Incidence of a Split \( \alpha_2 \)-Glycoprotein Band in the Electrophoretic Pattern for Serum of Adenocarcinoma Patients

Gerald B. Dermer,\(^1\) Lawrence M. Silverman,\(^1\) Sandra J. Gendler,\(^2\) and Zoltan A. Tokés\(^2\)

We electrophoresed serum samples on Mylar-backed cellulose acetate membranes and stained for glycoproteins with the periodic acid–Schiff reagent. The samples were from untreated adenocarcinoma patients, adenocarcinoma patients receiving chemotherapy, and patients with other malignancies, and also from patients with benign proliferative diseases, inflammatory diseases, and other nonmalignant conditions. Forty-five per cent of the sera from untreated adenocarcinoma patients and 80% of those from adenocarcinoma patients with progressive systemic disease exhibited a splitting of the \( \alpha_2 \)-glycoproteins into a fast and slow band. Such a pattern was seen in only 4% of the non-adenocarcinoma cancer patients and 4% of the control group. Serial studies indicated that electrophoretic patterns of \( \alpha_2 \)-glycoproteins change with clinical status. Non-cancer patients with high concentrations of acute-phase proteins in their serum did not exhibit two \( \alpha_2 \)-glycoprotein bands. Further characterization of serum proteins from the fast \( \alpha_2 \) region suggest that \( \alpha_1 \)-acid glycoprotein and haptoglobin beta chains are the principal components staining with periodic acid–Schiff reagent. These components are markedly less apparent in, or are absent from, the fast \( \alpha_2 \) region of normal sera.

Additional Keyphrases: cancer • electrophoresis on cellulose acetate • two-dimensional gel electrophoresis

Electrophoresis on cellulose acetate separates serum glycoproteins into \( \alpha_1 \), \( \alpha_2 \), \( \beta \), and \( \gamma \) regions (1). We have described (2) an alteration in the electrophoretic pattern of glycoproteins in serum from women with untreated breast cancer. As compared with normal sera, increased glycoprotein material was present between the \( \alpha_2 \) and \( \beta \)-globulin regions; after mastectomy this material decreased, and patterns became like those seen in women with benign breast lesions. These results were consistent with numerous reports (3-11) of increased glycoproteins in the serum of cancer patients. Our purposes in the present study were (a) to increase the resolution of cellulose acetate electrophoresis so that serum glycoprotein patterns could be evaluated more objectively, and (b) to try and identify the components responsible for altered electrophoretic patterns. The cellulose acetate electrophoretic system was chosen because of its simplicity and availability in most clinical laboratories. The types of proteins in our cancer group were broadened to include: (a) those with adenocarcinomas of other tissue types besides breast, (b) other types of solid and hematologic malignancies, and (c) cancer patients receiving chemotherapy. The control group was also expanded and included patients with conditions associated with increases in acute-phase proteins in the sera. We find that 45% of untreated adenocarcinoma patients and 80% of adenocarcinoma patients with active metastatic disease exhibit two \( \alpha_2 \)-glycoprotein bands, whereas other types of cancer or controls exhibit only one. A portion of this work was presented at an NIH Workshop on “The Immunodiagnosis of Human Cancer” (12).

Materials and Methods

Patient Population

Serum glycoprotein electrophoresis was performed on specimens from a total of 302 individuals. We tested 198 cancer patients: 45 had solid tumors with no previous therapy, 88 had solid tumors with proven metastatic disease and were receiving chemotherapy, 40 had hematologic malignancies and were receiving chemotherapy, and 25 appeared to be cured of their solid tumors and were receiving adjuvant chemotherapy. The 45 cancer patients with no previous therapy consisted of 20 individuals with breast carcinoma; 14 with adenocarcinomas of the colon, prostate, ovary, and lung; and 11 with other solid tumors such as squamous-cell carcinoma of the lung, transitional-cell carcinoma of the bladder, and sarcoma. The 88 individuals with solid tumors and metastatic disease who were receiving chemotherapy consisted of 48 patients with breast carcinoma, 20 with other adenocarcinomas, and 21 with other epithelial tumors. The 40 individuals with hematologic malignancies included 15 with leukemia, 15 with non-Hodgkin’s lymphoma, eight with myeloma and two with Hodgkin’s disease. The 25 individuals who were receiving adjuvant chemotherapy consisted of 18 patients with breast carcinoma and seven with other adenocarcinomas. The cancer patients were evenly divided between men and women except for those with cancer of the breast, ovary, or prostate. None of the cancer patients received radiation therapy or therapy with estrogens during this study.

We tested sera from a control group of 104 individuals, consisting mostly of patients admitted for elective surgery. Of these, 10 had benign breast lesions, 11 had benign prostatic hyperplasia, and most of the remaining 83 had various inflammatory disorders, including cervicitis, cholecystitis, cystitis, tonsillitis, arthritis, and Crohn’s disease. Disorders in the other patients included diabetes mellitus, cataracts, and orthopedic problems.

During 18 months we also tested the sera of 21 adenocarcinoma patients with widespread disease, who were receiving chemotherapy. The average number of specimens from each individual was 12. Of these patients, nine had adenocarcinomas of the breast, six of the ovary, five of the colon, and one of the lung. Seven patients expired during the testing interval (three breast-, two ovary-, and two colon-adeno-carcinoma patients). One breast-, one ovary-, and one lung-cancer patient remained in remission during the testing interval. Nine patients began the study in remission but relapsed later (four breast-, two ovary-, two colon-, and one lung-cancerer). Two patients (one breast- and one ovary-cancer) never responded to chemotherapy.

---

\(^1\) Departments of Pathology, Hospital of the Good Samaritan, 616 S. Witmer St., Los Angeles, CA 90017, and the University of Southern California School of Medicine, Los Angeles, CA 90033.

\(^2\) USC Comprehensive Cancer Center and Departments of Biochemistry, University of Southern California School of Medicine, Los Angeles, CA 90033.

Received Feb. 16, 1979; accepted Nov. 30, 1979.
Electrophoresis

Glycoprotein electrophoresis was done by a modification of our original method (2). Cellulose acetate membranes (Helena Labs., Beaumont, TX 77704) with Mylar backing ("Titan III" membranes) were wetted for 15 min before use in tria(hydroxymethyl)methylamino–barbital buffer (pH 8.9, 50 mmol/L). We placed 160 mL of this same buffer in the "Zip Zone" electrophoresis chamber (Helena). Serum stored at -20 °C was thawed and 5 μL was placed in a well in the application plate (Gelman Instrument Co., Ann Arbor, MI 48106). Additional specimens were similarly treated until seven of the eight wells were filled. The final position was filled with a positive control (2). Samples were applied to the wetted membrane at a point 4 mm cathodic from the midpoint of the strip. For optimal resolution, samples were applied by using four applications with a "Sepratek" (Gelman) applicator. The membrane was placed in the electrophoresis chamber on top of filter paper wicks wetted in the buffer. Electrophoresis was carried out for 27 min at 190 V, constant voltage.

After electrophoresis, membranes were fixed for 5 min in trichloroacetic acid (50 g/L), oxidized in periodate–acetate (24 g of sodium metaperiodate per liter of sodium acetate, 3 g/L) for 15 min, and stained in Schiff's reagent (HyceI, Inc., Houston, TX 77036) for 12 min. The stained membrane was then washed three times in dilute nitric acid (5 g/L) for 1 min per wash, followed by two washes in absolute methanol for 1 min per wash, and finally it was placed in clearing solution (glacial acetic acid/methanol, 1/4 by vol) for 4 min. The membrane was then hung to drain for 2 min before being dried for 4 min in an oven at 90 °C. Membranes were scanned in a CDS-100 scanning densitometer (Beckman Instruments, Inc. Fullerton, CA 92634) with a 520-nm interference filter.

Immunological and Biochemical Characterizations

The fast α2 region from three adenocarcinoma patients was eluted from strips of cellulose acetate and the eluate, mixed with complete Freund's adjuvant, was injected subcutaneously into rabbits. Three injections were made of fast α2 fractions derived from 20 μL of serum. The antiserum obtained was tested on sera from normal and adenocarcinoma patients by the Ouchterlony diffusion technique and by immunoelectrophoresis. Specific rabbit antiserum against human serum components were obtained from Behring Diagnostics, Somerville, NJ 08876.

High-resolution two-dimensional gel electrophoresis was used to identify serum components eluted from the α2 regions of normal individuals and four adenocarcinoma patients. We electrophoresed 50-μL portions of serum on cellulose acetate membranes and cut out the two α2 regions. These cut-out portions were eluted with a 10 g/L sodium dodecyl sulfate solution and the proteins were analyzed by the O'Farrell technique as previously described for human plasma proteins (13). The two-dimensional polyacrylamide gels were either stained with Coomassie Brilliant Blue (2 g/L solution) or with periodic acid–Schiff reagent (PAS).

Results

Two PAS-stained α2 bands were found in serum electropherograms of 45% of untreated adenocarcinoma patients and 80% of adenocarcinoma patients whose disease, in spite of chemotherapy, was progressing (Table 1). One component migrated faster (more anodically) and the other slower than the α2-glycoprotein band seen in the pattern for patients showing only a single α2 band (Figure 1). In every patient's pattern in which these "fast" and "slow" α2 components were observed, the slower band stained more intensely than the faster one. The presence of two α2 components was our sole criterion for classifying a patient as positive. No more than 8% of patients in any other cancer group exhibited this electrophoretic pattern (Table 1). However, many of the sera from these patients exhibited high amounts of α1- and α2-glycoproteins, as judged from the staining intensity of these bands. The split α2-glycoprotein pattern was seen in four of the 104 controls. Serum from patients with inflammatory diseases such as cervicitis and cholecystitis often exhibited high proportions of α1- and α2-glycoproteins, but had only a single, broad, heavily stained α2 band, even after dilution of the serum. This is illustrated in the electropherogram of serum from a patient with Crohn's disease (Figure 1), where high proportions of α1- and α2-glycoproteins were present, but no splitting of the α2 components into two PAS-stained bands was observed.

Densitometric tracings (Figure 2) of the PAS-stained electropherograms revealed information about the electrophoretic mobility of the α2 components. The α2-glycoprotein band in sera with a single α2 band (Figure 2b) was about equidistant between the α1- and β-glycoproteins. In adenocarcinoma patients with a split α2 pattern (Figure 2a), neither α2 peak appeared equidistant between the α1 and β peaks. The slow α2 band was closer to the β peak and the fast α2 band was closer to the α1 peak.

Table 1. Results of Serum Glycoprotein Electrophoresis

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Split α2 pattern present</th>
<th>a2 α1 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer—untreated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>16/34 (45%)</td>
<td></td>
</tr>
<tr>
<td>Other solid tumors</td>
<td>0/11</td>
<td></td>
</tr>
<tr>
<td>Cancer—chemotherapy (responding)</td>
<td>3/53 (6%)</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other solid tumors</td>
<td>0/21</td>
<td></td>
</tr>
<tr>
<td>Lymphoma and leukemia</td>
<td>1/31 (3%)</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma (adjuvant)</td>
<td>2/25 (8%)</td>
<td></td>
</tr>
<tr>
<td>Cancer—chemotherapy (not responding)</td>
<td>11/14 (80%)</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma and leukemia</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td>Non-malignant conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign breast</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>Benign prostate</td>
<td>1/11 (9 %)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>3/83 (4 %)</td>
<td></td>
</tr>
</tbody>
</table>

*No. of individuals tested whose serum showed the split α2 pattern/total no. in the group indicated.

![Fig. 1. Serum glycoprotein electropherograms](figure)

(a) Serum from patient with Crohn's disease, showing an intensely PAS-stained α2 region and only a single α2 band. (b) Serum from patient with untreated breast carcinoma, with evidence of a splitting of the α2 region into a fast and slow component. (c) Serum from patient with untreated ovarian adenocarcinoma with a split α2 band. (d) Normal serum, with a single α2 component.
In our study of serially collected sera from 21 patients (not included in the data of Table 1), 19 exhibited glycoprotein patterns consistent with the patient's clinical status. Serum from one patient with ovarian adenocarcinoma exhibited a fast $\alpha_2$ band two months before she was judged to have progressive disease. The other 18 exhibited patterns that consistently were in agreement with their clinical status. Two patients who were not responding to therapy consistently displayed a normal glycoprotein pattern.

Figure 3 shows the PAS pattern obtained with high-resolution two-dimensional electrophoresis of normal serum. More than 15 glycoproteins are visible. Samples eluted from the $\alpha_2$ regions of patterns for normal serum on cellulose acetate membranes contained four major PAS-staining components, which, with this system electrophoresed to positions of $\alpha_1$-acid glycoprotein, haptoglobin $\beta$ chains, $\alpha_2$-HS glycoprotein, and $\alpha_1$-antitrypsin. Components from the $\alpha_2$ region that were stained by Coomassie Blue but not PAS included $\alpha_2$-macroglobulin, ceruloplasmin, $\alpha_1$-antichymotrypsin, hemopexin, and haptoglobin $\alpha$ chains. Fast $\alpha_2$ regions from adenocarcinoma patients contained two major PAS-positive components—$\alpha_1$-acid glycoprotein and $\beta$ chains of haptoglobin—and two minor components, $\alpha_2$-HS glycoprotein and $\alpha_1$-antitrypsin. Equal amounts of protein (1.1 mg per sample) eluted from the fast $\alpha_2$ region of the pattern for normal serum exhibited only trace quantities of $\alpha_1$-acid glycoprotein and $\beta$ haptoglobin chains.

Antisera, raised in rabbits, to fast $\alpha_2$ regions from adenocarcinoma patients recognized four serum components in both patients' and control sera by immunoelectrophoresis. The major antigenic component was $\alpha_2$-macroglobulin. In Ouchterlony diffusion plates, anti-$\alpha_2$-macroglobulin and anti-$\alpha_2$ fast region antisera used against unfractionated sera from an adenocarcinoma patient and a normal individual produced immunoprecipitation curves indicating antigenic identity.

Discussion

Eighty percent of the sera from patients with progressive adenocarcinomas that we examined exhibited a unique electrophoretic pattern for glycoproteins as compared to patients in remission or those with some other types of cancer. Sera from patients with benign proliferative diseases, inflammatory diseases, or other non-malignant disorders did not produce this electrophoretic pattern. The pattern is characterized by the presence of two $\alpha_2$-glycoprotein bands, which we have
called the “fast” and “slow” components because of their electrophoretic mobilities. The Beckman “Microzone” cellulose acetate electrophoretic procedure used in an earlier study (2) did not resolve the α2 region into two distinct components.

The split α2 pattern is observed most frequently in adenocarcinoma patients with widespread disease, and our data suggest that serum glycoprotein electrophoresis would be useful in monitoring the clinical status of such patients. In our serial study, 19 of 21 patients (90%) were correctly evaluated by this technique. One of the 19 exhibited a fast α2 band two months before any other evidence of relapse was observed. In nine, a single α2 band changed during the period of observation to a split pattern, and the change correlated with evidence of tumor growth.

Two-dimensional electrophoresis demonstrated that components which electrophoresed to positions of α1-acid glycoprotein, haptoglobin β chains and, to a lesser extent, α2-HS glycoprotein and α1-antitrypsin were responsible for the PAS staining of fast α2 regions in patterns for sera from adenocarcinoma patients. Immunologic studies are in progress to verify the exact nature of the specific glycoproteins migrating in the fast α2 region. The major antigenic component of the fast α2 region appeared to be α2-macroglobulin since it was the primary component recognized by antisera to fast α2.

We cannot now explain the altered electrophoretic migration of several glycoproteins in the serum of adenocarcinoma patients. It is known that there are increased amounts of “acute-phase” proteins (3, 8)—such as the fast α2 components, α1-acid glycoprotein, and haptoglobin β chains—in the sera of cancer patients. Acute-phase proteins are glycoproteins that normally are present in serum and which electrophorese as normal α1 and α2 components, but are increased (14) in infectious, inflammatory, and malignant diseases. Some cancer patients did exhibit intensely stained α1- and α2-glycoprotein bands but not the split α2 pattern. High proportions of α1- and α2-glycoproteins were also seen in most sera from individuals with inflammatory diseases, but they and other controls did not exhibit two components.

Our data suggest that the fast α2 band may be due to the presence of some particular one (or ones) of the acute-phase proteins. There is evidence (15) that suggests that the carbohydrate content of serum glycoproteins is modified in cancer patients. This is compatible with altered amounts of various glycosyl transferases in the serum of animals (16, 17) and humans (18-20) with cancer. Changes in carbohydrate content could produce glycoproteins with altered electrophoretic properties. Because human tumors synthesize and release glycoproteins (21, 22) and serum proteins (23), it would not be surprising if components derived from malignant cells enter the circulation (24, 25) and contribute to the fast α2 band. The eventual identification of specific glycoprotein abnormalities in adenocarcinoma patients may result in further improvement in diagnostic assays.

We thank Helena Laboratories for technical assistance and support in this undertaking and Glenn Tisman, M.D., for sera and diagnostic information. Part of this work was supported by NIH Grant No. CA-24545.

References