Isolation and Properties of a Human Pancreatic Adenocarcinoma-associated Antigen, DU-PAN-2

Michael S. Lan, Olivera J. Finn, Philip D. Fernsten and Richard S. Metzgar

ABSTRACT

This work describes the molecular properties of a human pancreatic adenocarcinoma-associated mucin-like antigen defined by a murine monoclonal antibody (DU-PAN-2). DU-PAN-2 antigen is a large molecule, readily detected in the body fluids of patients with pancreatic adenocarcinoma and sensitive to neuraminidase treatment and alkaline reduction. The antigen binding activity of the DU-PAN-2 immunoglobulin M antibody is markedly reduced when coupled to an insoluble matrix. Therefore, the antigen was partially purified from the serum and ascites of patients with pancreatic adenocarcinoma by ammonium sulfate fractionation and Affi-Gel-Blue chromatography to remove most of the serum proteins. Noncovalently associated proteins were further separated on CsCl/CsBr density gradients and noncovalently associated lipids removed by organic solvent extraction. DU-PAN-2 antigen was monitored by a solid-phase competition radioimmunoassay. We have been able to detect antigen reactivity and analyze its electrophoretic pattern following transfer from 1% agarose gel onto nitrocellulose paper and immunoblotting with DU-PAN-2 antibody. Antigen was also labeled metabolically with various radioactive monosaccharides and sulfates.

Radioimmunoprecipitation of labeled antigen with DU-PAN-2 antibody showed two distinct broad antigen bands consistent with the immunoblotting signals. The heavily glycosylated and polydisperse nature of this antigen and the results of various enzyme treatments suggest that the DU-PAN-2 epitope is expressed on a mucin-like molecule.

INTRODUCTION

Malignant transformation of a cell can be accompanied by a redistribution or alteration of cellular molecular components. The structures expressed on the surface of neoplastic versus normal human cells have been the subjects of intensive research. Mucins are normally found in the secretions of many epithelial glands, and alterations in mucin antigen expression in neoplastic mucosa have been demonstrated in human colonic adenocarcinomas (3, 10).

A mucin-antigen detected by monoclonal antibody 19-9 has been found in the sera of patients with gastrointestinal and pancreatic cancer (12). In addition, we have reported previously (14) that a murine monoclonal antibody DU-PAN-2, produced against the human pancreatic adenocarcinoma cell line, recognizes a heavily glycosylated antigen. This antigen is present on some normal adult and fetal secretory epithelial cells. It is expressed on both acinar and ductal cells in the fetal pancreas but is detectable only on ductal cells in the adult. The antigen is also present on adenocarcinoma cells of pancreatic and nonpancreatic origin (4), and can be found both on the surface and in the secretions of pancreatic tumors and cell lines. It can also be detected in patients’ body fluids, and a competitive inhibition RIA has been developed to monitor the antigen levels in patients’ sera and ascites (15).

Sputum tissue culture media and ascitic fluids from pancreatic cancer patients provide rich sources of this antigen for the study of its molecular properties (15). In this report, we used density gradient centrifugation, agarose gel electrophoresis, and immunoblotting to purify and monitor the DU-PAN-2 antigen. Biosynthetic labeling and radioimmunoprecipitation provided evidence that DU-PAN-2 antigen consisted of 2 broad bands in horizontal agarose gel electrophoresis. Based on the present data, the epitope defined by DU-PAN-2 monoclonal antibody is thought to be expressed on glandular cell secretory products which exhibit molecular microheterogeneity in structure. The epitope and molecule recognized by DU-PAN-2 monoclonal antibody appear different from the CA125, 19-9, Tn, B72.3, and Lewis blood group antigens (a and b).

MATERIALS AND METHODS

Antigens and Antibodies. DU-PAN-2 murine monoclonal antibody (IgM) was produced in our laboratory (14). OC125 monoclonal antibody (IgG1) and CA125 antigen associated with epithelial ovarian cancer were obtained from Dr. R. C. Bast (Dana-Farber Cancer Institute, Boston, MA). Monoclonal antibodies 19-9, 129.3-B3.9, and 151.6-A7.9, which are directed against gastrointestinal and pancreatic cancer-associated antigen, and Lewis a and b antigens were obtained from Dr. Z. Stepniowski (The Wistar Institute, Philadelphia, PA). Tn and Tn, general carcinoma autoantigens, were obtained from Dr. G. F. Springer (Northwestern University, Evanston, IL). Monoclonal antibody B72.3 (IgG2), detecting a breast cancer-associated antigen, was obtained from Dr. J. Schlim (NIH, Bethesda, MD). Reactivities of other antigens and antibodies with DU-PAN-2 were determined by competition RIA (15) and immunoblotting as described later.

Partial Purification of DU-PAN-2 Antigen from Patient’s Ascitic Fluid. Ascites of patients with pancreatic adenocarcinoma containing high titers of antigen activity were collected and stored at -20° until used. Antigen purification by immunoaffinity column chromatography was unsuccessful, since the DU-PAN-2 IgM antibody shows poor antigen binding when coupled to insoluble matrix. Therefore, ammonium sulfate fractionation (50 to 75%) was used as the first step in antigen purification in order to concentrate samples into small volumes and to remove some of the proteins. Human serum albumin in the ascites fluid coprecipitated with DU-PAN-2 antigen in the 50 to 75% saturated ammonium sulfate fraction. The precipitates were dialyzed against phosphate-buffered sa-
line (0.01 M sodium phosphate buffer, pH 7.4, containing 0.85% sodium chloride) and chromatographed on an Affi-Gel-Blue (Bio-Rad) affinity column to remove the albumin which accounts for up to 80% of total proteins present in the ascitic fluid. The effluent fraction which contained about 85% of the original antigen activity was dialyzed against water and lyophilized as a crude antigen preparation.

**CsBr Density Gradient Ultracentrifugation.** Crude antigen preparations from above were subjected to cesium bromide (Fluka) density gradient ultracentrifugation according to the procedure of Creeth et al. (7). Briefly, lyophilized samples were dissolved in deionized water (1.2 mg/ml) containing 42% (w/w) CsBr at a loading density of 1.43 g/ml and stirred overnight at 4°C. The well-mixed solution was transferred to cellulose nitrate centrifuge tubes. The samples were centrifuged at 30,000 rpm for 72 hr at 10°C in a Beckman SW41 Ti rotor. After centrifugation, tubes were punctured at the bottom, and fractions of 1.3 ml each were collected. CsBr was removed by dialysis against water, and each fraction was assayed for antigen reactivity by competition RIA (15) as well as protein concentration (5). Densities were determined by weighing a 100-μl aliquot of each fraction.

**Lipid Extraction.** In the above CsBr density gradient, fractions with densities lower than 1.4 g/ml were pooled and lyophilized. The lyophilized antigen was subjected to delipidation based on a procedure described by Esselman et al. (8). Extractions were performed twice each with chloroform:methanol (2:1) and chloroform:methanol (1:2) in a small Eppendorf centrifuge tube. The delipidated antigen was dried and redissolved in deionized water with 42% CsBr.

**Agarose Gel Electrophoresis and Immunoblotting.** DU-PAN-2 antigen from lyophilized crude antigen preparations and density gradient fraction was subjected to horizontal gel electrophoresis and immunoblotting as described previously (15). The antigen was electrophoresed in 1% agarose (SeaKem, ME, Agarose) in the presence of 0.1% SDS using 0.1 M Tris-boric acid:EDTA buffer (pH 8.2) at 100 V for 4 hr. Antigen was transferred to nitrocellulose paper (Bio-Rad) by electroblotting (30 V, 16 to 24 hr, 4°C). The nitrocellulose was then incubated with 5% bovine serum albumin in 10 mM Tris:0.9% NaCl solution (saline) (pH 7.4) at 40°C, followed by DU-PAN-2 antibody or a control antibody at room temperature for 1 hr with continuous rocking. After washing, the paper was further incubated for 1 hr at room temperature with rabbit anti-mouse IgM antibody (Bionetics), followed by a 1-hr incubation with 125I-labeled Staphylococcus aureus Protein A (Sigma Chemical Co., St. Louis, MO) at 10 000 cpm/ml. The blots were washed extensively, blotted dry, and exposed to Kodak XAR-5 X-ray film with an intensifying screen at -70°C for an appropriate time interval.

**Preparation of Labeled Cell Extracts.** Radiolabeling and immunoprecipitation were performed according to slight modifications of the technique of Melchers (13). Before labeling, the cells were cultured for 2 days in glucose-free minimum essential medium supplemented with 10% dialyzed fetal calf serum to deplete the intracellular glucose pool. The culture was then supplemented with 1 mM of ϕ-2-[3H]glucosamine hydrochloride (New England Nuclear, Boston, MA; >10 CI/mmol) and incubated for 2 days at 37°C. The labeled cells were harvested by centrifugation, washed, and extracted by detergent solubilization in 1-ml buffer containing 0.5% Nonidet P-40 (Sigma), 0.01 M Tris-HCl, pH 7.6, 1 mM MgCl2, and 0.1 mM phenylmethylsulfonyl fluoride (Sigma). The suspension was incubated on ice for 30 min, followed by centrifugation at 12,000 rpm for 15 min. The supernatant fraction was recovered, dialyzed, concentrated, and stored at -70°C until use. Different radiolabeled amino acids and monosaccharides (125I]leucine, [35S]methionine, [35S]cysteine, [3H]mannose and [35S]sulfate), were used to label HPAF cells with the same procedures described above, except for using different amino acid or sulfate-free media depletion of their internal pool for 1 hr, and incorporation of labeled precursors for 20 hr. Cell lysate and concentrated tissue culture supernatant (100 μl) were separately incubated with DU-PAN-2 monoclonal antibody (100 μg) at 37°C for 2 hr, followed by 80 μl of goat anti-mouse antisera at 37°C overnight. Two μl of normal mouse serum were then added for further incubation overnight at 4°C. The immunoprecipitated complexes were collected by centrifugation and washed extensively with the following buffers: 3 times in a buffer of 0.15 M NaCl:5 mM EDTA:50 mM Tris-HCl, pH 7.5, supplemented with 1% Nonidet P-40 and NaCl to give a final concentration of 0.5 M; twice in the same buffer with the Nonidet P-40 concentration reduced to 0.5%; and twice in 0.15 M NaCl:5 mM EDTA:50 mM Tris-HCl, pH 7.5 buffer. The pellets were dissolved in running buffer without 2-mercaptoethanol and boiled for 15 min before electrophoresis on 1% agarose (0.1% SDS) slab gels as described above. The gel was soaked in methanol for 1 hr and then soaked in 10% PPO solution in methanol for 3 hr to enhance radiolabeled signals, then dried and exposed to Kodak XAR-5 X-ray film with an intensifying screen at -70°C for an appropriate time interval.

**CsCl Density Gradient Ultracentrifugation.** Small amounts of [35S]sulfate-labeled tissue culture supernatant were added to 40% (w/w) CsCl in the presence and absence of 4 M guanidine-HCl, as discussed by Carlstedt et al. (6), at a loading density of 1.42 g/ml. Gradients were formed by centrifugation in a Beckman SW41 Ti rotor at 30,000 rpm for 72 hr at 10°C. Fractions were collected and assayed for antigenic reactivities and [35S]sulfate radioactivities. Fractions with the highest antigen reactivity were concentrated and subjected to 1% agarose gel electrophoresis, and the gel processed, dried, and fluorographed as described above.

**Enzyme Digestions.** Endoglycosidase H (Streptomyces plicatus), and endoglycosidase D (Diplococcus pneumoniae) were obtained from Miles Laboratories, Inc. Elkhart, IN. Chondroitinase ABC (Proteus vulgaris) was obtained from Sigma. Hyaluronidase (bovine testicular, 300 units/ml) and collagenase (Clostridium histolyticum, 125 units/mg) were obtained from Worthington Biochemical Corp., Freehold, NJ. Neuraminidase (Vibrio cholerae) was obtained from Calbiochem-Behring, San Diego, CA. All digestions were carried out at 37°C. Hyaluronidase digestion was carried out in 0.15 M NaCl, 0.10 M sodium phosphate buffer, pH 5.3, and endoglycosidase D and endoglycosidase H digestions, in 50 mM sodium citrate buffer at pH 6.5 and 6.0, respectively. Neuraminidase digestion was carried out in 0.15 M NaCl:50 mM sodium acetate:0.1% CaCl2 buffer, pH 5.5; collagenase digestion, in 50 mM Tris-HCl:0.1% CaCl2 buffer, pH 7.5; and chondroitinase ABC, in 50 mM Tris HCl buffer, pH 8.0. Activity of the chondroitinase ABC preparation was confirmed by the method of Yamagata, et al. (18). Activity of the collagenase preparation was confirmed using azocoll (Calbiochem) as substrate, of the hyaluronidase preparation, by reduction of the viscosity of 5 mg/ml, hyaluronic acid, and of the neuraminidase preparation, using the thioribarbituric acid method of Aminoff (1) to detect release of free sialic acid from fetuin.

**Gel Filtration Chromatography of DU-PAN-2 Antigen.** Gel filtration was carried out on a 2.5 x 95-cm column of Sepharose CL-2B pre-equilibrated with phosphate-buffered saline (0.15 M NaCl:0.01 M sodium phosphate, pH 7.4). Partially purified antigen or antigens after enzyme treatments were applied to the column separately. Fractions were collected and assayed by competition RIA (15).

**RESULTS**

**Radiolabeling and Identification of DU-PAN-2 Antigen.** HPAF cells were metabolically labeled with [3H]glucosamine, [3H]mannose, [3H]leucine, and [35S]sulfate. Cell lysates and culture supernatants were collected as labeled antigen sources for radioimmunoprecipitation. Molecules precipitated specifically with DU-PAN-2 antibody and those precipitated by the control antibody were run on 1% agarose gels in the presence of SDS. As shown in Fig. 1, [35S]sulfate, [3H]glucosamine, and [3H]mannose (Fig. 1, A, B, and C) are all capable of labeling the DU-PAN-2 antigen. The labeled antigen appears as 2 broad bands of an apparently slow migration on 1% agarose, 0.1% SDS gels. The antigen precipitated from the cell lysate (Fig. 1) comigrates and otherwise appears the same as the antigen precipitated from...
Fig. 1. Radioimmunoprecipitation of DU-PAN-2 antigen from cell lysates and tissue culture supernatants. HPAF cells were labeled with [35S]sulfate (A), [3H]-glucosamine (B), [3H]mannose (C), and [3H]leucine (D). Radiolabeled antigens were immunoprecipitated with DU-PAN-2 antibody (left lane) or control culture supernatants P3x63Ag8 (right lane) and run on 1% agarose gel in the presence of SDS.

Purification of DU-PAN-2 Antigen by Density Gradient Ultracentrifugation. Ammonium sulfate fractionation and Affi-Gel-Blue chromatography of patient's ascitic fluid allow only partial purification of DU-PAN-2 antigen. In addition, antibody affinity chromatography did not appear feasible because DU-PAN-2 antibody loses its antigen binding property when immobilized on cyanogen bromide-activated Sepharose-4B column. Therefore, we used CsBr density gradient centrifugation, which has been shown to efficiently separate polysaccharides from proteins, to separate the heavily glycosylated DU-PAN-2 antigen from the rest of the contaminating proteins. Competitive inhibition RIA (15) and immunoblotting were used to monitor the presence of the antigen in fractions collected after centrifugation. As shown in Chart 1, A and B, the antigenic reactivities appeared distributed along the entire gradient including densities typical of proteins and glycoproteins ranging from 1.2 to 1.3 g/ml, as well as those of heavily glycosylated glycoproteins with densities exceeding 1.4 g/ml. It has been demonstrated previously that the presence of noncovalently associated lipids may give rise to different buoyant densities exhibited by the glycoproteins due to the
variations in lipid binding (17). In order to test the possibility that DU-PAN-2 may be lipid associated, Fractions 4 to 10 (Chart 1B) were pooled, delipidated, and then rerun on a CsBr density gradient. The results of this experiment (Chart 1C) indicated that most of the DU-PAN-2 antigenic reactivities were relocated to high-density fractions greater than 1.4 g/ml. Two broad bands typical of the antigen pattern seen by specific immunoprecipitation were also observed by immunoblotting of the first 3 fractions of the gradient, and will be referred to as slow- and fast-migrating DU-PAN-2 antigens when discussed later. However, we understand that the migration distance of heavily glycosylated proteins does not necessarily reflect the true molecular weight on the SDS-agarose gels as indicated by the protein markers (thyroglobulin, M, 669,000; ferritin, M, 440,000; and catalase, M, 232,000) (15). In Chart 1A, the amount of slow-migrating antigen appears greater in the A1 fraction than in the A2 and A3. There is no slow-migrating antigen present in the other fractions. The fast-migrating antigen can be seen in each fraction (A1 to A10), although less intensely in A1 in contrast to the slow-migrating antigen. In Chart 1, B and C, the lowest density fraction (A10), although less intensely in A1 in contrast to the slow-migrating antigen, consistently retained some of the antigenic reactivity even after delipidation.

Following the demonstration that DU-PAN-2 antigen has a density typical of polysaccharides and can thus be separated away from most of the associated proteins, we used small amounts of labeled cell supernatants to determine the density of DU-PAN-2 antigen in CsCl gradients which gave a better separation than CsBr gradients. [35S]sulfate-labeled HPAF tissue culture supernatant was applied onto a CsCl density gradient. When fractions were analyzed, the antigenic reactivity detected by competition RIA resided in the fraction containing the highest level of radioactivity (Chart 2). The antigen density in CsCl alone exceeded 1.5 g/ml (Chart 2A) as compared to 1.45 g/ml in CsCl containing 4 M guanidium chloride (Chart 2B). Samples which have the highest antigenic reactivities from both gradients (A1 and B2) also show the gel patterns as described above. No other labeled molecules are detected in the DU-PAN-2-containing fractions. Although the base line of radioactivity was maintained at a level of 9000 cpm, this level of background might mask the presence of trace amounts of other labeled molecules. The diffuseness and intensity of the bands of the radioautograph may prevent the detection of other labeled molecules in Fractions A1 and B2.

Enzymatic Digestions and Gel Filtration Chromatography of DU-PAN-2 Antigen. Partially purified antigen as described above was subjected to various enzyme digestions. All digestions were carried out at 37°, after which samples were heated to 100° for 15 min to inactivate the enzymes. DU-PAN-2 antigen is stable at 100° for more than 15 min. Samples were then diluted and tested in the RIA. As shown in Table 1, the results indicate that neuraminidase treatment virtually abolished the capacity of DU-PAN-2 antigen to block in the RIA but that the antigenic reactivity was not quantitatively affected by other enzymes used. The sensitivity of DU-PAN-2 antigen to neuraminidase suggests that sialic acid is a component of the antigenic determinant or is responsible for maintaining its conformation, and that the DU-PAN-2 epitope may be expressed on a carbohydrate moiety. The possibility that the various enzyme treatments cleaved the antigen to smaller units without destroying its antigenic determinants was tested by gel filtration chromatography.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Inhibition RIA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>75</td>
</tr>
<tr>
<td>Endoglycosidase H, 5 milliunits</td>
<td>70</td>
</tr>
<tr>
<td>Endoglycosidase D, 10 milliunits</td>
<td>70</td>
</tr>
<tr>
<td>Chondroitinase ABC, 0.1 unit</td>
<td>72</td>
</tr>
<tr>
<td>Collagenase, 200 μg</td>
<td>83</td>
</tr>
<tr>
<td>Hyaluronidase, 200 μg</td>
<td>80</td>
</tr>
<tr>
<td>Neuraminidase, 10 milliunits</td>
<td>17</td>
</tr>
<tr>
<td>Neuraminidase, 10 milliunits</td>
<td>NaOH (0.5 N) + NaBH4 (1.0 M)</td>
</tr>
<tr>
<td>Neuraminidase, 10 milliunits</td>
<td>NaOH (0.1 N) + NaBH4 (1.0 M)</td>
</tr>
<tr>
<td>Neuraminidase, 10 milliunits</td>
<td>1.0 M NaOH (pH 8.0)</td>
</tr>
</tbody>
</table>

The enzyme inhibition was calculated as

\[
\% \text{ of inhibition of RIA} = 1 - \frac{\text{Net cpm for test sample}}{\text{Net cpm for RIA buffer}}
\]
HUMAN PANCREATIC ADENOCARCINOMA ANTIGEN DU-PAN-2

Chart 3. Gel filtration chromatography. Partially purified DU-PAN-2 antigen was subjected to Sepharose CL-2B chromatography (2.5 x 95 cm). Each fraction (4 ml/tube) was collected and assayed for antigen reactivity by competition RIA.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>DU-PAN-2 (units/ml)</th>
<th>Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DU-PAN-2</td>
<td>1:500</td>
<td>41,500</td>
<td>+</td>
</tr>
<tr>
<td>CA-125</td>
<td>Undiluted</td>
<td>165</td>
<td>+</td>
</tr>
<tr>
<td>T</td>
<td>Undiluted</td>
<td>37</td>
<td>+</td>
</tr>
<tr>
<td>Tn</td>
<td>Undiluted</td>
<td>60</td>
<td>+</td>
</tr>
</tbody>
</table>

Reactivities of DU-PAN-2 antibody with other partially purified antigens by competition RIA

Reactivities of other monoclonal antibodies with DU-PAN-2 antigen using immunoblotting

Comparison of Other Antigens and Antibodies with DU-PAN-2. We have reported previously that purified carcinoembryonic antigen, ABO, and Lewis blood group antigens gave less than 5% inhibition in the RIA (15). Extending this comparison, DU-PAN-2 epitope appears different from epitopes recognized by monoclonal antibodies OC125 (2), 19-9 (10), and B72.3 (16), and from Lewis blood group antigens a and b shares no similarity with T and Tn antigens, as tested by competition RIA and/or immunoblotting (Table 2). In addition, there was no reactivity of DU-PAN-2 antibody with over 15 ganglioseries gangliosides tested.

DISCUSSION

DU-PAN-2 antigen from ascitic fluid of a patient with pancreatic adenocarcinoma has been partially characterized, purified, and monitored by competitive inhibition RIA (15). We have been unable to run the antigen on conventional polyacrylamide gels and have attributed this difficulty to its large molecular size.

Consequently, gel electrophoresis in agarose (11) with large pore size for separation of larger molecular weight molecules was used to characterize the molecular properties of DU-PAN-2 antigen. Two broad bands with antigen reactivities were detected by radioimmunoprecipitation or immunoblotting after transfer onto nitrocellulose paper. The heterogeneity of antigen migration on the gel indicated that the monoclonal antibody DU-PAN-2 recognizes multiple molecules which bear the same epitope. We used 3% polyacrylamide isoelectric focusing with antigens applied to both the acidic as well as basic end of the gel. The antigen failed to reach the equilibrium point even when focused over 4800-V hr. It is likely that the heavily glycosylated DU-PAN-2 antigen interacted with the polyacrylamide which interfered with its migration. The large molecular weight and the high carbohydrate content which binds less efficiently to SDS caused the antigen to migrate much slower than the protein markers (15). Therefore, the molecular weight of DU-PAN-2 antigen cannot be correctly estimated by SDS-agarose gel electrophoresis. However, the high molecular weight of DU-PAN-2 antigen was confirmed in the gel filtration chromatography.

CsBr density gradients can be used for separating proteins from heavily glycosylated antigens. Although CsBr has a lower resolving power than CsCl (7), dispersal in CsBr prevents gelation of the antigen and allows quantitative separation of the protein and heavily glycosylated glycoprotein components. The antigenic reactivities which showed in the lower bands on immunoblotting (Chart 1A) fractionated at far lower densities than expected. These alterations in antigen density were shown to be due to noncovalently linked lipids which were removed as described in "Materials and Methods" and as shown in Chart 1C. On the other hand, it is not known why a small amount of antigen present in the lowest density fraction was resistant to delipidation. When a small amount of [35S]sulfate-labeled tissue culture supernatant was subjected to density gradient separation in CsCl, the density of labeled antigen was shown to be greater than 1.5 g/ml in CsCl alone and 1.45 g/ml in CsCl containing 4 M guanidinium chloride. This could be explained either by a lower binding of CsCl to negative groups due to competition by the guanidinium ion and/or a higher degree of hydration of the macromolecules in 4 M guanidinium chloride (6).

Enzymatic digestions of DU-PAN-2 antigen revealed that the antigen is sensitive to neuraminidase and alkaline reduction but resistant to heat treatment. In addition, our preliminary chemical composition data obtained from purified DU-PAN-2 antigen (9) indicated that serine and threonine comprise about one-third of the residues and, together, serine, threonine, proline, glycine, and alanine constitute more than 60% of the total amino acid content. The resistance of the antigen to endoglycosidases D and H (Table 1) suggests that the DU-PAN-2 epitope is not expressed on the "high mannose" type of carbohydrate N-glycosidically linked to a polypeptide core. In addition, the resistance of the DU-PAN-2 antigen to chondroitinase ABC and hyaluronidase suggests that the epitope is not expressed on a chondroitin sulfate proteoglycan. The resistance of DU-PAN-2 antigen to collagenase and hyaluronidase also indicates that the molecular size and heterogeneity of the DU-PAN-2 antigen are not a result of noncovalent interactions with collagen or hyaluronic acid. Treatment of DU-PAN-2 antigen under alkaline conditions in the presence or absence of sodium borohydride resulted in a significant loss of blocking activity. The properties are
consistent with the evidence indicated by histological studies (4) which suggested that DU-PAN-2 might be a mucin-like antigen. Mucins contain many carbohydrate chains and are differentially glycosylated. This could explain the microheterogeneity of the molecule as indicated by the broad bands in gel patterns (Fig. 1).

The heavily glycosylated and polydisperse nature of this antigen, the results of various enzyme treatments, and preliminary chemical composition data indicated that it is a mucin-like antigen. It can be labeled by [3H]glucosamine, [3H]mannose, and [35S]sulfate. Successful labeling with mannose is surprising, since mannose is usually not present in the mucins. However, [3H]mannose may undergo reduction to form deoxymonosaccharide [3H]fructose, or may convert to other monosaccharides which are then incorporated into the oligosaccharide chains of DU-PAN-2 antigen. This possibility will be examined by analyzing the recovered radioactive sugar from [3H]mannose-labeled antigen. We have been unable to label DU-PAN-2 antigen with the radioactive amino acids thus far tested. Since the protein content is much lower than polysaccharide in the mucin molecules (6), this signal may not be strong enough to be detected. However, radiolabeling of heavily glycosylated antigen with [35S]sulfate provides informative data as well as approaches to the study of this antigen. We have used it to show that density gradient purified antigen has no labeled low molecular weight components in agarose gel electrophoresis (Chart 2). In addition, internally radiolabeled DU-PAN-2 antigens with [35S]sulfate provide a sensitive probe which might be used to dissect the epitope associated with human pancreatic adenocarcinoma. Purified antigen is currently being used to produce "second generation" murine monoclonal antibodies and polyclonal rabbit antibodies which could be directed to different epitopes on the same molecule and possibly exhibit greater binding affinities for the antigen.

ACKNOWLEDGMENTS

The authors thank Dr. Bernard Kaufman, Dr. Robert Wheat, Dr. Peter Cresswell, Dr. Jeffrey Dawson, and Dr. James Moore for helpful advice and discussions and for critical reading of the manuscript. They also wish to thank Melissa Gaillard for helping to prepare radiolabeled cell extracts and appreciate the secretarial help of Kathy Greenwell and Teresa Hylton.

REFERENCES

Isolation and Properties of a Human Pancreatic Adenocarcinoma-associated Antigen, DU-PAN-2

Michael S. Lan, Olivera J. Finn, Philip D. Fernsten, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/45/1/305

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.