Identification of the Gastrointestinal and Pancreatic Cancer-associated Antigen Detected by Monoclonal Antibody 19-9 in the Sera of Patients as a Mucin

John L. Magnani, Zenon Steplewski, Hilary Koprowski, and Victor Ginsburg

National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, NIH, Bethesda, Maryland 20205 [J. L. M., V. G.], and The Wistar Institute, Philadelphia, Pennsylvania 19104 [Z. S., H. K.]

ABSTRACT

Monoclonal antibody 19-9, produced by a hybridoma prepared from spleen cells of a mouse immunized with a human colon carcinoma cell line, detects an antigen in the serum from most patients with gastrointestinal and pancreatic cancer (M. Hertlyn, H. F. Sears, Z. Steplewski, and H. Koprowski, J. Clin. Immunol., 2: 135-140, 1982). The epitope of this antibody is a carbohydrate with the sugar sequence

\[
\text{NeuNAc} \alpha_2 \text{Gal} \beta_3 \text{GlcNAc} \beta_1 \text{Gal} \beta_3 \text{Glc} \beta_1 \text{Gal} 
\]

in which NeuNAc is N-acetylneuraminic acid, Gal is galactose, GlcNAc is N-acetylgalactosamine, and Fuc is fucose. In the colon carcinoma cell line and many gastrointestinal and pancreatic cancers, this sequence occurs in a monosialoganglioside containing a sialylated Le\(^a\)-active pentasaccharide (sialylated lacto-N-fucopentaose II, IV\(^3\)-\(\alpha\)-NeuNAc-III\(^4\)-\(\alpha\)-Fuc-LcOse\(_4\), in which LcOse\(_4\) is Gal\(^1\)-3GlcNAc\(^1\)-3Gal\(^1\)-4Glc) (J. L. Magnani ef al. J. Biol. Chem., 257: 14365-14369, 1982). However, the antigen in the sera of patients occurs mainly as a mucin, not a ganglioside, based on the following evidence. Little antigen is extracted by organic solvents from sera, and that which is extracted remains at the origin under conditions of thin-layer chromatography where the ganglioside antigen migrates up the plate. Upon gel filtration of serum on Sephacryl S-400, the antigen is eluted in the void volume, indicating a molecular weight of \(\geq 5 \times 10^6\). Incubation for 5 hr at 37\(^\circ\) in 0.1 N NaOH destroys the serum antigen but does not affect the ganglioside antigen. The density of the serum antigen as determined in a CsCl gradient is 1.50 g/ml, while in 4 M guanidine. HCl its density is 1.43 g/ml. Finally, antigen affinity purified by antibody 19-9 from the serum of a cancer patient belonging to the Le\(^a\)-positive blood group contains Le\(^a\) antigen, consistent with the multiple antigenic specificities exhibited by mucins.

INTRODUCTION

Glycolipids and glycoproteins often share the same carbohydrate antigens. For example, the antigens of the human ABO and Lewis blood group systems are specific carbohydrate sequences that occur in glycolipids as well as in high-molecular-weight, carbohydrate-rich glycoproteins. For example, the antigens of the human ABO and Lewis blood group systems are specific carbohydrate sequences that occur in glycolipids as well as in high-molecular-weight, carbohydrate-rich glycoproteins. Mucins

\[\text{NeuNAc} \alpha_2 \text{Gal} \beta_3 \text{GlcNAc} \beta_1 \text{Gal} \beta_3 \text{Glc} \beta_1 \text{Gal} \]

are also found in the serum of patients with cancer or with pseudomucinous ovarian cysts (22).

Monoclonal antibody 19-9 derived from spleen cells of a mouse immunized with human colon adenocarcinoma cell line SW1116 (18) is directed against the sugar sequence

\[
\text{NeuNAc} \alpha_2 \text{Gal} \beta_3 \text{GlcNAc} \beta_1 \text{Gal} ... \]

as described by Magnani et al. (20). This antibody detects its antigen in the serum of most patients with gastrointestinal and pancreatic cancer (14). The antigen in the cell line used for immunization is mainly a ganglioside containing a sialylated Le\(^a\)-active pentasaccharide (sialylated lacto-N-fucopentaose II, IV\(^3\)-\(\alpha\)-NeuNAc-III\(^4\)-\(\alpha\)-Fuc-LcOse\(_4\)) (20). Based on the data presented in the present paper, however, the antigen in the serum of patients is mainly a mucin, not a ganglioside.

MATERIALS AND METHODS

**Materials.** Monoclonal antibodies 19-9 (IgG\(_1\) isotype) and 10c17 (IgM isotype) are produced by hybridomas prepared from spleen cells of mice immunized with a human colorectal carcinoma cell line (18). Spent supernatant fluids from hybridoma cell cultures containing approximately 10 \(\mu\)g of antibody per ml were used for all experiments except for the radioimmunoassay of affinity-purified antigen, in which antibody 19-9 was first purified on a Protein A-Sepharose column (7).

Human serum samples were obtained from the Wistar Institute, Philadelphia, Pa., and the NIH Blood Bank.

Affinity-purified goat anti-mouse IgM and IgG were purchased from Kirkegaard and Perry (Gaithersburg, Md.) and iodinated with lodogen (Pierce Chemical Co., Rockford, Ill.) and Na\(^{125}\)I (ICN, Irvine, Calif.) as described in Ref. 23.

The monosialoganglioside antigen detected by antibody 19-9 was purified from the colorectal carcinoma cell line SW119 as previously described (20).

**Fractionation of Serum.** Whole serum was centrifuged at 12,000 \(\times\) g for 15 min. Three ml of supernatant fluid were applied to a Sephacryl S-400 column (1.6 \(\times\) 95 cm) equilibrated in either phosphate-buffered saline (0.15 M NaCl 0.01 M sodium phosphate, pH 7.4), containing 0.1% sodium azide, or the same buffer containing 4 M guanidine·HCl. Fractions (3 ml) were collected and assayed for antigen by solid-phase radioimmunoassay for protein by absorbance of light at 280 nm and for hexose content by phenol-sulfuric acid assay (6).

Total lipid extracts of sera were prepared as described by Svennerholm and Fredman (26).

**Radioimmunoassays.** The binding of antibody to serum antigen was measured by solid-phase radioimmunoassay as previously described.

---

1This work was presented in part at the 74th Annual Meeting of the American Society of Biological Chemistry (21) and supported in part by Grants CA-10815, CA-25874, CA-21124, and RR-05540 from the NIH.

Received May 20, 1983; accepted August 3, 1983.
J. L. Magnani et al.

(20) with minor modifications. Aliquots (30 μl) of fractions from the Sephacryl column were added to wells of a round-bottomed polyvinyl chloride microtiter plate (Dynatech, Alexandria, Va.) and incubated overnight at 4°C. The wells were emptied and filled with phosphate-buffered saline containing 1% bovine serum albumin and 0.1% sodium azide (Buffer A). After 2 hr at 22°C, the wells were emptied, and 30 μl of monoclonal antibody solution diluted 1:10 with Buffer A were added to each well. The plate was covered with Parafilm, incubated for 4 to 5 hr at 22°C, and washed once with Buffer A; and about 50,000 cpm of 125I-labeled goat anti-mouse IgM or IgG in 30 μl of Buffer A was added to each well. After 16 hr, the wells were washed 6 times with cold phosphate-buffered saline, cut from the plate, and analyzed in a gamma counter.

The antigen defined by antibody 10c17 was detected in the antibody 19-9 affinity-purified material as follows. Purified monoclonal antibody 19-9 (50 ng/ml) in 30 μl of phosphate-buffered saline was added to wells of a microtiter plate and incubated overnight at 4°C. After a wash and incubation with Buffer A for 2 hr at 22°C, the wells were emptied, and 30 μl of column-fractionated antigen (Chart 1, Fraction 34) were added. After 5 hr at 22°C, the material that specifically bound to wells coated with antibody 19-9 was assayed for binding by antibody 10c17 using the solid-phase radioimmunoassay described above.

Antigens were detected directly on thin-layer chromatograms by autoradiography as described previously (20).

Density Gradient Ultracentrifugation. Ultracentrifugation of the antigen isolated from serum after chromatography on Sephacryl S-400 was done in 5 ml cesium chloride isopycnic density gradients in either phosphate-buffered saline or phosphate-buffered saline containing 4 M guanidine-HCl following procedures developed to study proteoglycans (13) and more recently used to study mucins (5). Gradients were formed by centrifugation in a Beckman SW50.1 rotor at 33,000 rpm for 72 hr at 10°C. Fractions (0.2 ml) were collected and assayed for antigen by the radioimmunoassay described above.

RESULTS AND DISCUSSION

The ganglioside antigens in lipid extracts of the SW1116 carcinoma cell line after thin-layer chromatography as revealed by autoradiography are shown in Fig. 1, Lane 4. The most prominent band, which has the mobility of GM1, is the ganglioside containing sialylated lacto-N-fucopentaose II (20). The slower-migrating, minor band is probably a ganglioside containing an octasaccharide with the same terminal sequence of sugars as that found in sialylated lacto-N-fucopentaose II (19). In contrast to the extraction of antigen from the colorectal carcinoma cell line, little antigen is extracted from the sera of cancer patients by organic solvents, and that which is extracted remains at the origin after thin-layer chromatography (Fig. 1, Lanes 2 and 3). No antigen is detected in lipid extracts of normal individuals by this procedure (Fig. 1, Lane 1).

Fractionation of 9 different antigen-positive sera from cancer patients on a Sephacryl S-400 column revealed antigen eluting in the void volume, indicating a molecular weight of >5 × 10^6. An example is shown in Chart 1. This procedure separates the antigen from most serum proteins. The same results were obtained when the fractionation was carried out in 4 M guanidine-HCl, suggesting that the high molecular weight of the serum antigen is not due to immune complexes or aggregation. The ganglioside antigen elutes from the Sephacryl S-400 column in the included volume when fractionated by the same procedure.

The antigen and the ganglioside antigen also differ in their lability to alkali; the ganglioside antigen is stable in 0.1 N NaOH for 5 hr at 37°C, whereas the serum antigen is destroyed by this treatment (Chart 2).

Density Gradient Ultracentrifugation. Ultracentrifugation of the antigen isolated from serum after chromatography on Sephacryl S-400 was done in 5 ml cesium chloride isopycnic density gradients in either phosphate-buffered saline or phosphate-buffered saline containing 4 M guanidine-HCl following procedures developed to study proteoglycans (13) and more recently used to study mucins (5). Gradients were formed by centrifugation in a Beckman SW50.1 rotor at 33,000 rpm for 72 hr at 10°C. Fractions (0.2 ml) were collected and assayed for antigen by the radioimmunoassay described above. The density of each fraction was determined by weighing a known volume in an analytical balance.

RESULTS AND DISCUSSION

The ganglioside antigens in lipid extracts of the SW1116 carcinoma cell line after thin-layer chromatography as revealed by autoradiography are shown in Fig. 1, Lane 4. The most prominent band, which has the mobility of GM1, is the ganglioside containing sialylated lacto-N-fucopentaose II (20). The slower-migrating, minor band is probably a ganglioside containing an octasaccharide with the same terminal sequence of sugars as that found in sialylated lacto-N-fucopentaose II (19). In contrast to the extraction of antigen from the colorectal carcinoma cell line, little antigen is extracted from the sera of cancer patients by organic solvents, and that which is extracted remains at the origin after thin-layer chromatography (Fig. 1, Lanes 2 and 3). No antigen is detected in lipid extracts of normal individuals by this procedure (Fig. 1, Lane 1).

Fractionation of 9 different antigen-positive sera from cancer patients on a Sephacryl S-400 column revealed antigen eluting in the void volume, indicating a molecular weight of >5 × 10^6. An example is shown in Chart 1. This procedure separates the antigen from most serum proteins. The same results were obtained when the fractionation was carried out in 4 M guanidine-HCl, suggesting that the high molecular weight of the serum antigen is not due to immune complexes or aggregation. The ganglioside antigen elutes from the Sephacryl S-400 column in the included volume when fractionated by the same procedure.

The antigen and the ganglioside antigen also differ in their lability to alkali; the ganglioside antigen is stable in 0.1 N NaOH for 5 hr at 37°C, whereas the serum antigen is destroyed by this treatment (Chart 2).

The density of the antigen obtained from serum by Sephacryl S-400 chromatography was determined by equilibrium ultracentrifugation in cesium chloride (13). As shown in Chart 3, the antigen sediments with a density of 1.5 g/ml in cesium chloride alone (Chart 3A) and at 1.43 g/ml in cesium chloride containing 4 M guanidine-HCl (Chart 3B). These values are characteristic of mucins (5, 25). The densities of typical proteins and glycoproteins range from 1.2 to 1.3 g/ml, whereas those for proteoglycans, which are mainly carbohydrate, including those made by

![Chart 1](chart1.png)
human colon carcinoma (15), exceed 1.5 g/ml (13).

Mucins contain hundreds of carbohydrate chains, some of which are blood group antigens (16, 27). As shown in Table 1, the antigen affinity purified by antibody 19-9 from the serum of a cancer patient with the Le(a−b+) blood type also contains the Leα antigen as detected by monoclonal antibody 10c17 (3).

Taken together, the foregoing results indicate that the antigen detected by antibody 19-9 in the sera of most patients with pancreatic and gastrointestinal cancer is a mucin. Differences in the immunochemical properties and carbohydrate composition of various mucins from normal and malignant colon have been described (2, 8, 10). Also, a large-molecular-weight glycoprotein, possibly a mucin, has been detected in the sera of patients with colorectal and pancreatic cancer using polyclonal antisera produced in goats (24).

In a previous study, antigen defined by antibody 19-9 was not detected by solid-phase radioimmunoassay in extracts from normal adult tissues. By immunoperoxidase labeling of normal tissue sections, however, antigen was found in a layer of ductal cells in normal pancreas and a layer of cells in normal salivary glands and bronchial epithelium that secrete mucins (1); and by autoradiography low levels of ganglioside antigen were detected.
in extracts of normal pancreas (12). The antigen is also found in salivary mucin from most normal individuals belonging to the Le(a+b-) or Le(a-b-) blood group and is not found in salivary mucin from normal individuals belonging to the Le(a-b-) blood group and is not found in salivary mucin from normal individuals belonging to the Le(a-b-) blood group (12). The antigen is also found in extracts of normal pancreas (12).

Table 1
Detection of the human Le\(^b\) blood group antigen by solid-phase radiomunoassay in affinity-purified cancer-associated antigen from the serum of a patient

<table>
<thead>
<tr>
<th>Immobilized antibody</th>
<th>Serum</th>
<th>Test antibody</th>
<th>(^{125})I bound (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19-9(^a)</td>
<td>Patient B</td>
<td>10c17(^b)</td>
<td>2,420</td>
</tr>
<tr>
<td>19-9</td>
<td>Patient B</td>
<td>TJ132(^c)</td>
<td>430</td>
</tr>
<tr>
<td>19-9 Patient B</td>
<td>None(^d)</td>
<td></td>
<td>410</td>
</tr>
<tr>
<td>19-9 Normal</td>
<td>10c17</td>
<td></td>
<td>540</td>
</tr>
<tr>
<td>19-9 Normal</td>
<td>TJ132</td>
<td></td>
<td>500</td>
</tr>
<tr>
<td>19-9 Normal</td>
<td>Normal</td>
<td></td>
<td>460</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

\(^a\) Antibody 19-9 is an IgG1 (18).
\(^b\) Antibody 10c17 is directed against the human Le\(^b\) blood group antigen and is an IgM (3).
\(^c\) Antibody TJ132 binds a protein not related to mucin and is an IgM.
\(^d\) Wells are filled with 1% bovine serum albumin in phosphate-buffered saline alone.

Acknowledgments

We thank Dr. Vincent Hascall and Dr. Masaki Yanagishita of the NIH for their help in performing the equilibrium ultracentrifugation experiments. We also thank Juli Maltagliati for typing the manuscript.

References

Identification of the Gastrointestinal and Pancreatic Cancer-associated Antigen Detected by Monoclonal Antibody 19-9 in the Sera of Patients as a Mucin

John L. Magnani, Zenon Steplewski, Hilary Koprowski, et al.