Cell Surface Membrane Protein Changes during the Differentiation of Cultured Human Promyelocytic Leukemia HL-60 Cells

Ronald L. Feistel, Suresh K. Gupta, Constance J. Glover, Steven A. Fischkoff, and Robert E. Gallagher

ABSTRACT

The human promyelocytic leukemia cell line HL-60 was induced to differentiate in vitro by treatment with dimethyl sulfoxide or retinoic acid. Morphological maturation was accompanied by a total loss of transferrin binding and a 7-fold increase in the percentage of cells reducing nitro blue tetrazolium. Cell surface membrane proteins and glycoproteins were labeled with \(^{125}\)I by the lactoperoxidase-\(\text{H}_2\text{O}_2\) or 1,3,4,6-tetrachloro-3a,6a-diphenyl-glycoluril (lodox-Gen) methods and analyzed by two-dimensional isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. A minimum of 12 cell surface proteins were unchanged, 3 proteins (\(M_r, 95,000, 87,000, \) and 77,000) were lost, and up to 7 new proteins (\(M_r, 270,000, 240,000, 150,000, 135,000, 58,000, 56,000,\) and 50,000) appeared during HL-60 cell differentiation. The kinetics of disappearance of one major labeled cell surface protein (\(M_r, 95,000\)) within two days during treatment with retinoic acid correlated with the loss of cellular transferrin binding. This protein was identified as the transferrin receptor by affinity absorption of extracts of \(^{125}\)I-labeled protein-labeled cells to transferrin-Sepharose beads. The affinity-purified component had molecular weights of 190,000 and 95,000 under nonreducing and reducing conditions, respectively, confirming its dimeric structure. Twodimensional electrophoresis of cell surface membrane-labeled proteins of normal human granulocytes confirmed the absence of the transferrin receptor and identified cell surface proteins with molecular weight and pi values corresponding to three of the new cell surface proteins which appeared during HL-60 maturation. The most intensely labeled of these had a molecular weight of about 55,000, and was confirmed as being identical to the corresponding \(M_r, 58,000\) HL-60 cell surface membrane protein by one-dimensional peptide-mapping analysis. This prominent new \(M_r, 55,000\) to 58,000 protein increased continuously throughout retinoic acid-induced maturation and was identified as a major terminal myeloid differentiation cell surface membrane protein.

INTRODUCTION

The definitive analysis of specific cell surface membrane changes that occur during myeloid cell differentiation has been facilitated by the recent establishment of a number of myeloid leukemic cell lines which are blocked at different stages of maturation. By treatment with the appropriate chemical agents (5, 7, 19), specific cell surface membrane changes which accompany this maturation include the loss of a \(M_r, 88,000/86,000\) antigenic doublet (17), the loss of the cell surface transferrin receptor (18, 21), and the appearance of a \(M_r, 83,000\) antigen (17), as detected by immunoprecipitations with either antigranulocyte, antiproganulocyte, or antimonocyte antisera, respectively.

In the absence of specific antisera, cell surface membrane changes of this type have been specifically analyzed by surface radiolabeling of whole cells, followed by one-dimensional SDS-polyacrylamide gel electrophoresis of total cell extracts, and visualization of the labeled membrane components by fluorography or autoradiography (10, 11). For example, oligosaccharide radiolabeling and one-dimensional electrophoreses revealed the apparent loss of a \(M_r, 160,000\) glycoprotein and the concomitant appearance of glycoproteins of \(M_r, 155,000\) and 130,000 during the induction of HL-60 differentiation (11). We previously used this approach to compare the cell surface proteins from the leukemic cell lines K-562 and HL-60 after specific surface radiolabeling with \(^{125}\)I. However, we found that an electrophoretic separation based only on molecular weight did not adequately resolve the complex assembly of macromolecules found on most cell surfaces. Therefore, we analyzed the \(^{125}\)I-labeled surface proteins by 2-dimensional isoelectric focusing and SDS-polyacrylamide gel electrophoresis (8). This 2-dimensional analysis, based on charge and molecular weight, provided for the simultaneous analysis of many more prominent cell surface components than was possible by one-dimensional analysis. In this report, we have applied these methods to examine the cell surface membrane protein changes that occur during HL-60 granulocyte differentiation induced by dimethyl sulfoxide and retinoic acid. While most of the labeled HL-60 surface proteins persisted during cellular maturation, at least 3 proteins were lost, and as many as 7 new surface proteins appeared. One of the proteins lost during cell differentiation was identified as the transferrin receptor. The most intensely labeled new protein appearing during cellular differentiation was also identified as a major surface membrane protein in normal human granulocytes, and was designated as a terminal myeloid differentiation-depending cell surface membrane protein.

MATERIALS AND METHODS

Preparation of Cells. Human promyelocytic leukemic HL-60 cells (passage level, 14 to 30) were cultured in Roswell Park Memorial Institute Medium 1640 (Grand Island Biological Co., Grand Island, N. Y.) containing 100 IU of penicillin per ml and 100 \(\mu\)g of streptomycin per ml (Grand Island Biological Co.). The human promyelocytic leukemia cell line HL-60 was induced to differentiate in vitro by treatment with dimethyl sulfoxide and as many as 7 new surface proteins appeared. One of the new \(M_r, 58,000\) protein increased continuously throughout retinoic acid-induced maturation and was identified as a major terminal myeloid differentiation cell surface membrane protein.

1 To whom requests for reprints should be addressed.

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Membrane Changes during HL-60 Differentiation

Island Biological Co.), and either 10% fetal bovine serum (Sterile Sys-
tems, Logan, Utah) or 5 μg of human transferrin per ml (Sigma Chemical
Co., St. Louis, Mo.), and 5 μg of bovine insulin per ml (Sigma) at 37° in
a humidified atmosphere with 5% carbon dioxide. The cultures were
seeded at initial cell densities of 2.5 to 5.0 x 10⁶ cells/ml and incubated
for either 5 to 8 days with no additions (unstimulated), 7 days with 1.25%
dimethyl sulfoxide (Mallinkrodt Chemical Works, St. Louis, Mo.) (7), or
up to 5 days with 1 μM all-trans-retinoic acid (Sigma) (5). Viable cells
were isolated from cell cultures that were less than 90% viable (trypan
blue exclusion) by Ficoll-Hypaque (Bionetics Laboratory Products, Ken-
nington, Md.) centrifugation (1). Human granulocytes were purified from
peripheral blood by sequential Ficoll-Hypaque and dextran sedi-
mentation, as described previously (2). The contaminating red cells were
then removed by a 45-sec hypotonic lysis, and the granulocytes were re-
covered by centrifugation at 280 x g for 10 min at 5° in a Sorval RC3
refrigerated centrifuge (Du Pont, Wilmington, Del.). Final preparations
consisted of greater than 98% neutrophils with less than 2% eosinophils
and essentially no basophils, as determined by the Wright-Giemsa stain
(Dade Diagnostic Inc., Agua, Puerto Rico). Immediately before labeling,
the HL-60 cells were washed 4 times in Dulbecco’s PBS-Ca²⁺ (Grand
Island Biological Co.), while purified granulocytes were washed with
isotonic HEPES (Sigma) by centrifugation as described above. Cell
counts were determined in a Model ZBI Coulter Counter (Coulter Elec-
tronics, Inc., Hialeah, Fla.).

Nitro Blue Tetrazolium Reduction. The development of HL-60
maturation-dependent oxidase activity was measured by the reduction of
nitro blue tetrazolium to formazan by the method of Mendelsohn et
al. (16).

Cell-binding Studies. The binding of transferrin to intact HL-60 cells
was measured as described (21). Human transferrin (Sigma) was ra-
diiodinated with 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril (Iodo-Gen;
Pierce Chemical Co., Rockford, Ill.) as described (9). Cells were
incubated with 10⁻⁵ M transferrin for 1 h at 37° in a humidified
atmosphere with 5% carbon dioxide. The cultures were
subjected to one-dimensional electrophoresis in the presence of Sfapri-
lene as described above. The paper backing was removed and the gels
were subjected to two-dimensional gel electrophoresis and autoradiography,
using a representative plot shown in figure 3.

Extraction of Cell Surface-labeled Proteins and Glycoproteins. The
labeled and washed cells were treated with disopropylfluorophosphate
and extracted with ice-cold 2% Triton X-100 (Sigma) in Dulbecco’s PBS-
Ca²⁺ containing 0.1% glucose and 5 mM KCl, by centrifugation.

Preparation of Labeled Cell Surface Proteins for Isoelectric Focus-
ning. Labeled cell surface membrane proteins were prepared for iso-
electric focusing as previously described (9) by bringing the 2% Triton X-100
extracts to approximate final concentrations of 9.5 μM urea, 2% amphot-
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Table 1

Growth and differentiation of retinoic acid-treated HL-60 cells

| Time (days) after retinoic acid induction | Without retinoic acid (cells/ml × 10^6) | With retinoic acid (cells/ml × 10^6) | Cell growth | Differential cell counts in presence of retinoic acid
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td>Myeloblasts or promyelocytes</td>
</tr>
<tr>
<td>1</td>
<td>0.73 ± 0.095a</td>
<td>0.66 ± 0.055</td>
<td>180</td>
<td>Myelocytes</td>
</tr>
<tr>
<td>2</td>
<td>0.67 ± 0.053</td>
<td>0.80 ± 0.214</td>
<td>190</td>
<td>Metamyelocytes to segmented</td>
</tr>
<tr>
<td>3</td>
<td>1.05 ± 0.059</td>
<td>0.94 ± 0.091</td>
<td>108</td>
<td>Differential</td>
</tr>
<tr>
<td>4</td>
<td>1.86 ± 0.180</td>
<td>0.80 ± 0.035</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Values are average of duplicate determinations on 200 cells. The differential cell count after 4 days in the absence of retinoic acid was: myeloblasts or promyelocytes, 177; myelocytes, 17; and metamyelocytes to segmented cells, 8.

Percentage of myelocytes, metamyelocytes, and banded and segmented cells as determined by cytological evaluation of Wright-stained preparations.

Mean ± S.D.

after 7 days were again about one-half of that in the absence of inducer (data not shown). Greater than 90% of the cells were differentiated into myelocytes, metamyelocytes, and banded and segmented cells after either 4 days of culture with retinoic acid (Table 1), or after 7 days of culture with dimethyl sulfoxide (data not shown). Examples of HL-60 cells cultured in the absence or presence of dimethyl sulfoxide are shown in Fig. 1, A and B, respectively. The morphological differentiation of HL-60 cells correlated with an increase in nitro blue tetrazolium reduction and a decrease in cell surface membrane binding of human transferrin (Table 2).

Cell Surface Membrane Changes during HL-60 Differentiation. HL-60 cells were surface membrane-labeled with 125I before and after induction of differentiation with retinoic acid or dimethyl sulfoxide. Two-dimensional gel electrophoretic autoradiographs of labeled cells revealed numerous radioactive proteins which appeared as horizontal smears and/or as closely spaced multiply charged chains of similar molecular weight (8). At least 12 of these labeled protein complexes were unchanged during HL-60 differentiation. Cell maturation was accompanied by a marked decrease in a major labeled protein complex with a molecular weight of 95,000 (Protein 8) (Fig. 2; Charts 1 and 2). The loss of Protein 8 was accompanied by the apparent total loss of less intensely labeled protein complexes of M, 86,000 (Protein 10) and 77,000 (Protein 11) (Fig. 2; Charts 1 and 2). Concomitantly, retinoic acid induction resulted in the appearance of 7 new protein complexes (Proteins G1 to G7). Two of these (Proteins G3 and G5) were extensively labeled, compared to the other 5 less intensely labeled new proteins. Nevertheless, the appearance of these lightly labeled new proteins was confirmed in at least 4 different retinoic acid-stimulated HL-60 cell cultures. Five of the same 7 labeled proteins appeared in at least 6 separate HL-60 cell cultures induced with dimethyl sulfoxide (Proteins G1 to G5) (Table 3).

Correlation of Cell Surface Membrane Protein Changes with HL-60 Functional and Morphological Differentiation. The time course for the disappearance and appearance of 2 of the

Fig. 1. Morphological differentiation of dimethyl sulfoxide-induced HL-60 cells. A, undifferentiated HL-60 promyelocytes; B, HL-60 cells after 7 days in the presence of 1.25% dimethyl sulfoxide. Wright-Giemsa stain, × 330.

Table 2

Functional differentiation of retinoic acid-treated HL-60 cells

<table>
<thead>
<tr>
<th>Time (days) after retinoic acid induction</th>
<th>Nitro blue tetrazolium reduction (% of positive cells)</th>
<th>125I-Transferrin bound to cells (cpm/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10 ± 1.7b</td>
<td>2104 ± 858</td>
</tr>
<tr>
<td>1</td>
<td>18 ± 4.9</td>
<td>1159 ± 508</td>
</tr>
<tr>
<td>2</td>
<td>27 ± 6.8</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>43 ± 5.7</td>
<td>1775 ± 206</td>
</tr>
<tr>
<td>4</td>
<td>69 ± 1.7</td>
<td>8 ± 0.6</td>
</tr>
</tbody>
</table>

Cellular transferrin binding was determined as the radioactivity of 125I-transferrin bound per 10^6 cells less a background cell binding of 1639 ± 454 (n = 8) determined in the presence of a 200-fold excess of unlabeled transferrin. Mean ± S.D. of 3 determinations.

No statistical difference (p = 0.55) between 125I-transferrin binding HL-60 cells cultured in the absence of retinoic acid for 0 and 2 days, respectively.

ND, not detectable.

G5 were extensively labeled, compared to the other 5 less intensely labeled new proteins. Nevertheless, the appearance of these lightly labeled new proteins was confirmed in at least 4 different retinoic acid-stimulated HL-60 cell cultures. Five of the same 7 labeled proteins appeared in at least 6 separate HL-60 cell cultures induced with dimethyl sulfoxide (Proteins G1 to G5) (Table 3).

Correlation of Cell Surface Membrane Protein Changes with HL-60 Functional and Morphological Differentiation. The time course for the disappearance and appearance of 2 of the

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Membrane Changes during HL-60 Differentiation

Chart 1. Tracing of HL-60 cell surface 125I-labeled 2-dimensional autoradiograph typical of that shown in Fig. 2A. Arabic numerals indicate previously described HL-60 surface proteins (8). Darkened spots, those surface proteins which disappeared or were markedly reduced during chemically induced HL-60 differentiation. The complex of spots identified as Protein G5 indicates a surface protein which was substantially enhanced during the chemical induction of HL-60 myeloid differentiation. Dashed spot, labeled proteins which corresponded to visible Coomassie brilliant blue-stained proteins. Dashed line at bottom, tracking dye front for the second dimension of electrophoresis. The tracing is representative of at least 4 different experiments analyzed at several exposures.

Chart 2. Tracing of retinoic acid-induced HL-60 cell surface 125I-labeled 2-dimensional autoradiograph typical of that shown in Fig. 2B. The protein complex identified with the arabic numeral 8 was substantially reduced compared to that in unstimulated HL-60 cells. Darkened spots (G1 to G7), those surface proteins which appeared during retinoic acid-induced HL-60 differentiation. Dashed spots, labeled proteins which corresponded to visible Coomassie brilliant blue-stained proteins. Dashed line at bottom, tracking dye front for the second dimension of electrophoresis. The tracing is representative of at least 4 different experiments analyzed at several exposures.

Table 3

<table>
<thead>
<tr>
<th>Surface protein</th>
<th>Retinoic acid induced</th>
<th>Dimethyl sulfoxide induced</th>
<th>Normal human granulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apparent M&lt;sub&gt;r&lt;/sub&gt; (×10&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>Apparent pl</td>
<td>Apparent M&lt;sub&gt;r&lt;/sub&gt; (×10&lt;sup&gt;3&lt;/sup&gt;)</td>
</tr>
<tr>
<td>G1</td>
<td>270</td>
<td>6.6 - 7.0</td>
<td>265</td>
</tr>
<tr>
<td>G2</td>
<td>240</td>
<td>6.4 - 6.7</td>
<td>230</td>
</tr>
<tr>
<td>G3</td>
<td>150</td>
<td>6.5 - 6.9</td>
<td>140</td>
</tr>
<tr>
<td>G4</td>
<td>135</td>
<td>5.7 - 6.1</td>
<td>130</td>
</tr>
<tr>
<td>G5</td>
<td>58</td>
<td>5.5 - 5.9</td>
<td>57</td>
</tr>
<tr>
<td>G6</td>
<td>56</td>
<td>5.1 - 5.5</td>
<td>56</td>
</tr>
<tr>
<td>G7</td>
<td>50</td>
<td>5.3 - 5.7</td>
<td>50</td>
</tr>
</tbody>
</table>

* Proteins G1 through G7 represent groups of multiply charged surface membrane proteins which appear during HL-60 differentiation and/or are present on normal human granulocytes.

Identification of Protein 8 as the Cell Surface Transferrin Receptor. The differentiation-dependent loss of HL-60 cell transferrin-binding activity was correlated with the loss of the major Mr 95,000 cell surface membrane protein (Protein 8). Protein 8 was identified as the cell transferrin receptor by absorption of...

Fig. 2. Two-dimensional polycrylamide gel electrophoresis of surface membrane proteins from HL-60 cells lactoperoxidase-H<sub>2</sub>O<sub>2</sub>-labeled with <sup>129</sup>I. The first (horizontal) dimension was isoelectric focusing with the acid pH on the left. The second (vertical) dimension was SDS electrophoresis. A, autoradiograph of undifferentiated HL-60 cells; B, autoradiograph of HL-60 surface proteins after 4 days of culture in the presence of 1μM retinoic acid. The photographs represent the best compromise with respect to overall autoradiograph exposures. Heavily labeled regions of the gel were clarified by examining underexposed films, while lightly labeled components were confirmed in overexposed films.
Charts. Kinetics of the disappearance and appearance of 2 major surface proteins during retinoic acid-induced HL-60 myeloid differentiation. The changes were quantified by cutting appropriate labeled surface proteins from the gels and counting in a gamma counter. The disappearance of Protein 8 was expressed as the ratio of the radioactivity remaining in Protein 8 to the radioactivity in unchanged labeled surface Protein 5: O, cpm Protein 8/cpm Protein 5; the appearance of Protein G5 is expressed as the ratio of the radioactivity in Protein G5 to the radioactivity in unchanged labeled surface Protein 5: ●, cpm Protein G5/cpm Protein 5.

Fig. 3. Autoradiograph of 2-dimensional polyacrylamide gel electrophoresis of the surface 125I-labeled transferrin receptor (Protein 8; see Fig. 2A and Chart 1) from HL-60. The first (horizontal) dimension was isoelectric focusing and the second (vertical) dimension was SDS electrophoresis.

Fig. 4. Autoradiograph of the SDS-polyacrylamide gel electrophoresis of the surface 125I-labeled transferrin receptor (Protein 8) from HL-60 in the presence (reduced) and absence (nonreduced) of β-mercaptoethanol. Electrophoresis was from top to bottom.

Comparison of Cell Surface Membrane Proteins in Differentiated HL-60 Cells and Normal Human Granulocytes. Cell surface membrane proteins of normal human granulocytes were analyzed as described above and compared to chemically induced HL-60 cells (Fig. 5; Chart 4). The surface membrane proteins lost during HL-60 induction (Proteins 8, 10, and 11) were also absent in mature granulocytes. In addition, human granulocytes had 3 labeled membrane proteins with molecular weights and pI ranges corresponding to 3 of the 7 new membrane proteins found in differentiated HL-60 cells (Proteins G2, G3, and G5, Table 3). Furthermore, that Protein G5 in differentiated HL-60 cells is apparently related to a major surface protein of normal mature granulocytes was confirmed by one-dimensional peptide-mapping analysis (Fig. 6).

DISCUSSION

Cell surface radioiodination, followed by 2-dimensional isoelectric focusing and SDS-polyacrylamide gel electrophoresis and...
other labeled cell surface membrane proteins within 2 days of culture in the presence of retinoic acid, before any significant change in cell growth. Protein 8 was identified as the cell membrane transferrin receptor subunit by its dimeric molecular structure, its specific absorption to transferrin-Sepharose, by similar kinetics for the loss of cell surface transferrin-binding capacity, and by its disappearance from 2-dimensional autoradiographs during retinoic acid induction. This identification is similar to the loss of the transferrin receptor from dimethyl sulfoxide-treated HL-60 cells, as previously shown with monoclonal antibodies (18, 21). Since the transferrin receptor is an obligatory factor of cell division, its down regulation is an important early step during cell maturation (18). Protein 8 is also analogous to the transferrin receptor identified in K-562 cells (8), and it is possibly identical to the cancer-associated antigen found in a wide variety of tumors (3, 4).

The loss of transferrin receptor (Protein 8) was accompanied by the simultaneous loss of cell membrane proteins of approximately $M_\text{r}$ 87,000 (Protein 10) and 77,000 (Protein 11). Protein 10 is possibly identical to the $M_\text{r}$ 88,000/86,000 antigen which was previously identified in $[^{35}\text{S}]$methionine-labeled HL-60 cells with an antigranulocyte serum (17). This would be consistent with the observation that both Protein 10 and the antigenic doublet are lost during dimethyl sulfoxide induction of HL-60 differentiation. A cell surface protein analogous to Protein 11 was previously described in K-562 cells, and was tentatively identified as fetal bovine serum transferrin which remained tightly associated with the cell transferrin receptor (Protein 8) after cell washing (8). This is consistent with the association of trace amounts of Protein 11 with transferrin-Sepharose affinity-purified Protein 8 (Fig. 3), and can be explained by the biospecific absorption to the transferrin-Sepharose resin of both free labeled dimeric transferrin receptor and transferrin receptor which has one of its 2 subunits bound to labeled Protein 11.

Cell maturation also may result in the loss of a protein complex of $M_\text{r}$ 130,000 (Protein 6) (Figs. 1 and 2). This decrease was noted in several experiments, and in some it appeared to be accompanied by an increase in Protein 7 ($M_\text{r}$ 110,000). A possible
interconversion relationship between these 2 protein complexes was previously noted during neuraminidase treatment (8). However, considerable background exposure in the region of the gel surrounding Protein 6 in most experiments precludes a definitive conclusion at this time.

As many as 7 new surface membrane proteins appeared during HL-60 differentiation. After 4 days of incubation with retinoic acid, the most intensely labeled of these new proteins (Protein G5) increased 2- to 7-fold relative to 6 other membrane proteins that remained unchanged throughout HL-60 maturation. Although Protein G5 is expressed early, in contrast to the transferrin receptor, it continues to accumulate throughout cell maturation. A similar surface membrane protein of apparently identical molecular weight and pl range was identified in normal human granulocytes. Confirmation of the identity of this granulocyte cell surface protein as Protein G5 by peptide mapping is consistent with the suggestion that Protein G5 is a late myeloid differentiation-dependent surface protein. It is also consistent with the expected induction of progranulocytic leukemic HL-60 cells into mature myeloid-like cells. The presence of small quantities of Protein G5 in unstimulated HL-60 control cells can be explained by the occurrence of 5 to 10% spontaneously differentiated cells in immature HL-60 cultures (7).

Dimethyl sulfoxide-induced differentiation has been shown to be accompanied by the appearance of glycoproteins of $M_c 130,000$ (gp130) and $M_c 155,000$ (gp155), and the concomitant loss of a prominent $M_c 160,000$ glycoprotein (gp160) in neuraminidase-galactose oxidase-NaB$_3$H$_4$ surface-labeled HL-60 cells (11). Although a precise comparison of glycoprotein molecular weights observed in different laboratories is not possible due to anomalies relative to protein standards (20), the appearance of gp130 and gp155 may correspond to the appearance of proteins with molecular weights of $130,000$ to $135,000$ (Protein G4) and $140,000$ to $150,000$ (Protein G3), respectively, in 2-dimensional autoradiographs of $^{125}$I-labeled, differentiated HL-60 cells. We did not observe the loss of a higher-molecular-weight $^{125}$I-labeled protein corresponding to the loss of tritiated gp160. This may be because of steric blockage or the absence of amino acid side chains in gp160, susceptible to chemical or enzymatic iodination. Alternately, since myeloid differentiation is characterized by significant changes in the quantity and branching of surface membrane oligosaccharides (10), it is possible that the apparent loss of tritium-labeled gp160 merely reflects changes in the susceptibility of oligosaccharide structure to the specific penultimate galactose-labeling methodology used. This could explain the observation of the same authors that no difference was seen between uninduced and induced HL-60 cells labeled on their terminal sialic acid sugars by the periodate-NaB$_3$H$_4$ method (11). It also suggests that the apoprotein portion of gp160 may not be significantly diminished. Thus, assuming that the change in oligosaccharide structure does not significantly alter the molecular weight and pl of the glycoprotein, it is possible that gp160 is identical to the $^{125}$I-labeled $M_c 165,000$ or $173,000$ proteins (Proteins 3 and 5, respectively), which were not significantly changed during HL-60 differentiation.

The 12-O-tetradecanoylphorbol-13-acetate induction of HL-60 cells along the monocyte-macrophage pathway resulted in the appearance of a $^{[35]}S$methionine-labeled surface antigen of $M_c 83,000$ to antimonocyte serum (17). Similar surface component was not evident in the present study after induction of HL-60 cells along the granulocyte series. This may reflect the expected phenotypic differences between granulocytic and monocytic maturation, or it may merely reflect the detection limitations of the present methodology, namely, sufficient quantities of susceptible radiodinitatable amino acid side chains.

In this study, the functional and morphological changes accompanying HL-60 differentiation have been correlated with specific changes in cell surface membrane proteins and/or glycoproteins. Cell surface iodination, 2-dimensional gel electrophoresis, and autoradiography have been used to resolve and visualize a number of specific new proteins which appear to be directly or indirectly involved in leukemic progranulocytic maturation. In addition, the presence of these new proteins in normal mature granulocytes suggests their importance in defining the normal mature cell phenotype. Because of their close relationship with cell maturation, an understanding of the identity, structure, and/or functional relationships of these membrane proteins may provide important new insights into the mechanism of leukemic and normal myeloid cell differentiation.

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REFERENCES

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