Journal of Supramolecular Structure 11:503–515 (1979)
Tumor Cell Surfaces and Malignancy 345–357

Human Placental Cell Surface Antigens: Expression by Cultured Cells of Diverse Phenotypic Origin

Thomas A. Hamilton, H. Garrett Wada, and Howard H. Sussman

Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, California 94305

The present work examined the expression of cell surface glycoprotein antigens in cultured human cell lines. The set of glycoproteins studied was defined by their immunoreactivity with antiserum developed to Triton-solubilized extracts of placental brush border membranes. Studies were performed using cell lines of trophoblastic (BeWo, JEG-3) and nontrophoblastic (Chang liver cells) origin, as well as diploid fibroblast cell lines (WI-38, GM-38).

Antiplacental brush border antiserum reacts with at least 19 distinct antigens present in placental membrane preparations, each of which can be resolved and identified in two-dimensional electrophoresis. The subunit molecular weight and isoelectric point for all components were defined by their positions in the two-dimensional matrix. Thirteen of these could be detected among the five cell lines examined by lactoperoxidase-catalyzed cell surface iodination.

One of these 13 antigens has been identified as the placental isoenzyme of alkaline phosphatase (PAP). The expression of this component is limited to choriocarcinoma cells and Chang liver cells and it is not present in diploid fibroblasts. Under normal circumstances expression of PAP is unique to the differentiated placenta but has been frequently demonstrated in both trophoblastic and nontrophoblastic neoplasms.

Two other antigens are variably expressed among the different cell types examined in the present study and their presence or absence was independent of the trophoblastic, epithelial nontrophoblastic, or fibroblastic origin of the cells.

Ten surface antigens were expressed in all five cell lines. Six of these had previously been found common to membranes from three adult differentiated tissues, including liver and kidney, as well as placenta (Wada et al, J Supramol Struc 10(3):287–305, 1979). The presence of this set of antigens in cultured cells as well extends the possibility that these are ubiquitously expressed on human cell surfaces. Two other antigens observed in all cultured cells had been found in both placental and either kidney or liver membranes and may represent common functions shared by many tissues which are also necessary for growth in vitro. The two remaining placental antigens seen in all cultured cells have previously been shown to be absent in adult tissues. Their presence in cultured cells but not in the membranes of resting differentiated tissues may signify the expression of glycoproteins characteristic of trophoblasts in all cells adapted to growth in culture.

Received March 18, 1979; accepted August 1, 1979.

0091-7419/79/1104-0503$02.60

Key words: glycoproteins, two-dimensional electrophoresis, differentiation

Cell surface glycoproteins have been the subject of intense study in recent years because of their direct interaction with the cell's immediate external environment. Consequently, they may play a major role in dictating cellular behavior in response to specific environmental stimuli. Trophoblastic and neoplastic cells share some behavioral properties including invasiveness [1], escape from immunosurveillance [2,3], and cell surface charge properties [4,5] which may be determined by their surface glycoproteins. The identification of the placental isoenzyme of alkaline phosphatase on both trophoblastic and neoplastic cell surfaces [6] provides evidence of biochemical similarity as well. Other presumptive placental specific membrane antigens have been demonstrated on the surface of several tumor cell lines grown in tissue culture [7], but precise identification of such components is necessary for their further analysis. Our interest in this area has focused on the brush border membrane of the human placenta with the intent of defining the tissue distribution of glycoprotein components found in this organ.

Previous work from this laboratory described a set of 33 sialoglycoprotein subunits in purified brush border membranes utilizing high-resolution two-dimensional electrophoresis in polyacrylamide gel slabs [8]. Antiserum prepared against detergent-extracted membranes (anti-placental brush border serum [APBB]) in combination with two-dimensional electrophoresis demonstrated 18 of the original 33 presumptive glycoproteins to be antigenic [9]. For comparison with other tissues, the glycoprotein antigen content of human liver and kidney membranes were similarly examined. This analysis identified four glycoprotein subunits which were present only in the placental membranes. In addition, we identified ten of the original 18 subunits in liver or kidney, and seven of these were found in all three tissues examined. Those studies thus defined three groups of placental surface antigens: a) those which appear unique to placenta; b) those which exhibit limited but not unique tissue distribution; and c) those which appear common to all tissues and which may perform essential functions.

The present study considers the frequency with which glycoproteins that fit into these three categories are expressed in selected human cell lines. Using the xenogenic antiserum described above to compare the glycoprotein antigen content of trophoblastic and nontrophoblastic tumor cells or diploid fibroblastic cells, we can evaluate how the expression of such surface components relates to the specific cellular phenotype. In this study, we used two cell lines (BeWo, JEG-3) developed from human gestational choriocarcinomas which have been shown to retain a variety of biochemical properties characteristic of the differentiated trophoblast [10–13], the Chang liver cell line, which is nontrophoblastic in origin and has been in continuous culture for 25 years [14], and two diploid fibroblastic lines (WI-38, GM-38). A remarkably similar set of placental membrane antigens was expressed by these distinct cell lines. In concert with previous work, the present study defines a set of antigenic glycoproteins which may be ubiquitously expressed on human cell surfaces.

METHODS

Cell Culture

BeWo cells were obtained from the American Type Culture Collection ([ATCC], CCL 98). JEG-3 cells were kindly provided by Dr. Saul Rosen of the National Institutes of Health. Both cell lines were grown as previously described [15]. Chang liver cells were pro-

vided by Dr. R. S. Chang of the University of California at Davis, and were grown as described earlier [16]. WI-38 cells were obtained from the ATCC (CCL 75) and GM-38 were a gift from Dr. Jon Williams of the Department of Pathology at Stanford University. Both fibroblast cells were subcultured at intervals of 3–4 days by 1:2 splits in minimum essential medium (MEM) and 10% fetal calf serum. Antibiotic-Antimycotic (Gibco) was present at a 1:100 dilution. Both fibroblasts were used at passage levels of 15–25. All cells were grown until confluent (3–4 days following subculture) before use. Cell culture media and fetal calf serum were from Gibco. Cells were grown in polystyrene T-flasks (Corning).

Lactoperoxidase-Catalyzed Cell Surface Labeling

Monolayers of cells were washed twice with Earle's balanced salt solution without Ca^{++} and Mg^{++} followed by two washes with 10mM Na phosphate-buffered saline (PBS), pH 7.5. The monolayers were then thoroughly drained and 1 ml of PBS containing 50 μg lactoperoxidase (Sigma Chemical Co.) and 100–200 μCi of ^{125}I (New England Nuclear, carrier-free) was added. Iodination was started by addition of 10 μl of 250 μM hydrogen peroxide followed by an additional 10 μl after 5 min. Following a total incubation period of 10 min at room temperature, the labeling reaction was terminated by addition of 50 μl of 0.5 M NaI followed by two washes with PBS containing 50 mM NaI. A final wash of the monolayer was done with PBS. Examination of viability by Trypan Blue exclusion showed such cells to be $>$ 90% viable. After the flasks were drained of excess PBS, the cells were scraped from the plastic surface with a rubber policeman into 1 ml of PBS per T-75 flask. The resulting cell suspension was made 1% in Triton X-100 and extracted at 4°C for 10 min. The extracts were centrifuged at 1000g for 10 min to remove nuclei and cell debris and at 110,000g for 60 min. The trichloroacetic acid-precipitable radioactivity in the resulting supernatant was measured in a gamma counter.

Immunoprecipitation and Two-Dimensional Electrophoresis

The preparation and characterization of APBB serum as well as adsorption of this antiserum with liver microsomes has been previously described [9]. Between 200,000 and 400,000 cpm of iodinated cell extracts were reacted with 20 μl of APBB serum or 100 μl of liver-adsorbed APBB serum in the presence of 0.1% sodium dodecyl sulfate (SDS). After 1 hr at 4°C, the APBB-bound labeled antigens were precipitated by the addition of 200 μl of a 10% suspension of heat-killed, glutaraldehyde-fixed S. aureus according to Kessler [17]. After 1 h at 4°C, the mixture was centrifuged at 6000g for 10 min and the resulting pellet was washed twice in NET buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCL, pH 7.2, 0.5% v/v Triton X-100) containing 1 mg/ml ovalbumin (Sigma Chemical Co.) and 50 mM NaI. A final wash of the pellet was done in NET buffer. The washed pellet containing specific APBB-adsorbed antigens was extracted with 5 mM Na phosphate (pH 8.1), 8 M urea, and 2% Triton X-100 for 10 min at room temperature. The extracted material was subjected to sequential isoelectric focusing and SDS polyacrylamide gel electrophoresis as fully described in Wada et al [8].

Alkaline Phosphatase Assay

Alkaline phosphatase activity was measured in unlabeled crude sonicates of cells according to Bessey et al [18] and the placental isoenzyme was identified in Triton X-100 extracts with a previously described immunochemical assay [19]. Protein was measured by the method of Schaffner and Weissmann [20].

RESULTS

Figure 1 is a schematic representation of a two-dimensional electropherogram of placental brush border glycoproteins selected by immunoprecipitation with anti-placental brush border antiserum. Eighteen distinct components are present. Those components represented by open spots are potentially specific to placental tissue (P) by virtue of their absence in either liver or kidney. Those represented by filled spots are found in liver and kidney as well as placenta (PKL). The crosshatched spots are common to kidney and placenta but were not observed in liver plasma membranes (PK). The numbering system used in this figure and throughout this report corresponds to the originally identified components present in the total placental brush border membrane preparation [8,9]. Table I gives the subunit molecular weight (m_T) and isolectric point (pI) for each component in Figure 1.

The components described in Figure 1 were initially identified by radiolabeling of sialic acid moieties using [^{3}H] sodium borohydride reduction of periodate oxidized mem-

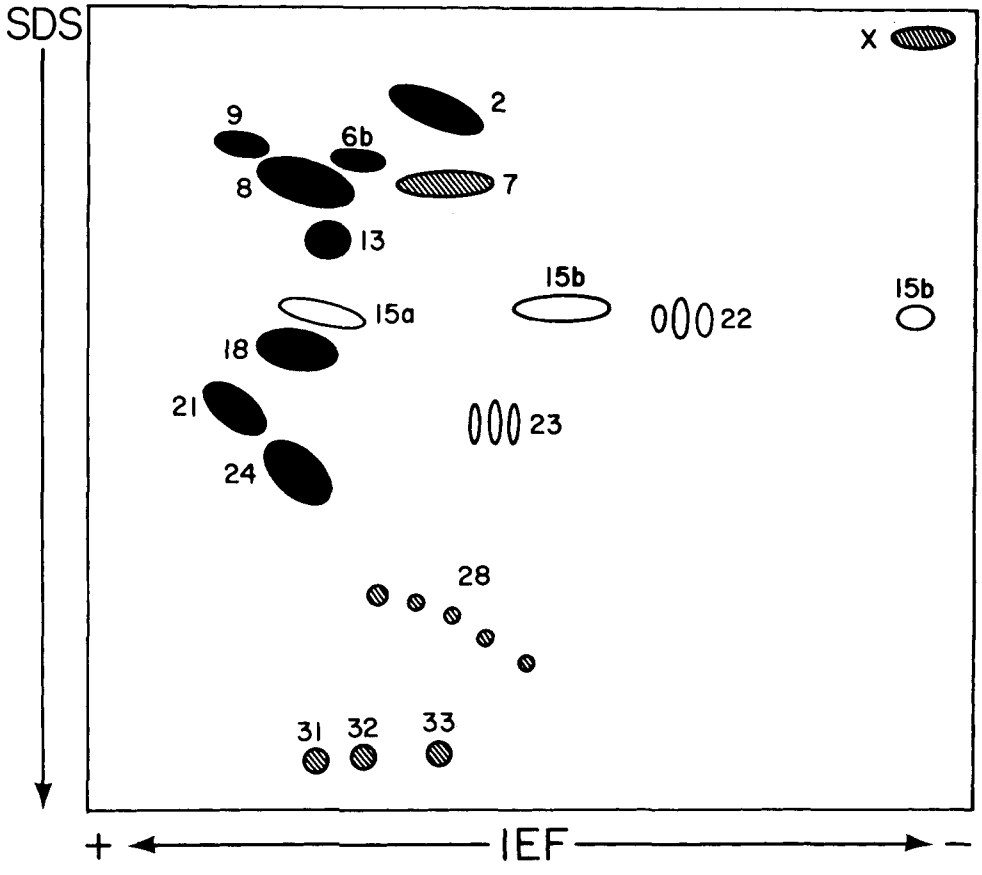


Fig. 1. Schematic diagram of two-dimensional electropherogram of placental brush border glycoprotein antigens. The relative positions and number assignments as well as the indicated tissue distribution are derived from a previous publication. Solid spots: subunits common to placenta, liver, and kidney; cross-hatched spots: subunits common to placenta and kidney only; open spots: subunits potentially specific to placenta.

branes. All except 28 and X were also labeled by lactoperoxidase-catalyzed incorporation of ^{125}I. Each component that labeled with both ^{3}H and ^{125}I was considered to be a glycoprotein. Component P23 was shown by coelectrophoresis of $^{32}P_i$ and ^{3}H-labeled membranes to be placental alkaline phosphatase [8]. Component P22 was identified as membrane-bound human transferrin by coelectrophoresis and radioimmunoassay [9]. Of the 18 components in Figure 1, only 12 will be considered below: PK X and 28 are not labeled with ^{125}I; 31, 32, and 33 are not adequately resolved for evaluation; and P22 is not an integral membrane protein.

Initially, we wished to examine a cell line of trophoblastic origin known to express placenta-specific functions. For this purpose, we selected the BeWo line, originally grown by serial transplantation of a human gestational choriocarcinoma in the hamster cheek pouch [10] and later adapted to growth in tissue culture [11]. As a nontrophoblastic cell, we chose the Chang liver cell line [14] because it is known to make at least one placental membrane glycoprotein (placental alkaline phosphatase) [16]. Lactoperoxidase-catalyzed iodination was used to label cell surface proteins and it was demonstrated that the incorporated iodine label banded at the same buoyant density as the alkaline phospatase-containing plasma membrane vesicles in sucrose density gradients. This indicated the cell surface specificity of the labeling method (data not shown).

TABLE I. Coordinates of Placental Brush Border Sialoglycoprotein Subunits in Two-Dimensional Maps

Spots	Apparent pI	Apparent M_r (k)
X[a]	8.15	308.0
2	5.80	235.0
6b	5.68	170.0
7[b]	5.88	161.0
8	5.68	148.0
9	5.18	175.0
13	5.86	123.0
15a	5.82	100.0
b	6.57	100.0
18	5.65	92.4
21	4.60	72.5
22a	7.10	74.6
b	7.19	74.6
23a	6.22	66.2
b	6.30	66.2
c	6.39	66.2
24	5.60	64.2
28a	5.79	47.0
b	5.89	46.0
c	6.00	44.0
d	6.12	42.0
e	6.22	41.0
31	4.60	<40.0
32	5.45	<40.0
33	5.66	<40.0

[a]X was only detectable after immunoprecipitation with APBB serum.
[b]7 was only detectable after immunoprecipitation with liver-adsorbed APBB serum.

Figure 2A shows a two-dimensional map of iodinated BeWo cell surface components immunoprecipitated with APBB serum. Nine of the 12 components under consideration could be identified in this gel. All three putative placenta-specific subunits are expressed by this cell line. Components P15a and P15b are easily seen. However, component P23 (placental alkaline phosphatase) is not seen in BeWo cells by surface labeling with ^{125}I. Previous studies using two-dimensional electrophoresis of $^{32}P_i$ active center labeled subunits have demonstrated this enzyme in membranes of BeWo cells [15]. Table II gives the specific activity of the placental isoenzyme of alkaline phosphatase in each of the cell lines examined in this study. In addition to the P-type subunits, six of eight PKL-type subunits were positively identified (PKL 26b, 8, 9, 21, 24), while 13 and 18 were not seen. Component 2, whose tissue distribution was previously undefined, is also present. Two components not previously described are observed in this autoradiogram; one (7a) focusing at a pI of 5.9 and having a M_r of 130,000 daltons, and a second (2a) which runs directly above component 2. Subunit 7a is observed at low levels in placental membranes labeled with ^{125}I.

Figure 2B presents a two-dimensional map of iodinated Chang liver cell surface components precipitated with APBB. This cell line expresses 11 of 12 original placental brush border antigens as well as 7a. The only apparent difference between the trophoblastic BeWo line and the nontrophoblastic Chang cell line is the presence of PKL 18 and the absence of 2a in Chang. All three putative placental subunits (P15a, P15b, and P23) are expressed in Chang cells. P15a is easily identified. A subunit which runs at the same M_r as P15b (100,000) but which does not enter the focusing gel is seen as the basic pole of the map. We have tentatively designated this component as P15b because it appears to be immunochemically related to P15b (see below) and in placental membrane preparations P15b has shown a similar failure to migrate from the basic pole in the isoelectric focusing dimension. The failure of this subunit to enter the focusing gel may be due to higher structure order which is not sufficiently disrupted by the 8 M urea present in the first-dimension buffer. P23 is not easily seen in Figure 2B but could be detected in the original autoradiogram. Previous studies have clearly identified this specific enzyme in Chang liver cells [16]. Table II shows that the placental alkaline phosphatase specific activity is nearly ten times higher in Chang cells than in BeWo cells.

The identity of the glycoprotein subunits present either in BeWo or Chang cells is confirmed by two-dimensional electrophoretic analysis of iodinated material immunoprecipitated with APBB serum that has been adsorbed four times with human liver microsomes. As shown in Figure 2 (C,D), components also found in liver membranes are either eliminated or reduced in intensity (PKL 6b, 8, 9, 21, 24), while those not previously observed in liver are unaffected or relatively enhanced (PK 7, P15a, 15b). Antibodies to PAP (P23) are not removed by absorption [9], but this component is very difficult to visualize when present at the low levels seen in these cultured cells. Component 7a is also eliminated by liver adsorption. The enhanced intensity of the 100,000-dalton polypeptide at the basic pole supports our suggestion that this corresponds to P15b. Because both 2 and 7a are removed by adsorption we conclude that these components are also present in liver tissue. The intensity of PKL 18 is seen to be unreduced in the Chang cell map using adsorbed antiserum (Fig. 2D). PKL 18 is found in liver and kidney membranes but antibodies to this antigen are not removed from APBB serum by adsorption with liver or kidney tissue or with cultured cells which contain this component (data not shown). This suggests that the antigenic determinants of PKL 18 recognized by APBB serum are not exposed when this glycoprotein is in the membrane.

The remarkable similarity demonstrated between BeWo and Chang liver cells in the results described above prompted our examination of other cultured cell lines in order to determine whether the expression of placental antigens is a common phenomenon in cells adapted to growth in vitro. We have examined the surface antigen composition of a second choriocarcinoma cell (JEG-3, a clonal cell line isolated independently of BeWo but from the same transplantable tumor [13]), as well as two fibroblastic cell lines of embryonic (WI-38) and adult (GM-38) origin. Table III provides a summary of the glycoprotein antigen composition for each cell line examined. Figure 3A–C shows three autoradiograms of APBB-precipitated ^{125}I-labeled material from these cells. Figure 3A is a map of JEG-3 which demonstrates that the two choriocarcinoma cell lines (BeWo and JEG-3) contain a qualitatively identical set of placental membrane antigens, an observation which is not unexpected considering their identical origin (see also Table II).

Figure 3 (B and C) shows electropherograms of iodinated cell surface antigens from WI-38 and GM-38 cells, respectively. These cell lines also expressed most of the placenta-related surface antigens. Six of eight PKL-type subunits and two of the three P-type subunits were detected. PKL 13 is seen only in WI-38. PKL 24 is not seen in this photograph of the WI-38 map (Fig. 3B) but was seen in other gels. P23 was not present, either in the map or by enzymatic assay (see Table II), but both 15a and 15b were readily visible for either cell line. The identity of the various antigenic subunits was confirmed in each of these additional cell lines (WI-38, GM-38, JEG-3) by means of two-dimensional gels of liver microsome-adsorbed APBB immunoprecipitates, as had been done for BeWo and Chang cell membrane antigens (see Fig. 2C, D). In addition, all gel patterns were reproducible using cells grown and labeled on separate occasions.

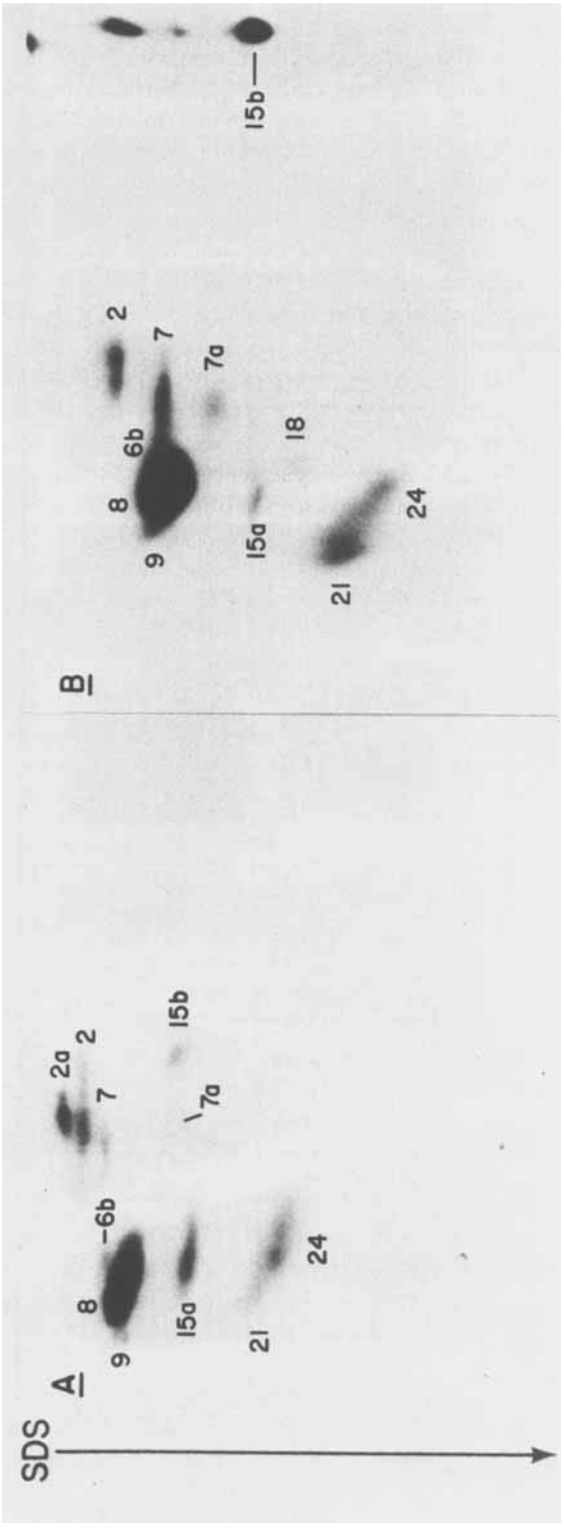
TABLE II. Placental Alkaline Phosphatase Specific Activities

Cell line	Specific activity (Units × 10^{-3}/mg protein)
BeWo	23
Chang	170
JEG-3	7
WI-38	-
GM-38	-

TABLE III. Summary of Plasma Membrane Antigen Compositions

Tissue or cell line	Antigens												
	2	6b	7a	7	8	9	13	15a	15b	18	21	23	24
Placenta	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	–	+	+	+	–	–	+	+	–	+
Kidney	–	+	ND	+	+	+	+	–	–	+	+	–	+
BeWo	+	+	+	+	+	+	–	+	+	–	+	+	+
JEG-3	+	+	+	+	+	+	–	+	+	–	+	+	+
Chang	+	+	+	+	+	+	–	+	+	+	+	+	+
WI-38	+	+	+	+	+	+	+	+	+	–	+	–	+
GM-38	+	+	+	+	+	+	–	+	+	–	+	–	+

ND – Not determined.



2

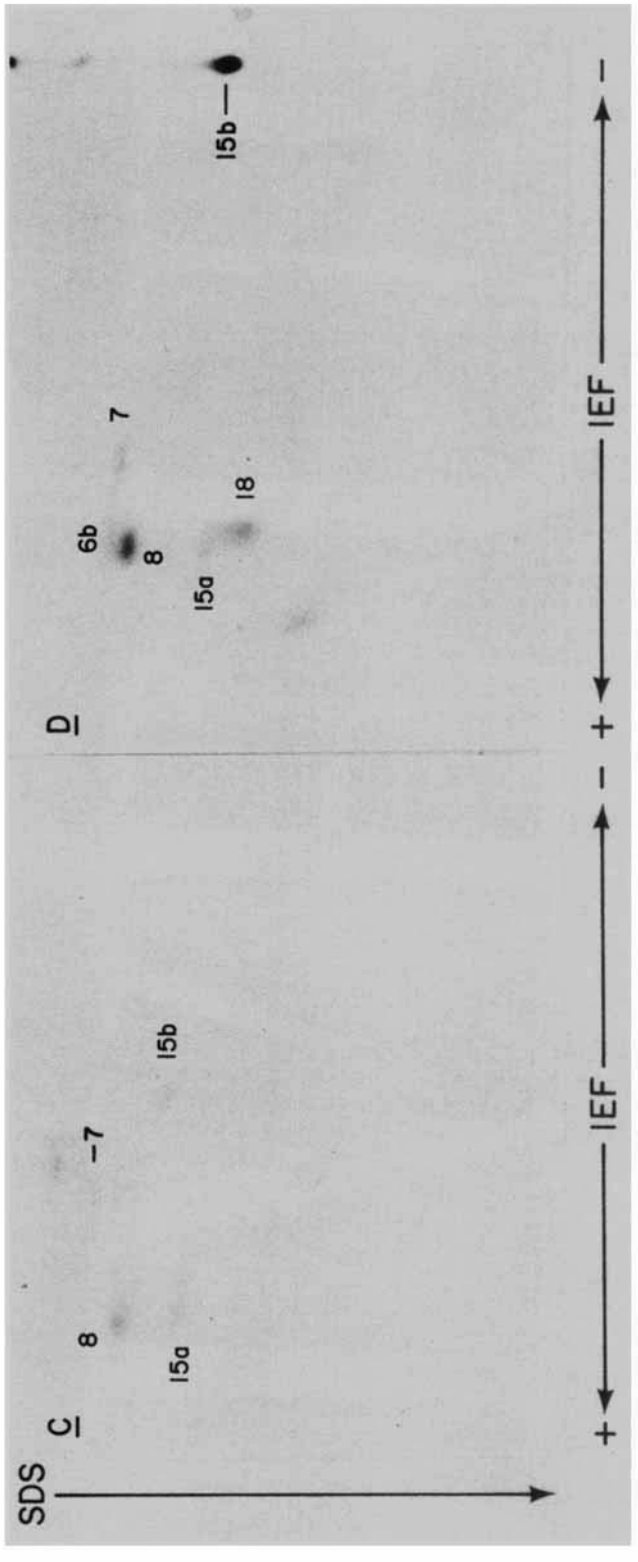


Fig. 2. Two-dimensional autoradiographs of cell surface labeled antigens from BeWo and Chang liver cells. A confluent T-75 flask of either cell type was iodinated as described in Experimantal Procedures; 300,000 acid-precipitable cpm of iodinated detergent-solubilized proteins was then precipitated with either APBB serum or liver-adsorbed APBB serum and finally subjected to two-dimensional electrophoresis. Gels were dried and exposed for four days at −76°C using Dupont "Lightening-Plus" intensifying screens. A) BeWo vs APBB; B) Chang liver vs APBB; C) BeWo vs liver-adsorbed APBB; D) Chang liver vs liver-adsorbed APBB.

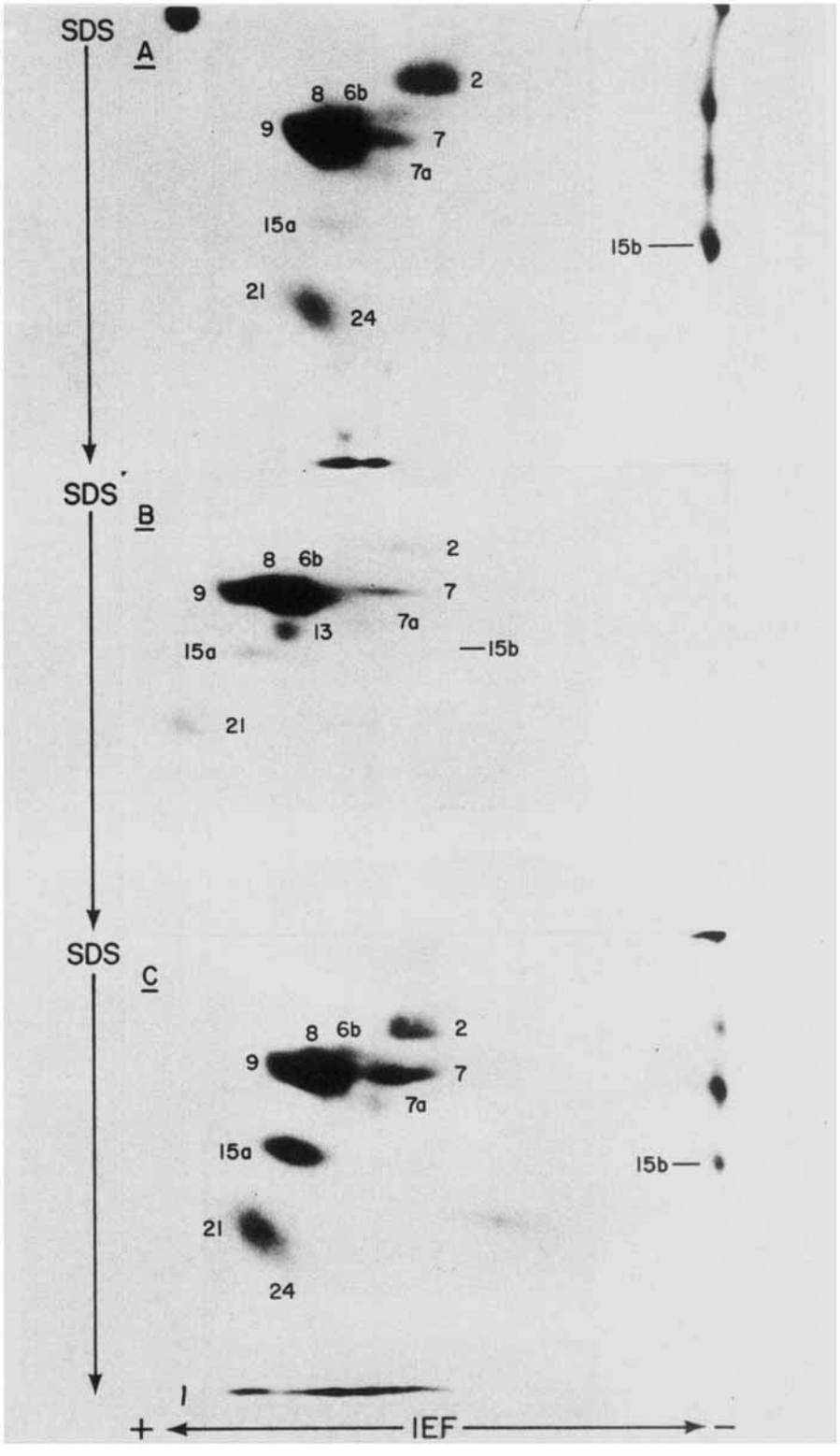


Fig. 3. Two-dimensional autoradiographs of cell surface antigens of JEG-3, WI-38, and GM-38. JEG-3 (300,000 cpm) and WI-38 or GM-38 (200,000 cpm) were immunoprecipitated with APBB serum. Two-dimensional electrophoretic analysis was as described in Figure 2. A) JEG-3; B) WI-38; C) GM-38.

DISCUSSION

The experiments described in the present report focus on the expression of a defined set of surface antigens in cell lines derived from gestational choriocarcinoma as well as in other transformed or diploid cells growing in culture. The application of lactoperoxidase-dependent ^{125}I-labeling in the present study convincingly demonstrates that nearly all of the antigens previously identified in placental brush border membranes are in fact located on the exterior surface of the cell and are in direct contact with the environment. These cell surface components were also shown to be glycoproteins by virtue of their coincident labeling with reagents directed toward both the protein and carbohydrate moiety of the molecules [8,9]. Whether PK 28, X, 31, 32, and 33 are located on the plasma membrane could not be assessed because they were either not labeled by ^{125}I or could not be adequately resolved. Components 2 and 7a, which labeled with ^{125}I, had not been demonstrated previously by specific labeling of sialic acid groups, and consequently they may not be sialoglycoproteins. Both components may also be present in liver membranes because adsorption of APBB serum with liver microsomes removes these components from immunoprecipitates.

The resolving potential of two-dimensional electrophoresis has been thoroughly documented [21]. Because glycoproteins especially exhibit heterogeneity in both focusing and SDS dimensions, the individual spots have unique morphology, facilitating identification in the two-dimensional matrix. The certainty of individual component identification is further improved by the application of immunologic criteria. By using a multivalent antiserum to preselect the antigenic proteins and then adsorbing this antiserum with a membrane-enriched fraction of human liver, each component considered must fit these four objective criterial before identity is confirmed (ie, pI, M_r, antigenicity, loss of immunoreactivity following adsorption of the antiserum).

The two-dimensional autoradiographic technique used to separate and visualize the membrane antigens is a qualitative rather than a quantitative method. However, we are able to provide an estimate of its lower limit of detection by using placental alkaline phosphatase as a specific example. In Chang liver cells this enzyme is barely visible by autoradiography at a level of approximately 150–300 ng/mg total cell protein as estimated both from enzyme activity measurements and radioimmunoassay (Hamilton and Sussman, unpublished data). Hence, any cell surface component which represents 0.015–0.03% or more of the total cell protein should be detectable in the current system. Such sensitivity is of course also dependent upon the relative intensity of radiolabeling for the component under consideration.

Consideration of the antigenic complement of all five cell lines examined here shows them to be remarkably similar. All five express 10 of 14 components in common (2, 7, 7a, 6b, 8, 9, 15a, 15b, 21, 24). The only major distinctions are: 1) the presence of placental alkaline phosphatase in BeWo, JEG-3, and Chang detected by enzymatic immunochemical assay, and its absence in WI-38 and GM-38; 2) the expression of PKL 13 in WI-38, which was not detected in either choriocarcinoma cells or in Chang liver cells; and 3) the expression of PKL 18 in Chang cells but not in choriocarcinoma or fibroblasts.

Our previous work showed that six (7a, 6b, 8, 9, 21, 24) of these common antigens were also found in human kidney and liver membranes (see Wada et al [9]; and unpublished data). With the exception of human lymphocytes and lymphoblastoid cell lines (Hamilton et al [21a]), these components appear to be ubiquitously expressed on human cell surfaces. Two other components among the 10 common antigens (PKL 2 and PK 7) are also present on liver and kidney membranes, respectively. These may represent

relatively common entities expressed by most but not all tissues whose functions are also necessary for in vitro growth. It has been observed that xenogenic antisera prepared against human membrane-bound antigens detect many common antigens [22,23]. The predominance of commonly expressed components among these antigens bound by APBB serum may result from this phenomenon. These common antigens may be minor components in some cell types and consequently would be masked in whole-membrane extracts by the major membrane components. However, the use of immunoprecipitation enhances their detectability.

Neville and Glossman [24, 25] have studied the plasma membrane protein and glycoprotein composition of three adult rat tissues in one-dimensional SDS polyacrylamide gel electrophoresis and observed that the pattern for each tissue was largely distinct. In our previous study the two-dimensional maps of total plasma membrane glycoproteins from human placenta and liver also demonstrated distinct patterns [9]. These results suggested that cell surface phenotypes may result principally from a display of a unique combination of components on the plasma membrane. However, quantitative expression of common antigens as indicated by relative spot intensity varies considerably among different cell and tissue types. This variation could be partially responsible for some phenotypic differences between highly differentiated cell types.

Several laboratories have identified common human cell surface antigens in cultured cells and defined the chromosome segments coding for their expression [23, 26, 27]. For the most part, these antigens have been demonstrated and studied by immunologic methods and their molecular characteristics have not been determined. Consequently, the relationship between these antigens and those described in the present report remains to be determined.

Several of the antigens commonly expressed in the cultured cell lines studied here were not detected at any level in kidney or liver membranes (P15a and P15b). The presence of these two components on all five cell lines examined could be accounted for by the stringent selective pressures applied to cells adapting to growth in vitro. The re-expression of functions repressed during differentiation and the loss of luxury functions normally expressed in the original differentiated tissue locale are commonly observed phenomena in cultured cells [28].

The present study clearly defines a set of commonly expressed human cell surface antigens according to the criteria of M_r and pI. Additionally, components which may show a more limited tissue distribution are identified and characterized. This work provides a firm data base for the further study of both the tissue distribution and functional identity of each glycoprotein entity. This approach should prove to be of value in the understanding of molecular events occurring at the cell-environment interface.

ACKNOWLEDGMENTS

This work was supported by contract CB74086 and grant CA13533 from the National Cancer Institute of the National Institutes of Health. T.A.H. was supported by a postdoctoral traineeship from the National Institutes of Health. We wish to express our appreciation to Agnes Tin for her technical assistance in cell culture, and to Philip Hass for his technical assistance in electrophoretic analysis.

REFERENCES

1. Billington WD: In Bishop MWH (ed): "Advances in Reproductive Physiology." New York: Academic, 1971, vol 5, No. 2, pp 27–66.
2. Beer AE, Billingham RE: Sci Am 230:36, 1974.
3. Nicolson GL: Biochim Biophys Acta 458:1, 1976.
4. Gasic G, Baydak T: In Brennan MJ, Simpson WL (eds): "Biological Interactions in Normal and Neoplastic Growth." Boston: Little, Brown, 1961, pp 709–719.
5. Hause LL, Pattillo RA, Sances A Jr, Mattingly RF: Science 169:601, 1970.
6. Sussman HH: In Ruoslahti E, Engvall E (eds): "Chemistry of Tumor-Associated Antigens." Scand J Immunol (Suppl 6), 1978, pp 126–140.
7. Faulk WP, Temple WP, Lovins RE, Smith N Proc Natl Acad Sci USA 75:1947, 1978.
8. Wada HG, Górnicki S, Sussman HH: J Supramol Struct 6:473, 1977.
9. Wada HG, Hass PE, Sussman HH: J Supramol Struct 10(3):287–305, 1979.
10. Hertz R: Proc Soc Exp Biol Med 102:77, 1959.
11. Pattillo RH, Gey GO: Cancer Res 28:1231, 1968.
12. Pattillo RH, Story MT, Hershman JM, Delfs E, Mattingly RF: In Anderson NG, Coggin JH Jr, Cole E, Holleman JW (eds): "Embryonic and Fetal Antigens in Cancer." Proceedings of the Second Conference, Springfield, VA: National Technical Information Service, vol 2, 1972, pp 45–52.
13. Kohler PO, Bridson WE: J Clin Endocrinol Metab 32:683, 1971.
14. Chang RS: Proc Soc Exp Biol Med 87:440, 1954.
15. Hamilton TA, Tin AW, Sussman HH: Proc Natl Acad Sci USA 76:323, 1979.
16. Ludueña MA, Iverson GM, Sussman HH: J Cell Physiol 91:119, 1977.
17. Kessler SW: J Immunol 115:1617, 1975.
18. Bessey OA, Lowry OH, Brock MJ: J Biol Chem 164:321, 1946.
19. Sussman HH, Small PA, Cotlove E: J Biol Chem 243:160, 1968.
20. Schaffner W, Weissmann C: Anal Biochem 56:502, 1973.
21. O'Farrell PH: J Biol Chem 250:4007, 1975.

21a. Hamilton TA, Wada HG, Sussman HH: Scand J Imm (in press).

22. Williams AF: In Ada GL, Porter RR (eds): "Contemporary Topics in Molecular Immunology," 1977, vol 6, pp 93–116.
23. Barnstable CJ, Bodmer WF, Brown G, Galfre G, Milstein C, Williams AF, Ziegler A: Cell 14:9, 1978.
24. Neville DM, Glossman H: J Biol Chem 246:6335, 1971.
25. Glossman H, Neville DM: J Biol Chem 246:6339, 1971.
26. Buck DW, Bodmer WF: Cytogen Cell Gen 14:257, 1974.
27. Kao FT, Jones C, Puck TT: Somatic Cell Gen 3:421, 1977.
28. Schwarz RI, Bissell MJ: Proc Natl Acad Sci USA 74:4453, 1977.