Jpn. J. Cancer Res. 87, 488–496, May 1996

A Novel Monoclonal Antibody Specific for Sialylated MUC1 Mucin

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Development of a new monoclonal antibody (mAb) MY.1E12 which reacts with sialylated MUC1 mucins is described. The mAb did not react with any component in the lysates of COS-1 cells, whereas it bound to sialylated MUC1 mucins produced by COS-1 cells transiently transfected with MUC1 mucin cDNA, strongly suggesting that the expression of the epitope of mAb MY.1E12 depends on the presence of the MUC1 mucin core peptide. The requirement of sialyl residues for antibody recognition was established by Western blotting analysis of extracts of various carcinoma cells and *in situ* desialylation. In all cases, the mAb binding of electrophoretically separated MUC1 mucin diminished after desialylation by mild acid hydrolysis. When Capan-1 pancreatic carcinoma cells were pretreated with benzyl-*N*-acetylgalactosaminide in culture, the MUC1 mucins produced under these conditions, which were detected by core peptide-specific mAbs, did not react with mAb MY.1E12. These results suggest that *O*-linked carbohydrate chains are important for the mAb binding.

Key words: MUC1 mucin — Glycosylation — Carcinoma-associated epitope — Monoclonal antibody — Sialic acid

Mucins are high-molecular-weight glycoproteins consisting of an extended core polypeptide and many oligosaccharides *O*-linked through serine or threonine residues. In humans, eight distinct epithelial mucin genes have been identified.[1–8] MUC1 mucin is a transmembrane glycoprotein with an extracellular domain consisting mostly of tandem repeats of 20-amino-acid sequences,[2,9] and a cytoplasmic tail of 69 amino acids.[1,10,11] MUC1 mucin, like other epithelial mucins, seems to be expressed in an organ-specific manner,[12] and often shows structural and quantitative alterations in epithelial tissues under pathological conditions. Furthermore, carcinoma-associated MUC1 mucin has been found in the sera of cancer patients and is used as a marker for certain types of cancer.[13–15]

The structural differences between MUC1 mucins produced by normal epithelia and carcinoma tissues appear to be at least in part due to the differential glycosylation of its core peptide. In mammary carcinomas, the carbohydrate side chains of MUC1 mucin are thought to be shorter or less densely distributed than those in normal cells.[16] Such changes would result in the exposure of cryptic polypeptide epitopes which are recognized by T and B cells.[17–20] Carcinoma-specific cytotoxic lymphocytes recognize MUC1 mucin core peptides, such as Ala-Pro-Asp-Thr-Arg, within the tandem repeat portion. These peptide epitopes also serve as antigenic determinants for many anti-MUC1 mucin monoclonal antibodies.[9] Furthermore, mucin-specific humoral immune response to this epitope has been observed in mammary carcinoma patients.[21] Such immune responses may be used as a basis for the immunotherapy of mammary carcinomas.

In colorectal carcinomas, MUC1 mucin does not seem to function as a immunogenic epitope. The level of MUC1 mucin expression is high in colorectal carcinomas at advanced clinical stages.[22] Such a profile of MUC1 mucin expression in colorectal carcinoma does not necessarily conflict with the observation with mammary carcinomas because the clinical observations on colorectal carcinomas are based on studies using a monoclonal antibody (mAb) specific for glycosylated MUC1 mucins, HMFG-1. It is possible that the peptide epitopes in the highly glycosylated MUC1 mucins produced by colon carcinoma are masked. Furthermore, highly glycosylated MUC1 mucin may facilitate the dissemination of carcinoma cells by preventing hemophilic aggregation through various adhesive receptors.[23] These anti-adhesive interactions may also be dependent on the sialylation of MUC1 mucins. However, mAbs which preferentially recognize MUC1 mucins with sialylated carbohydrate chains were not previously available. In this paper, we report the preparation of a mAb useful for the identification of MUC1 mucin bearing sialylated carbohydrate chains. This mAb reacted with MUC1 mucin produced by a variety of carcinoma cells and that isolated from human urine. It did not react with human colonic sulfomucin, bovine submaxillary mucin (BSM), ovine submaxillary

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mucin (OSM), porcine gastric mucin (PGM), human glycophorin, or bovine fetuin. These and other results strongly suggest that the epitope of mAb MY.1E12 contains both the carbohydrate portion of MUC1 mucin and the MUC1 mucin core peptide.

MATERIALS AND METHODS

Cell lines and cell cultures Myeloma line SP2/0 was grown in Dulbecco's modified Eagle's minimum essential medium (DMEM) containing a high concentration of glucose (4.5 g/ml) and 10% fetal calf serum (FCS) in a humidified atmosphere with 5% CO_2 at 37°C. A549 lung carcinoma cells, Capan-1 pancreatic adenocarcinoma cells, HeLa epitheloid carcinoma cells, HT29-LMM colon carcinoma cells, HT1080 fibrosarcoma cells, KATO III gastric carcinoma cells, LC-1sq lung carcinoma cells, and MKN-1 stomach adenosquamous carcinoma cells were obtained from the Cell Bank of Japan. SN12 renal carcinoma cells were provided by Dr. Isaiah J. Fidler of the University of Texas M. D. Anderson Cancer Center, Houston, TX. These cells were grown in a 1:1 mixture of DMEM and Ham's F-12 medium (DMEM/F12), supplemented with 10% FCS in a humidified atmosphere with 5% CO_2 at 37°C. In some experiments, Capan-1 cells were treated with benzyl-*N*-acetylgalactosaminide (Bzl-GalNAc, Sigma, St. Louis, MO) added to culture media to inhibit the extension of *O*-linked carbohydrate chains. Cells (7×10^6) in a 10-cm culture dish were treated with 4 m*M* Bzl-GalNAc for 48 h as previously described by Kuan and co-workers.[24]

Immunization procedures Human milk fat globule (HMFG) membranes rich in MUC1 mucin purified according to the method of Hanisch *et al.*[25] were provided by Dr. Ryuichiro Tanaka, Yakult Institute for Microbiological Research, Tokyo. Eight-week-old female BALB/c mice were immunized by intraperitoneal injection of 100 μg of HMFG membranes emulsified in Freund's complete adjuvant. One week later, a second immunization with 100 μg of HMFG membranes in Freund's incomplete adjuvant was given followed by three additional injections at weekly intervals. Three months after the last immunization, the mice were boosted by i.v. injections with 200 μg of HMFG membranes performed twice. Five days after the last booster injection the mice were killed and their spleens were removed.

Cell fusion and isolation of hybridoma cells Splenocytes from the immunized mice were fused with SP2/0 myeloma cells at a 5:1 cell ratio in the presence of 50% polyethyleneglycol (Hybri max, Sigma). Cell suspensions were distributed into wells of eight 96-well plastic plates in DMEM containing hypoxanthine, aminopterin, thymidine and 20% FCS and incubated for 8 days. Subsequently, the cells were cultured in DMEM containing hypoxanthine, thymidine and 10% FCS for 8 days. Supernatants of these hybridoma cultures were first screened for binding to HMFG membranes by ELISA. Hybridoma supernatants containing antibodies to HMFG membranes were then tested for their binding to MUC1 mucin in Capan-1 cell lysates by Western blotting analysis as described below. Hybridoma cells producing anti-MUC1 mucin antibodies were cloned twice by the limiting dilution method in DMEM containing 20% FCS and oxaloacetate/pyruvate/bovine insulin-media supplement (Sigma). Immunoglobulin subclasses of the antibodies were determined by the Isotype Ab-Stat kit (Sang Stat Medical, Menlo Park, CA). To produce ascitis antibodies, mice were intraperitoneally injected with pristine (0.5 ml/mouse), followed by hybridoma cells (2×10^5) after 7 days. The ascitis fluids were harvested and freed of blood cells by centrifugation.

Binding assay for antibodies Ninety-six-well plastic plates were coated with 10 μg/ml of HMFG membranes (suspended in 200 m*M* $NaHCO_3$, pH 9.6; 50 μl/well) by incubating them overnight at 4°C. Excess antigen was removed, then the wells were filled with 3% bovine serum albumin (BSA) and incubated for 2 h at room temperature to block nonspecific binding sites. The wells were washed with 0.05% Tween-20 in phosphate-buffered saline (PBS) and 100 μl of hybridoma culture supernatant was added to each well. The plates were incubated at room temperature for 2 h and washed 3 times with 0.05% Tween-20 in PBS. One hundred microliters of 1:1000 diluted alkaline phosphatase-goat anti-mouse IgG (Zymed, So. San Francisco, CA) was added to each well and incubated for 1 h at room temperature, then washed 5 times with 0.05% Tween-20 in PBS. Finally, *p*-nitrophenyl phosphate (100 μg/100 μl, Sigma) with 10% diethanolamine in 0.5 m*M* $MgCl_2$ (pH 9.8) was added to each well, and absorption at 405 nm was measured with a Multiscan microtiter reader. Binding of mAbs to various mucins was also tested by ELISA assays. Human colonic sulfomucin[26] (20 μg/ml), OSM, BSM, or PGM (500 μg/ml), human urine MUC1 mucin (2.5 mg/ml), or human erythrocyte glycophorin A (2.5 mg/ml) was suspended in 200 m*M* $NaHCO_3$, pH 9.2; 100 μl/well and used to coat plastic multi-well plates. mAb binding to these plates was tested. To determine the inhibitory activity of oligopeptides and other potential haptenic inhibitors for mAbs, the test substance at increasing concentrations was incubated with mAbs (at a concentration sufficient to give half-maximum binding), then the binding was tested by ELISA.

mAbs mAb HMFG-1 (mouse IgG, specific for glycosylated MUC1 mucin),[27] HMFG-2 (mouse IgG, specific for underglycosylated MUC1 mucin)[27] and mAb SM-3 (mouse IgG, specific for core peptide of MUC1 mucin)[28] were provided by Dr. Joy Burchell, ICRF, London, UK.

mAb 115D8 (mouse IgG, specific for glycosylated MUC1 mucin)[29] was provided by Dr. John Hikens, Netherlands Cancer Center, Amsterdam, Netherlands.

Western blotting analysis Cells were lysed in a buffer containing 0.5% NP-40 as previously described.[30] One hundred microliters of cell lysate was electrophoretically separated on 4% polyacrylamide gels in the presence of 0.1% sodium dodecyl sulfate (SDS-PAGE) and the separated components were transblotted onto polyvinylidene difluoride (PVDF) membranes (Immobilon, Millipore, Boston, MA). The membranes were treated with PBS containing 2% BSA at 4°C overnight and then incubated with hybridoma culture supernatants for 2 h at room temperature. The membranes were washed with PBS containing 0.1% Tween-20 and incubated at room temperature for 1 h with 100 μl of 1:1000 diluted peroxidase-labeled rabbit anti-mouse IgG (Zymed). The membranes were again washed with Tris-buffered saline containing 0.1% Tween-20 and the bound antibodies were visualized with ECL (Amersham, Buckinghamshire, UK). In some experiments, sialic acid was removed from the glycoproteins by mild acid treatment prior to the application of the first antibody. For example, Capan-1 cell lysates were separated by SDS-PAGE on 4% gels and electrophoretically transblotted onto PVDF membranes as described above. The membranes were treated with 10% acetic acid at 80°C for 1 h, washed with PBS at 4°C, and blocked with PBS containing 2% BSA at 4°C overnight prior to treatment with mAb as described above.

Transient transfection of COS-1 cells with MUC1 mucin cDNA The plasmid pDKOF, containing a nearly full-length MUC1 mucin cDNA missing only 18 base pairs at the 5′end involving the 22 tandem repeat portion,[19] was a gift from Dr. Olivera J. Finn, University of Pittsburgh, Pittsburgh, PA. *Escherichia coli* JM109 was transformed with this plasmid. The plasmid DNA was purified from *E. coli* using a Plasmid Maxi Kit (Quiagen, Hilden, Germany). The purity of the plasmid was confirmed by electrophoresis after *Hin*d III digestion. COS-1 cells (6×10^5 cells in 6-cm dish) were transfected with 15 μg of MUC1 plasmids by the calcium phosphate method. After transfection, the cells were incubated at 37°C for 20–24 h, rinsed with PBS and further incubated for 24 h before use.

Immunohistochemical distribution of sialylated MUC1 mucins in colorectal carcinoma Immunohistochemical localization of the antigen recognized by mAb MY.1E12 was investigated with two primary adenocarcinomas. Four-micron sections were prepared from formalin-fixed and paraffin-embedded permanent pathological specimens. The sections were deparaffinized and treated with 0.03% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity. The sections were rehydrated and washed with PBS and then incubated overnight at 4°C with 2% BSA in PBS. The sections were then incubated with mAb MY.1E12 (hybridoma culture supernatant diluted 1:2 in PBS containing 2% BSA) for 2 h at room temperature, washed several times with PBS and treated with biotinylated goat anti-mouse IgG (Vector, Burlingame, CA) for 1 h. After repeated washing with Dulbecco's phosphate-buffered saline, the sections were incubated with a solution of avidin-biotinylated peroxidase complex (Vector) followed by a mixture of 1 ml of 0.4% 3-amino-9-ethylcarbazole dissolved in *N,N′*-dimethylformamide, 100 ml of 20 m*M* sodium acetate, pH 5.2, and 70 ml of 30% hydrogen peroxide. The sections were mounted with gelatin-glycerin-mounting agent.

RESULTS

Production and selection of hybridoma cells Hybridoma cells produced by the use of splenocytes from a mouse immunized with HMFG membranes rich in MUC1 mucin were screened for the ability of antibodies in the supernatants to bind to HMFG membranes. Hybridoma cells that secreted antibodies having strong reactivity with HMFG membranes were further screened for the antibody's reactivity with MUC1 mucin produced by Capan-1 pancreatic carcinoma cells by Western blotting analysis. Three hybridoma clones were selected, and after recloning, one stable hybridoma clone was obtained. The antibody produced by this clone was designated as MY.1E12, which was of the IgG_{2a} isotype.

Reactivity of mAb with human carcinoma cell components revealed by Western blotting analysis To examine further the reactivity of mAb MY.1E12 with carcinoma-associated MUC1 mucin, lysates of various carcinoma cell lines were examined by Western blotting analysis using mAb MY.1E12 and three other anti-MUC1 mucin mAbs (Fig. 1). The electrophoretic mobility of MUC1 mucin of these carcinoma cells was unique to each cell line. For example, Capan-1 (lane 2), HeLa (lane 3), KATO III (lane 6), and MKN-1 (lane 8) cells show expression of MUC1 mucin, the electrophoretic mobility of which was apparently dependent on polymorphism regarding the number of tandem repeats.[31] Lysates of HT29-LMM (lane 4), HT1080 (lane 5), and SN12 (lane 9) did not contain any component having reactivity with these anti-MUC1 mucin mAbs. The four anti-MUC1 mucin mAbs tested differed slightly in their ability to bind MUC1 mucin in cell lysates of different cells, although all of them recognized the high-molecular-weight components corresponding to MUC1 mucin. Comparison of the binding patterns indicated that mAb MY.1E12 reacted with MUC1 mucin of Capan-1 cells. The electrophoretic behavior of components reacting with mAb MY.1E12 and mAb 115D8 were similar suggesting that

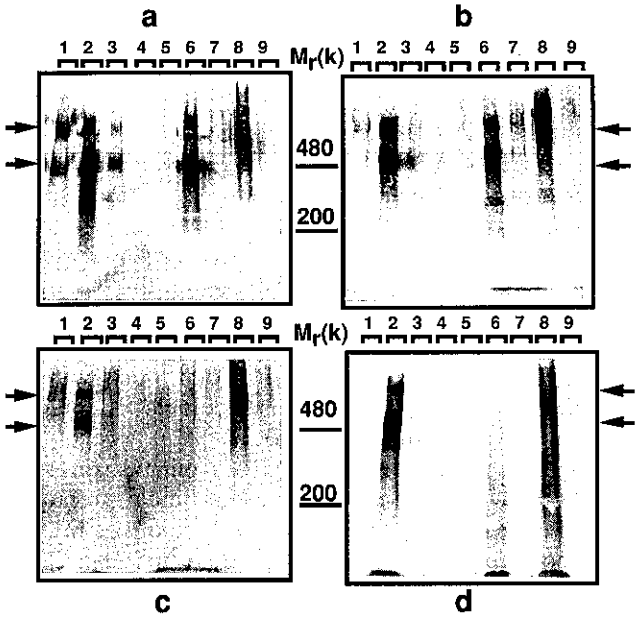

Fig. 1. Binding of various anti-MUC1 mucin mAbs to lysates of human carcinoma cell lines as revealed by Western blotting analysis. Lysates of A549 (lane 1), Capan-1 (lane 2), HeLa (lane 3), HT29-LMM (lane 4), HT1080 (lane 5), KATO III (lane 6), LC-1sq (lane 7), MKN-1 (lane 8), and SN12 (lane 9) cells were subjected to SDS-PAGE on 4% gels and transblotted onto PVDF membranes. The membranes were processed as described in "Materials and Methods." a, 115D8; b, MY.1E12; c, HMFG-1; d, HMFG-2. Arrows indicate the approximate position of MUC1 mucin of Capan-1 cells.

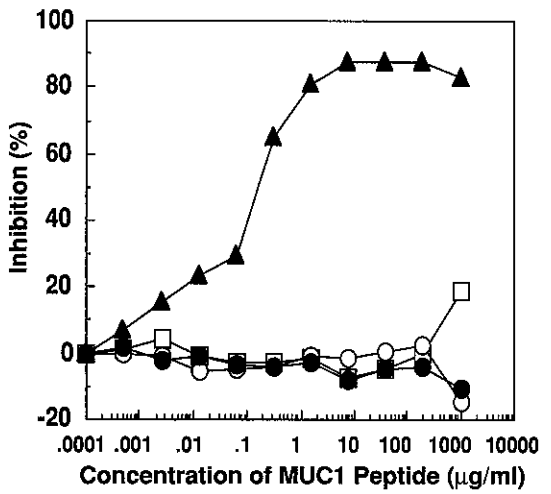

Fig. 2. Inhibition of the binding of various anti-MUC1 mucin mAbs to HMFG by synthetic oligopeptides in ELISA. mAbs MY.1E12 (open circles), 115D8 (closed circles), HMFG-1 (open squares), and HMFG-2 (closed triangles) were used.

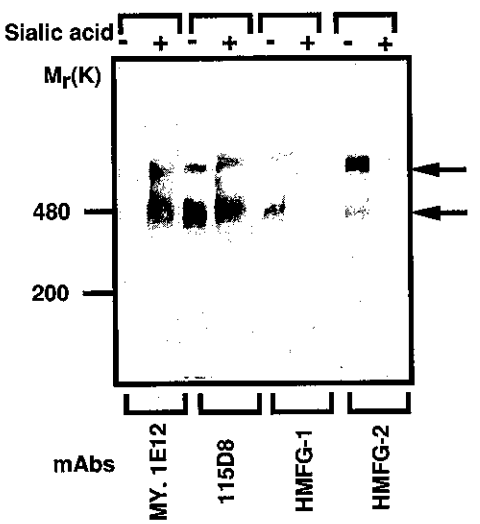

Fig. 3. Binding of mAbs to Capan-1 cell lysates before and after removal of sialic acid by mild acid hydrolysis *in situ*. The Capan-1 cell lysates were subjected to SDS-PAGE using 4% gel and then transblotted onto PVDF membranes. The membrane strips were treated with mild acid to hydrolyze sialic acid (lanes marked −; lanes 1, 3, 5, 7), or were untreated (lanes marked +; lanes 2, 4, 6, 8) and then incubated with antibodies as described in "Materials and Methods." Arrows indicate the approximate position of MUC1 mucin of these cells.

the epitope recognized by mAb MY.1E12 is closely related, but not identical, to that recognized by mAb 115D8.

Inhibition of mAb binding to HMFG membranes by an oligopeptide A synthetic oligopeptide representing a portion of MUC1 mucin tandem repeats (PDTRPAPG-STAPPAC) was tested for inhibitory activity against anti-MUC1 mucin mAbs. Increasing concentrations of this peptide effectively inhibited the binding of mAb HMFG-2 to HMFG membrane antigen, while the binding of mAb MY.1E12 or 115D8 was not inhibited (Fig. 2). In other experiments, mAb MY.1E12 did not bind to this peptide immobilized on plastic wells (data not shown). These results indicate that this stretch of the MUC1 mucin peptide alone, which is known to be recognized by many anti-MUC1 mucin mAbs,[9, 32, 33] was not recognized by mAb MY.1E12.

Effect of removal of sialic acid from MUC1 mucin on the binding of mAbs To test whether sialic acid at the terminal of carbohydrate chains of MUC1 mucin was required for antibody binding, electrophoretically separated sialoglycoproteins of Capan-1 cells were desialylated by mild acid hydrolysis before Western blotting analysis. As shown in Fig. 3, the reactivity of mAb MY.1E12 was diminished by this treatment, indicating

that the antigenic epitope for this mAb contained sialylated carbohydrate chains or is modulated by sialic acid. The reactivity of mAb 115D8 did not significantly change after this treatment (Fig. 3), whereas the reactivities of mAb HMFG-1 and mAb HMFG-2 with MUC1 mucin increased (Fig. 3).

Effect of Bzl-GalNAc on binding of mAbs To determine whether the epitope of this mAb resided on *O*-linked carbohydrate chains, the effect of inhibitors of extension of *O*-linked carbohydrate chains on mAb MY.1E12 binding was tested. Capan-1 cells in culture were treated with Bzl-GalNAc for 48 h, then the lysates of these cells were electrophoretically separated, transferred to PVDF membranes, and blotted with four anti-MUC1 mucin mAbs. Interestingly, the apparent molecular weight of MUC1 mucin became greater than that of MUC1 mucin of untreated cells, as shown by electrophoretic analysis (Fig. 4). Four mAbs, HMFG-1, HMFG-2, SM-3, and 115D8, bound to these modified mucin components, whereas mAb MY.1E12 did not bind to the low-mobility MUC1 mucin synthesized in the presence of Bzl-GalNAc. The reduced electrophoretic mobility is due to decreased sialylation of *O*-linked carbohydrate, resulting in reduced SDS-PAGE mobility, as previously shown for MUC1 mucin from HEp-2 cells.[34] The position after the electrophoretic separation of these products also corresponded to the position of a processing intermediate of MUC1 mucin as described by Hilkens and Buijs.[35] Two mAbs, HMFG-2 and SM-3, strongly reacted with modified MUC1 mucin, in agreement with their known binding to underglycosylated MUC1 mucin core peptides.[27, 28] In contrast, mAb 115D8 and mAb MY.1E12 either reacted weakly or not at all with the slowly migrating components produced in the presence of Bzl-GalNAc. These results suggest that mAb MY.1E12 recognizes *O*-linked carbohydrates chains having sialic acid at their nonreducing terminals.

Reactivity of mAb MY.1E12 with various mucin-like glycoproteins mAbs MY.1E12 was tested for reactivity with various purified mucins and mucin-like glycoproteins in ELISA. This mAb reacted strongly with MUC1 mucin purified from human urine. It did not show reactivity with OSM, BSM, PGM, human colonic sulfomucin, or human glycophorin A (Fig. 5).

Binding of mAb MY.1E12 to MUC1 mucin expressed in COS-1 cells To test whether the binding of mAb MY.1E12 was dependent on the presence of the MUC1 mucin core polypeptide, we transiently transfected COS-1 cells with MUC1 mucin cDNA. In Western blotting analysis, the transfected COS-1 cells apparently expressed mAb MY.1E12 epitope (Fig. 6, lane 8). Lysates of untransfected COS-1 cells or mock transfectant cells did not contain any component reactive with this mAb (lanes 6, 7). Lysates of the transfectant cells were also tested for the binding of another anti-MUC1 mucin mAb, HMFG-2. As can be seen in Fig. 6 (lane 4), this mAb bound to two components in the electrophoretically separated lysates of transfectant cells. From the migration positions, the bottom band was estimated to be a nonglycosylated precursor of MUC1 mucin. The upper band, having M_r 380,000, probably corresponded to the glyco-

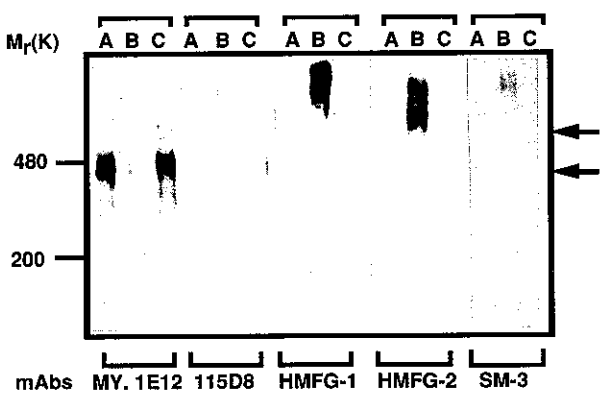

Fig. 4. Binding of mAbs to Capan-1 cell lysates prepared from untreated or Bzl-GalNAc treated cells. The Capan-1 cells were untreated (lane A), treated with Bzl-GalNAc (lane B), or treated with Bzl-GlcNAc as negative control (lane C) for 48 h. The cell lysates were subjected to SDS-polyacrylamide gel electrophoresis using 4% gel and transblotted onto PVDF membranes. The membranes were incubated with various anti-MUC1 mucin mAbs as described in "Materials and Methods." Arrows indicate the approximate positions of MUC1 mucin produced by untreated Capan-1 cells.

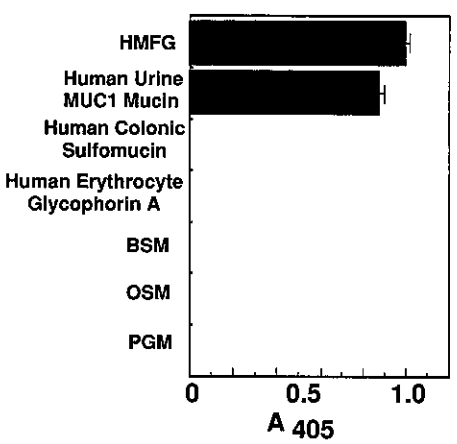

Fig. 5. Binding of mAb MY.1E12 to various mucin-like glycoproteins in ELISA. HMFG, human urine MUC1 mucin, human colonic sulfomucin, human erythrocyte glycophorin A, OSM, BSM, or PGM were used to coat microtiter plates as described in "Materials and Methods." Values are means ± SD as indicated by bars from triplicate experiments.

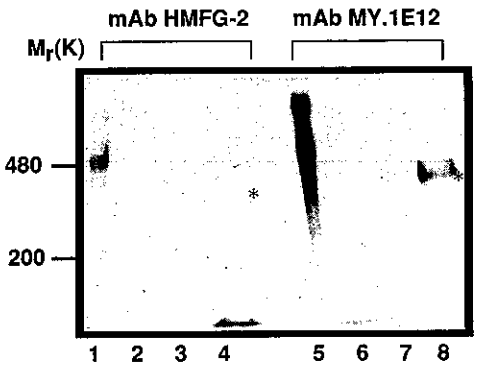

Fig. 6. Binding of mAbs HMFG-2 and MY.1E12 to electrophoretically separated lysates of COS-1 cells transfected with the MUC1 mucin cDNA. COS-1 cells were untreated (lane 2, 6), transfected with empty vectors (lane 3, 7), or transfected with MUC1 mucin cDNA (lane 4, 8). These cell lysates and lysates of Capan-1 cells (lane 1, 5) were subjected to SDS polyacrylamide gel electrophoresis using 4% gels and transblotted onto PVDF membranes. The PVDF membranes with transblotted samples were processed for the detection of MUC1 mucin. An M_r 380,000 component detectable with these two mAbs is indicated by an asterisk.

sylated MUC1 mucin. This M_r 380,000 component was the only band with which mAb MY.1E12 reacted. These results strongly suggested that this component was a highly sialylated and mature form of MUC1 mucin. After treatment of the PVDF membranes with mild acid to remove sialic acid from the mucins and other glycoproteins, mAb MY.1E12 did not react with this component (data not shown). Because the components involved in the biosynthesis of the carbohydrate chains in COS-1 cells are not likely to be modified by the transfection of MUC1 mucin core polypeptide cDNA, the mAb MY.1E12 reactivity probably requires the presence of MUC1 mucin core polypeptides translated from the transfected cDNA. These results strongly suggest that mAb MY.1E12 recognizes an epitope consisting of MUC1 mucin core peptide and sialylated *O*-linked carbohydrate chains.

Immunohistochemical localization of sialylated MUC1 mucin in sections of colorectal carcinoma tissues The immunohistochemical localization of MUC1 mucins expressed in colorectal carcinomas was investigated using mAb MY.1E12. Morphologically normal mucosa was present in the sections and a typical mAb MY.1E12 staining pattern is shown in Fig. 7. In this particular specimen, the antibody binding was not uniform among carcinoma cells with different morphology. This antibody bound more strongly to the poorly differentiated carcinoma cells than to moderately well-differentiated carcinoma cells forming glandular structures. Normal intestinal mucosas were not stained by mAb MY.1E12, consistent with previous reports showing that mAbs specific for glycosylated MUC1 mucins do not react with normal colonic mucosa.[22, 36, 37]

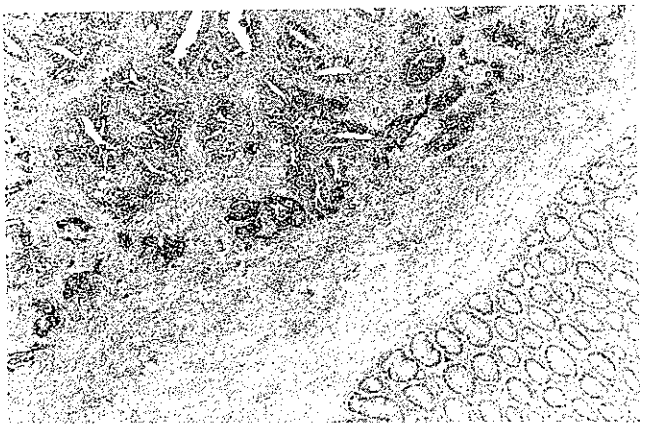

Fig. 7. Immunohistochemical staining of human colon carcinoma tissue sections with mAb MY.1E12. The sections were surgical specimens from a stage D patient with poorly differentiated colon carcinoma. Materials to which mAb MY.1E12 bound are seen at the luminal surfaces and in secretions.

DISCUSSION

A new mAb MY.1E12 reacting with MUC1 mucin containing sialylated *O*-linked oligosaccharides was produced. A mAb having such a specificity has not been previously reported. Either the sialylated oligosaccharides alone or the MUC1 mucin core polypeptide alone does not have affinity for this mAb. For example, mAb MY.1E12 did not react with ^{3}H-glucosamine-labeled oligosaccharides isolated from culture supernatants of Capan-1 cells incubated in the presence of Bzl-GalNAc. This mAb strongly reacted with MUC1 mucin produced by human laryngeal carcinoma cell line HEp-2 cells. Bardales and co-workers reported that the structures of the *O*-linked oligosaccharides of MUC1 mucins purified from HEp-2 cells resembled those of human erythrocyte glycophorin A and bovine fetuin.[38] However, since neither glycophorin A nor fetuin bind to mAb MY.1E12, the carbohydrate chains alone are insufficient to constitute the epitope.

It is not known whether this mAb directly recognizes the MUC1 mucin core peptide or whether the peptide backbone is necessary simply to present multiple carbohydrate chains as a single epitope. An alternative hypothesis is that the mAb MY.1E12 binds directly to both MUC1 mucin core protein and sialylated oligosaccharides. It is not likely that the interaction of a carboxyl

group of sialic acid with a positively charged amino group of the peptide backbone is necessary to formulate an epitope, because HMFG maintains its reactivity with mAb MY.1E12 after acetylation of the free amino group with acetic anhydride. Further studies are necessary to elucidate the minimum epitope for mAb MY.1E12.

This mAb should be useful to identify a unique subgroup of MUC1 mucin because sialic acid at the terminus of carbohydrate chains is known to influence the biological behavior of carcinoma cells. For example, the cells expressing sialomucin were shown to be less sensitive to cytolysis by human lymphokine-activated killer lymphocytes.[39–42] Recently Wesseling and co-workers showed that high levels of MUC1 mucin were able to reduce integrin-mediated extracellular matrix interaction.[23] Thus, MUC1 mucin might facilitate the invasion and dissemination of carcinoma cells. For these reasons, high levels of cell surface sialomucin might result in increased chances of survival and metastatic colonization of carcinoma cells.[42, 43]

Many experimental and clinical studies have demonstrated that carcinoma cells at advanced stages are different from cells at early stages in their expression of cell surface glycoproteins.[43, 44] The phenotypes associated with advanced carcinoma should therefore influence the biological behavior of metastatic carcinoma cells. Metastatic tumor cells are known to express a variety of phenotypes that collectively facilitate dissemination, invasion and survival at distant organ sites. The MUC1 mucin is likely to be one of these determinants that are characteristic of advanced carcinoma. These mechanisms are likely to be dependent on the sialylation of MUC1 mucin. It would be an interesting subject for future investigations to determine whether there is a strong correlation between malignant behavior of carcinoma cells and levels of sialylated MUC1 mucin as determined by the use of mAb MY.1E12.

ACKNOWLEDGMENTS

We thank Dr. Olja J. Finn, University of Pittsburgh for her gift of plasmid pDKOF containing MUC1 cDNA. We also thank Ms. Yoko Tsuchiya for her assistance in the preparation of this manuscript. This work was supported by Grants-in-Aid from the Ministry of Education, Science, and Culture of Japan (04454591, 05151018, 05152037, 05274101, 05557104, 05671813, 06282213) and the Ministry of Health and Welfare, as well as grants from the Japan Health Science Foundation, the Research Association for Biotechnology, the Terumo Foundation, the Uehara Foundation, and the Princess Takamatsu Foundation for Cancer Research.

(Received November 27, 1995/Accepted February 6, 1996)

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