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

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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-3$\alpha$,6$\alpha$-diphenylglycoluril (lodo-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, 95,000, 87,000, and 77,000) were lost, and up to 7 new proteins (M, 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, 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 surface 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. Two-dimensional 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, 58,000 HL-60 cell surface membrane protein by one-dimensional peptide-mapping analysis. This prominent M, 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 (7, 12–15). One of these, the promyelocytic HL-60 cell line, can be induced to terminally differentiate into cells with many of the characteristics of mature granulocytes or macrophages by treatment with the appropriate chemical agents (5, 7, 19). Specific cell surface membrane changes which accompany this maturation include the loss of a $M$, 88,000/86,000 antigenic doublet (17), the loss of the cell surface transferrin receptor (18, 21), and the appearance of a $M$, 83,000 antigen (17), as detected by immunoprecipitations with either antigranulocyte, antiperoxidase, 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 $M$, 160,000 glycoprotein and the concomitant appearance of glycoproteins of $M$, 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-dependent 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.) growth medium containing 10% fetal bovine serum (Grand Island Biological Co.) and antibiotics.
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Island Biological Co.), and either 10% fetal bovine serum (Sterile Systems, 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°C in a humidified atmosphere with 5% carbon dioxide. The cultures were seeded at initial cell densities of 2.5 to 5.0 × 10^6 cells/ml and incubated for either 5 to 8 days with no additions (unstimulated), 7 days with 1.25% dimethyl sulfoxide (Mallinckrodt 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, Kensington, Md.) centrifugation (1). Human granulocytes were purified from peripheral blood by sequential Ficoll-Hypaque and dextran sedimentations, as described previously (2). The contaminating red cells were then removed by a 45-sec hypotonic lysis, and the granulocytes were recovered by centrifugation at 280 × g for 10 min at 5°C in a Sorval RC-3 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., Aguada, Puerto Rico). Immediately before labeling, the HL-60 cells were washed 4 times in Dulbecco's PBS-3Ca (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 ZB1 Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.).

Nitro Blue Tetrazolium Reduction. The development of HL-60 maturation-dependent oxidative 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 radiiodinated with 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril (Iodo-Gen; Pierce Chemical Co., Rockford, Ill.) as described (9).

Cell Surface Labeling. Cells were labeled by the lactoperoxidase-H_2O_2 or Iodo-Gen methods as described (8). Identical cell surface membrane patterns were obtained with both labeling procedures.

Briefly, immediately after washing the cells, lactoperoxidase-H_2O_2 cell surface labeling was performed on 2 × 10^6 cells with 0.5 mM Na_125I (350 mCi/ml; carrier-free, 17 Ci/mg) (New England Nuclear, Boston, Mass.) and 0.014 mg lactoperoxidase (83 units/mg protein) (Sigma) by centrifugation as described above. The resulting gels were then dried and autoradiographed as described below. Two additional equal-sized portions of the transferrin-Sepharose beads were suspended in equal volumes of 4% SDS and 20% glycerol in 0.08 M Tris-HCl, pH 6.8, in the presence (reduced) or absence (nonreduced) of 20% β-mercaptoethanol. After 5 min at 100°C, the SDS-treated beads and supernatant solutions were transferred to a one-dimensional SDS slab gel, and electrophoresed as described below.

Polyacrylamide Gel Electrophoresis. One- and 2-dimensional polyacrylamide electrophoreses were performed as described previously (8).

Molecular Weight and Isoelectric Point Determination. The isoelectric point calibration of 2-dimensional gels was accomplished by running a blank isoelectric focusing gel with every isoelectric-focusing experiment. After isoelectric focusing, the gel was cut into 0.5-cm sections, soaked for 4 hr in 0.5 ml of deionized water, and pH measurements were made at room temperature. The molecular-weight calibration of the second-dimension electrophoresis was accomplished by a mixture of high-molecular-weight standard proteins (myosin, M, 200,000; β-galactosidase, M, 116,000; phosphorylase B, M, 92,500; bovine serum albumin, M, 69,000; and ovalbumin, M, 45,000) (Bio-Rad Laboratories).

Surface Membrane Protein Electrophoresis Kinetics. The kinetics of HL-60 surface protein changes was quantified by comparing the ratios of labeled proteins 8 and Protein G5 to 7 other apparently unchanged labeled proteins (Proteins 1, 2, 3, 5, 6, 7, and 9) on each of 4 days following retinoic acid treatment. Two-dimensional gel electrophoresis and autoradiographs were prepared from cells labeled by the Iodo-Gen method. Labeled proteins were located on the gel by means of autoradiography, cut from the gel, and quantitated by counting the radioactivity in a gamma counter. In all cases, the plots of the change of ratios of Proteins 8 or G5 to each of the respective unchanged labeled surface proteins was essentially identical to the representative plot shown in Chart 3.

Peptide Mapping. Protein G5 from retinoic acid-stimulated HL-60 cells and normal granulocytes was cut from corresponding 2-dimensional gels as described above. The paper backing was removed and the gels were swelled for 10 to 30 min in 0.125 M Tris-HCl, pH 6.8, 0.1% SDS, and 1 mM EDTA at room temperature. The swollen gel pieces were then subjected to one-dimensional electrophoresis in the presence of Staphylococcus aureus V-8 (Miles Laboratories, Inc., Elkhart, Ind.) on 2.25-mm slab gels of 15% acrylamide-0.8% bisacrylamide as described previously (8). The resulting gels were then dried and autoradiographed as described below.

Autoradiography. Slab gels were dried with a slab gel dryer (Bio-Rad Laboratories) and autoradiographed with Kodak XAR-5 X-ray film (Eastman Kodak Co., Rochester, N. Y.) with Lightening-Plus intensifying screens (Du Pont) at −70°C.

RESULTS

Morphological and Functional Differentiation of HL-60 Cells. After 4 days of culture in the presence of 1 μM retinoic acid, the cell count of human leukemic HL-60 cells was about half that of the absence of retinoic acid (Table 1). Whereas the initial growth rate in the presence of 1.25% dimethyl sulfoxide was greater than in the presence of retinoic acid, cell counts...
Table 1

<table>
<thead>
<tr>
<th>Time (days) after retinoic acid induction</th>
<th>Cell growth</th>
<th>Differential cell counts in presence of retinoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without retinoic acid (cells/ml × 10^6)</td>
<td>With retinoic acid (cells/ml × 10^6)</td>
</tr>
<tr>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
<td>0.73 ± 0.095</td>
<td>0.66 ± 0.055</td>
</tr>
<tr>
<td>2</td>
<td>0.67 ± 0.053</td>
<td>0.80 ± 0.214</td>
</tr>
<tr>
<td>3</td>
<td>1.05 ± 0.059</td>
<td>0.94 ± 0.091</td>
</tr>
<tr>
<td>4</td>
<td>1.66 ± 0.180</td>
<td>0.80 ± 0.035</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
- 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.

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

<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></td>
<td>With retinoic acid</td>
<td>Without retinoic acid</td>
</tr>
<tr>
<td>0</td>
<td>10 ± 1.7</td>
<td>10 ± 1.7</td>
</tr>
<tr>
<td>1</td>
<td>18 ± 4.9</td>
<td>ND</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>9 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>69 ± 1.7</td>
<td>ND</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
- 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. of 3 determinations.

There was 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.

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
Membrane Changes during HL-60 Differentiation

5.0
6.0
7.0

pH

MOLECULAR WEIGHT (X 10^3)

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.

major HL-60 cell surface membrane proteins with molecular weights of 95,000 (Protein 8) and 58,000 (Protein G5), respectively, was followed by determining the ratio of radioactivities of these changing labeled surface proteins relative to radioactive surface proteins that remained constant on each of 4 days following retinoic acid induction (Chart 3). The kinetics of the disappearance of cell surface Protein 8 after retinoic acid induction (6- to 10-fold) was very similar to the loss of cell surface transferrin-binding capacity (Chart 3; Table 2). Also, the gradual increase in cell surface Protein G5 (2- to 7-fold) was very similar to the progressive accumulation of nitro blue tetrazolium-reducing cells. These effects suggest a close correlation between the change in cell surface proteins and the functional activities which change during HL-60 maturation.

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

Table 3
Correlation of cell surface membrane proteins in terminally differentiated HL-60 cells and in normal human granulocytes

<table>
<thead>
<tr>
<th>Differentiated HL-60 cells</th>
<th>Retinoic acid induced</th>
<th>Dimethyl sulfide oxide induced</th>
<th>Normal human granulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface protein*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Apparent M&lt;sub&gt;p&lt;/sub&gt; (&lt;x10&lt;sup&gt;4&lt;/sup&gt;)</td>
<td>Apparent pl</td>
<td>Apparent M&lt;sub&gt;p&lt;/sub&gt; (&lt;x10&lt;sup&gt;4&lt;/sup&gt;)</td>
</tr>
<tr>
<td>G1</td>
<td>270</td>
<td>6.6-7.0</td>
<td>285</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></td>
</tr>
<tr>
<td>G7</td>
<td>50</td>
<td>5.3-5.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> 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.
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 pl 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 radiiodination, followed by 2-dimensional isoelectric focusing and SDS-polyacrylamide gel electrophoresis and
Fig. 5. Two-dimensional isoelectric focusing and polyacrylamide gel electrophoresis autoradiograph of membrane proteins from human granulocytes labeled with $^{125}$I by the lactoperoxidase-H$_2$O$_2$ method. Electrophoresis was carried out as described in Fig. 2. The autoradiograph is representative of at least 4 different granulocyte preparations.

Fig. 6. SDS-polyacrylamide gel electrophoresis of $^{125}$I-labeled Protein G5 from retinoic acid-stimulated HL-60 cells (left lane) and human granulocytes (right lane) after partial digestion with S. aureus V8 protease.

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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 $M_r$, 87,000 (Protein 10) and 77,000 (Protein 11). Protein 10 is possibly identical to the $M_r$, 88,000/86,000 antigen which was previously identified in $^{35}$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) during 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 $M_r$, 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 ($M_r$, 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 pi 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 Mr 130,000 (gp130) and 155,000 (gp155), and the concomitant loss of a prominent Mr 160,000 glycoprotein (gp160) in neuraminidase-galactose oxidase-NaB3H4 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 125I-labeled, differentiated HL-60 cells. We did not observe the loss of a higher-molecular-weight 125I-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-NaB3H4 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 pi of the glycoprotein, it is possible that gp160 is identical to the 125I-labeled Mr 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 [35S]methionine-labeled surface antigen of Mr 83,000 to antimonocyte serum (17). A 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 radiiodinated 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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