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Mucin-carbohydrate directed monoclonal antibody

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To raise monoclonal antibodies recognizing cancer-associated alterations of the carbohydrate structure of glycoproteins, Balb/c mice were immunized with human colonic cancer cells (LS 180 from ATCC). One of the generated hybridomas produced a monoclonal antibody that bound to the carbohydrate moiety of mucin-type glycoproteins from LS 180. The antibody did not bind to glycoproteins from another colonic cancer cell line, SW 1116, or to glycolipids from any of the colonic cancer cell lines. The antibody bound to ovine and bovine submaxillary mucins (OSM and BSM). NeuAcα2→6GalNAc seemed to be involved in the epitope.

Monoclonal antibody; Colonic cancer; Cancer-associated carbohydrate antigen; Mucin

1. INTRODUCTION

Alterations of carbohydrate chains of glycoconjugates are often associated with malignant transformation of cells. A number of cancer-associated carbohydrate antigens have been identified by the use of monoclonal antibodies [1]. Most of the antigens recognized are glycolipids and are often related to blood group antigens. Structural studies of the epitopes have also been performed using glycolipids, but the alterations specifically expressed on sugar chains of glycoproteins have not been studied extensively.

By injecting human cancer cells (LS 180 cells) into mice and selecting hybridomas which secrete antibodies reacting with glycopeptides from the cancer cells, we have obtained five monoclonal antibodies recognizing carbohydrate moiety of glycoproteins (Fukui, S. et al., to be published elsewhere). Here, we describe the properties of one such monoclonal antibody, designated MLS 102.

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2. MATERIALS AND METHODS

2.1. *Materials*

Human colonic cancer cells, LS 180 and SW 1116, were obtained from ATCC, Rockville. NeuAcα2→3lactose, NeuAcα2→6lactose and NeuAcα2→3Galβ1→3(NeuAcα2→6)GalNAcβ1→4Galβ1→4Glc from bovine colostrum were gifts from Dr Y. Uchida (Marukin Shoyu Co.). *N*-Acetylneuraminic acid was from Nakarai (Kyoto) and *N*-glycolylneuraminic acid from Sigma (St. Louis, MO).

2.2. *Methods*

Ovine and bovine submaxillary mucins were prepared according to Tettamanti and Pigman [2], and porcine submaxillary mucin as described by De Salegui and Plonska [3].

The cell membrane glycoproteins were isolated from LS 180 cells according to Funakoshi and Yamashina [4] and extensively digested with pronase. The digest was fractionated by gel filtration on Sephadex G-50 to obtain mucin-type glycopeptides (G-50I) and serum-type glycopeptides (G-50II) [5]. The glycolipid fraction was separately

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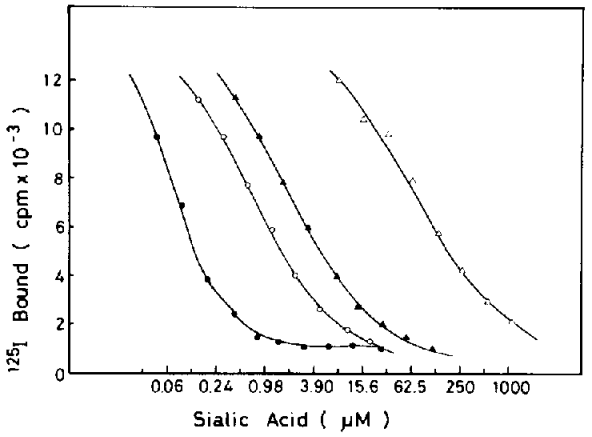

Fig.1. Activities of glycoproteins inhibiting the binding of MLS 102 to G-50I. (Abscissa) Glycoprotein concentrations expressed as sialic acid; (ordinate) amounts of MLS 102 bound to the wells expressed as bound protein A. (●) OSM, (○) G-50I, (▲) BSM, (△) PSM.

prepared by extracting the packed cells with chloroform/methanol.

Reactivities of mucin-type glycoproteins and several sugars with MLS 102 were determined by measuring their ability to inhibit the binding of MLS 102 to G-50I. Wells of a polyvinyl chloride plate (96 wells, Corster) were coated with G-50I (about 500 ng in each well), then the uncoated area of the wells was covered by 1% bovine serum albumin (BSA) in 20 mM phosphate-buffered saline (PBS), pH 7.2. An appropriate amount of MLS 102 in PBS containing 0.1% BSA (10 μl) and aqueous solution of glycoproteins or sugars (10 μl) were added successively. The plate was left standing overnight at 4°C. The wells were washed with PBS containing 1% BSA, then ^{125}I-labelled protein A (12.5 ng with about 100000 cpm in 50 μl PBS containing 0.1% BSA) was added and incubated at room temperature for 2 h. The wells were washed with PBS (150 μl) three times, then each well was cut out from the plate, and radioactivities were counted in a gamma spectrophotometer (Gamma-5500, Beckman).

3. RESULTS AND DISCUSSION

One of the monoclonal antibodies raised against LS 180 cells, designated MLS 102, appeared to recognize cancer-associated antigens in cancers of intestine, esophagus and ovary as assessed from histological studies using fluorescein-labelled anti-mouse IgG rabbit IgG. The antibody bound to both the cells and their secretions. MLS 102 reacted with G-50I, but not with G-50II. Sialidase treatment of G-50I abolished the reactivity.

No reaction was observed with glycolipids from LS 180 or another colonic cancer cell line, SW 1116, or with glycolipids from various sources such as bovine brain and human meconium. Thus,

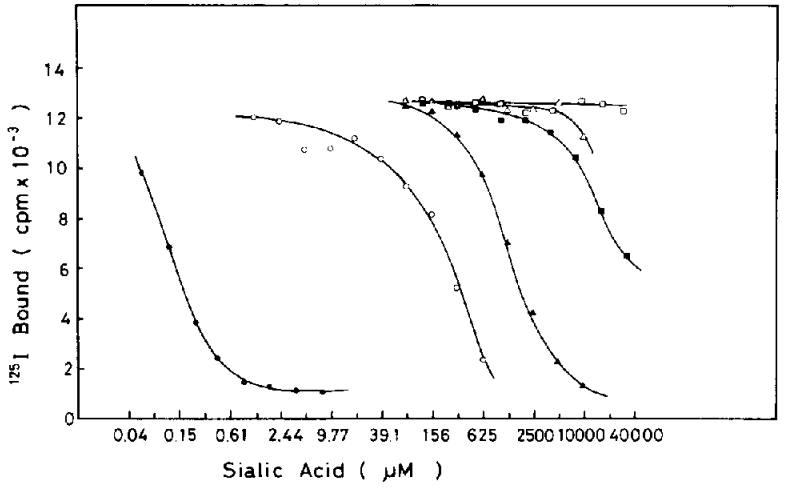

Fig.2. Reactivities of sialic acids and sialyl oligosaccharides with MLS 102 determined by inhibition of binding of MLS 102 to G-50I. (○) NeuAcα2→6GalNAc, (▲) NeuAcα2→6lactose, (■) *N*-acetylneuraminic acid, (△) NeuAcα2→3lactose, (□) *N*-glycolylneuraminic acid, (●) OSM as a reference.

MLS 102 appeared to differ from the antibodies recognizing sialylated terminal structures of gangliosides as exemplified by the sialyl Le^a structure [6]. Glycopeptides, both mucin and serum types, prepared from SW 1116 did not react with MLS 102. The antibody did not react with seminal fluid, amniotic fluid, cord serum, colostrum, maternal serum and saliva from human sources. The only immunoreactive glycopeptides or glycoproteins of human origin other than G-50I so far tested were a mucin from meconium and a rectal adenocarcinoma glycoprotein [7] (not shown). Only about 10% of G-50I was reactive with MLS 102, as determined by immunoaffinity chromatography using a column constructed by coupling MLS 102 to Sepharose 4B.

Interestingly, MLS 102 reacted with OSM and BSM with less activity towards porcine submaxillary mucin (fig.1). On fractionation using the immunoaffinity column, about 90% of OSM, based on sialic acid content, was retained and eluted, whereas about 40% of BSM was retained.

Most of the carbohydrate moiety of OSM is known to be NeuAcα2→6GalNAcα1→ [8]. The disaccharide could be released from OSM by alkaline-borohydride treatment as a sugar alcohol or by hydrazine treatment followed by re-*N*-acetylation as a reducing sugar (unpublished). The disaccharide-alcohol had no inhibitory activity towards the reaction between MLS 102 and G-50I, but the reducing disaccharide had significant activity, as shown in fig.2. Unlike Hanganutziu-Deicher antigen [9], *N*-glycolylneuraminic acid did not react with MLS 102. The inhibitory activity seemed to be confined to NeuAcα2→6GalNAc (or NeuAcα2→6Gal to some extent), and practically no activity was found for 2→3-linked sialic acid residue. However, when the *N*-acetylgalactosamine to which *N*-acetylneuraminic acid is linked at C-6 is substituted at C-3, the reactivity seems to be lost since the following oligosaccharide had no activity: NeuAcα2→3Galβ1→3(NeuAcα2→6)-GalNAcβ1→4Galβ1→4Glc isolated from bovine colostrum. This may be applied to glycolipids as well. Disialoganglioside having the colostrum oligosaccharide structure, described above, as the carbohydrate moiety was not reactive with MLS 102. Glycolipids with the terminal NeuAcα2→6GalNAc structure would be reactive, but the occurrence of such glycolipid has so far not been reported.

The reactivity of OSM was, however, much higher compared to that of NeuAcα2→6GalNAc (fig.2). This would probably be due to the occurrence of a cluster of the disaccharide on the polypeptide as was found for the Ca antigen [10].

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