Cell Membrane Sialoglycopeptides of Corticoid-sensitive and -resistant Lymphosarcoma P1798

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SUMMARY

Cells of corticoid-sensitive (CS) and corticoid-resistant (CR) lymphosarcoma P1798 were labeled in vivo with either [14C]- or [3H]fucose before and after treatment with 9-α-fluoroprednisolone (9-FP). Labeled glycopeptides, derived from isolated, Pronase-digested cell membranes of both tumors were separated by gel filtration on Bio-Gel P-10 by a double-label technique. Elution profiles of CS and CR fractions showed significant differences in early eluting material. Desialylation of glycopeptides by neuraminidase lowered the molecular weight of both CS and CR fractions, and altered 1H:14C ratios indicated that CS and CR sialoglycopeptides are different. 9-FP treatment for 7 hr increased the density of isolated P1798-CS and -CR cell membranes. All CS and CR glycopeptides from treated tumors eluted faster than did those of untreated preparations. Both CS and CR sialoglycopeptides were altered, although differences in CS and CR profiles persisted. Histochemical investigations indicated that negative charge, present on surfaces of untreated CS cells, is lost between 6 and 8 hr after exposure in vivo to 9-FP. CR cells had no or few anionic sites on their surfaces before and after steroid administration. We demonstrated that glycopeptides of both CS and CR tumors contain sialic acid, although only CS cells carry a surface-exposed negative charge that is lost after 9-FP treatment. Glucocorticoids alter both P1798-CS and -CR sialoglycopeptides, but the consistent differences between their chromatographic patterns suggest that steroid-induced changes in cell membranes of the two tumors are not identical. Cell death or survival of glucocorticoid-treated P1798 cells may, therefore, be influenced by specific structural characteristics involving cell surface sialoglycoproteins.

INTRODUCTION

Evidence is accumulating that cell membrane glycoproteins, which are unique for different cells, are associated with a variety of biological phenomena. The carbohydrate components of these proteins are exposed at the cell surface and are of major importance in physiological events (16, 18, 39). The various monosaccharide units and the different linkages among them offer the potential of numerous combinations and permutations with specific informational content. Sugars that naturally occur in glycoproteins have been reported to alter cell growth and metabolism selectively (8). Much attention has been given to the negatively charged sialic acids on the cell surface. These strongly acidic sugars have been implicated in events such as the homing of circulating lymphocytes (12), implantation of ova (11), the capacity of malignant tumors to metastasize (24), and the secretion of insulin by pancreatic β-cells (15).

Sialic acids have been shown to contribute most of the negative surface charge on normal and leukemic lymphocytes (2, 7). We demonstrated that CS cells of P1798 and 6C3HED lymphomas carry a negative surface charge, whereas cells of CR strains of these tumors have no or few anionic sites on their exposed surfaces (4). Glucocorticoid hormones have been shown to alter electrophoretic mobility in hepatoma cells by decreasing negative surface charge (3). These changes require antibiotic-, pH-, and temperature-sensitive synthetic steps. In CR lymphoid tissues, the inhibition of nutrient transport into the cell by corticosteroids (21, 22, 28, 29, 32) involves similar synthetic events (23), although no involvement of the cell surface has yet been reported.

After administration of glucocorticoids to mice bearing P1798, CS tumors shrink, due to cell lysis, whereas CR tumors continue to grow. The purpose of this study was to compare labeled cell membrane glycopeptides of P1798-CS and -CR before and after treatment with 9-FP. CS and CR tumors were labeled in vivo with either L-[14C]- or L-[3H]fucose as plasma membrane marker. Pronase-digested-labeled glycopeptides were separated by gel filtration on Bio-Gel P-10 before and after treatment with neuraminidase. Possible steroid-induced effects on cell surface charge were examined histochemically.

MATERIALS AND METHODS

Animals and Tumors. CS and CR cell lines of lymphosarcoma P1798 were maintained in female BALB/c mice (NIH Mammalian Genetics and Animal Production Section, Drug Research and Development, National Cancer Institute, Bethesda, Md.) (31). Tumor transplant generations used in this

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3 To whom requests for reprints should be addressed.

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study were tested for corticoid sensitivity with 9-FP as described by Stevens et al. (33).

Isolation of Plasma Membranes. Tumors, ranging from 1 to 2 g, were removed from the animals and processed for the isolation of plasma membranes under ice-cold conditions. The tissue was minced in Hanks' BSS, pH 7.4, and filtered through 4 layers of surgical gauze. A packed cell pellet was obtained by centrifugation of the cell suspension at 150 × g for 5 min. For the removal of RBC, the pellet was resuspended in a mixture of 1 volume of Hanks' BSS and 1 volume of 0.85% ammonium chloride, pH 7.4 (adjusted with 1 M Tris). The suspension was again centrifuged (at 150 × g for 5 min), and hemoglobin and RBC ghosts were removed with the supernatant. The tumor cell pellet was processed for the isolation of cell membranes by a method similar to that described by Ray (26). The packed cell volume was resuspended in 2 volumes of hypotonic buffer (1 mM NaHCO3, 2 mM CaCl2, pH 7.5). The cells were allowed to swell for 10 min and were then homogenized in a Dounce homogenizer (Pestle B) by 20 to 30 gentle downstrokes. Breakage (at least 95%) of cells was monitored by phase microscopy. The homogenate was diluted to 100-fold the initial cell volume with hypotonic buffer and left standing for 10 min. Subsequent steps are shown schematically in Chart 1.

The crude membrane pellet was resuspended in a small volume of hypotonic buffer and homogenized in a 7-ml Dounce homogenizer (Pestle A) by 1 to 2 gentle downstrokes to break up small clumps. The suspension was diluted with 60% sucrose to a final concentration of 48% sucrose. Portions of the membrane suspension (7.5 ml) were placed in Beckman cellulose nitrate tubes (1-inch di-
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were made up in 5 mM Tris containing 5 mM CaCl₂, pH 7.4. The tubes were centrifuged for 2 hr at 25,000 rpm in an SW27 rotor at 4°. Membrane fractions appeared at the interfaces between 37 and 41% (L1), as well as between 41 and 45% sucrose (L2) in the form of a white, compact sheet. The membrane layers were removed and washed free of sucrose by repeated suspension and centrifugation in 50 ml of 0.85% NaCl solution.

Chemical Assays. Protein was determined by the method of Lowry et al. (19). Succinate dehydrogenase activity was assayed by the method of Reid (27). β-Nitrophenol-phosphatase, glucose-6-phosphatase, and Mg⁺⁺-dependent ATPase activities were determined as described by Ray (26), and the P₁ released (by glucose-6-phosphatase and Mg⁺⁺-activated ATPase) was measured by the method of Chen et al. (6). The sialic acid content of homogenates and cell membrane fractions was determined as described by Jourdain et al. (17).

Labeling and Steroid Treatment. Groups of 10 to 12 each of CS and CR tumor-bearing animals were selected for differential labeling in vivo. Each animal of an experimental group was given an i.p. injection of either 12.5 μCi of L-[¹⁴C]fucose or 25 μCi of L-[1,5,6-³H]fucose in 0.5 ml of 0.85% NaCl solution. For labeling studies after steroid treatment, animals received i.p. injections of 9-FP (25 mg/kg of body weight) 2 or 3 hr before the administration of radioactive fucose. After 4 hr of in vivo labeling, cell membranes of treated and untreated CS and CR tumors were isolated as described above.

Preparation of Labeled Plasma Membranes for Gel Filtration. Appropriate portions of isolated P1798-CS and -CR cell membranes (pooled L1 and L2 fractions containing 2 to 6 mg protein), which had been differentially labeled with [¹⁴C]- and [³H]fucose, were mixed and solubilized in 1 ml of 1% sodium dodecyl sulfate at 60° for 45 min to 1 hr. The solubilized membrane proteins were diluted to a total volume of 15.5 ml with 0.2 M sodium phosphate buffer, pH 7.8, containing 1.5 mM CaCl₂ and 10 mg Pronase (K & K Laboratories, Inc., Plainview, N. Y.). Pronase digestion and dialysis of Pronase-digested material were carried out as described by van Beek et al. (35). The nondialyzable material was lyophilized and then dissolved in 10 ml of 0.05 M sodium acetate buffer, pH 5.5, containing 6.8 mM CaCl₂. The mixture was divided into 2 equal portions. To one of the samples 50 units of Vibrio cholerae neuraminidase (General Biochemicals, Chagrin Falls, Ohio) were added; the other portion served as the control. Both samples were incubated for 2 hr at 37° on a gyratory shaker. The preparations were then lyophilized and stored at −20° until fractionation by chromatography.

Gel Filtration. The lyophilized material was dissolved in 1 ml elution buffer (0.1 M Tris-sodium acetate, pH 9, containing 0.1% sodium dodecyl sulfate, 0.01% EDTA, and 0.1% mercaptoethanol). Blue dextran 2000 (Pharmacia Fine Chemicals, Piscataway, N. J.) and phenol red were added to serve as high- and low-molecular-weight markers, respectively. The samples (³H, 4000 to 9000 cpm; ¹⁴C, 4000 to 8000 cpm) were fractionated on Bio-Gel P-10, 200 to 400 mesh (Bio-Rad Laboratories, Richmond, Calif.) (35). The column measured 100 × 11 mm, and the samples were eluted in 0.8-ml fractions at a rate of 3 ml/hr. ³H and ¹⁴C radioactivity was counted in Aquasol (New England Nuclear, Boston, Mass.) with either a Packard Tri-Carb Model 3380 or an Intertechnique SL-30 liquid scintillation spectrometer. Counting efficiencies were 25% for ³H and 52% for ¹⁴C. All fractionation experiments were repeated with reversed radioisotope labeling.

Labeled Compounds. L-[¹⁴C]Fucose (56.2 mCi/mmmole) and L-[³H]fucose (3.73 Ci/mmmole) were obtained from New England Nuclear. The purity of radioactive fucose was confirmed by thin-layer chromatography on 20 × 5-cm cellulose plates (J. T. Baker Chemical Co., Phillipsburg, N. J.) which were developed in 1-propanol: H₂O:ethyl acetate (7:2:1) and in butanol:ethanol: H₂O (10:1:2). The position of the radioisotopes was determined with a Packard Model 7201 radiochromatogram scanner and compared with the Rₚ of nonradioactive fucose.

Electron Microscopy. P1798-CS and -CR tumors were treated in vivo with 9-FP (25 mg/kg of body weight). At 0, 3, 6, and 15 hr after treatment, cells of both tumors were fixed in the presence of the cationic dye, Alcian blue 8GX, and prepared for electron microscopy as described previously (4).

The above procedure was also carried out on CS and CR cell suspensions after the removal of dead cells, free nuclei, and BRC by centrifugation on Hypaque:Ficoll. Hypaque:Ficoll was prepared by mixing 10 parts of 33.9% Hypaque (Winthrop Laboratories, New York, N. Y.) and 24 parts of 11% Ficoll (Pharmacia Fine Chemicals, Piscataway, N. J.). The final density was 1.082 at 23°. CS and CR cell suspensions (10⁶ cells in 1 ml of Hanks’ BSS, pH 7.4) were layered on 2 ml of the above Hypaque:Ficoll mixture and centrifuged at 100 × g for 5 min. Viable cells (as judged by trypan blue exclusion) at the Hypaque:Ficoll-Hanks’ BSS interface were aspirated, washed in 10 ml Hanks’ BSS, and prepared for electron microscopy (4).

Isolated cell membranes were fixed in 1% OsO₄ in 0.1 M sodium cacodylate, pH 7.3, for 30 min at 0°, dehydrated in ethanol, and embedded in Epon 812 (20). Thin sections for electron microscopy were cut either on an MT-2 Porter-Blum or an LKB ultramicrotome and stained with uranyl acetate and lead citrate (37). Electron micrographs were taken with a Phillips 300 electron microscope at 80 kV.

RESULTS

Flotation through a discontinuous sucrose gradient yielded plasma membranes of acceptable purity from both P1798-CS and -CR which appeared at the interfaces between 37 and 41% (L1) and between 41 and 45% sucrose (L2). Figs. 1 and 2 show electron micrographs of L1 and L2 sections, respectively. Both L1 and L2 membranes showed a trilamellar structure that is typical of cell membranes (Fig. 3). Total recoveries of cell-membrane protein (L1 and L2) ranged from approximately 3 to 5% of starting protein (Table 1). Most of the membrane material was found in the L1...
fraction (more than 80% of total membrane protein). Enzyme activities are given in Table 2. 5'-Nucleotidase and Na⁺:K⁺-activated, Mg²⁺-dependent ATPase activities in both CS and CR cell membrane fractions were too low to be measured accurately. Apparently, these enzymes cannot be considered plasma membrane marker enzymes in most leukemic lymphocytes (25, 38). p-Nitrophenylphosphatase (a plasma membrane marker) also showed low, but measurable, activity in all (CS and CR) L1 and L2 fractions. The only enzyme (of those tested) that showed a high concentration in plasma membranes was Mg²⁺-activated ATPase. In CS samples, the activity of this enzyme was significantly lower in L1 fractions than in L2 fractions, whereas no marked difference in activity was found in CR L1 and L2 samples. Plasma membranes are known to consist of distinct areas with respect to density, composition, and enzyme activity (9). The absence of succinate dehydrogenase (a mitochondrial enzyme) activity in L1 and L2 membranes was consistent with the absence of mitochondria in electron micrographs of these fractions (Figs. 1 and 2). Glucose-6-phosphatase (a marker of the endoplasmic reticulum) showed low activity in all L1 and L2 samples, comparable to those reported by other authors for lymphocyte membranes (1, 36). Some contamination of L1 and L2 preparations with membranes of the endoplasmic reticulum cannot be excluded.

Sialic acid was most concentrated in L1 fractions of both CS and CR preparations (Table 3). Values for L2 samples did not differ significantly from those obtained for homogenates. The mean sialic acid content based on membrane protein was lower in CR samples than in CS samples; however, these differences were not statistically significant.

### Table 1

<table>
<thead>
<tr>
<th>Protein content of homogenate (100 to 150 mg) = 100%</th>
<th>Percentage of recovery of plasma membrane protein</th>
</tr>
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<tbody>
<tr>
<td>% of recovery</td>
<td></td>
</tr>
<tr>
<td>CS-L1</td>
<td>4.0 ± 0.79b (6)b</td>
</tr>
<tr>
<td>CR-L1</td>
<td>3.7 ± 0.42 (6)</td>
</tr>
<tr>
<td>CS-L2</td>
<td>0.6 ± 0.28 (6)</td>
</tr>
<tr>
<td>CR-L2</td>
<td>0.7 ± 0.38 (6)</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

b Numbers in parentheses, numbers of determinations.

### Table 2

<table>
<thead>
<tr>
<th>Specific activities of selected enzymes in homogenates and plasma membrane fractions of lymphosarcoma P1798b</th>
</tr>
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<tbody>
<tr>
<td>Preparation</td>
</tr>
<tr>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Homogenate-CS</td>
</tr>
<tr>
<td>Homogenate-CR</td>
</tr>
<tr>
<td>CS-L1</td>
</tr>
<tr>
<td>CR-L1</td>
</tr>
<tr>
<td>CS-L2</td>
</tr>
<tr>
<td>CR-L2</td>
</tr>
</tbody>
</table>

* All enzyme activities are expressed in μmoles of product produced per mg protein per hr.

** Mean ± S.E.

b Numbers in parentheses, numbers of experiments.

Chromatographic Patterns before and after Treatment with 9-FP. Radioactivity incorporated into the cell membrane fraction 4 hr after injection of L-[¹⁴C]- or L-[³H]fucose was found to be sufficient for this study. Approximately 13 to 15% of both ¹⁴C- and ³H-labeled material was lost from all membrane preparations during dialysis after Pronase digestion. Recovery of radioactivity after filtration through Bio-Gel P-10 ranged from 90 to almost 100% for all samples.

Chart 2A shows the elution profile of differentially labeled P1798-CS and -CR samples before exposure in vivo to 9-FP. Radioactivity was eluted in 2 major regions. Both CS and CR glycopeptide preparations contained early eluting, high-molecular-weight fractions (Peak a) that accounted for 18 to 20% of total radioactivity in CR samples, compared to only 7 to 9% in CS samples. Fractions of lower molecular weight, making up the largest portion of labeled glycopeptides (Peak b), were present in smaller amounts in CR preparations than in CS preparations. A 3rd low-molecular-weight shoulder was barely discernible in both CS and CR chromatographic profiles.

Removal of glycopeptide-bound sialic acid by V. cholerae neuraminidase resulted in an overall shift of both P1798-CS and -CR-derived fractions to an apparently lower molecular weight (Chart 2B). ³H:¹⁴C ratios were altered (Chart 5A), which was reflected in differences in the degree of shift of CS and CR desialylated glycopeptides in the chromatographic profile (compare Chart 2B with Chart 2A). The small shoulder between Fractions 44 and 55 (Chart 2A) had disappeared. The neuraminidase used in this study was found to remove 80% of sialic acid from unlabeled CS and CR cell membranes. No fucose was released, as determined by a method described by Spiro (30). Removal of some hexoses...
by contaminating enzymes cannot be excluded. Elution profiles similar to those described were reproduced in 2 additional experiments. The percentage of variability of recovered radioactivity for Peaks a and b is given in Table 4.

No significant change in glycopeptide fractions was observed 6 hr after in vivo 9-FP treatment (Chart 3A), with the exception that incubation with neuraminidase produced a new low-molecular-weight peak (Chart 3B) that is barely visible in Chart 3A.

Seven hr after 9-FP treatment, the isolated labeled cell membranes of both P1798-CS and -CR showed a change in density. As compared to untreated samples and membranes isolated 6 hr after steroid administration, a significant portion of L1 fractions (approximately 50%), usually found at the 37%-41% sucrose interface, had shifted to the 41%-45% sucrose interface. The chromatographic profiles of glycopeptides derived from these membranes showed a general shift of both CS and CR fractions to the left (Chart 4A), suggesting an increase in molecular weight. Desialylation

Chart 2. Elution profiles of L-fucose-containing glycopeptides of sodium dodecyl sulfate-solubilized and Pronase-digested cell membranes from differentially labeled P1798-CS and -CR, following filtration through Bio-Gel P-10 before (A) and after (B) treatment with neuraminidase. •, ³H; ○, ¹⁴C. The radioactivity of the fractions is reported as percentage of total radioactivity eluted from the column. B.D., blue dextran.

Chart 3. Elution profiles of L-fucose-containing glycopeptides of sodium dodecyl sulfate-solubilized and Pronase-digested cell membranes from differentially labeled P1798-CS and -CR 6 hr after treatment in vivo with 9-FP. Samples were filtered through Bio-Gel P-10 before (A) and after (B) treatment with neuraminidase. •, ³H; ○, ¹⁴C. The radioactivity of the fractions is reported as percentage of total radioactivity eluted from the column. B.D., blue dextran.

Table 4

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Peak a</th>
<th>Peak b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>18.0–20.0 (3)</td>
<td>70.4–73.1 (3)</td>
</tr>
<tr>
<td>P1798-CR</td>
<td>7.7–9.7 (3)</td>
<td>78.0–81.6 (3)</td>
</tr>
<tr>
<td>P1798-CS</td>
<td>8.5–9.9 (2)</td>
<td>84.0–84.6 (2)</td>
</tr>
<tr>
<td>6 hr after 9-FP</td>
<td>19.5–20.6 (2)</td>
<td>72.9–73.0 (2)</td>
</tr>
<tr>
<td>P1798-CR</td>
<td>8.7–8.9 (2)</td>
<td>79.5–83.2 (2)</td>
</tr>
<tr>
<td>P1798-CS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of determinations.
induced the appearance of an additional low-molecular-weight peak (Chart 4B) similar to but much more prominent and of higher molecular weight than that found 6 hr after exposure to 9-FP (Chart 3B). Neuraminidase altered $^3$H:$^{14}$C ratios in both treated (6 and 7 hr after 9-FP administration) preparations (Chart 5, B and C). The distribution of radioactivity obtained with reversed radioisotope labeling (after steroid treatment) was similar to that described (Table 4).

**Effects of Centrifugation on Hypaque:Ficoll.** Electron microscopic examination of P1798 cells that had been exposed to Hypaque:Ficoll and subsequently stained with Alcian blue revealed alterations of the cell membrane. CS cells showed vesiculation of membranous material and loosening of dye complexes from their surfaces (Fig. 4). The Alcian blue-positive membrane components appeared to be associated with the outer leaflet of the trilaminar cell membrane (Fig. 4, inset). Contrary to our previous results (4), P1798-CR cells, after contact with Hypaque:Ficoll, bound Alcian blue, and dye-complexed material, were observed to detach from the cell surface (Fig. 5). Numerous small vesicles, presumably derived from membrane fragments that had resealed, were mostly Alcian blue negative. Some cells showed a patchy, discontinuous distribution of reaction product on the remaining plasma membrane (Fig. 5): others retained a continuous dense layer after the removal of surface material. Larger magnifications of Alcian blue-stained CR cell surfaces had a fuzzy appearance (Fig. 5, inset) and did not clearly indicate with which layer of the cell membrane dye-binding material is associated.

**Cell Surface Changes after Steroid Treatment.** A visible cell coat, resulting from Alcian blue binding, was observed on P1798-CS cell membranes (not exposed to Hypaque:Ficoll) up to 6 hr after 9-FP treatment *in vivo* (Fig. 6). The morphological appearance of dye complexes was not significantly different from that described previously for untreated cell preparations (4). Between 6 and 8 hr after exposure (*in vivo*) to corticosteroids, anionic sites disappeared from the surface of CS cells (Fig. 7). 9-FP-treated
P1798-CR cells showed little or no Alcian blue binding, similar to those of untreated CR tumors (4).

**DISCUSSION**

The aim of this investigation was to compare cell surface glycopeptides of lymphosarcoma P1798-CS and -CR and to study possible alterations of these molecules by the action of glucocorticoids.

Histochecmical cell surface differences between P1798-CS and -CR cells, reported earlier (4), were substantiated by the demonstration of biochemical differences between cell membrane components of the 2 tumors. CS cell membranes (L1) showed lower Mg+-activated ATPase activities than did CR cell membranes. Possible correlations between this difference and corticoid sensitivity were not investigated. Sialic acids were found to be constituents of both CS and CR cell membranes, although CS cells carry no or little negative surface charge (4). Apparently, in CR cell membranes, the ionized carboxyl groups of these sugars are located at deeper sites. This possibility was supported by the Alcian blue-binding capacity of CR cells after Hypaque: Ficoll-induced surface alterations. The Hypaque: Ficoll density centrifugation method (5) is routinely used in research laboratories to separate different cell types found in peripheral blood. The discovery that this procedure results in membrane changes points to the need for caution in the interpretation of experimental results, whenever cells have been exposed to such treatment.

Chromatographic profiles of P1798-CS and -CR cell-membrane glycopeptides differ in the distribution of radioactivity before and after treatment in vivo with 9-FP. Neuraminidase-induced changes in 3H:4C ratios suggest that sialylation of glycopeptides of CS and CR preparations are structurally dissimilar. The effect of sialic acids on the speed of migration can be recognized by the significant shift of desialylated CS and CR fractions to the lower-molecular-weight region in the elution profile. It has been shown that the migration distance of sialoglycoproteins cannot be correlated directly with their molecular weight but rather with their molecular dimensions (11). The negatively charged carboxyl groups of the terminal sialic acids (that are either N-acyl or O-acyl derivatives of neuraminic acid) (14) give rise to extended shapes of carrier molecules and thereby increase their viscosity (13).

9-FP-induced alterations in CS and CR cell membrane glycopeptides are similar but not identical. Changes were evident 6 hr after in vivo treatment and became more pronounced 7 hr after exposure to the steroid. Glycopeptides of both treated tumors (7 hr) eluted faster, which could be the result of an increase in molecular weight and/or an increase in molecular dimensions due to unfolding of oligosaccharide chains. Removal of glycopeptide-bound sialic acid produced low-molecular-weight material that was absent in untreated tumors, suggesting alterations in both CS and CR sialoglycopeptides. Seven hr after 9-FP administration both CS and CR cell membranes increased in density. Although changes in lipid metabolism were not investigated, a decrease in the membrane lipid:protein ratio is conceivable. Studies by Story et al. (34) indicate that glucocorticoids inhibit phospholipid synthesis in mouse lymphoma cells. 9-FP binding to the cell membrane fraction is unlikely, since no increase in density was observed 6 hr after steroid administration.

Obvious glucocorticoid-mediated histochemical changes occurred only in P1798-CS. Cells of this tumor lost the capacity for Alcian blue binding between 6 and 8 hr after treatment in vivo with 9-FP. CR cell surfaces did not bind the dye before or after treatment. The unexpected, steroid-mediated chromatographic alterations of P1798-CS CR glycopeptides, as well as the membrane density changes, are difficult to explain. Glucocorticoids do not induce inhibition of nutrient transport or regression in this tumor. However, as in P1798-CR, corticosteroids have been shown to produce cell-membrane alterations in hepatoma cells that are not related to a cytotoxic process (3). The question of whether glucocorticoids affect the cell membrane of P1798 cells directly or indirectly could be answered by in vitro studies which were unsuccessful in our hands.

Our previous (4) and present observations support the possibility that the cytotoxicity of corticosteroid hormones in P1798 tumors is influenced by specific cell membrane characteristics involving the structure and conformation of negatively charged sialoglycoproteins.

**ACKNOWLEDGMENTS**

We wish to thank John Bodnaruk of the department of Chemical Engineering, City College, City University of New York, for his generosity and cooperation in the use of the electron microscope facility.

**REFERENCES**


**Fig. 1.** L1 fraction of P1798 cell membranes. No mitochondria are present. × 41,000.

**Fig. 2.** L2 fraction of P1798 cell membranes. Some contaminants, possibly ribosomes (arrows), but no mitochondria are seen. × 41,000.

**Fig. 3.** Trilaminar structure of P1798 cell membranes. × 307,000.

**Fig. 4.** P1798-CS cell surface, stained with Alcian blue after centrifugation on Hypaque:Ficoll. Alcian blue-positive material is loosening from the surface. Small vesicles (arrows), presumably derived from fragmented membranes, show little or no dye binding. × 50,000. Inset, magnified area of Fig. 4 showing that Alcian blue-positive material is associated with the outer layer of the trilaminar cell membrane. × 230,500.

**Fig. 5.** P1798-CR cell surface, stained with Alcian blue after centrifugation on Hypaque:Ficoll. Dye-complexed material is detaching from the cell membrane, leaving a patchy, discontinuous Alcian blue-positive layer on the cell surface. Small vesicles (arrows), presumably derived from fragmented membranes, show little or no dye binding. × 50,000. Inset, magnified area of Fig. 5. A trilaminar structure, seen in cross-section of vesiculated material (arrow), cannot be clearly distinguished in Alcian blue-stained area of the cell membrane. × 230,500.

**Fig. 6.** P1798-CS cell surface 6 hr after treatment in vivo. The cell membrane shows a distinct electron-dense coat. Alcian blue, × 30,000.

**Fig. 7.** Alcian blue-stained P1798-CS cell surface 6 hr after treatment with 5-FU. No Alcian blue-positive material is visible on the cell surface. × 30,000.
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