Jpn. J. Cancer Res. **89**, 1267–1275, December 1998

Expression of Mucin-associated Sulfo-Le^a Carbohydrate Epitopes on Human Colon Carcinoma Cells

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The level of sulfo-Le^a (SO_3-3Galβ1-3(Fucα1-4)GlcNAc) epitope recognized by monoclonal antibody (mAb) 91.9H in hepatic metastasis of colon carcinoma is known to be lower than at the primary sites. We examined 19 human colon carcinoma cell lines for their production of this epitope. Sixteen cell lines were found to produce high M_r components that metabolically incorporated [^{35}S]sulfate and were resistant to heparitinase I and chondroitinase ABC, and 8 of them were reactive with mAb 91.9H as shown by western blotting analysis. These were all of the 4 cell lines derived from well differentiated primary tumors (HCCP-2998, LS174T, GEO, and CBS), 2 of 10 cell lines (DLD-1 and HCT116) from moderately to poorly differentiated primary tumors, and 2 of 5 cell lines (SW480 and HCC-M1544) from metastases. Incubation of LS174T cells with benzyl-*N*-acetyl-α-D-galactosaminide abrogated the incorporation of [^{35}S]sulfate and the reactivity of mAb 91.9H with high M_r components in the cell lysates. Sodium chlorate, which inhibits the formation of 3′-phosphoadenosine 5′-phosphosulfate, also inhibited the [^{35}S]sulfate incorporation and reactivity with mAb 91.9H. These treatments did not change the incorporation of [^{14}C]threonine into high M_r components. These results indicated that sulfo-Le^a epitopes were expressed on *O*-linked carbohydrate chains in sulfomucins. Immunohistochemical studies of tumor tissues in nude mice indicated that sulfo-Le^a was expressed at the site of orthotopic transplantation in the cecum. The expression appeared to be suppressed in liver metastatic foci in nude mice.

Key words: Colon carcinoma cell — Sulfo-Le^a — *O*-Linked sugar chain — Metastasis

Colonic epithelial cells are known to produce sulfated mucins that can be identified by high iron diamine histochemical staining.[1)] The presence of colonic sulfomucins was also revealed by metabolic incorporation of [^{35}S]sulfate. Similar sulfated high M_r components are found in colon carcinoma tissues to a much smaller extent.[2)] Synthesis of sulfomucins by metastatic cells was at a lower level than in tumors at primary sites. Filipe and co-workers[3, 4)] also reported that there is a decrease in sulfomucin production during colonic carcinogenesis. The molecular and cellular basis for such variation of sulfated products by colon carcinoma cells is not well understood.

We previously prepared mAb 91.9H using sulfomucin partially purified from normal colonic mucosa.[5)] This mAb reacted with high M_r components from extracts of normal mucosa, colorectal primary carcinoma, and metastasis, in that order of magnitude.[5–8)] Moreover, immunohistochemical studies using mAb 91.9H showed that expression of sulfomucins decreased with increasing depth of invasion of colon carcinomas.[9)] Vavasseur and co-workers[10)] reported that human colon carcinoma cells with high reactivity to 91.9H were less tumorigenic in nude mice than cells with low reactivity to 91.9H. The epitope structure for mAb 91.9H was shown to contain sulfated groups, at least in normal colonic mucins.[7)] Further investigation using oligosaccharides with defined structures indicated that the epitope structure for mAb 91.9H involves sulfo-Le^a carbohydrate chains.[11, 12)]

In the present study, at least 8 out of 19 human colon carcinoma cell lines were found to be reactive with mAb 91.9H specific for sulfo-Le^a in high M_r components. The LS174T cell line was chosen as a model to evaluate further the importance of *O*-linked carbohydrate chains as a backbone in the epitope for mAb 91.9H. The present paper shows that sulfo-Le^a antigen was expressed on the major high-molecular-weight sulfated glycoprotein produced by LS174T cells in a sulfated *O*-linked carbohydrate chain-specific manner. When transplanted into nude

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Abbreviations used: BSA, bovine serum albumin; Bzl-GalNAc, benzyl-*N*-acetyl-α-D-galactosaminide; DMEM/F12, 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium; EDTA, ethylenediaminetetraacetate; Le^a, Galβ1-3(Fucα1-4)GlcNAc; mAb, monoclonal antibody; M_r, relative molecular mass; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; SDS, sodium dodecyl sulfate; sulfo-Le^a, SO_3-3Galβ1-3(Fucα1-4)GlcNAc.

mice, these cells appeared to express the sulfo-Le^a antigen at the primary site, but the expression appeared to be suppressed in metastatic tumors in the liver.

MATERIALS AND METHODS

Cell lines The human colon carcinoma cell lines LS174T, HCT-15, SW480, SW620, DLD-1, LoVo, and HT-29 were obtained from the American Type Culture Collection (Rockville, MD). HCC-M1544, HCC-M1410,[13] MOSER, GEO, RKO, C, CBS,[14, 15] HCCP-2998, HCT116, KM12C, and Omega cells were kindly provided by Dr. Reuben Lotan (The University of Texas M. D. Anderson Cancer Center, Houston, TX). DiFi cells[16] were from Dr. Marsha Frazier (The University of Texas M. D. Anderson Cancer Center). All cells were grown in DMEM/F12 supplemented with 10% heat-inactivated fetal calf serum in a humidified atmosphere with 5% CO_2 at 37°C.

Metabolic labeling of colon carcinoma cells Radiolabeling of sulfated residues in macromolecules of cells was performed as described previously.[17] Subconfluent cells in 10-cm tissue culture dishes were incubated with [^{35}S]sulfate-labeling media, DMEM/F12 with nine-tenths concentration of free inorganic sulfate containing 50 μCi/ml [^{35}S]Na_2SO_4 (Carrier free; ARC, St. Louis, MO) for 48 h. For radiolabeling of polypeptide moieties of mucins and of macromolecules containing polypeptide portions, the subconfluent cells in 10-cm tissue culture dishes were incubated with 5 ml of [^{14}C]threonine-labeling media, DMEM/F12 containing 1 μCi/ml L-[^{14}C(U)]threonine (NEN Life Science Products, Boston, MA) for 48 h.

Preparation of lysates of cultured colon carcinoma cells Cells were rinsed with PBS and incubated with PBS containing 0.02% EDTA and 0.05% trypsin for 5 min. The cells were rinsed twice with PBS, then suspended in 4 volumes of 10 m*M* Tris-HCl buffer (pH 7.4) containing 0.5% Nonidet P-40, 1 m*M* EDTA, and 0.5 m*M* phenylmethylsulfonyl fluoride. After incubation on ice for 1 h, the solution was centrifuged at 15,000 rpm for 30 min and the supernatants were used as cell lysates. Protein concentration was assessed by using a BCA protein assay kit (Pierce, Rockford, IL). To remove sulfated glycosaminoglycans, [^{35}S]sulfate-labeled cell lysate corresponding to 36 μg protein was mixed with 0.003 U of heparitinase I (EC No. 4.2.2.7, Seikagaku Corp., Tokyo), and 0.03 U of chondroitinase ABC (EC No. 4.2.2.4, Seikagaku Corp.) in 20 m*M* sodium acetate, 2 m*M* calcium acetate, pH 7.4, and incubated at 37°C overnight.

Polyacrylamide gel electrophoresis and western blotting analysis The cell lysates were treated with 67.25 m*M* Tris-HCl buffer (pH 6.8) containing 1% SDS, 2.5% 2-mercaptoethanol, 5% glycerin, and 1 m*M* EDTA at 100°C for 5 min. In some cases, lysates were heated at 100°C for 5 min to remove proteins, then centrifuged at 15,000 rpm for 15 min, and the supernatants were used as samples for electrophoretic analysis. They were separated by PAGE on 4% gels in the presence of 0.1% SDS, according to the method of Laemmli.[18] Gels were dried on Whatman 3MM paper (Whatman International Ltd., England), and the locations of [^{35}S]SO_4-radiolabeled materials were identified by a BAS-2000 imaging analyzer (Fuji Film, Tokyo). For western blotting analysis, electrophoretically separated proteins were electroblotted onto a PVDF membrane (Immobilon-Psq Transfer Membrane, Millipore, Bedford, MA) using a Milli Blot-SDE system (Millipore). Membranes were blocked in PBS containing 3% BSA (Seikagaku Corp.) at 4°C 18 h, followed by incubation with ascites of mAb 91.9H diluted at 1:1000 in 50 m*M* citrate buffer (pH 5.5) containing 0.5% BSA and 0.05% Tween 20 at room temperature for 2 h with gentle shaking. After having been washed three times with 50 m*M* citrate buffer (pH 5.5) containing 0.1% Tween 20, each membrane was incubated at room temperature with horseradish peroxidase-conjugated goat anti-mouse IgG (Zymed, South San Francisco, CA) diluted at 1:1000 in 50 m*M* citrate buffer (pH 5.5) containing 0.05% Tween 20 for 1 h. Glycoproteins were visualized with ECL western blotting detection reagents (Amersham, Buckinghamshire, UK), and the locations of [^{35}S]SO_4-radiolabeled materials were identified by a BAS-2000 imaging analyzer. In some cases, membranes were incubated overnight in blocking solution from a Digoxigenin Detection Kit (Boehringer Mannheim Biochemica, Indianapolis, IN) at 4°C, followed by incubation with ascitic fluid containing mAb 91.9H diluted at 1:1000 in 50 m*M* citrate buffer (pH 5.5) containing 0.5% BSA and 0.1% Tween 20 at room temperature for 2 h with gentle shaking. The membranes were washed three times with 50 m*M* citrate buffer (pH 5.5) containing 0.1% Tween 20, then incubated at room temperature with anti-mouse Ig-digoxigenin, F(ab′)2-fragment (Boehringer Mannheim GmbH, Mannheim, Germany) diluted at 1:30 in 50 m*M* citrate buffer (pH 5.5) containing 0.1% Tween 20 for 1 h. Glycoproteins were visualized with a Digoxigenin Detection Kit (Boehringer Mannheim GmbH).

Treatments of cells with inhibitors of sulfation or extension of *O*-linked carbohydrate chains To block the sulfation of carbohydrate residues, 1×10^7 LS174T cells in a 100-cm dish were incubated overnight, then the medium was switched to 5 ml of [^{35}S]sulfate-labeling medium or [^{14}C]threonine-labeling medium containing 0 or 50 m*M* sodium chlorate and incubation was continued for another 48 h.[19] For the inhibition of *O*-glycosylation, the medium was changed to 5 ml of [^{35}S]sulfate-labeling medium or [^{14}C]threonine-labeling medium containing 4 m*M* Bzl-GalNAc (Sigma, St. Louis, MO) and incubation was continued for another 48 h. The total protein concentration of each lysate was adjusted to 9.5 mg/ml by using

a BCA protein assay kit. The lysates were heated at 100°C for 5 min to denature proteins, then centrifuged at 15,000 rpm for 15 min. Aliquots (38 μl) of the supernatants were loaded in each lane, separated by SDS-PAGE, and electroblotted onto a membrane, and the radioactivity levels associated with high M_r components were compared.

Immunohistochemical analysis of LS174T cells growing *in vivo* after orthotopic transplantation Cells were harvested by treatment with 0.02% EDTA and 0.05% trypsin. Viable single cells (1×10^6 cells in 50 μl of Hanks' solution) were injected into the subserosal space of the cecum of anesthetized athymic BALB/c nude mice (5 weeks old, male) (Japan SLC Inc., Shizuoka). Nine mice were used. Six weeks later, the mice were killed by administration of an overdose of pentobarbital. All had tumors at the site of injection and 4 had multiple liver metastases. Tumors at the site of transplantation (3 specimens) and those growing in the liver (4 specimens) were removed, fixed in 4% paraformaldehyde and processed for histochemical examinations. Four-micrometer sections were prepared and deparaffined sections were coated with PBS containing 2% normal goat serum and 3% BSA at room temperature for 10 min. The sections were then incubated with antibodies. Hybridoma culture supernatant was used for mAb 91.9H. mAb 7LE, obtained from Seikagaku Co., Ltd., was used at 10 μg/ml. As a control, mouse IgG was used at 10 μg/ml. Incubations were performed at room temperature for 40 min. Unbound antibodies were removed by washing 5 times with PBS for 3 min each. The sections were incubated with alkaline phos-

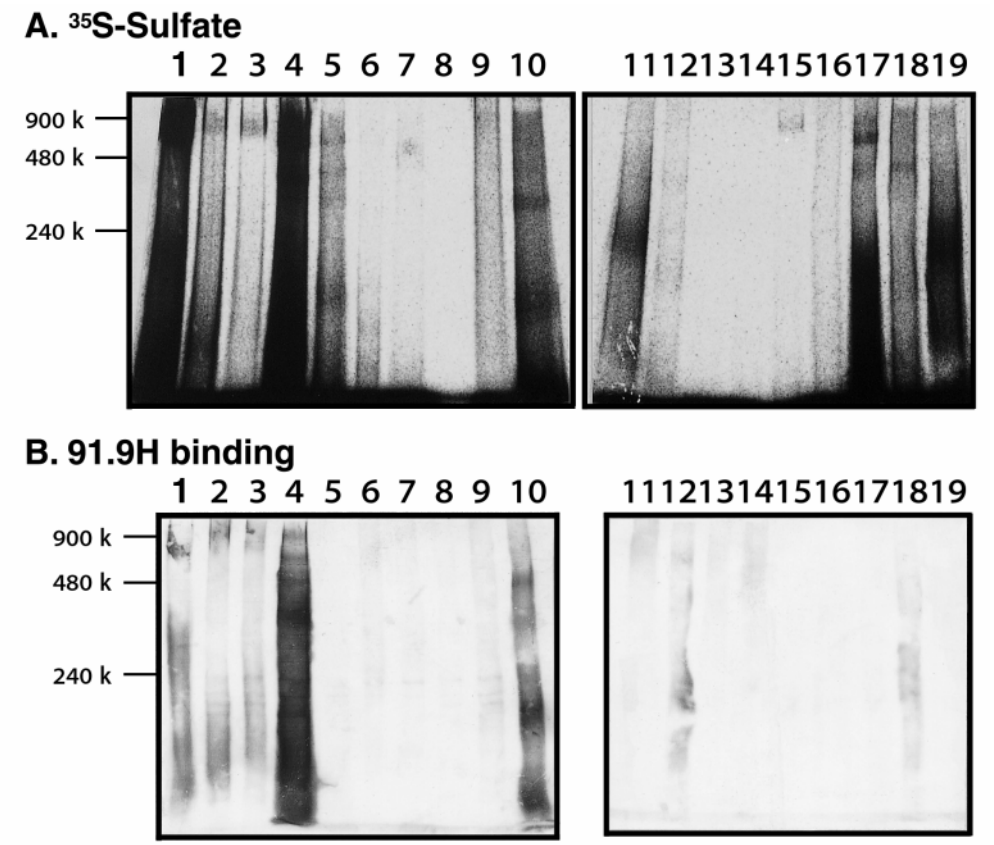

Fig. 1. (A) Electrophoretic profiles of metabolically [^{35}S]sulfate-labeled proteins and glycoproteins from 19 human colon carcinoma cell lines. [^{35}S]Sulfate-labeled cell lysates corresponding to 36 μg protein were treated with heparitinase I and chondroitinase ABC as described in "Materials and Methods," mixed with SDS-PAGE sample buffer with 2-mercaptoethanol, loaded on 4% gels and separated by electrophoresis. Radioactive components were detected as described in "Materials and Methods." Cell lines in lanes 1–19 are: 1, HCCP-2998; 2, LS174T; 3, GEO; 4, CBS; 5, HCT-15; 6, HT-29; 7, KM12C; 8, MOSER; 9, C; 10, DLD-1; 11, HCT116; 12, SW480; 13, RKO; 14, DiFi; 15, SW620; 16, LoVo; 17, HCC-M1410; 18, HCC-M1544; 19, Omega. (B) Western blotting analysis of electrophoretically separated lysates of the 19 human colon carcinoma cell lines. [^{35}S]Sulfate-labeled cell lysates of the 19 human colon carcinoma cell lines were separated by SDS-PAGE on 4% gels, electroblotted onto PVDF membranes, and reacted with mAb 91.9H. Equal amounts of protein (42 μg) were loaded per lane. Bound antibody was visualized by the use of F(ab′)2-fragments of anti-mouse Ig-digoxigenin, and a Digoxigenin Detection Kit.

phatase-labeled goat anti-mouse IgG (Zymed Lab., Inc., San Francisco, CA) at room temperature for 20 min, washed 5 times with PBS for 2 min each, and visualized with HistoMark Red alkaline phosphatase substrate kit (Nichirei, Tokyo). The relative staining intensity was evaluated in terms of the number of cells associated with alkaline phosphatase reaction products. When >10% of the cells reacted with an antibody, the section was judged as positive with the epitope. Counterstaining was performed with Mayer's hematoxylin. The high-iron diamine method was also applied according to the procedure of Spicer.[20)]

RESULTS

Sulfomucins and sulfo-Le^a epitopes produced by human colon carcinoma cell lines Nineteen human colon carcinoma cell lines were incubated with [^{35}S]sulfate for the metabolic labeling of sulfate groups in macromolecules. Subconfluent cells in 10-cm tissue culture dishes were incubated with 10 ml of [^{35}S]sulfate-labeling medium for 72 h. Cell lysates were separated by SDS-PAGE on 4% gels under reducing conditions and ^{35}S-labeled materials were identified (data not shown). All 19 cell lines incorporated [^{35}S]sulfate into relatively high M_r components (M_r>200,000). The cell lysates were extensively treated with heparitinase I and chondroitinase ABC, then separated by SDS-PAGE on 4% gels. Electrophoretic profiles of radiolabeled materials are shown in Fig. 1A. After treatment with a combination of heparitinase I and chondroitinase ABC, 16 out of 19 cell lines still contained high M_r sulfated components. They are HCCP-2998, LS174T, GEO, CBS, HCT-15, HT-29, KM12C, C, DLD-1, HCT116, SW480, SW620, LoVo, HCC-M1410, HCC-M1544, and Omega cells. These results indicated that sulfated macromolecules other than heparan sulfate or chondroitin sulfate proteoglycans, probably sulfomucins, were produced by these cells.

The cell lysates were separated by SDS-PAGE on 4% gels and binding of mAb 91.9H (specific for sulfo-Le^a carbohydrate chains) was examined by western blotting analysis. Among 16 cell lines which produced sulfated macromolecules resistant to heparitinase I and chondroitinase ABC, at least 8 cell lines, HCCP-2998, LS174T, GEO, CBS, DLD-1, HCT116, SW480, and HCC-M1544 produced the epitope for mAb 91.9H (Fig. 1B). Table I shows the histological types of carcinomas from which cell lines were derived.[21)] All 4 cell lines derived from well-differentiated tumor tissues, HCCP-2998, LS174T, GEO, and CBS, reacted strongly with mAb 91.9H. The apparent M_r of the major component reactive with this mAb varied among different cell lines. Electrophoretic migration of components having reactivity with mAb 91.9H also suggested that not all sulfated mucins contained sulfo-Le^a epitopes. A sulfated component having an apparent M_r 900,000 that was reactive with mAb 91.9H was observed with HCCP-2998, LS174T, GEO, and CBS cells. Diffuse multiple bands were also observed with M_r values ranging between 100,000 and 500,000.

Table I. Human Colon Carcinoma Cell Lines Used in This Study

Cell line	Derived from	
	Site of tumor	Histological grade[a)]
HCCP-2998	Primary	W
LS174T	Primary	W
GEO	Primary	W
CBS	Primary	W
HCT-15	Primary	W to M
HT-29	Primary	M
KM12C	Primary	M
MOSER	Primary	M
C	Primary	M
DLD-1	Primary	P to M
HCT116	Primary	P
SW480	Primary	P
RKO	Primary	P
Omega	Primary	P
DiFi	Peritoneal metastasis	
SW620	Lymph node metastasis	P
LoVo	Metastasis	M
HCC-M1410	Liver metastasis	
HCC-M1544	Liver metastasis	

a) W, well differentiated; M, moderately differentiated; and P, poorly differentiated.

Inhibition of biosynthesis of LS174T sulfomucins with inhibitors of sulfation or extension of *O*-linked carbohydrate chains of mucins A colon carcinoma cell line LS174T was used as a model to investigate the nature of the sulfo-Le^a antigen recognized by mAb 91.9H. Sodium chlorate is a known inhibitor of protein and carbohydrate sulfation.[19, 22)] Aryl-*N*-acetyl-galactosaminides were initially used as potential competitors of the extending glycosylation of GalNAc residues linked to the core protein in LS174T cell lines.[23)] Thus, we tested the effects of these compounds on mucin sulfation and biosynthesis of the mAb 91.9H epitope. As shown in Fig. 2A, both chlorate treatment and Bzl-GalNAc treatment significantly reduced the incorporation of [^{35}S]sulfate into high M_r glycoprotein. To examine whether these treatments influence the biosynthetic rate of the polypeptide portion of mucins by LS174T cells, cells were metabolically labeled with [^{14}C]threonine and treated with sodium chlorate or Bzl-GalNAc under the same conditions as described above. Fig. 2B shows the radioactivity associated with high M_r components electroblotted on the membrane. The results

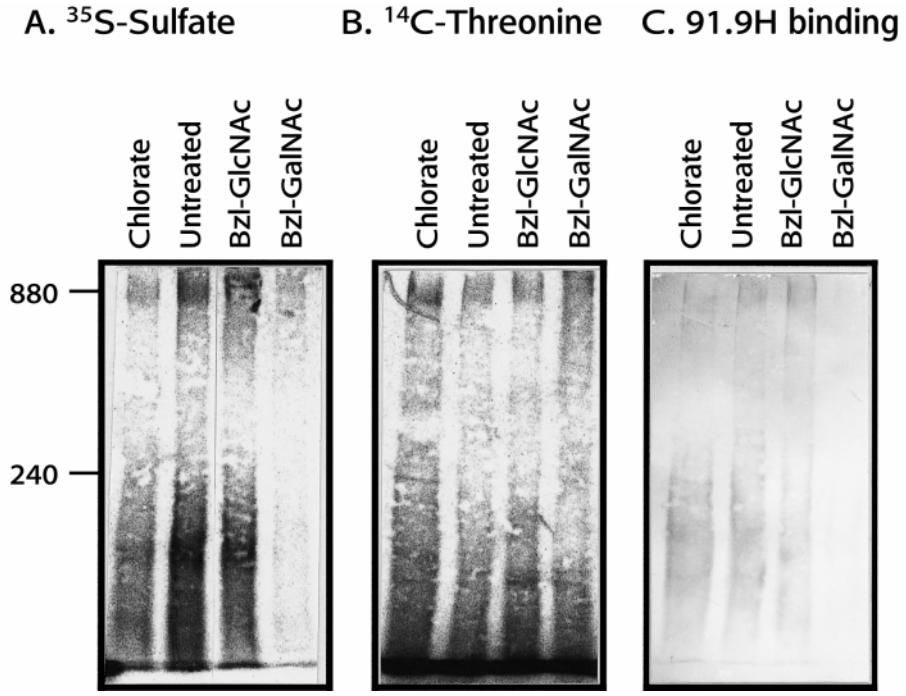

Fig. 2. (A) Electrophoretic profiles of lysates of [^{35}S]sulfate-labeled LS174T cells untreated or treated with chlorate or Bzl-GalNAc in culture. Subconfluent cells were incubated for 48 h in 5 ml of media containing 50 m*M* sodium chlorate or 4 m*M* Bzl-GalNAc or 4 m*M* benzyl-α-*N*-acetyl-D-glucosaminide (negative control for Bzl-GalNAc treatment) prior to the preparation of lysates. Equal amounts of aliquots of boiled lysates were loaded, separated by SDS-PAGE, and electroblotted onto PVDF membranes. Radioactivity associated with high M_r components was visualized by autoradiography. (B) Electrophoretic profiles of lysates of [^{14}C]threonine-labeled LS174T cells untreated or treated with chlorate or Bzl-GalNAc. Methods were the same as for (A), except that metabolic labeling was performed with [^{14}C]threonine instead of [^{35}S]sulfate. (C) mAb 91.9H binding to lysates of LS174T cells untreated or treated with chlorate or Bzl-GalNAc. Bindings of mAb 91.9H to [^{14}C]threonine-labeled LS174T cell lysates, as in panel (B), are shown.

indicated that these treatments did not reduce the incorporation of [^{14}C]threonine into high M_r components that migrated at approximately M_r 900,000. The incorporation seemed to increase slightly after chlorate or Bzl-GalNAc treatment. There was no significant difference apparent in the electrophoretic migration distance of this component as revealed by [^{14}C]threonine incorporation after chlorate treatment. After Bzl-GalNAc treatment, a diffuse band ranging between 400,000 and 900,000 was observed, possibly due to reduced size of the carbohydrate chains. To analyze whether any sulfate residue or *O*-linked carbohydrate chain is involved in the sulfo-Le[a] epitope recognized by mAb 91.9H, binding of this antibody with [^{14}C]threonine-labeled materials was tested on the same membrane, as shown in Fig. 2B. Fig. 2C illustrates the result of this western blotting analysis. The amount of mAb 91.9H bound to an area corresponding to the position of sulfomucins was significantly smaller when the cells were treated with sodium chlorate, indicating that biosynthesis of sulfo-Le[a] antigen is blocked by chlorate. A decrease in the amount of mAb 91.9H binding was also observed when LS174T cells were treated with Bzl-GalNAc. Because Bzl-GalNAc was shown to inhibit the extension of *O*-linked carbohydrate chains in LS174T cells,[23] these results strongly suggested that the sulfo-Le[a] epitope in high-molecular-weight glycoproteins is carried predominantly by *O*-linked carbohydrate chains in these cells. It is not known whether *O*-glycans predominantly carry sulfo-Le[a] epitopes in other colon carcinoma cell lines expressing this epitope.

Immunohistochemical analysis of the mAb 91.9H epitope in LS174T cells growing in nude mice LS174T cells were orthotopically transplanted into the subserosal space of the cecum in nude mice. All 9 mice used had tumors at the site of injection and 4 mice had liver metastases. Three primary tumors and 4 metastatic foci were examined to evaluate the immunohistochemical localization of binding sites for mAb 91.9H (Fig. 3). When tumors in the cecum were examined, binding of mAb 91.9H was detected only to LS174T human colon carcinoma cells. Epithelial cells of the mouse cecum were not stained. LS174T cells growing in the primary tissues

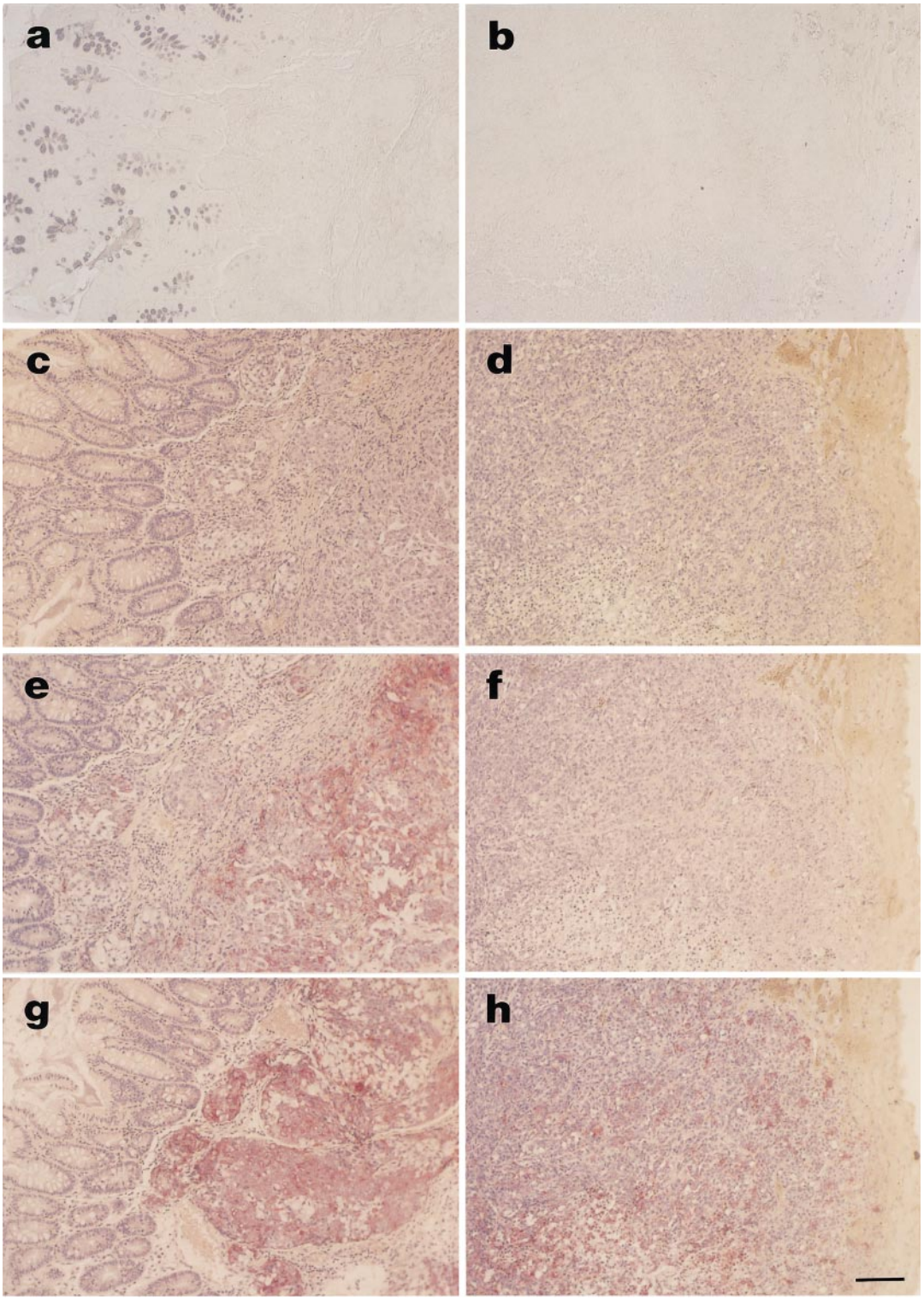

Fig. 3. Histochemical analysis of orthotopically transplanted LS174T cells growing at the primary site (a, c, e, and g) and in a liver metastasis (b, d, f, and h) in nude mice. High-iron diamine staining (a and b). Control staining with mouse IgG (c and d). Immunohistochemical staining with mAb 91.9H (anti-sulfo-Le^a: e and f). Immunohistochemical staining with mAb 7LE (anti-Le^a: g and h). The bar indicates 100 μm (applicable to all panels).

contained >10% cells that reacted with mAb 91.9H. However, not all of the LS174T cells reacted. Thus, the expression of sulfo-Le^a carbohydrate chains in LS174T cells growing *in vivo* seemed to be partially suppressed. In the liver metastases, carcinoma cells rarely reacted with mAb 91.9H (Fig. 3f). Interestingly, the nonsulfated counterpart of sulfo-Le^a, the Le^a epitope, revealed by mAb 7LE, was detected both at the primary tumors and in liver metastases (Fig. 3, g and h). These results suggested that sulfation of type 1 precursor carbohydrate chains was suppressed when LS174T human colon carcinoma cells were growing in the liver in nude mice. Such differences in the staining of primary tumors and metastases were also observed when DLD-1 cells were orthotopically transplanted, though the incidence of metastases was very low. However, the intensity of high iron diamine staining of LS174T cells growing at the primary site was very low and the level was similar to that in the metastatic foci (Fig. 3, a and b). Other carbohydrate epitopes that were decreased in the metastatic foci of LS174T cells are sialyl-Le^a (mAb CA19-9) and sialyl-Le^X (mAb KM93). Carbohydrate epitopes that were increased or unchanged in the metastatic foci are Le^b, Le^X and Le^Y (data not shown).

DISCUSSION

Recently, we have found using oligosaccharides and glycolipids with defined structures that the epitope of mAb 91.9H is sulfo-Le^a carbohydrate chains.[11, 12] However, the molecular basis for the decreased expression of this epitope in metastatic foci compared to the cells in primary sites was not previously known. When sulfate incorporation and mAb 91.9H reactivity to macromolecules of 19 human colon carcinoma cell lines were compared, 8 out of 16 cell lines, which incorporated sulfate groups into high M_r glycoproteins, produced components reactive with mAb 91.9H. The relative intensity of the binding did not correspond to the degree of sulfate incorporation, confirming that this antibody does not recognize all sulfated glycoproteins. Among cell lines derived from a moderately to poorly differentiated primary tumor, only DLD-1 cells produced sulfated high M_r components apparently reactive with mAb 91.9H. Few cell lines derived from poorly differentiated primary tumor or metastasis produced sulfated components. These components were only weakly reactive with mAb 91.9H, indicating that there is a weak but significant association between the sulfo-Le^a epitope and the status of differentiation of colon carcinoma cells.

In our previous work, cell surface expression of sialyl-Le^X and sialyl-Le^a antigens was examined in these 19 human colon carcinoma cell lines by flow-cytometric analysis.[24] Sialyl-Le^a was always detected on the cells expressing mAb 91.9H epitopes. Therefore, type 1 chains on mucin backbones seem to be converted into both sulfated and sialylated forms in these cells. Sulfated Le^X was recently reported to comprise the major carbohydrate chains in mucin glycoproteins of a variant of LS174T cells.[25] Thus, LS174T cells seem to have a capacity to synthesize sulfo-3Galβ1-4(Fucα1-3)GlcNAc and sulfo-3Galβ1-3(Fucα1-4)GlcNAc.

The core polypeptide of normal colonic sulfomucin was previously proposed to be encoded by the *MUC2* gene.[7] This hypothesis, however, might need to be reevaluated as far as colon carcinoma cell lines are concerned, because our studies on the mucin core peptide mRNA products detected by reverse transcription-polymerase chain reaction in 19 human colon carcinoma cell lines indicated that some cell lines expressing sulfomucin recognized by mAb 91.9H did not produce the MUC2 mucin core polypeptide.[24] Thus, a sulfo-Le^a epitope might be expressed on several different mucin core polypeptide backbones.

Immunohistochemical studies on LS174T cells transplanted into nude mice indicated that the sulfo-Le^a epitope recognized by mAb 91.9H was expressed at the site of orthotopic transplantation. The level of antibody binding was much lower in hepatic metastasis than in the tumor tissue at the site of initial transplantation in the cecum. These patterns are consistent with the antibody binding profiles observed with clinical materials.[5–9] We are currently conducting additional studies to clarify whether the downregulation of the sulfo-Le^a epitopes in LS174T tumors growing in the liver of recipient mice was due to the selective growth of sulfo-Le^a-negative populations. Alternatively, the expression of sulfo-Le^a could be downregulated through a paracrine mechanism in the liver. It would be of particular interest to see if the same phenomenon is observed with SW480 and HCC-M1544 cells, because they were derived from the metastatic region of colon carcinomas. Down-regulation of carbohydrate epitopes by microenvironmental factors in the liver was previously observed with the sialyl Le^X carbohydrate antigens expressed by HT-29 human colon carcinoma cells.[26]

Changes in sulfomucins induced by nonmalignant diseases such as cystic fibrosis and ulcerative colitis have also been documented.[27–29] Their causal relationship to the pathogenesis of these diseases has not been established. Partial carbohydrate structures of sulfomucins were previously reported in tracheobronchial mucins or respiratory mucins from cystic fibrosis patients.[30–34] It is not known whether these sulfomucins contain sulfo-Le^a epitopes.

The biological functions of sulfo-Le^a carbohydrate chains in relation to the behavior of colon carcinoma cells are not known, though some reports have indicated that sulfo-Le^a and other sulfated chains are directly recognized by L-selectin, E-selectin, or P-selectin.[35–39] Therefore, adhesive behavior of cells expressing sulfo-Le^a carbohydrate chains recognized by mAb 91.9H will be an interesting subject for further investigations.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan (05274101, 05557104, and 07407063), the Ministry of Health and Welfare, the Japan Health Science Foundation, the Research Association for Biotechnology, PROBRAIN and New Energy Development Organization. Hitomi Tsuiji is the recipient of a Hayashi Memorial Foundation Fellowship for Female Natural Scientists. We thank Ms. Chizu Hiraiwa for her assistance in preparing this manuscript.

(Received June 17, 1998/Revised September 14, 1998/Accepted September 21, 1998)

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