[CANCER RESEARCH 48, 2214–2220, April 15, 1988]

Preparation and Characterization of Monoclonal Antibodies Directed to the Tumor-associated O-linked Sialosyl-2→6 α-*N*-Acetylgalactosaminyl (Sialosyl-Tn) Epitope[1]

Thomas Kjeldsen,[2] Henrik Clausen,[3] Setsuo Hirohashi, Tomoya Ogawa, Hiroyuki Iijima, and Sen-itiroh Hakomori[4]

The Biomembrane Institute, and Department of Pathobiology, University of Washington, Seattle, Washington 98119 [T. K., H. C., S. Hakomori]; National Cancer Center Research Institute, Tsukiji, Tokyo, Japan [S. Hirohashi]; and Institute for Chemistry and Physics (Riken), Wako, Saitama, Japan [H. I., T. O.]

ABSTRACT

Two monoclonal antibodies, TKH1 and TKH2, directed toward the sialosyl-Tn structure (NeuAcα2→6GalNAcα1→O-Ser or Thr), which display a remarkable immunohistological tumor specificity, were generated by immunization with ovine submaxillary mucin. The reactivity of these antibodies was monitored by solid phase enzyme-linked immunosorbent assay with different native and glycosidase-treated mucins and glycoproteins. Binding of the antibody to ovine submaxillary mucin glycoprotein was strongly inhibited by the O-linked disaccharide NeuAcα2→6GalNAcα1→O-serine, less strongly by NeuAcα2→6GalNAcβ1→O-propyl, and weakly by the monosaccharide GalNac. The reactivity was compared with previously established anti-Tn antibodies B72.3, NCC-Lu-35, and NCC-Lu-81. The antibody B72.3 was prepared previously after immunization with metastatic breast adenocarcinoma and its epitope was claimed to be GalNAcα1→O-Ser (or - Thr) by Springer and associates [Springer, G. F., *et al. In:* T. Dao, *et al.* (eds.), Tumor Markers and Their Significance in the Management of Breast Cancer, pp. 47–70. New York: A. R. Liss, 1986]. The antibody was found to show very similar reactivity as that of TKH1/TKH2, and its reactivity to ovine submaxillary mucin was inhibited specifically by NeuAcα2→6GalNAcα1→O-serine, indicating that the antibody is clearly directed to sialosyl-Tn antigen. Immunohistological study of the distribution of this antigen in various normal human tissues and carcinomas by TKH1/TKH2 antibodies, as well as B72.3 and monoclonal antibodies NCC-Lu-35/81, which are directed to GalNAcα1→O-Ser or Thr (Tn), was performed. The sialosyl-Tn antigen was not found in normal tissue except for a weak expression in Leydig cells of the testis, goblet cells of the colon, and parietal cells of the stomach. In contrast, the sialosyl-Tn antigen was strongly expressed in a large number of adenocarcinomas. As expected from the specificity studies, B72.3 shows the same reactivity as TKH1 and TKH2. Thus, both sialosyl-Tn (NeuAcα2→6GalNAcα1→O-Ser/Thr) and Tn (GalNAcα1→O-Ser/Thr) are good tumor markers, and combined use of antibodies directed to these structures might be useful in the screening and classification of cancer.

INTRODUCTION

Oncogenic transformation is often associated with changes in glycosylation in either glycolipids or glycoproteins in cell membranes (1, 2). Incomplete synthesis associated with precursor accumulation is frequently observed, and one of the clearest examples of this phenomenon is found in O-linked (mucin type) glycosylation in which the core structure, T (Galβ1→3GalNAcα→Ser/Thr) or Tn (GalNAcα1→Ser/Thr) (see Table 1), is exposed probably due to incomplete synthesis. These structures are normally cryptic in mucin of human tissue and secretions due to sialylation and/or chain elongation and branching by addition of other sugar residues.

The Tn antigen has been regarded as an A-like antigen since Dahr *et al.* (3) detected this structure by various lectins that agglutinate A-erythrocytes after sialidase treatment of erythrocytes. Subsequently, Springer *et al.* (4) recognized this structure as a tumor-associated antigen. Recently, this concept has been supported by studies of monoclonal antibodies obtained by immunizing with a lung carcinoma cell line that cross-reacts with blood group A-antigens and have been identified as being directed to the Tn antigen (5).

Biosynthetic studies of mucin-type carbohydrate chains have revealed that the β-galactosyltransferase adding the galactosyl residue to the GalNAcα1→Ser/Thr (Tn) to make the T-antigen does not catalyze the addition of Gal to GalNAc if α2→6 sialylation of the GalNAc residue has preceded it (6). Changes in the glycosylation pattern of the O-linked carbohydrate core (T/Tn) may not only be a result of incomplete synthesis, but could also be caused by changes in sialylation pattern, where accumulation of the sialosyl-Tn structure is accomplished by premature sialylation (7).

Recently, two monoclonal antibodies, B72.3 (8–11) and MSL102 (12, 19), have been established after immunization with human metastatic breast cancer and a colonic cancer cell line, respectively, which show highly specific reactivity with various human cancers and restricted reactivity with normal tissue. In previous studies, the antigenicity defined by B72.3 antibody was shown to be sensitive to sialidase (11), whereas Springer *et al.* (13) showed that B72.3 antibody agglutinates Tn erythrocytes and assumed it to be directed to Tn antigen. Both B72.3 and MSL102 seem to be directed to the sialosyl-Tn epitope using solid-phase binding assays and inhibition of antibody binding by synthetic authentic oligosaccharide conjugates.

The aim of the present study was to develop monoclonal antibodies defining the sialosyl-Tn epitope utilizing mucin glycoprotein enriched in sialosyl-Tn as an immunogen, to determine the cross-reactivity with Tn, and to study tissue distribution and tumor specificity of the sialosyl-Tn epitope. Further characterization of the nature of the antibody B72.3 was also performed.

MATERIALS AND METHODS

OSM[5] was isolated from ovine submaxillary glands as described previously (14). BSM and glycophorin A were purchased from Sigma Chemical Co. (St. Louis, MO). BALB/c mice were immunized with OSM coated on heat-inactivated *Salmonella minnesota*. Approximately 90% of carbohydrate chains on OSM and 50% on BSM consist of

Received 9/14/87; revised 1/12/88; accepted 1/22/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by a research grant from the National Cancer Institute (OIG CA42505).

[2] Supported by a fellowship from the Danish Medical Research Council and Hjort's Legat, Denmark.

[3] Supported by Ingeborg og Leo Dannin Fonden, Vera og Carl Johan Michaelsons Legat, and Arkitekt Holger Hjortenberg og Hustru, Dagmar Hjortenbergs fund, Denmark.

[4] To whom requests for reprint should be addressed, at The Biomembrane Institute, 201 Elliott Ave. W., Seattle, WA 98119.

[5] The abbreviations used are: OSM, ovine submaxillary mucin; BSM, bovine submaxillary mucin; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline.

Table 1 *Structures of carbohydrate epitopes and specificity of monoclonal antibodies*

Antigen	Structure	Monoclonal antibodies					
		TKH1	TKH2	B72.3	Lu35/81[a]	CA3239	HH8
Tn	GalNAcα1→O	−	−	−	+	+	−
Sialosyl-Tn	NeuAcα2→6GalNAcα1→O	+	+	+	+	−	−
T	Galβ1→3GalNAcα1→O	−	−	−	−	−	+

[a] Specificity of NCC-Lu-35/81, previously identified as anti-Tn (5), showed a strong binding with native OSM as well as sialidase-treated OSM. In contrast, CA3239 reacted with sialidase-treated OSM but not native OSM. It is judged, therefore, that NCC-Lu-35/81 antibodies may recognize both Tn and sialosyl Tn. This tentative assumption must be confirmed by inhibition studies with oligosaccharides.

sialosyl-Tn (15, 16). Spleen cells of immunized mice were fused with SP-2 myeloma cells, and hybridomas were screened for reactivity with OSM and neuraminidase-treated OSM, BSM, and glycophorin A. The B72.3 hybridoma was obtained from the American Type Culture Collection and grown in RPMI 1640 + 10% fetal calf serum (9). The monoclonal antibody CA3239 (anti-Tn)[6] was used as ascites fluid and HH8 (anti-T), as culture supernatant (17).

For direct ELISA, antigens were coated on Falcon 3915 ELISA plates (Beckton-Dickinson, Lincoln Park, NJ) by incubation of antigen solution in PBS for 1 h at room temperature. After three successive washes with PBS, additional protein-binding sites were blocked with 1% bovine serum albumin in PBS for 1 h. Mucins and glycoproteins were desialylated by treatment with 0.1 units/ml of neuraminidase from *Clostridium perfringens* type X (Sigma). Wells were incubated with hybridoma supernatant for 1 h. Plates were washed 3 times with PBS, followed by incubation with peroxidase-conjugated goat anti-mouse antibodies (Boehringer Mannheim Biochemicals). After three washes, wells were incubated with enzyme substrate (0.4 mg/ml *O*-phenylenediamine; 0.05% H_2O_2 in 50 mM citric acid buffer). The enzyme reaction was terminated by addition of 2 N H_2SO_4.

The reactivity of the monoclonal antibodies was inhibited with both monosaccharides and disaccharides. The following monosaccharides were used: D-*N*-acetylgalactosamine, D-*N*-acetylglucosamine, D-galactose, D-glucose, and L-fucose (Sigma). They were double diluted in PBS from a starting concentration of 500 mM. After 1 h preincubation of monosaccharides and monoclonal antibodies, the reaction mixture (50 μl) was transferred to ELISA plates coated with OSM (0.5 μg/ml) and incubated for 1 h. Thereafter, the procedure was as described for direct ELISA. Two oligosaccharide conjugates, NeuAcα2→6GalNAcα1→O-Ser and NeuAcα2→6GalNAcβ1→O-propyl, were chemically synthesized. Details of this chemical synthesis will be published elsewhere.[7] Inhibition with the oligosaccharides NeuAcα2→6GalNAcα1→O-Ser, NeuAcα2→6GalNAcβ1→O-propyl, and lactose was performed in the same manner as with the monosaccharides except that the reaction volume was 10 μl, OSM at 0.1 μg/ml, and the oligosaccharide concentration was 7 mM. For inhibition assays, TKH1 and B72.3 supernatants were diluted 1/40, and TKH2 was diluted 1/300 in PBS containing 1% bovine serum albumin.

Immunohistology was performed as previously described using a complex of avidin and biotinyl-peroxidase (5). Briefly, specimens were fixed in formalin and embedded in paraffin for detection of antigens. Monoclonal antibodies were applied to deparaffinized tissue sections and incubated overnight at 4°C. Detection of bound monoclonal antibodies was performed with biotinyl-anti-mouse IgG + IgM and biotinyl-peroxidase complex (vectastain ABC kit; Vector Laboratories, Inc.). Enzyme activity was demonstrated by 0.05 M Tris containing 0.02% deaminobenzidine tetrahydrochloride and 0.005% hydrogen peroxide.

RESULTS

Establishment of TKH1 and TKH2. Immunization of mice with OSM containing a high concentration of sialosyl-Tn followed by selection of hybridoma by reactivity with native OSM and sialidase-treated or sialidase-galactosidase-treated OSM, BSM, and glycophorin A resulted in establishment of two hybridomas, TKH1 and TKH2, producing IgM and IgG antibodies, respectively. The antibodies were characterized by preferential reactivity with native OSM, and the reactivity of both antibodies was greatly reduced after sialidase treatment (see Fig. 1). Periodate oxidation but not treatment with α-L-fucosidase and α-*N*-acetylgalactosaminidase of OSM abolished the reactivity with TKH1. A similar preferential reactivity with native OSM was observed with another antibody directed to a tumor-associated antigen, B72.3, which was originally obtained after immunization with breast carcinoma cells (8–11). Although the antibody has previously been designated as an anti-Tn antibody (13), its reactivity strongly suggested that it is directed to sialosyl-Tn. Native OSM showed a very weak reactivity with anti-Tn (CA3239); however, the reactivity was greatly enhanced after sialidase treatment. No reactivity was observed with HH8 before or after treatment with sialidase (see Fig. 2). These reactivities indicate that OSM does not express T- or Tn antigen (or does so very weakly) but strongly expresses sialosyl-Tn.

Specificity of TKH1 Antibody in Comparison with B72.3 Antibody. In order to establish the specificity of TKH1 antibody in comparison with B72.3, the reactivities of the antibodies with OSM after various enzymatic degradations were compared using anti-Tn (CA3239) and anti-T (HH8) antibodies as mon-

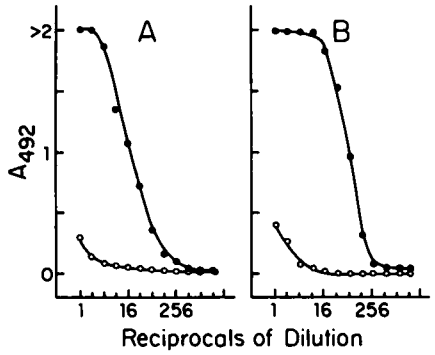

Fig. 1. Reactivity of monoclonal antibody TKH1 to solid-phase OSM (1.0 μg/ml) as described in "Materials and Methods." *A*, reactivity of TKH1 with OSM (1.0 μg/ml) at various concentrations of TKH1 supernatant. *B*, reactivity of TKH1 with various concentrations of OSM; beginning concentration, 5.0 μg/ml. Reactivity with native OSM (●) and with neuraminidase-treated OSM (○).

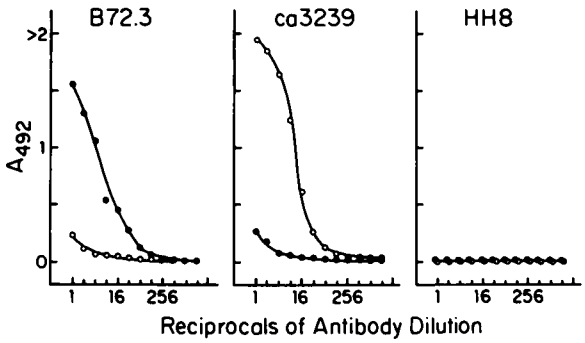

Fig. 2. Reactivity of B72.3, CA 3239, and HH8 with OSM (1.0 μg/ml) absorbed onto the solid phase of ELISA plates. Reaction with native OSM (●) and with sialidase-treated OSM (○).

[6] Springer, G. F., unpublished data.
[7] Iijima, H., and Ogawa, T., unpublished data.

itors. Both TKH1 and B72.3 react strongly with OSM, and the reactivity was abolished by sialidase treatment, whereas reciprocal reactivity was demonstrated with anti-Tn, *i.e.*, OSM showed anti-Tn reactivity only after sialidase treatment (Fig. 2). The reactivity of B72.3 with OSM can only be inhibited by TKH1 and not by anti-Tn (CA3239) or anti-T (HH8) antibodies (see Fig. 3). Neither TKH1, B72.3, nor anti-Tn (CA3239) reacted with glycophorin A or its desialylated derivative, whereas HH8 showed strong reactivity with desialylated glycophorin A, indicating exposure of T-antigen but not Tn or sialosyl-Tn in glycophorin A after desialylation (see Fig. 4). TKH2 were also unreactive with native and desialylated glycophorin A.

Inhibition of Reactivity of TKH1, TKH2, and B72.3 Antibodies by Mono- and Disaccharides. Various monosaccharides and a few disaccharides have been tested for inhibition of TKH1, TKH2, and B72.3 antibodies binding to solid-phase OSM and BSM. Only the monosaccharide GalNAc was able to inhibit the binding of these antibodies to OSM and BSM, whereas other monosaccharides, L-fucose, D-galactose, D-glucose, and D-*N*-acetylglucosamine, were unable to inhibit the reactivity (see Figs. 5–7).[8] The disaccharides NeuAcα2→6GalNAcα1→O-serine and NeuAcα2→6GalNAcβ1→O-propyl also inhibited the reactivity of TKH1, TKH2, and B72.3, whereas lactose was not inhibitory (see Figs. 5–7).

Specificity of Antibody Binding to Various Tissues by Immunohistology. A large number of human cancers have been screened with TKH1 and TKH2 in comparison with B72.3 and anti-Tn NCC-Lu-35 and -81. As shown in Table 2, the antibodies showed a remarkable specificity to a large variety of human carcinomas, particularly adenocarcinomas of gastric and colonic origin. In contrast, normal tissues showed very little, if any, positive reactivity with the antibodies, although a few locations such as goblet cells of intestinal epithelia, which also were positive with B72.3 and anti-Tn antibodies, showed a weak reactivity (Table 3). This staining pattern is, in general, similar to that of anti-Tn antibody, as previously described (5). A typical example of positive staining in tumors and negative staining in surrounding apparently normal tissue with TKH1 and TKH2 is shown in Figs. 8 and 9, respectively.

It is surprising that TKH1 reacted only with 1 of 8 pancreas tumors whereas TKH2, B72.3, and NCC-Lu-35 reacted with all 8 tested pancreas tumors. The reason for this difference in reaction pattern with pancreas tumors is unknown but could be related to different affinity (or susceptibility to steric hindrance).

DISCUSSION

The Galβ1→3GalNAcα (T) and GalNAcα (Tn) antigens have been widely recognized as tumor-associated antigens in human carcinomas (18), and their expression in normal adult tissues is highly restricted. The T/Tn structures constitute the core of O-linked carbohydrate chains α-glycosidically linked to serine or threonine in polypeptide chains. These core structures are generally sialylated, as on human glycophorin A [NeuAcα2→3Galβ1→3(NeuAcα2→6)GalNAcα1→Ser/Thr], or masked by addition of other sugar residues, producing, for example, the ABH type 3 chain antigens (H type 3: Fucα1→2Galβ1→3GalNAcα1→Ser/Thr). Interestingly, it appears that the O-

[8] NeuAc could inhibit binding reactivities of TKH1, TKH2, B72.3, and other unrelated antibodies such as anti-Le^a (SH1) and anti-T (HH8) with their respective antigens under similar conditions. Therefore, inhibition of monoclonal antibody activity by NeuAc is most likely due to a nonspecific effect when used in concentrations necessary to inhibit solid-phase reactivity.

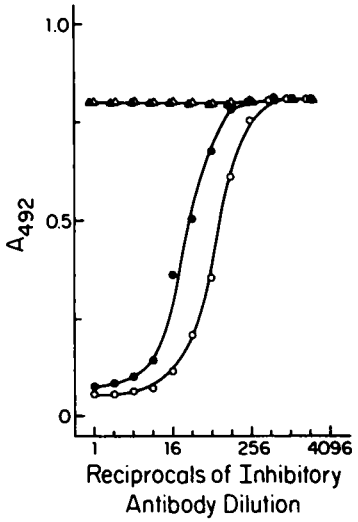

Fig. 3. Inhibition of the reactivity of peroxidase-conjugated B72.3 with OSM (1.0 μg/ml) by different monoclonal antibodies: B72.3 (○), TKH1 (●), CA 3239 (▲), and HH8 (△). Inhibition was determined by solid-phase ELISA as described in "Materials and Methods." Peroxidase-conjugated B72.3 was used at 2.0 μg/ml; the inhibiting antibodies B72.3, TKH1, HH8 were used as supernatants, and CA 3239 was used as ascites at an initial concentration of 1/500.

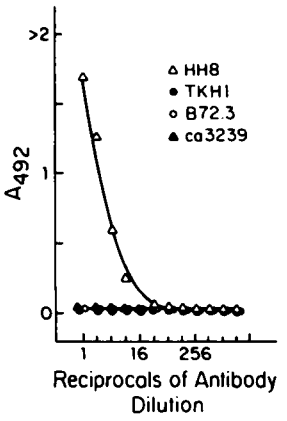

Fig. 4. Reactivity of the antibodies HH8, TKH1, B72.3, and CA 3239 with sialidase-treated glycophorin A (5.0 μg/ml) using solid-phase ELISA, as described in "Materials and Methods."

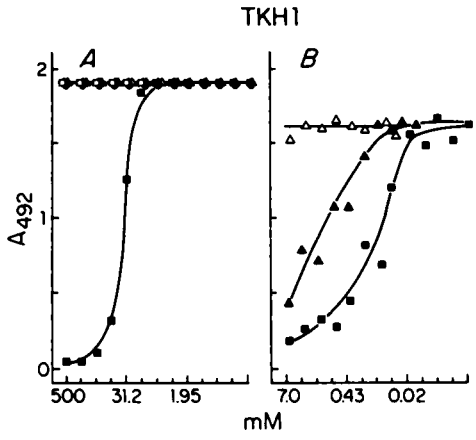

Fig. 5. Inhibition of TKH1 binding activity to OSM with different saccharides. *A*, inhibition of TKH1 binding with the monosaccharides fucose (□), galactose (○), glucose (▲), *N*-acetylglucosamine (△), and *N*-acetylgalactosamine (■). All monosaccharides were used at an initial concentration of 500 mM. *B*, inhibition of TKH1 with the disaccharides NeuAcα2→6GalNAcα1→O-serine (■), NeuAcα2→6GalNAcβ1→O-propyl (▲), and lactose (△). Inhibition was determined by solid-phase ELISA using TKH1 supernatant diluted 1/40 and OSM at 0.1 μg/ml.

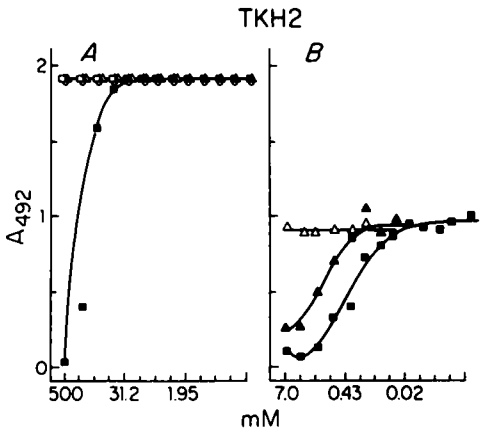

Fig. 6. Inhibition of TKH2 (1/300) reactivity to OSM by different saccharides. *A*, inhibition of TKH2 binding with the monosaccharides fucose (□), galactose (○), glucose (▲), *N*-acetylglucosamine (△), and *N*-acetylgalactosamine (■). *B*, inhibition of TKH2 reactivity by the disaccharides NeuAcα2→6GalNAcα1→O-serine (■), NeuAcα2→6GalNAcβ1→O-propyl (▲), and lactose (△). Conditions were as described in "Materials and Methods" and for Fig. 5.

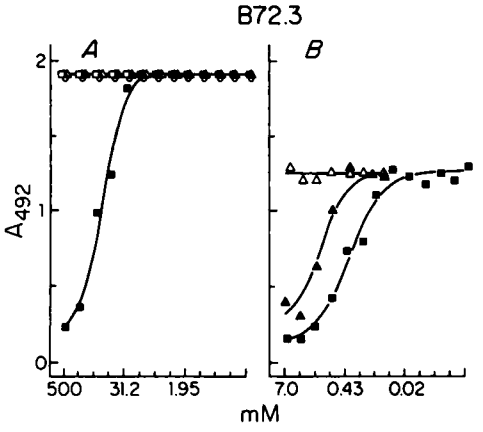

Fig. 7. Inhibition of B72.3 (1/40) binding to OSM by mono- and disaccharides. *A*, inhibition of B72.3 reactivity with the monosaccharides fucose (□), galactose (○), glucose (▲), *N*-acetylglucosamine (△), and *N*-acetylgalactosamine (■). *B*, inhibition of B72.3 reactivity with the disaccharides NeuAcα2→6GalNAcα1→O-serine (■), NeuAcα2→6GalNAcβ1→O-propyl (▲), and lactose (△). Conditions were as described in "Materials and Methods" and as for Figs. 5 and 6.

Table 2 *Immunohistochemical reactivity of monoclonal antibodies TKH1, TKH2, B72.3, NCC-Lu-35, and NCC-Lu-81 with tissue sections from various human cancers*

Cancer type	TKH1	TKH2	B72.3	NCC-Lu-35	NCC-Lu-81
Lung	2/9	3/9	3/9	3/9	2/9
Liver	0/10	0/10	0/10		0/10
Stomach	7/10	10/10	10/10		10/10
Colon	8/10	10/10	10/10		8/10
Breast	1/2	2/2	1/2	1/2	1/2
Pancreas	1/8	8/8	8/8	8/8	7/7

linked core in normal human tissue contains mostly T-structure and not Tn. The T-structure can be demasked by desialylation in many normal tissues, but only minute amounts of Tn can be demasked. Thus, expression of sialosyl-Tn, common in tumor cells due to incomplete synthesis and/or premature α2→6 sialylation, is highly restricted in normal tissue in man.

Based on the dominant occurrence of sialosyl-Tn in OSM (15), two monoclonal antibodies, TKH1 and TKH2, were generated by immunizing mice with OSM rather than tumor cell membranes or derived product (8–12). Specificities of both antibodies are well defined by antibody binding to solid-phase OSM and by their sensitivity to inhibition by NeuAcα2→6GalNAcα1→O-Ser. The antibody binding activity was inhibited to a lesser extent by NeuAcα2→6GalNAcβ1→O-propyl.

Table 3 *Immunohistochemical reactivity of monoclonal antibodies with TKH1, TKH2, B72.3, NCC-Lu-35, and NCC-Lu-81 with normal tissues*

Tissue	TKH1	TKH2	B72.3	NCC-Lu-35	NCC-Lu-81
Spinal cord	–	–	–	–	–
Adrenal	–	–	–	–	–
Kidney	–	–	–	–	–
Testis	–	+[a]	+[a]	–	–
Salivary gland	–	–	–	+	–
Pancreas	–	–	–	+	–
Heart	–	–	–	–	–
Liver	–	–	–	–	–
Spleen	–	–	–	–	–
Prostate	–	–	–	–	–
Lung	–	–	–	–	–
Thyroid	–	–	–	–	–
Gall bladder	–	–		–	–
Endothelium	–	+	+	–	–
Colon	–	+[b]	+[b]	+[b]	–
Stomach	–	+[c]	+[c]	–	–

[a] Leydig cells.
[b] Goblet cells.
[c] Parietal cells.

The antibodies may recognize α-anomeric conformation of the disaccharide or may recognize the structure including the serine residue. The antigen bearing the B72.3 epitope has been shown to be a high molecular weight mucin-like material, and the reactivity of B72.3 is sialidase sensitive (11), although B72.3 has previously been reported by Springer and associates to be directed toward the Tn antigen (13). The results of the present study using solid-phase ELISA indicate that B72.3, in contrast to other anti-Tn antibodies, reacts with native OSM and not with sialidase-treated OSM, and that the reactivity of TKH1 with OSM was only inhibited by B72.3 but not by anti-Tn antibodies. These results clearly indicate that the epitope defined by B72.3 must be similar to that defined by TKH1 and TKH2, *i.e.*, sialosyl-Tn.

Antibodies NCC-Lu-35 and -81 were identified as being directed to Tn (5). These antibodies, however, react with native as well as sialidase-treated OSM. The reason for this reaction pattern is unknown at present, but it is suggested that NCC-Lu-35 and -81 may recognize a certain structure mainly limited to the α-GalNAc residue and only influenced to a minor degree by sialosyl-α2→6 substitution of the GalNAc. This is in contrast to the antibody CA3239, which binds to OSM only after sialidase treatment, *i.e.*, CA3239 may react only with α-GalNAc residue without influence by sialosyl substitution.

The results of immunohistological screening of various normal and tumor tissues clearly indicate that the presence of the antigen defined by TKH1 and TKH2 in normal tissue is highly restricted to a few locations, such as goblet cells of intestinal epithelia, which nonetheless showed very weak staining. In contrast, a high incidence of intense staining was observed in adenocarcinomas of stomach and colon. A close comparison of staining of tissues with TKH1, TKH2, B72.3, and NCC-Lu-35 and -81 indicate that expression of all of these antigens in tissues is closely related. The intensity and incidence of staining in tumors was higher with TKH2, B72.3, and NCC-Lu-35; however, TKH1 showed a highly restricted staining of normal tissue as compared with other anti-Tn or sialosyl-Tn antibodies. It is also of interest to note that antigen distribution in tissues is very similar for sialosyl-Tn defined by TKH1, TKH2, and B72.3 and Tn defined by NCC-Lu-35 and -81. Coexpression of these two antigens in the same mucin-type glycoprotein has been observed, and tissue distribution of these antigens should be identical. It is possible that application of TKH1 and TKH2 in combination with anti-Tn or other antibodies in tumor imaging or specific drug delivery to tumors would be effective for diagnosis or treatment of cancer.

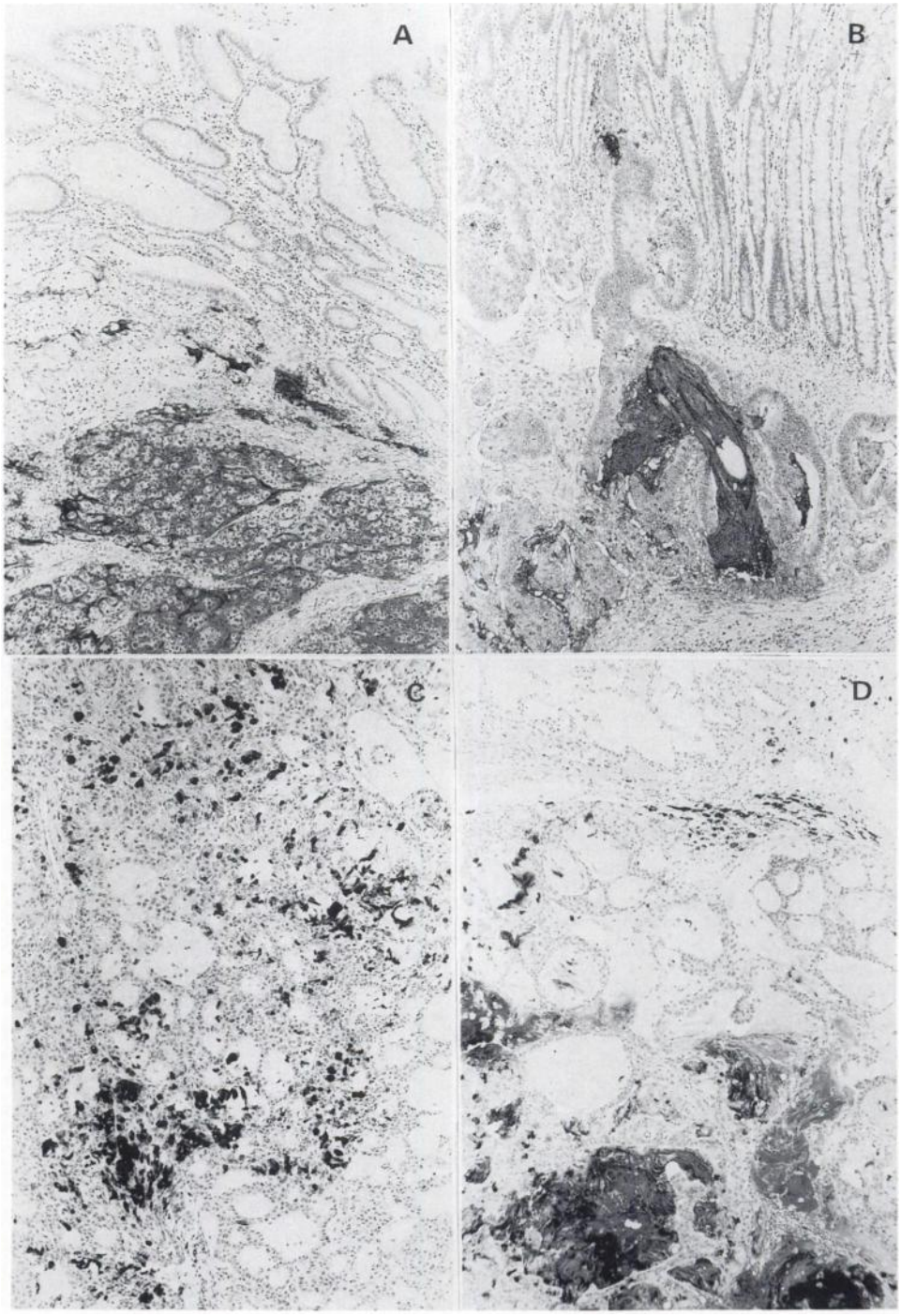

Fig. 8. Immunoperoxidase staining of formalin-fixed paraffin-embedded malignant tumors using TKH1 monoclonal antibody. *A*, TKH1 reactivity with a stomach adenocarcinoma whereas normal stomach are unstained (× 90). *B*, TKH1 staining of moderately differentiated colon adenocarcinoma, surrounding normal cells, and nonreactive stroma. *C*, lung adenocarcinoma showing heterologous TKH1 staining of cancer cells (× 90). *D*, TKH1 staining of acinar lung adenocarcinoma; normal lung tissue are unstained.

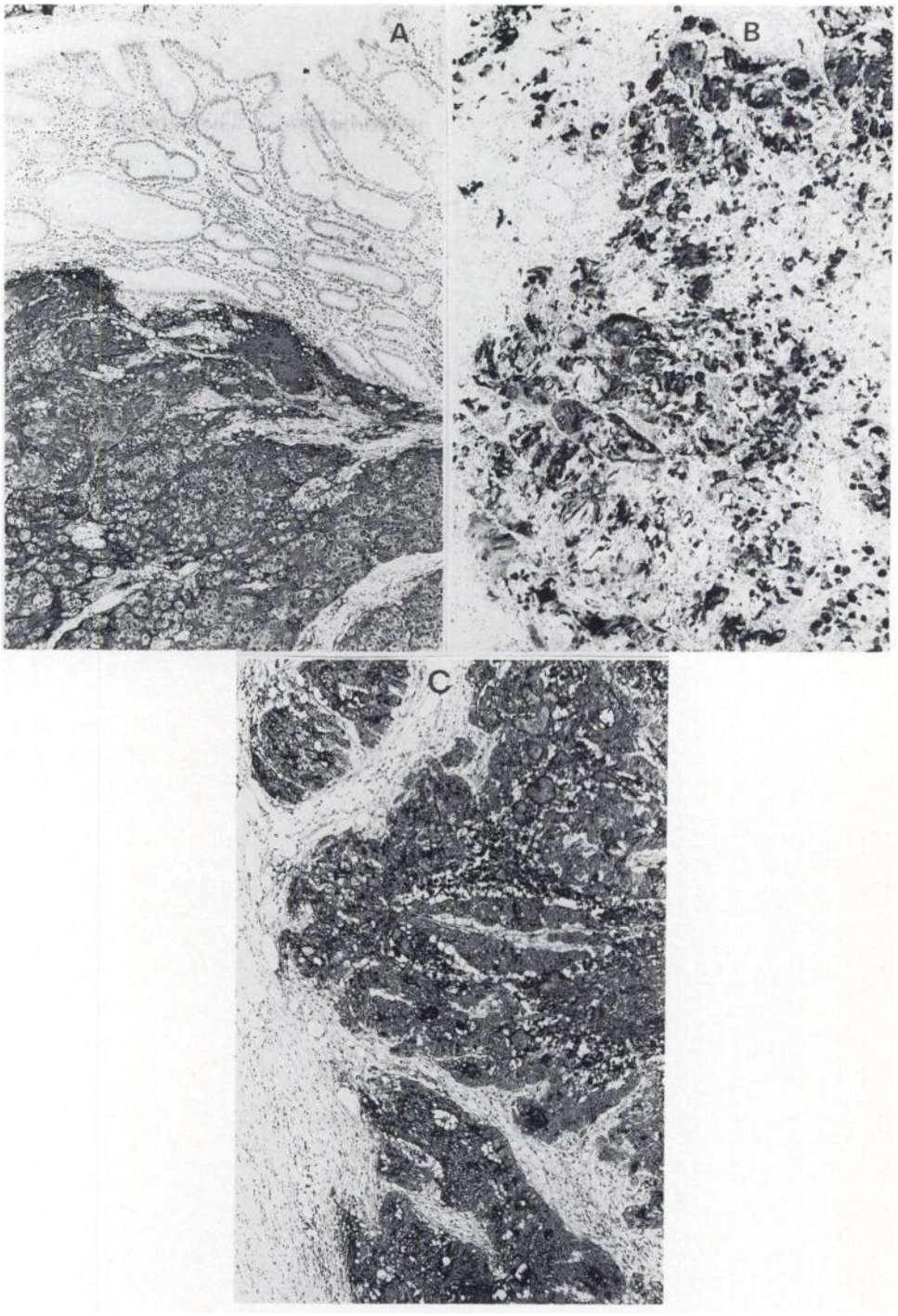

Fig. 9. Immunoperoxidase staining of formalin-fixed paraffin-embedded malignant tumors using TKH2 monoclonal antibody. *A*, TKH2 reactivity with a stomach adenocarcinoma (same area as in Fig. 8*A*). (× 40). *B*, TKH2 staining of a lung adenocarcinoma (same area as in Fig. 8*C*) (× 90). *C*, TKH2 reactivity with a moderately differentiated colon adenocarcinoma; normal stroma are unreactive (× 90).

ACKNOWLEDGMENTS

We are grateful to Dr. G. F. Springer for the donation of the anti-Tn antibody (CA 3239).

REFERENCES

1. Hakomori, S. Blood group glycolipid antigens and their modifications as human cancer antigens (Philip Levine Award Lecture). Am. J. Clin. Path., *82:* 635–648, 1984.
2. Hakomori, S. Aberrant glycosylation in cancer cell membranes as focused on glycolipids: overview and perspective. Cancer Res., *45:* 2405–2414, 1985.
3. Dahr, W., Uhlenbruck, G., Gunson, H. H., and Hart, M. V. D. Molecular basis of Tn-polyagglutinability. Vox Sang., *29:* 36–50, 1975.
4. Springer, G. F., Desai, P. R., and Bawatwala, I. Blood group MN specific substances and precursors in normal and malignant human breast tissue. Naturwissenschaften, *61:* 457–458, 1974.
5. Hirohashi, S., Clausen, H., Yamada, T., Shimosata, Y., and Hakomori, S. Blood group A cross-reacting epitope defined by monoclonal antibodies NCC-Lu-35 and -81 expressed in cancer of blood group O or B individuals: its identification as Tn antigen. Proc. Natl. Acad. Sci. USA, *82:* 7039–7043, 1985.
6. Schachter, H., McGuire, E. Y., and Roseman, S. Sialic acids XIII: a uridine diphosphate D-galactose:mucin galactosyl-transferase from porcine submaxillary gland. J. Biol. Chem., *246:* 5321–5328, 1971.
7. Korosaka, A., Nakayima, H., Fuwakoshi, I., Matsuyama, M., Nagayo, T., and Yamashiwa, I. Structures of major oligosaccharides from a human rectal adenocarcinoma glycoprotein. J. Biol. Chem., *285:* 11594–11598, 1983.
8. Colcher, D., Horan Hand, P., Nuti, M., and Schlom, J. A spectrum of monoclonal antibodies reactive with human mammary tumor cells. Proc. Natl. Acad. Sci. USA, *78:* 3199–3203, 1981.
9. Nuti, M., Teramoto, Y. A., Mariani-Constantini, R., Hand, P. H., Colcher, D., and Schlom, J. A monoclonal antibody (B72.3) defines patterns of a novel tumor-associated antigen in human mammary carcinoma cell populations. J. Inst. Cancer, *29:* 539–545, 1982.
10. Thor, A., Ohuchi, W., Szpak, C. A., Johnston, W. W., and Schlom, J. Distribution of oncofetal antigen tumor-associated glycoprotein-72 defined by monoclonal antibody B72.3. Cancer Res., *46:* 3118–3124, 1986.

11. Johnson, V. G., Schlom, J., Paterson, A. J., Bennett, J., Magnani, J. H., and Colcher, D. Analysis of human tumor-associated glycoproteins (TAG-72) identified by monoclonal antibody B72.3. Cancer Res., *46:* 850–857, 1986.
12. Kurosawa, A., Fukui, S., Kitagawa, H., Nakeda, H., Namata, Y., Fumakoshi, I., Kawasashi, T., and Yamashiwa, I. Mucin-carbohydrate directed monoclonal antibody. FEBS Lett., *215:* 137–139, 1987.
13. Springer, G. F., Desai, P. R., Robinson, M. K., Tegtmeyer, H., and Scanlon, E. F. The fundamental and diagnostic role of T and Tn antigens in breast carcinomas at earliest histologic stage and throughout. *In:* T. Dao, A. Brodie, and C. Ip (eds.), Tumor Markers and Their Significance in the Management of Breast Cancer, pp. 47–70. New York: A. R. Liss, 1986.
14. Tettamanti, G., and Pigman, W. Isolation and characterization of glycoproteins from ovine submaxillary mucin: purification and characterization of bovine and ovine submaxillary mucins. Arch. Biochem. Biophys., *124:* 45–50, 1968.
15. Pigman, W., and Gottschalk, A. Submaxillary gland glycoproteins. *In:* A. Gottschalk (ed.), Glycoproteins, Vol. 5, pp. 434–445. New York: Elsevier, 1966.
16. Tsuji, T., and Osawa, T. Carbohydrate structures of bovine submaxillary mucin. Carbohydr. Res., *151:* 391–402, 1986.
17. Clausen, H., Levery, S. B., Nudelman, E., Stroud, M., Parker, J., White, T., and Hakomori, S. Isolation and characterization of blood group A-associated type 3 chain glycosphingolipids: galactosyl-A and its sialyl derivative. *In:* Proc. IXth Intl. Symp. on Glycoconjugates, p. F49. Tourcoing, France: Lerouge, 1987.
18. Springer, G. F. T and Tn, general carcinoma autoantigens. Science (Wash. DC), *224:* 1198–1206, 1984.
19. Nakada, H., Kitagawa, H., Kurosaka, A., Fukui, F., Numata, Y., Funakoshi, I., Kawasaki, T., and Yamashima, I. Monoclonal Antibodies recognizing mucin-type glycoproteins. *In:* Proc. IXth Intl. Symp. on Glycoconjugates, p. F49. Tourcoing, France: Lerouge, 1987.