Characterization of Mucin Antigens Recognized by Monoclonal Antibodies Raised against Human Colon Cancer Cells

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ABSTRACT

Two monoclonal antibodies, MLS 102, which recognizes cancer-associated mucin antigens, and MLS 103, which recognizes normal mucin, were used to isolate, by immunofluorescence chromatography, the corresponding antigens from cell lysates and spent medium of a human colorectal carcinoma cell line, LS 180. The MLS 102 antigen contained serine, threonine, and proline as major amino acids. The carbohydrate chains of the MLS 102 antigen were composed of O-linked NeuAcα2→6GalNAc (56%), N-acetylgalactosamine (25%), and longer oligosaccharide chains. The MLS 102 antigen differed from the MLS 102 antigen in both amino acid and carbohydrate composition. Most O-linked oligosaccharides of the MLS 103 antigen were longer than the disaccharide found in the MLS 102 antigen.

Immunostaining of LS 180 cells using MLS 102 and MLS 103 revealed that the cells are heterogeneous with respect to the expression of the antigens.

INTRODUCTION

We have raised many monoclonal antibodies which recognize the carbohydrate moieties of the mucin-type glycoproteins on human colon cancer cells, using a human colorectal carcinoma cell line, LS 180, as an immunogen and glycopeptides as probes for screening hybridomas. One of the monoclonal antibodies, designated as MLS 102, reacted immunohistochemically with the cancer cell surface and mucinous glycoproteins secreted by the cells, but not with normal mucosal epithelium (1). We have already demonstrated that MLS 102 reacted with ovine, bovine, and porcine submaxillary mucins and that the reactivity toward these mucins varied in parallel with the NeuAcα2→6GalNAc-Ser/Thr content. The antigenic determinant was then identified as a cluster of NeuAcα2→6GalNAc residues on the polypeptide chain (2, 3).

Another monoclonal antibody, designated as MLS 103, strongly reacted with colon cancer cells as well as normal mucosal epithelium, as revealed in immunohistochemical studies (1). The antigenic determinant has not been identified, but it was suggested that a fucose residue may be involved (1).

This paper reports the isolation and molecular characterization of a colon cancer-associated antigen (MLS 102 antigen), in comparison with normal glycoproteins recognized by MLS 103, and the staining properties of LS 180 cells with these two antibodies.

MATERIALS AND METHODS

Abbreviations used are: NeuAc, N-acetylneuraminic acid; GalNAc, N-acetylgalactosamine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline.

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substituted for 1% Triton X-100. Antigens retained on the column were then eluted with 50 mM diethylamine-HCl buffer, pH 11.5, containing 0.1% Triton X-100. Antigens retained on the column were then eluted with 50 mM diethylamine-HCl buffer, pH 11.5, containing 0.1% Triton X-100.

Chemical Analysis. Amino acids and hexosamine were determined with a Hitachi 835 amino acid analyzer after hydrolysis of samples in 6 M HCl at 110°C for 24 h and in 6 M HCl at 100°C for 16 h, respectively. Sialic acid was determined by the thioctic acid method of Warren (9).

SDS-PAGE. SDS-PAGE was performed in a 7.5% acrylamide gel by the procedure described by Laemmli (10). Fluorography, to detect radioisotope-labeled antigens, was carried out according to the method of Bonner and Laskey (11).

RESULTS

Isoxation of the MLS 102 and MLS 103 antigens. The MLS 102 and MLS 103 antigens derived from cell lysates and designated as the c-MLS 102 and c-MLS 103 antigens, respectively, and those from spent medium as the m-MLS 102 and m-MLS 103 antigens, respectively. Table 1 shows the distribution of the radioactivity in the affinity-purified antigens. As shown in Table 1, the 14C/3H ratios for the c- and m-MLS 102 antigens were lower than those for the c- and m-MLS 103 antigens, respectively. This would reflect the higher carbohydrate content of the MLS 102 antigen than that of the MLS 103 antigen. It appears that about one-tenth of each of the antigens was secreted into medium during 3 days of cultivation.

SDS-PAGE of the MLS 102 and MLS 103 Antigens. The electrophoretic profiles of the antigens are shown in Fig. 1, the c-MLS 102 as well as the m-MLS 102 antigen being separated into four distinct bands (Fig. 1, Lane A). In contrast, the c- and m-MLS 103 antigens migrated as broad bands with major bands at around 200,000 and 180,000, respectively.

The results of SDS-PAGE suggest that the MLS 102 antigens are different glycoproteins from the MLS 103 antigens. The electrophoretic profiles of the antigens are shown in Fig. 1, the c-MLS 102 as well as the m-MLS 102 antigen being separated into four distinct bands (Fig. 1, Lane A). In contrast, the c- and m-MLS 103 antigens migrated as broad bands with major bands at around 200,000 and 180,000, respectively.

Chemical Compositions of the MLS 102 and MLS 103 Antigens. The affinity-purified MLS 102 and MLS 103 antigens were subjected to amino acid, amino sugar, and sialic acid analyses. As shown in Table 2, both the c- and m-MLS 102 antigens were characterized by high contents of serine, threonine, and proline. The sum of these three amino acids comprised about 60% of the total amino acids. Furthermore, the MLS 102 antigens contained large amounts of galactosamine and sialic acid, but only a small amount of glucoseamine. These data are compatible with the mucin-type glycoprotein nature of the MLS 102 antigens, with a high degree of O-glycosylation. The MLS 103 antigens showed amino acid compositions distinctly different from those of the MLS 102 antigens. The sum of serine, threonine, and proline comprised only 25% of the total amino acids and the amino sugar content was low. However, mucin-type glycopeptides could be prepared from the c-MLS 103 antigen by sialidase digestion, followed by gel filtration on Sephadex G-50. All the carbohydrates, as judged from the 14C radioactivity derived from 14C-glucosamine, were recovered in the glycopeptide fraction (void volume fraction). The glycopeptides had amino acid and amino sugar compositions distinctly different from those of the MLS 102 antigens.
Table 2 Chemical compositions of the MLS 102 and MLS 103 antigens

<table>
<thead>
<tr>
<th>Residues 100/amino acid residues</th>
<th>c-MLS 102 antigen</th>
<th>m-MLS 102 antigen</th>
<th>c-MLS 103 antigen</th>
<th>m-MLS 103 antigen</th>
<th>glycopeptide obtained from asialo c-MLS 103 antigen</th>
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<td>Asp</td>
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<td>4.54</td>
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<td>Pro</td>
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<td>4.78</td>
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Fig. 2. Cross-reactivity of MLS 102 and MLS 103 with the respective antigens. The affinity-purified antigens were adsorbed on the wells of 96-well microtiter plates. The amounts of antibodies bound to the plates were determined with [125I]-labeled protein A. (A) Activity toward the MLS 102 antigen. (B) Activity toward the MLS 103 antigen.

Cross-Reactivity of MLS 102 and MLS 103. As shown in Fig. 2, MLS 102 and MLS 103 did not mutually inhibit their binding to the other’s antigenic glycoproteins. This indicates that the antigenic carbohydrate chains defined by MLS 102 and MLS 103 are different.

Analysis of O-linked Carbohydrate Chains. To characterize the carbohydrate chains of the antigenic glycoproteins defined by the monoclonal antibodies, MLS 102 and MLS 103, the affinity-purified antigens were submitted to reductive alkaline β-elimination and the carbohydrate chains released were fractionated by gel filtration on Sephadex G-25. As shown in Fig. 3a, carbohydrate alcohols released from the c-MLS 102 antigen were separated into at least four fractions, Fractions I–IV. Fraction III and IV were identified as NeuAcα2→6GalNAcol and GalNAcol, respectively, on descending paper chromatography (Fig. 4). Determination of galactosaminitol released on acid hydrolysis revealed that the molar ratio of O-linked carbohydrates in Fractions I plus II, III (NeuAcα2→6GalNAcol), and IV (GalNAcol) was 0.19:0.56:0.25.

The carbohydrate chains released from the c- and m-MLS 103 antigens, on fractionation, gave multiple peaks, but these could be pooled into two or three fractions, as shown in Fig. 3, b and c. We did not detect NeuAcα2→6GalNAcol and GalNAcol in any of the fractions. Determination of galactosaminitol in the fractions indicated that the molar ratio of O-linked carbohydrates in Fractions I plus II was 0.19:0.56:0.25.
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Fig. 4. Characterization of the oligosaccharides derived from the MLS 102 antigen. Fractions I, II, III, and IV from the Sephadex G-25 column were applied on Toyo No. SIA paper, followed by development with ethyl acetate:pyridine:acetic acid:water (5:5:1:3, v/v) for 24 h. The reference carbohydrates used were [3H]NeuAcO→6GalNAc (□) and [14C]GalNAc (●).

carbohydrates in Fractions I, II, and III of the c-MLS 103 antigen was 0.36:0.49:0.15.

Staining of LS 180 Cells with MLS 102 and MLS 103. To see how MLS 102 and MLS 103 antigens are expressed on the cells used as the immunogen, the cultured cells were stained with these two antibodies. For this, MLS 102 was conjugated to fluorescein and MLS 103 with rhodamine. As shown in Fig. 5, the cells were stained differently with the antibodies. Four types of the cells could be seen based on the staining properties: (a) stained only with MLS 102 (about 10% of the total cells); (b) stained only with MLS 103 (about 80%); (c) stained with both of the antibodies (about 5%); (d) not stained with any of the antibodies (2–3%). Interestingly, when a single cell with any staining property was grown to multiply to quite a number of cells, the staining properties of the progeny cells (four types of staining) were apparently the same as those of the parent cells. The results may imply characteristics of cancer cells. Namely, one cell line of cancer cells is composed of cells with different antigens on cell surfaces, and these cells are able to transform to each other reversibly.

DISCUSSION

With the use of immobilized glycopeptides we have raised many monoclonal antibodies, all of which are directed toward the carbohydrate moieties of glycoproteins (1, 12, 13–15). One such monoclonal antibody, designated as MLS 102, has been shown to react with colon cancer cells and their secretions, but not with normal colon cells (1). The epitopic carbohydrate for this antibody has been identified as NeuAcα2→6GalNAc (2, 3). MLS 102 showed extremely strong reactivity with ovine submaxillary mucin, which led us to propose that this antibody recognizes a cluster of this disaccharide attached to one and the same polypeptide chain.

To characterize antigenic glycoproteins, we have isolated the glycoproteins using an immunoaffinity column. For comparison, we have also isolated the antigenic glycoproteins for another monoclonal antibody, MLS 103, which binds to normal colon cells as well as to cancer cells. The affinity-purified MLS 102 antigen contains serine, threonine, and proline as major amino acids, which are characteristic of mucin-type glycopro-
proteins such as submaxillary mucins. The carbohydrate chains of the MLS 102 antigen are composed of $O$-linked NeuAc$\alpha_2\rightarrow$GalNAc (56%) and $O$-linked GalNAc (25%) residues. Thus, the MLS 102 antigen consists of $O$-linked NeuAc$\alpha_2\rightarrow$GalNAc in mucin-type glycoproteins. J. Biol. Chem., 263: 8724–8726, 1988.


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