocytes, surface labeled with biotin, affinity purified on various lectins, and stained as in Figure 3. **Lane 1**, Con A. **Lane 2**, DBA. **Lane 3**, LCH. **Lane 4**, PWM. **Lane 5**, RCA. **Lane 6**, PNA. **Lane 7**, SBA. **Lane 8**, WGA, **Lane 9**, UEA. Molecular weight standards as described in Figure 3. See Table I for names of lectins.

The greatest change observed with the induction of differentiation was with UEA surface glycoproteins. In Figure 6, equivalent numbers of cells were processed in lanes 1–6. After treatment of K562 cells with PMA, butyrate, or DMSO and treatment of HL-60 with PMA (or DMSO, data not shown) there was a striking decrease in UEA surface glycoproteins. In lanes 7–11 varying numbers of cells were processed in order to compare the remaining UEA surface glycoproteins of treated K562 cells with untreated cells. Surprisingly, the remaining bands bore little resemblance to those of untreated K562 cells. Hemin-treated K562 cells retained UEA-bound surface glycoproteins, but comparison of the molecular weights of the bands indicated that of the 46 distinct bands of untreated K562 cells, 36 decreased or disappeared and 34 bands increased or appeared after hemin treatment. A band at 44 kDa was lost upon treatment with all four agents, and bands at 90, 66, and 42 kDa were increased.

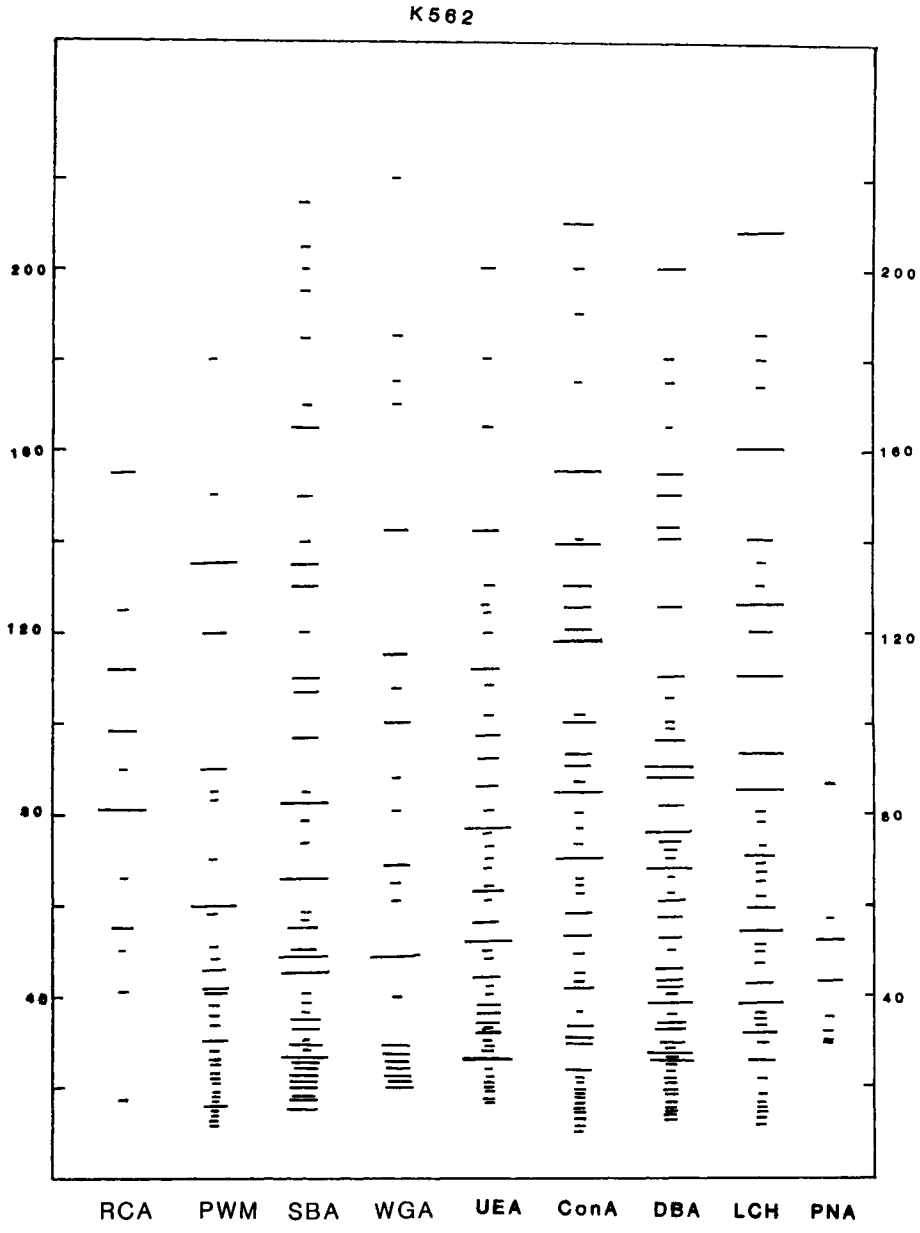

Fig. 5. A composite of visually scored bands and their calculated molecular weights from several different gels bands were scored as heavy (——), medium (—), or faint(–) and plotted against their apparent molecular weight for comparison. K562 cells were surface labeled with biotin and extracts were affinity purified on various lectins and analyzed as in Figure 3.

TABLE III. Consistent Changes After Induction of Differentiation of K562 Cells With PMA, Butyrate, DMSO, and Hemin

	kDa	
Lectin	Increases	Decreases
RCA	120	150, 125
PWM	None	18
WGA	None	None
DBA	200, 180, 175, 155	39
Con A	180, 165, 150, 97, 39	None
UEA	44	90, 66, 42

DISCUSSION

The use of sulfo-NHS-biotin, lectin affinity chromatography, and avidin-peroxidase staining offers a high-resolution method for fingerprinting cell surface glycoproteins. The results obtained by this technique are much more comprehensive than those obtained by other methods. Lactoperoxidase-catalyzed radioiodination only reveals surface proteins and glycoproteins with exposed tyrosine residues, and the resolution after autoradiography

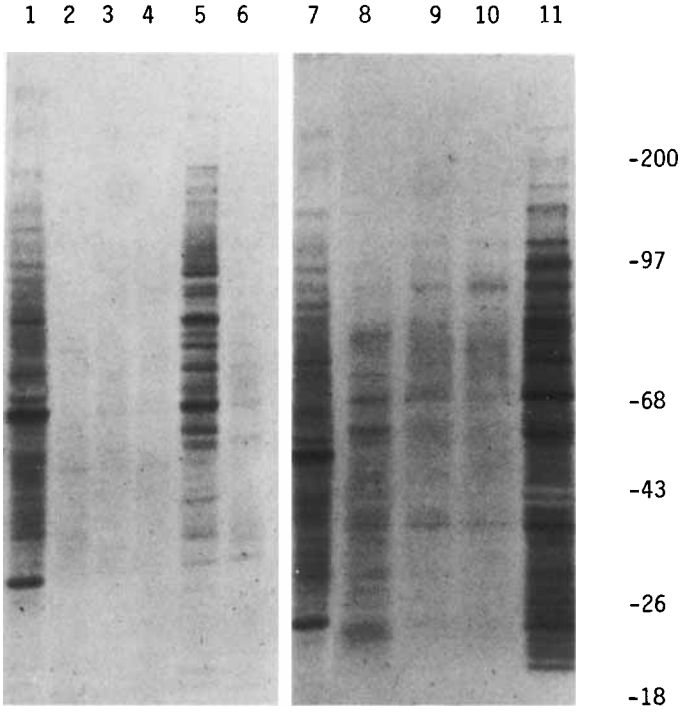

Fig. 6. Extracts of K562 cells surface labeled with biotin treated as in Figure 3 except that they were affinity purified on UEA. **Lanes 1** and **7**, K562 cells untreated. **Lanes 2** and **8**, K562 cells treated with PMA. **Lanes 3** and **9**, butyrate, **Lanes 4 and 10**, DMSO, **Lane 11**, hemin. HL60 cells were treated as above after culture without (**Lane 5**) or with PMA (**Lane 6**). Equivalent cells numbers were processed in **Lanes 1–6**, and increased numbers of treated cells were loaded in **Lanes 8–10** for comparison.

is usually relatively poor, with small numbers of broad bands. Periodate oxidation of sialic acid residues followed by reduction with NaB $(^3H)_4$ and fluorography leads to better resolution but is limited to the analysis of sialoglycoproteins. Likewise, the use of a similar procedure with galactose oxidase is limited to terminal galactose and N-acetylgalactosamine residues. Biosynthetic labeling does not discriminate between surface and internal glycoproteins. The use of sulfo-NHS biotin by itself leads to such a large number of labeled surface bands that identification of individual components is difficult. Lectin staining or lectin affinity chromatography alone again fails to distinguish between internal and external glycoproteins.

With the present method, each lectin appears to detect a subset of the total cell surface glycoprotein complement and thus allows better resolution of individual components. It is clear that this technique can be used to detect alterations in cellular glycoproteins related to differentiation and malignancy. Once specific components of interest are identified, they can simply be isolated by lectin affinity chromatography and SDS-PAGE, and the region bearing the component can be used as an immunogen to produce monoclonal antibodies. We have used this technique with a 210-kDa component and a 135-kDa component from K562 cells detected by PWM to produce monoclonal antibodies (Reading and Hickey, unpublished data). In both cases, the desired antibodies were obtained from a single fusion.

Changes in leukemia cells after the induction of differentiation have been analyzed in the past in an effort to detect the loss of malignancy-associated phenotypes as the cells become more "normal." In this study, the induction of differentiation in continuous leukemia cell lines and in freshly isolated leukemia cells was accompanied by major alterations in growth, morphology, cytochemistry, and surface phenotype. At the same time, major changes in cell surface glycoprotein profiles were detected.

With K562 cells, there appear to be two types of changes detected with this technique. The first type of alteration is the overall increase or decrease in glycoproteins bound by an individual lectin. This was seen as a decrease in RCA-bound glycoproteins after treatment with PMA, butyrate, or DMSO; increases in WGA-bound and PWM-bound glycoproteins after treatment with PMA or hemin; a decrease in DBA-bound glycoproteins after treatment with butryate or DMSO; and a virtual disappearance of UEA-bound glycoproteins after treatment with PMA, butyrate, or DMSO. The UEA-bound glycoproteins from HL-60 cells were also dramatically decreased after treatment with PMA or DMSO. This change in UEA-bound glycoproteins was not seen with an M2 AML (AML-1) sample after treatment with PMA or DMSO (data not shown). The specific alterations observed may well be related to the stage of differentiation arrest of the particular leukemia.

The second type of alteration observed was the substantial increase or decrease in particular glycoprotein bands after treatment with particular agents. Although a large number of bands were altered by the individual treatments, we concentrated on identifying those glycoprotein bands which were dramatically increased or decreased or lost after treatment with all four differentiation agents. These alterations, which were independent of the lineage toward which the K562 cells were differentiating, are more likely to represent bands which are related to undifferentiated proliferation, rather than lineage restriction. In particular, when K562 cells are induced to differentiate the following glycoproteins are lost or greatly decreased: 150- and 125-kDa bands bound by RCA; an 18-kDa band bound by PWM; a 39-kDa band bound by DBA; and 90-, 66-; and 42-kDa bands bound by UEA. It will be of interest to analyze fresh acute leukemia cells

for the presence of the bands which are lost or decreased upon differentiation of K562 cells. These glycoproteins may be related either to the undifferentiated state of both normal and leukemia cells or to the hyperproliferative state of leukemia cells. Comparisons with undifferentiated normal precursors will help to resolve this issue.

There were also glycoproteins which were substantially increased or appeared on the surface of K562 cells after the induction of differentiation with all four agents: a 120-kDa band bound by RCA; 200-, 180-, 175-, and 155-kDa bands bound by DBA; 180-, 165-, 150-, 97-, and 39-kDa bands bound by Con A; and a 44-kDa band bound by UEA. It is possible that some of these glycoproteins may exert negative controls on proliferation. It will be of interest to determine if any of these glycoproteins are uniformly expressed on normal differentiated hematopoietic cells. In a number of the differentiated elements analyzed in Figure 3, DBA-bound bands are prominent at 200, 180, and 175 kDa. We do not know yet if these components are identical to the increased and new DBA bands after induction of differentiation of K562 cells.

Our interpretation of the first class of changes is that upon induction of differentiation there are overall changes in particular patterns of glycosylation that either increase or decrease glycoproteins bound by a particular lectin. For the second class of changes, we favor the hypothesis that there is increased or decreased synthesis of the individual glycoprotein, per se. An alternative mechanism is the switching of glycosylation of individual components in a specific manner. We have not detected an increase at a particular molecular weight in the glycoproteins bound by one lectin, which corresponds to a loss in the same region in the glycoproteins bound by another lectin as a function of differentiation. In the cases of PWM or WGA, this might not be expected since an increase or decrease in poly-N-acetyllactosamine synthesis or the degree of sialylation of an individual component could greatly influence its migration. This question can be addressed by following the fates of particular glycoprotein components isolated by using monoclonal antibodies directed to the polypeptide region, followed by affinity chromatography and analysis on western transfers.

The changes observed with UEA-bound glycoproteins are the most complex. First of all, there was a major loss of UEA-bound glycoproteins with all treatments except hemin. Since K562 cells are classified as erythroleukemia cells and hemin induces erythroid differentiation, the continued presence of UEA-bound glycoproteins is consistent, except that the pattern after hemin treatment is quite different. It may be that fucosylation appropriate for UEA binding is a major erythroid glycosylation pathway, but that there are a relatively large number of changes in the surface glycoproteins as the leukemic erythroblasts mature. This glycosylation pattern seems to be greatly inhibited after treatment with PMA, butryate, or DMSO, and the remaining glycoproteins do not reflect the UEA-bound glycoproteins of untreated K562 cells. This may indicate the presence of a minor set of fucosylated glycoproteins that do not change with differentiation or it may represent limited fucosylation in major new glycoprotein bands.

The resolution to a number of these questions may be aided by the use of an image-digitizing system that will yield a quantitative density and normalized migration for each band [24]. Computer-assisted analysis coupled with studying the kinetics of the changes may lead to a better understanding of the surface glycoprotein changes associated with hematopoietic differentiation.

Although it may be of interest to identify the major glycoproteins that appear to increase or decrease dramatically with differentiation of leukemia cells, this approach may not elucidate the basic cell surface glycoproteins associated with the leukemia state.

Even though the HL-60 is a promyelocytic leukemia cell line, its surface glycoproteins bear little resemblance to those of enriched promyelocytes. When it was induced to granulocytic maturation with DMSO, the surface glycoproteins did not change to resemble those of enriched myelocytes and metamyelocytes (data not shown). In this study, promyelocytes, myelocytes, and metamyelocytes were isolated from a CML patient in the chronic phase. Although these cells are thought to be representative of their normal counterparts, their glycosylation patterns could be altered from those of normal cells. It has been reported that altered glycosylation may be correlated with premature marrow egress in AML [25]. Comparison of freshly isolated AML, LH-60, and K562 cells after monocytic differentiation with purified monocytes also failed to show changes in the glycoproteins of the less mature cells associated with mature monocytes (data not shown). A more valid comparison will be that of promyelocytic leukemia cells with normal promyelocytes, acute myeloblastic leukemia cells with purified normal myeloblasts, and erythroblastic leukemia with purified erythroblasts. We have developed techniques for purification of these elements from marrow but to date have not been able to obtain a sufficient yield for this analysis.

Preliminary studies indicate that perhaps a tenfold increase in sensitivity can be obtained by using avidin-alkaline phosphatase and the substrate 5-bromo-4-chloro-indolyl phosphate [26]. We are hopeful that this will enable us to analyze the more elusive normal components of marrow. This would allow detection of specific leukemia-associated glycoproteins and production of monoclonal antibodies that might be useful for diagnosis and certain therapeutic approaches, such as removal of residual leukemia cells in autologous bone marrow transplantation.

ACKNOWLEDGMENTS

We thank Drs. G. Ventura and J. Hester for assistance with pheresis samples, Dr. J. Trial for helpful discussions, and Le Foster for assistance with the manuscript and the figures.

Work supported by a grant from the NIH, CA-23077, and by a WHO Fellowship to W.Y.

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