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Development of monoclonal antibodies against bovine mucin core 2 β6 *N*-acetylglucosaminyltransferase

Cheng-Ming Li[13], Nirmal Joshee[2], Kenneth B. Adler[3] and Pi-Wan Cheng[1,2]*

[1] *Departments of Pediatrics and Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC*
[2] *Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE*
[3] *Department of Anatomy, Physiological Sciences & Radiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC*

Molecular cloning techniques have been used to produce abundant amounts of recombinant glycosyltransferases for biochemical studies. We recently cloned a cDNA which encoded bovine mucin core 2 β6*N*-acetylglucosaminyl transferase (C2TF). Poly-histidine-C2TF fusion protein was generated from the cloned cDNA in the E. coli Xpress system and used to produce monoclonal antibodies (MAbs). We obtained seven hybridomas which secreted MAbs against bovine C2TF in mouse ascites with titers ranging from 1:1280 to 1:40960 as assessed by immunofluorescence assay (IF). Isotyping revealed that all seven MAbs were IgG (4 IgG1, 2 IgG2b and 1 IgG2a). The affinity constants (M^{-1}) for these MAbs range from 5.4×10^7 to 1.2×10^9. These MAbs recognized bovine C2TF in tissue sections and on Western blottings. Six of these MAbs reacted with human core 2-M enzyme and one with both core 2-L and core 2-M enzymes on Western blottings. Therefore, These antibodies should be useful for further study of bovine and human core 2 enzymes.

Keywords: **core 2 *N*-acetylglucosaminyltransferase, glycosyltransferase, monoclonal antibodies, mucin**

Introduction

The oligosaccharides of glycoconjugates play crucial roles in many important biological processes, such as maintaining glycoproteins in circulation and regulating cell-cell interactions, differentiation, bacterial and viral adhesions, and protein solubility and stability [1,2]. In airway mucins, carbohydrate is the major constituent, which contributes to the rheological properties of the mucus gel that protects the respiratory tract from infection by airborne pathogens [3,4]. Mucin carbohydrates are very heterogeneous in size and structure, which are determined by sequential addition of different sugars as catalyzed by glycosyltransferases. N-Acetylglucosaminyltransferases belong to the group of glycosyltransferases which are responsible for elongation and branching of oligosaccharides [4–7]. The synthesis of branched mucin oligosaccharides, which include core 2, core 4, and blood group I structures [4–7], is determined solely by β6GlcNAc TF's. In bovine tracheal epithelium, all three GlcNAc structures can be synthesized by one β6GlcNAc transferase [6] while in human myloid cells these structures are synthesized by three different enzymes [8–10]. The core 2 GlcNAc transferase, which catalyzes the transfers of GlcNAc from UDP-GlcNAc to Galβ1-3GalNAc forming Galβ1-3(GlcNAcβ1-6) GalNAc structure, is one of the better characterized β6GlcNAc transferases. In competition with sialyltransferase (STGalNAc I/II), which catalyzes the transfer of NeuAc from CMP-NeuAc to Galβ3GalNAc forming Galβ3(NeuAcα2-6)GalNAc structure, C2TF is responsible for the elongation of mucin oligosaccharide from the C-6 of GalNAc. This concept has been elegantly demonstrated by Piller et al. [11] in the example of human T-cell activation, in which they showed that increase in mucin oligosaccharide chain length following activation of T-cells correlated with an increase in C2TF activity and a concomitant decrease in STGalNAc I/II activity [11]. Transfection of CHO cells [12] and human pancreatic cancer cells [13], which do not express core 2 enzyme, with C2TF cDNA confirms the important role of this enzyme in the elongation of mucin-type glycans. Increased C2TF activity has also been shown to be associated

*To whom correspondence should be addressed: Dr. Pi-Wan Cheng, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, 984525 Nebraska Medical Center, Omaha, NE 68198-4525. Tel.: (402) 559-5776; Fax: (402) 559-6650; E-mail: pcheng@unmc.edu

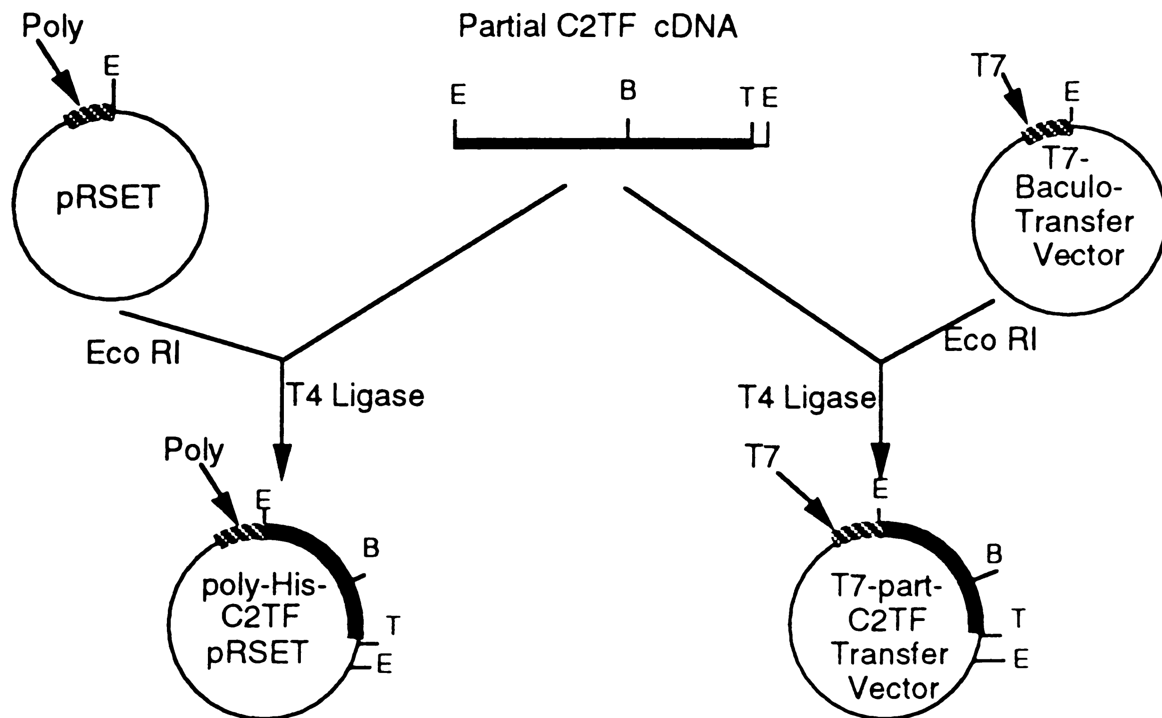

Figure 1. Construction of the recombinant C2TF expression plasmid. B, Bam HI site; E, Eco RI site; Poly, poly-His tag sequence region; T7, T7 tag sequence region; and T, termination point of bovine C2TF ORF.

with other biological processes, such as tumor progression [14], leukemia [15,16], and immunodeficiency associated with the Wiskott-Aldrich syndrome [17–19] and AIDS [8,20]. In spite of the potential importance of this enzyme in many biological processes, study of this enzyme was limited largely to the measurement of enzyme activity because of extreme instability of this enzyme and its low abundance in the tissue. A recent report of the cloning of human C2TF cDNA [8,9] prompted us to clone the bovine C2TF cDNA [21] and generate monoclonal antibodies against the recombinant bovine C2TF. In this report, we describe the production of MAb isotypes against bovine C2TF using the recombinant C2TF produced in E. coli as immunogen. We demonstrate that these MAbs recognize bovine C2TF in tissue sections and on Western blottings. In addition, some of these MAbs recognize human core 2-L, core 2-M, or both.

Materials and methods

Construction of expression plasmids containing C2TF cDNA insert

All DNA cloning procedures were performed as described by Sambrook et al. [22] unless otherwise specified. The E. coli Xpress system from the Invitrogen Inc. (San Diego, CA, USA) was used for the production of recombinant C2TF which in turn was used for immunization. The Baculovirus Express System from Clontech (Palo Alto, CA, USA) was used for the preparation of recombinant C2TF antigen-containing sf21 cells, which in turn were used for screening the presence of C2TF-specific MAbs by immunofluorescence (IF) assay. The cDNA of bovine C2TF was cloned from a bovine lung λgt 10 cDNA library (Clontech) [21]. This cDNA has an open reading frame of 1284 bp (with an Eco RI site at 97th nucleotide) coding 427 amino acids of bovine C2TF. Eco RI digestion resulted in a large Eco RI cDNA fragment which contained a partial C2TF coding sequence lacking the 5'-end 97 bp of bovine C2TF cDNA [21]. For expression cloning, this Eco RI fragment was inserted into the Eco RI site of the Xpress pRSET-A vector flanked by a poly-histidine leading sequence and under the control of T7 promoter (Fig. 1). To prepare sf21 cells which contain the recombinant C2TF antigen for MAb screening by IF, the recombinant baculovirus which contained the partial C2TF open reading frame (ORF) construct was used. In this construct, the larger Eco RI fragment was flanked by a 33 bp of T7 tag sequence and its expression was under the control of the baculoviral polyhedrin promoter.

Expression of a partial bovine C2TF

In Xpress system, the recombinant pRSET vector was transformed into competent E. coli cells (JM109) according to manufacturer's protocol. After transformation, the E.

coli colonies which contained the vector (C2TF-E.coli colonies) were picked from the agar plate and cultured in L.B. broth at 37°C with vigorous shaking (250 rpm). The growth of bacteria was monitored by OD600nm. When the OD reading reached 0.3, isopropyl-β-D-thiogalactopyranoside (IPTG) (Promega, Madison, WI) was added to the culture medium to a final concentration of 1 mM to induce protein expression. For monitoring the expression of the recombinant protein, the culture samples were collected at hourly intervals following IPTG addition and were centrifuged at 3,000 × g for 10 min to spin down the bacteria. Then, the bacteria pellets were solubilized in PAGE sample buffer and the proteins separated by SDS-10% PAGE [23]. The protein bands were visualized by Coomassie blue staining.

In the viral expression system, the recombinant baculovirus vector was co-transfected into sf21 insect cells with Bsu 36I-digested baculoviral DNA by the Lipofectin-transfection method as described in the manual provided by the manufacturer. The resulted recombinant virus was purified in the sf21 cells by limited dilution. The expressed fusion protein was analyzed by Western blotting using anti-T7 tag MAb from Novagen (Madison, WI, USA).

Antigen preparation and immunization

The expressed poly-His-C2TF fusion protein was purified from the recombinant bacteria using the Xpress purification kit from Invitrogen with some modifications. After induction for four hours with IPTG, 100 ml of C2TF-E. coli culture were harvested by low-speed centrifugation (3000 × g, 15 min). The bacterial pellet was lysed with 10 ml of the denature-lysing buffer (20 mM sodium phosphate, pH 7.8 containing 6 M guanidinium hydrochloride and 500 mM NaCl) for 30 min at room temperature and centrifuged at 25,000 × g at 4°C for 30 min. The supernatant was collected and slowly passed through a metal-ion-affinity column pre-equilibrated with denaturing buffer (20 mM sodium phosphate, pH 7.8 containing 8 M urea and 500 mM NaCl). The poly-His-C2TF fusion protein was bound to the column via its poly-histidine domain. After washing the column with 20 ml of washing buffer, the bound protein was eluted into microtubes (1 ml/tube) with 0.2 M glycine buffer at a pH 6.5 to 2.5 gradient. Each fraction was dialyzed against PBS, pH 7.2 and analyzed by SDS- PAGE. The fractions containing purified poly-His-C2TF fusion protein were stored at −20° C.

Balb/c mice were immunized intraperitoneally with 200 ng poly-His-C2TF antigen in complete Freund's adjuvant at day 0, followed by re-immunizations at days 30 and 60 with 100 ng antigen each in incomplete Freund's adjuvant. At day 102, mice were boosted by intravenous injection of 300 ng antigen without adjuvant.

MAb production

At day 3 following boosting, the spleen cells were collected and fused with SP 2/0 myeloma cells at an 8:1 ratio as described [24–26]. The fused cells were cultured in three 48-well plates in HAT medium at 37 °C under 7% CO_2 in a water-saturated environment for 14 days and then switched to HT medium. The hybridoma-conditioned media were assayed for C2TF antibodies by immunofluorescence (IF). The hybridoma cells which produced C2TF-reactive-MAb were purified twice by a limited-dilution subcloning technique.

Immunofluorescence assay

For the screening of C2TF-specific MAbs, an indirect immunofluorescence assay was established as follows: a) Preparation of antigen-coated slides - The sf21 cells were infected with the recombinant C2TF-baculovirus (wild-type baculovirus infected sf21 cells served as the control) and cultured at 27°C. Thirty-six hours later, the infected cells were spread directly onto clean glass slides and air dried. Each cell-spread spot was about 0.5 cm in diameter and each slide contained 10 spots. The slides coated with infected cells were stored at −20°C; b) IF assay - The slides were warmed up at room temperature and then fixed with acetone for 10 min. After fixation, they were washed with PBS three times (2 min/ washing) and air dried. The conditioned medium of the cultured hybridomas was added to the recombinant C2TF baculovirus-infected cell spots and the wild-type baculovirus-infected cell spots on the slides, which were incubated in 37 °C for 30∼60 min and washed three times as above. After drying, 1:200 fluorescein conjugated goat anti-mouse Ig (Cappel Inc., Durham, NC, USA) diluted with 0.01 % Evan's blue-PBS was added onto all of the cell spots and the slides were incubated at 37 °C for 30 min. Finally, the slides were washed five times as above, mounted in glycerol with coverslip, and examined under a fluorescence microscope.

For immunohistochemical study, frozen sections of bovine trachea were fixed with acetone and stained as described above.

Western blotting assay

The E. coli cell lysates in SDS-PAGE sample buffer were subjected to analysis by SDS-10% PAGE as described [23]. The resolved proteins were electroblotted onto nitrocellulose membrane, which was cut into 0.4 cm-wide strips. The Western blotting assay of the conditioned media with these strips was performed as described previously [27,28], but with some minor modifications. In the present study, the peroxidase-goat anti-mouse Ig conjugate from Cappel was used to replace the radio-labeled conjugate and the result was visualized with an Enhanced Chemiluminescence kit from Amersham (Arlington, IL, USA).

For characterization of the specificity of bovine core 2 MAbs against human core 2-L and core 2-M enzymes, human lung carcinoma cells, A549 and H292, were employed. A549 expressed only core 2-M and not core 2-L [29] while

H292 expressed both core 2-L and core 2-M (Cheng, unpublished observation). H292 and A549 cells grown to confluence in 10% FBS-supplemented RPMI 1640 and F12K media, respectively were subjected to Western blotting analysis with these MAbs as described above.

Isotyping of MAbs

The classes and subclasses of these MAbs were determined by enzyme immunoassay using the ImmunoPure MAb isotyping Kit I from the Pierce (Rockford, IL, USA).

Determination of the interactions between bovine C2TF and their MAbs

The BIAcore biosensor (Pharmacia Biosensor, Uppsala, Sweden), which uses surface plasmon resonance detection and permits real-time kinetic analysis of two interacting species [30], was employed for the measurement of the binding kinetics of MAbs and recombinant C2TF purified from E. coli. C2TF antigen was immobilized on a CM5 dextran sensor chip in 100 mM sodium acetate, pH 4.5 using the Amine Coupling kit (Pharmacia Biosensor). The dextran layer of the sensor chip was activated by injecting 35 μL of 0.05 M N-hydroxysuccinimide and 0.2 M N-ethyl-N-(3-diethylaminopropyl) carbodiimide. Next, 200 μL of 250 nM purified C2TF in 0.1 M acetate buffer, pH 4.5 was injected until a surface of 56 resonance units (RU) was realized. Excess reactive groups were blocked by injection of 35 μL of 1 M ethanolamine, pH 8.5. Binding experiments were performed in HBS buffer (10 mM HEPES, pH 7.4, 0.15 M NaCl, 3.4 μM EDTA, 0.005% surfactant P20) at a flow rate of 75 μL/min at 25°C. The surface was regenerated with 5 μL of 10 mM hydrochloric acid at a flow rate of 5 μL/min with no loss of activity. MAbs for binding analysis were purified by Protein G Sepharose (Pharmacia Biotech) to at least 90% purity and dialyzed into the HBS binding buffer. The kinetic constants, K_{on} and K_{off}, and affinity constants (K_a) were evaluated using the BIAevaluation 3.0.2 software supplied by the manufacturer where the experimental design correlated with the Langmuir 1:1 interaction model [31].

Results

Preparation of Antigens

Two different expression systems were used to prepare recombinant C2TF antigens: one for immunization and another one for immunoassay. Fig. 1 summarizes the strategies for the construction of the recombinant plasmid containing bovine C2TF cDNA. When E. coli was transformed with the recombinant pRSET plasmid, a large amount of the C2TF fusion protein with a poly-his tag was expressed in the bacteria following induction with IPTG. PAGE analysis showed that the protein expression reached a peak (approximately 50 μg of the recombinant C2TF protein/ ml culture) at 4 h post-induction. The apparent molecular weight of the fusion protein was ca. 51,000 Daltons (Fig. 2 A). The poly-His-C2TF fusion protein expressed in the bacteria was purified to apparent homogeneity by the metal-ion affinity column (Fig. 2B) and used as the antigen to immunize mice.

Western blotting analysis and immunofluorescence assays showed that the recombinant C2TF-T7 fusion protein had also expressed in sf21 insect cells infected with the

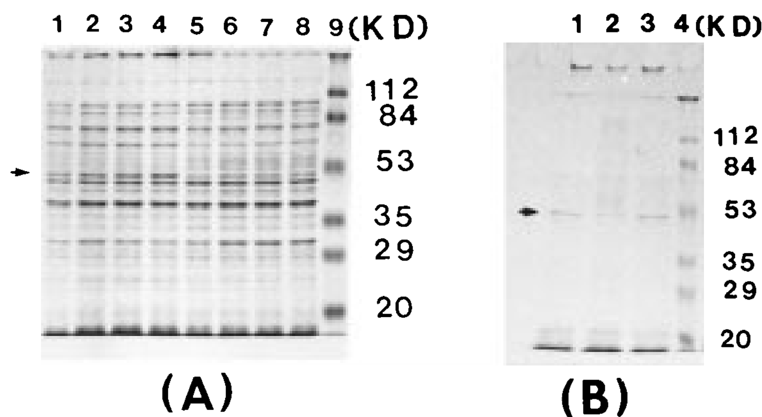

Figure 2. Analysis of the expressed (A) and purified (B) recombinant C2TF fusion proteins by SDS-10% PAGE. A) Expression of poly-His-C2TF in E. coli: Lanes 1~4 are lysates of the recombinant C2TF-pRSET transformed bacteria, lanes 5~8 are lysates of the pRSET (without insert) transformed bacteria, and lane 9 is protein markers (~2 ug protein/ per band). Lanes 1 and 5 are samples collected at 1 h after IPTG addition, and lanes 2 and 6, lanes 3 and 7, lanes 4 and 8 are lysates of the bacteria collected at 2, 3, and 4 hours post-IPTG addition, respectively. In the recombinant pRSET sample lanes (1~4), an expressed 51 kDa protein band was detected (arrow). The amount of the expressed protein increased with culture times, and protein expression reached the peak at ~50 μg/ ml culture at 4 h post-IPTG induction. B) Purification of the poly-His-C2TF fusion protein by metal-ion affinity column. Lanes 1~3 are the purified protein from three separated preparations, and lane 4 is the protein marker. Arrow points to the purified 51 kDa C2TF fusion protein.

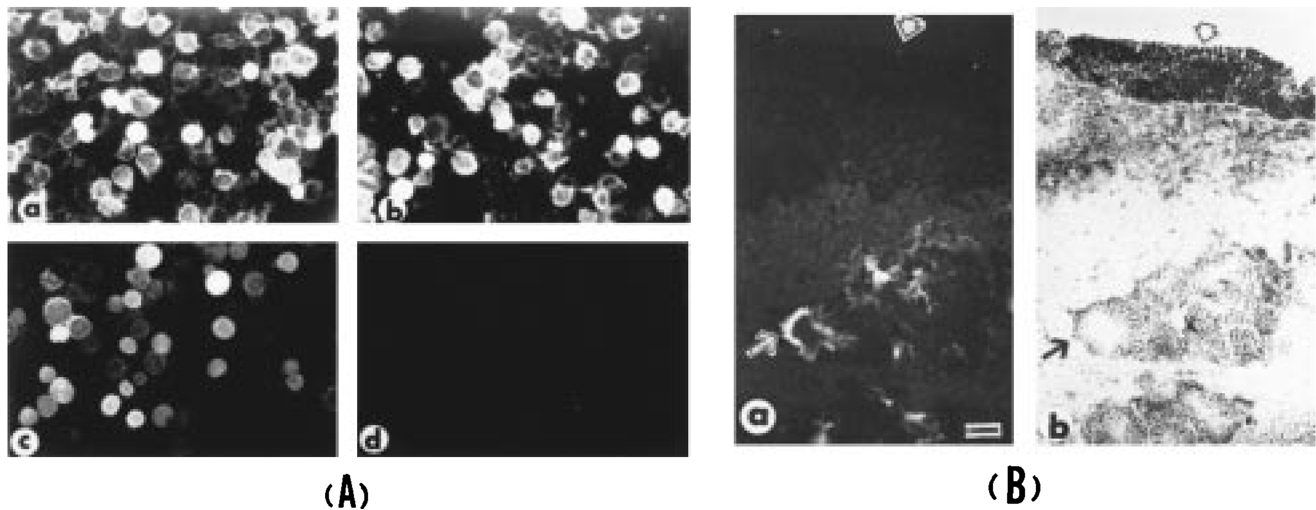

Figure 3. Immunofluorescence detection of C2TF with C2TF-MAbs. A) Detection of expressed C2TF in the sf21 cells infected with recombinant baculovirus: The a, b, and c are the recombinant virus infected cells as detected by MAbs 5, 79, and 93, respectively; d is the sf21 cells infected with wild-type baculovirus as stained by a mixture of these three MAbs. Strong IF reaction was detected in the recombinant virus infected cells (a, b, and c). B) Detection of native C2TF in bovine trachea epithelium: Frozen sections of bovine tracheal epithelium were treated with C2TF-MAb 79 followed by fluorescein-conjugated goat anti-mouse immunoglobulin antibodies and then examined under a) fluorescence and b) bright light microscope. Another tissue section, which serves as the control, was treated with MAb against other unrelated protein (Mycoplasma pneumoniae P1 protein). No fluorescence staining was observed in the control (data not shown). Fluorescence signal in MAb 79 treated section was localized mainly in the submucosal glands (arrow). Surface epithelial cells (arrow head) in this section were not stained with MAb 79. The size of the bar is 10 μm.

recombinant C2TF-T7 baculovirus. In comparison with pRSET-E. coli system, the yield of the recombinant C2TF-T7 fusion protein in the baculovirus expression system was relatively low and could not be detected by Coomassie blue stain on PAGE gel (data not shown). However, the amount of the recombinant C2TF-T7 fusion protein expressed in these cells gave a strong reaction with T7 MAb in both Western blotting (data not shown) and IF assays (see below).

Production of MAb-secreting hybridomas

Of the 144 wells plated with fused cells, 45 wells were found to have viable hybridoma cells. Analysis of the conditioned media of these hybridomas by IF assay in the sf21 cells infected with recombinant C2TF-T7 baculovirus revealed that 20 wells had anti-C2TF activity. After subcloning and continuous culturing of these hybridomas for three months, 7 of them still retained the capabilities of producing anti-C2TF MAbs. Fig. 3A shows that in IF assay, these MAbs can react with sf21 cells infected with recombinant baculovirus but not the cells infected with wild-type baculovirus.

Characterization of MAbs

All seven MAbs showed strong fluorescence in the IF assay using slides prepared with recombinant baculovirus-infected sf21 cells. The antibody titers ranged from 1:128 $\sim$ 1:2048 in the conditioned medium and 1: 1280 $\sim$ 1: 40960 in the ascites fluids. MAb 79 had the highest anti-C2TF activity (Table 1). The isotypes of these Igs were as follows: IgG1 (4 clones), IgG2b (2 clones), and IgG2a (1 clone). All of these MAbs raised by the E. coli recombinant C2TF-polyhis fusion protein could recognize the native bovine tracheal C2TF as indicated by immunofluorescence which was localized primarily in the submucosal glands of bovine tracheal sections (Fig. 3B). In addition, all seven MAbs could react with the expressed 51 kDa C2TF protein band by Western blotting analysis (Fig. 4). A lower molecular weight ($\sim$20,000 Da) band was also detected by this approach.

Western blotting analysis of the cell lysates of human lung carcinoma A549 and H292 showed that these MAbs can be grouped into 2 categories (Fig. 5 and Table 1). MAbs 5, 12, 17, 26, 79, and 93 reacted with human core 2-M but not core 2-L enzyme. MAb 48 recognized both core 2-L and core 2-M enzymes.

The affinity constants estimated from the kinetic constants of these MAbs range from 5.4×10^7 to 1.2×10^9 M^{-1} with increasing order of affinity for MAbs 12, 17, 48, 79, 26, and 5 (Table 1).

Discussions

Most glycosyltransferases are membrane-bound enzymes. Most of the membrane-bound enzymes become very labile when dissociated from the membrane [28], which is a major impediment when attempting purification. Another limiting factor is their low abundance in the cell. Therefore, it is

Table 1. Bovine C2TF MAbs: Immunoglobulin (Ig) subclasses, titers, affinity constants, and reactivities against human core 2-L and core 2-M

MAbs	Anti C2TF titers: Conditioned media	Anti C2TF titers: Ascites	Ig Subclasses	Kinetic constants*: K_{on} ($M^{-1}s^{-1}$)	Kinetic constants*: K_{off} (s^{-1})	Affinity constants K_a (M^{-1})	Reactivities against: Human core 2-L	Reactivities against: Human core 2-M
5	1:1024	1:20480	IgG2a	1.4×10^6	1.2×10^{-3}	1.2×10^9	−	+
12	1:1024	1:10240	IgG2b	1.4×10^5	2.6×10^{-3}	5.4×10^7	−	+
17	1:128	1:1280	IgG1	1.2×10^5	1.6×10^{-3}	7.3×10^7	−	+
26	1:512	1:10240	IgG1	2.9×10^5	2.9×10^{-3}	1.0×10^9	−	+
48	1:128	1:2560	IgG1	1.6×10^5	1.7×10^{-3}	9.6×10^8	+	+
79	1:2048	1:40960	IgG1	1.9×10^5	5.8×10^{-3}	3.3×10^9	−	+
93	1:2048	1:20480	IgG2b	ND#	ND	ND	−	+

*Obtained by BIAcore. Recombinant C2TF was immobilized on a CM5 dextran sensor chip.
#ND, not determined.

extremely difficult to prepare adequate amount of purified enzyme for antibody production. Recent successes in cloning of glycosyltransferase cDNA have offered a solution to this problem [28, 32–35]. Several glycosyltransferases have been prepared in large quantity by recombinant technology in E. coli as fusion proteins. The cloning strategy simplifies the screening and purification of the recombinant enzymes [28,32,33]. For example, human β1,4 galactosyltransferase [33] and α2,6-sialyltransferase [34] have been expressed and purified as β-galactosidase fusion proteins and rabbit GlcNAc transferase I as glutathione-S-transferase fusion protein [35]. These fusion proteins have been used to produce antisera, which can recognize native enzymes. However, these antisera also reacted with non-glycosyltransferase portion of the fusion proteins, which limited the utility of these antibodies [35]. Preparation of monoclonal antibodies which recognize only the glycosyltransferase portion of the fusion proteins should avoid this shortcoming. In the present study, we generated recombinant bovine C2TF as poly-His-C2TF fusion protein in Xpress-E. coli system and used this fusion protein purified by metal-ion-affinity column for imunization. To screen for the hybridomas which produce C2TF-specific MAbs, we utilized sf21 cells which had been infected with recombinant baculovirus containing same bovine C2TF cDNA insert. We were successful in generating seven C2TF-specific MAbs.

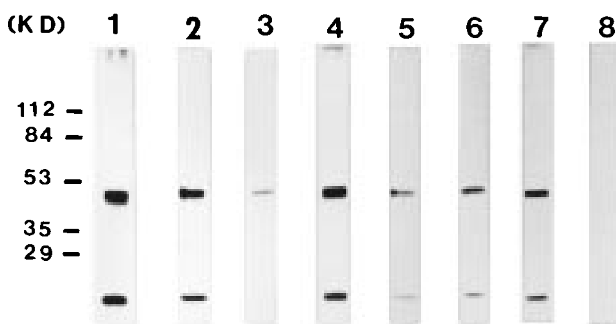

Figure 4. Western blotting analysis of C2TF-MAbs. Strips of recombinant poly-His-C2TF fusion protein from E. coli were detected by C2TF-MAbs (lanes 1~7) and strips from lysates of pRSET (no insert) transformed E. coli (control)(lane 8) was treated with a mixture of MAbs 5, 79 and 93. Lane 1 is MAb 5; lane 2, MAb 12; lane 3, MAb 17; lane 4, MAb 26; lane 5, MAb 48; lane 6, MAb 79; and lane 7, MAb 93. The ~51 kDa band was seen in all seven strips treated with C2TF-MAbs. A faster-moving band with a molecular weight of about 20 KDa was also seen in C2TF-MAb treated strips. This protein band, which may a degraded product of C2TF-poly-His fusion protein expressed in E. coli, was also seen in the recombinant C2TF fusion protein(s) purified by metal-ion-affinity colum (see Fig. 2).

The recombinant C2TF expressed either as a poly-His fusion protein in E. coli or as a partial C2TF (−33 amino acids at the N-terminus) in a baculovirus system had the expected masses but could not transfer GlcNAc from UDP-GlcNAc to the Galβ3-GalNAc acceptor (data not shown). This result suggests that the first 33 amino acids at the N-terminus are crucial for preserving the catalytic activity of bovine C2TF. Although these amino acids are separated by the stem region from the catalytic domain of this enzyme [9], their absence may lead to conformational changes in the catalytic domain which renders the enzyme inactive. This conclusion was confirmed by our observation that the recombinant C2TF expressed in the baculovirus expression system with the complete open reading frame of bovine C2TF cDNA showed the enzyme activity [21]. The failure of the recombinant protein expressed in E. coli to show enzyme activity could be the result of the lack of glycosylation since it is known that proteins expressed in E. coli are not glycosylated and human C2TF devoid of either one or both N-glycans loses enzyme activity [36]. Our results suggested that both the 33 amino acids at the N-terminus and the carbohydrate are important for the bovine core

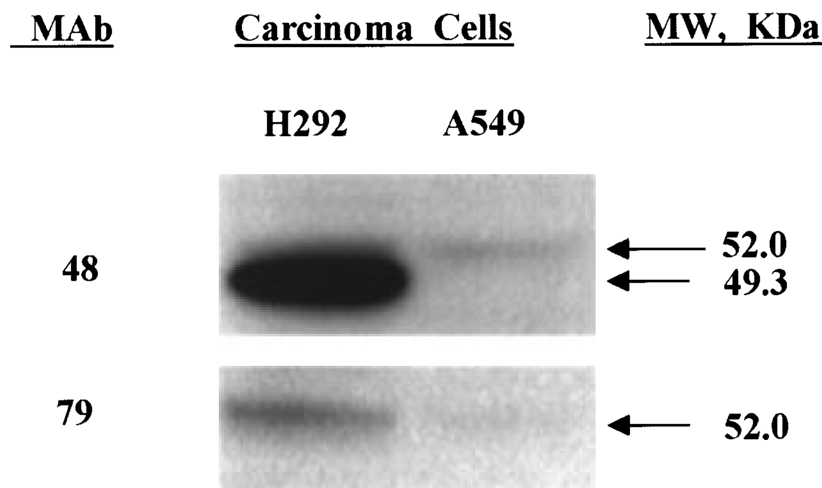

Figure 5. Western Blotting Analysis of Human Lung Carcinomas A549 and H292 with Bovine C2TF MAbs. MAb 48 reacts with both human core 2-L (49.3 KDa) and core 2-M (52.0 KDa) enzymes while MAbs 5, 12, 17, 26, 79, and 93 react with core 2-M but not core 2-L enzyme. Only the results of MAbs 48 and 79 are shown.

2 enzyme to maintain its catalytic activity. But, human C2TF lacking N-terminal 37 amino acids still retained enzyme activity [34]. It is worth noting that the MAbs generated using the inactive recombinant C2TF fusion protein could recognize bovine C2TF in tissue sections (Fig. 3) in addition to Western blottings [13,21]. Therefore, native enzyme antigen [35] is not required for producing MAbs that recognize native enzyme in the tissue.

Bovine tracheal mucins are present in both the submucosal glands and the surface epithelial goblet cells. However, the anti-C2TF MAbs stain primarily submucosal glands. The present results using MAbs suggest that this C2TF is present predominantly in the submucosal glands. This enzyme is probably different from the bovine tracheal epithelial β6GlcNAc transferase, which had a molecular weight of 66,000 Daltons and can form core 2, core 4 and I structures of mucin glycans [6]. The bovine cDNA encoding this β6GlcNAc TF remains to be cloned. Recently, Yeh et al. [29] and Schwientek et al. [37] have successfully cloned the cDNA encoding a human β6GlcNAc TF which shows similar acceptor specificity as does the bovine β6GlcNAc TF [6]. This enzyme is found primarily in the mucus-producing tissue and is called C2TF-M [29] while the other C2TF, which is found many other tissues, is called C2TF-L [29]. It will be of interest to compare the catalytic domain structures among this C2TF-M (29,37), C2TF-L (9), and β6GlcNAc TF which synthesizes the I structure [10].

IF assay has been a useful tool in many studies for the detection and localization of antigens in the cells and tissues. Comparing with other immunoassays, this assay is relatively simple and rapid, and can be completed within one hour. Therefore, it was used as a major approach for screening and analysis of MAb in this study. Because the recombinant bovine C2TF is generated only in the sf21 cells infected with recombinant baculovirus and not in cells infected with wild-type baculovirus, treatment of both cells with same MAb has helped us identify the C2TF-specific Mabs which can recognize C2TF on Western blots and tissue sections.

In summary, seven C2TF-MAbs have been raised with recombinant bovine C2TF as antigen. They can react with both the denatured and native bovine C2TF. Furthermore, most of them recognize human core 2-M while one recognizes both core 2-M and core 2-L. Therefore, these MAbs should be useful for studying the functions of bovine and human core 2 enzymes.

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