Murine Monoclonal Antibodies against Galactosyltransferase from the Ascites of Ovarian Cancer Patients

Sunil K. Chatterjee,2 Malaya Bhattacharya, and Joseph J. Barlow

Department of Gynecologic Oncology, Roswell Park Memorial Institute, Buffalo, New York 14263

ABSTRACT

Glycoprotein:galactosyltransferase is a promising enzyme marker for ovarian carcinoma. Five stable murine hybridoma monoclonals that produce homogeneous antibodies against galactosyltransferase from the ascites of ovarian cancer patients have been established. Three of the monoclonal antibodies produced were immunoglobulin G1 isotype, while two were immunoglobulin M. All the antibodies showed linear Scatchard binding plots and had very high affinity for galactosyltransferase with equilibrium dissociation constants (Kd) ranging between 0.16 x 10^-9 M and 0.97 x 10^-9 M. Two of the monoclonal antibodies recognized adjacent epitopes on the enzyme molecule, two antibodies recognized two other unique epitopes, while the epitope recognized by the fifth was uncertain. Following polyacrylamide gel electrophoresis of the purified enzyme, in the presence of sodium dodecyl sulfate, the separated proteins were transferred to nitrocellulose filters (transblotting) and galactosyltransferase was detected on the filters by immunoperoxidase staining after treatment with monoclonal antibodies. A band at M, 52,000 and M, 47,000 was detected by all of the monoclonal antibodies. One monoclonal antibody had a band at M, 54,000. Purified galactosyltransferase catalyzed the transfer of galactose to four types of acceptors: (a) alkali-stable N-glycosidic glycoproteins; (b) alkali-labile mucin-type acceptors; (c) N-acetylglucosamine; and (d) glucose in the presence of α-lactalbumin. All these transfer activities of the enzyme were present in the immunoprecipitates of the monoclonal antibodies. Transblotting of the enzyme from nondenaturing slab gels produced diffused stain patterns. Assay of the enzyme using the four types of acceptors in gel slices showed overlapping activity profiles, which coincided with the stained area on the filters, suggesting that the reactions are catalyzed by the same protein.

INTRODUCTION

Galactosyltransferases are the family of enzymes which transfer galactose from UDP-galactose to the nonreducing residues of oligosaccharides of various glycoconjugates as well as to monosaccharides. These enzymes are widespread in mammalian systems, present both as membrane-bound and soluble forms. Some of the galactosyltransferases have been demonstrated to be on the outer cell surface, where they have been implicated in a diverse biological phenomena, such as cell-cell recognition, morphogenesis, sperm-egg recognition, growth control, and malignant transformation (review in Ref. 14). A tumor-associated species of galactosyltransferase has also been detected (24). We have demonstrated that the specific activities of a galactosyltransferase (asialo-agalactofetuin galactosyltransferase) in the ovarian tumor homogenate were significantly higher than those from normal ovaries (2, 9). In patients with this type of cancer, the levels of this enzyme in the sera were also elevated compared to various controls, although such elevation is not cancer specific (23). We observed that, in ovarian cancer patients, serum levels of galactosyltransferase dropped after reduction of tumor mass by surgery, chemotherapy, or radiation, and these levels rise with tumor recurrence (8, 10). These observations have been confirmed recently (19, 28, 29). Galactosyltransferase thus appears to be a promising marker for the follow-up of ovarian cancer patients during treatment and may help the clinicians to choose the most effective therapy. Subsequently, we found that the ascites of the ovarian cancer patients are a rich source of this enzyme and purified it from these ascites over 100,000-fold by several steps of affinity chromatography (11). With a long-term goal of immune quantitation of this enzyme in situ in normal and malignant tissues, development of a sensitive immune assay for it, for the detection and isolation by immune affinity chromatography of any cancer-associated isoenzyme of galactosyltransferase, if such an isoenzyme exists in these ascites, we initiated the development of monoclonal antibodies directed against this purified preparation. In this communication, we described the production of 5 monoclonal antibodies directed against this enzyme and preliminary characterization of these monoclonal antibodies. A preliminary report of this work has appeared as an abstract (13).

MATERIALS AND METHODS

Materials. All tissue culture media were obtained from Grand Island Biological Laboratories, Grand Island, NY. Protein A-containing bacterial suspension, IgGso, was purchased from the Enzyme Center, Inc., Boston, MA. Rabbit anti-mouse immunoglobulin was from Dako Corporation, Santa Barbara, CA. Acrylamide, bisacrylamide, and SDS were from Bio-Rad Laboratories, Richmond, CA. [3H]Leucine was from New England Nuclear, Boston, MA.

Immunization and Fusion Protocol. Eight-week-old female BALB/c mice were immunized s.c. with 10 j/g of purified enzyme emulsified with Freund's complete adjuvant followed by a similar injection on the first and third weeks using Freund's incomplete adjuvant. Five days before fusion, the mice were boosted i.p. with 100 j/g of purified enzyme for 4 consecutive days. On the fifth day, the mice spleens were removed aseptically, and fusion was performed with P3-NS1-1-Ag4-1 myeloma suspension, IgGsorb, was purchased from the Enzyme Center, Inc., Biological Laboratories, Grand Island, NY. Protein A-containing bacterial material supematants was done by a solid-phase radioimmunoassay (3). The enzyme solution was diluted with PBS to 1 μg of protein/ml, and 20

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2 To whom requests for reprints should be addressed.
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of crude enzyme extract (0.05 milliliter) for 1 hr at room temperature, followed by 16 hr at 4°C. In a separate test tube, 50 mg of lyophilized IgGsorb were washed, suspended in 0.4 ml of PBST, and incubated with 100 µl of rabbit antimouse immunoglobulin for 3 hr at room temperature, while being mixed in a Roto-rack. The IgGsorb suspension was washed and finally suspended in 0.5 ml of PBST. To each tube containing the culture medium and the enzyme extract, 20 µl of this thoroughly mixed suspension were added and stirred at 4°C for 4 hr by means of a Roto- rack. This amount of second antibody was determined to be in excess in separate titration experiments. The suspension was centrifuged at 4°C, and the supernatant was saved. The pellet was washed twice, each time with 0.5 ml PBST, and finally suspended in 100 µl of PBST. Ten-µl aliquots from the supernatant or the suspended precipitate were assayed for enzyme activity.

Cloning of the Hybridomas. Selected hybridomas were cloned twice by the limiting dilution method (18) in the presence of peritoneal exudate cells from normal BALB/c mice as feeder layer.

Production of Antibodies in Vivo. Viable hybridoma cells (1 to 2 × 10^6) in PBS were injected i.p. into pristine-primed BALB/c mice for the in vivo production of the monoclonal antibodies.

Titration Curve with Mouse Ascites. Ascites were diluted with PBS containing bovine serum albumin (0.5 mg/ml). One hundred µl of diluted ascites were incubated with 25 µl of a crude enzyme extract (1.5 millilunits), and the immune complex was precipitated with IgGsorb for assay of galactosyltransferase.

Scatchard Analysis of Monoclonal Antibodies. Ascites were diluted 500- to 1000-fold with PBS containing bovine serum albumin (0.5 mg/ml), and 100-µl aliquots were incubated with 25 µl of enzyme extracts (0.4 to 2 millilunits) as described above. After IgGsorb precipitation, aliquots from the supernatant and suspended precipitates were assayed for galactosyltransferase activity. The activity in the precipitate represents the antibody-bound enzyme, while the residual activity in the supernatant represents the free enzyme. Total activity in each reaction was measured in a control incubation without ascites. Recovery of the enzyme from the pellet and supernatant was always over 90%. The molecular weight of galactosyltransferase was determined to be 47,600 by a Ferguson plot. The molarity of the enzyme in these experiments was determined from this molecular weight, and the recovery and specific activity data obtained during the purification of this enzyme.

Blocking of the Binding of Labeled Monoclonal Antibodies to Enzyme by Unlabeled Antibodies. For in vivo labeling of the monoclonal antibodies, ~1 × 10^6 cells from growing hybridoma cultures were washed with leucine-free Dulbecco's modified Eagle's medium and suspended in 1 ml of this Dulbecco's modified Eagle's medium. The cells were incubated for 20 hr in a CO2-air incubator after addition of dialyzed fetal calf serum (5%) and 5 µCi of [3H]leucine. The cells were removed by centrifugation, and the supernatant was dialyzed extensively against Hanks'-balanced salt solution. Blocking experiments were done as in Ref. 17 and are described briefly as follows. Microtiter plates were coated with 50 µl galactosyltransferase as described for solid-phase radioimmunoassay. The wells were then incubated with 50 µl of either unused medium or supernatants from cell cultures. After 18 hr of incubation at 4°C, the plates were washed and again incubated with 50 µl of labeled culture supernatant for 2 hr at 37°C, followed by 16 hr at 4°C. For each labeled supernatant, 3 separate wells were used. The plates were washed, and the radioactivity was determined after elution with 100 µl of 5% acetic acid-0.1% Nonidet P-40.

Polyacrylamide Gel Electrophoresis. Samples were heated at 100°C for 5 min in a buffer containing 0.0625 M Tris-HCl (pH 6.8), 5% (v/v) β-mercaptoethanol, 10% (w/v) glycerol, and 2.3% SDS. The proteins were resolved using Laemmli's system on 10% polyacrylamide gels. The composition of the nondenaturing slab gels was as described by Davis for disc electrophoresis (15). Separation gel was 12 cm high, on which 4 cm of the stacking gel were allowed to polymerize. Wells were formed on the stacking gel with a 2.5-cm-high plastic comb. Electrophoresis at 4°C was for 16 hr with a constant current of 3 mA and using a single slab gel.

Other Procedures. Galactosyltransferase was assayed using macro-molecular acceptors as described previously (9). Assay with GlcNAc acceptor was done as in Ref. 4, while lactose synthetase was assayed according to the method in Ref. 5. Transblotting of proteins from polyacrylamide gels to nitrocellulose sheets was done as described by Towbin et al. (27). Silver staining of the gels after Coomassie Brilliant Blue staining and immunoblotting of the nitrocellulose filters were done using Bio-Rad kits.

RESULTS

Production of Monoclonal Antibodies. Galactosyltransferase is weakly immunogenic in mice. We failed to induce the production of any monoclonal antibody by using 2 to 3 different immunization protocols but were successful following the immunization protocol described here. Even then the immunized mice had a very low titer of anti-galactosyltransferase antibody in their serum. After fusion of the spleen cells with myeloma cells, hybrid cells grew in 2 weeks in 95% of the seeded wells. For a quick screening, a solid-phase radioimmunoassay was used. Only clones with at least 10-fold radioactivity compared to the control were selected for further experiments (Table 1). Since our enyme preparation may not be 100% pure, monoclonal antibodies may be produced against highly immunogenic minor contaminants. Therefore, a second line of screening was done on the basis of the ability of the antibodies to precipitate galactosyltransferase. We detected various levels of galactosyltransferase activity in the culture supernatants from these hybrid clones. No such enzyme activity was found in the unused culture medium, suggesting that some galactosyltransferase is being shed by these hybrid cells into the media. However, the enzyme shed by the hybrid mouse cells cannot be precipitated by the rabbit antimouse immunoglobulin absorbed to IgGsorb and therefore did not affect our assay when immune precipitates were assayed only for enzyme activity. This background galactosyltransferase activity obviously will affect the assay of the residual enzyme activity after immunoprecipitation. Heating at 56°C for 30 min

<table>
<thead>
<tr>
<th>Culture</th>
<th>cpm in wells coated with the galactosyltransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td>7,321</td>
</tr>
<tr>
<td>6°C</td>
<td>5,558</td>
</tr>
<tr>
<td>10°C</td>
<td>3,268</td>
</tr>
<tr>
<td>100°C</td>
<td>2,104</td>
</tr>
<tr>
<td>111°C</td>
<td>6,684</td>
</tr>
<tr>
<td>P3-Ne1/Ag4-1</td>
<td>224</td>
</tr>
<tr>
<td>Medium</td>
<td>194</td>
</tr>
</tbody>
</table>

*cpm are average values from 3 different wells. S.D. were within 10%. The assay procedure is described in "Materials and Methods." Total cpm added per well were about 50,000 cpm.

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completely destroyed the activity of galactosyltransferase released into the media by the hybrid cells. The heating does not appear to affect the enzyme-binding capacity of the monoclonal antibodies, since the activities of the immune precipitates by both heated and unheated culture supernatants were the same.

Assay of the immune precipitate was also very sensitive. The activity of the positive clones varied from 10 to 120-fold compared to the controls. Assay of the residual enzyme activity after immune precipitation cannot detect reliably the positive clones, unless they have high activity as shown by clones 4C6 and 6C2 (Chart 1). The assay of the residual activity, however, is necessary at least for the initial screening, since enzyme activity will not be detectable in an immune precipitate formed by a monoclonal antibody directed against the active site of the enzyme.

Immunoprecipitates formed by all the monoclonal antibodies described here showed appreciable enzyme activity. This suggests that none of these antibodies are directed against the active site of the enzyme. This was further confirmed when no inhibition was detected when the enzyme activity was determined without any immune precipitation in the presence of these culture supernatants (data not shown).

Isotype of the Monoclonal Antibodies. Monoclonality of the hybrid cells were established by 2 cycles of limiting dilution. The immunoglobulin isotypes of these monoclonal antibodies were determined by immunodiffusion of 20-fold-concentrated culture supernatants against rabbit antiserum specific for mouse IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA (Littion-Bionetics, Kensington, MD). In each case, a single precipitin line was obtained. Clones 4C6, 6C2, and 11D1 were IgG1, type while 10A3 and 10D4 were IgM type. For the latter 2 clones, low cpm were obtained in both types of screening procedures (Table 1; Chart 1). This is probably due to our using rabbit anti-mouse immunoglobulin, which are mostly directed against IgG type and contain low titer against IgM isotype.

All the 5 monoclones remained stable for over 1 year in the culture medium and can be grown as ascites in syngenic BALB/c mice.

Titration of the Monoclonal Antibodies in the Ascites of Mice Given Injections of Hybrid Cells. These monoclonal antibodies have very high affinity for galactosyltransferase and can precipitate the enzyme from crude extracts or purified preparations at high dilutions (Chart 2). A control experiment with another IgG1 monoclonal antibody ID3, which recognizes an antigen from ovarian mucinous tumor (3), failed to precipitate galactosyltransferase from these extracts. The 2 clones 10A3 and 10D4 had very low titers and cannot be diluted more than 10-fold.

The IgG from these ascitic fluids were purified by DEAE-Affigel blue chromatography (6). On SDS-gel electrophoresis, all of them showed only one type of heavy chain (M, 56,000) and one type of light chain (M, 23,000) together with some faintly detectable contaminants at the M, 68,000 and M, 74,000 region (data not shown).

Analysis of these antibodies by Scatchard plot showed high binding affinities (Kd ranging from 0.16 to 0.97 × 10^{-9} M). The linearity of these curves suggests antibody homogeneity (Chart 3).

Blocking of the Binding of Labeled Monoclonal Antibodies to Galactosyltransferase by Various Unlabeled Monoclonal Antibodies. In order to determine whether these antibodies recognize the same or adjacent epitopes or unique epitopes, cross-blocking experiments were done. Each antibody was labeled in vivo with [3H]leucine and dialyzed extensively to remove free leucine. About 30 to 50% of the radioactivity after this treatment was precipitated by trichloroacetic acid. The cpm with clone 10D4 were too low to be useful in 2 separate experiments. The data of this cross-blocking experiment, summarized in Table 2, suggest that clones 4C6 and 11D1 recognize the same or adjacent determinants on the enzyme molecule. The epitopes
recognized by 6C2 and 10A3 were unique. The epitope recognized by 10D4 is probably different at least from those recognized by 6C2 and 11D1.

Immunoblotting of Galactosyltransferase with 4C6 Monoclonal Antibody. Galactosyltransferase was purified over 100,000-fold by 3 steps of affinity chromatography from the ascites of ovarian cancer patients. SDS-polyacrylamide gel electrophoresis of this material followed by staining with Coomassie Brilliant Blue showed a single protein band. However, when these gels were counterstained with silver staining, other minor bands can be seen (Fig. 1, Lane G). Although our monoclonal antibodies can specifically precipitate the enzyme from crude extracts, it can be still argued that these monoclonal antibodies are directed against some of these contaminants, and the enzyme is being coprecipitated from the extracts by being tightly bound to these contaminants. Wilson et al. (30) demonstrated that human immunoglobulins remain tightly bound to galactosyltransferase throughout various affinity chromatographic steps. The following experiment was done to rule out this possibility. Enzyme extracts at various stages of purification were electrophoresed in SDS-polyacrylamide gels followed by staining of one gel with Coomassie Brilliant Blue and silver staining (Fig. 1, Lane G). Proteins from second gel run in parallel were electrophoretically transferred to nitrocellulose filter, treated with monoclonal antibody 4C6 and stained with peroxidase using a Bio-Rad immunoblot kit (Fig. 1, Lanes A' to G'). For better resolution, the amount of protein applied per well was kept within 100 μg. For Lanes A and B, this corresponded to less than 2 ng of galactosyltransferase where no immunoband could be detected (Lanes A', B'). When the enzyme was purified by one step of affinity chromatography (>2,000-fold), a band at 47,000 was developed immediately during staining (Fig. 1, Lane C'). This band became more intense with increasing purification. Two additional bands at M, 52,000 and M, 54,000 regions appeared when the enzyme passed through one more step of affinity chromatography (>34,000-fold purification. Lane E'). The final preparation, which was more than 114,000-fold purified with respect to the ascites, showed all 3 bands and an additional faint band at M, 44,000 (Fig. 1, Lane G').

Results of the immunoblotting experiment with all 5 monoclonal antibodies and purified galactosyltransferase are shown in Fig. 2. All of them showed the M, 47,000 major band, but M, 52,000 and M, 54,000 components were detectable only with 4C6 monoclonal antibody. Antibody HDn, which appeared to recognize the same epitope on the enzyme molecule as does 4C6 (Table 2), did not show these bands. This observation led us to conclude that the epitopes recognized by 4C6 and 11D1 may be adjacent but not the same.

| Table 2 |
|------------------|------------------|------------------|------------------|------------------|
| **Blocking of the binding of labeled monoclonal antibodies to galactosyltransferase by various unlabeled monoclonal antibodies** | **cpm with following unlabeled antibodies for blocking** |
| **3H-Antibody** | None | 4C6 | 6C2 | 11D1 | 10A3 | 10D4 |
| 4C6 | 1208 ± 92 | 688 ± 6 | 1039 ± 59 | 871 ± 24 | 1112 ± 56 | ND |
| 6C2 | 591 ± 48 | 559 ± 12 | 416 ± 11 | 524 ± 31 | 549 ± 14 | 565 ± 17 |
| 11D1 | 942 ± 20 | 584 ± 14 | 937 ± 75 | 754 ± 21 | 915 ± 43 | 921 ± 26 |
| 10A3 | 675 ± 55 | 632 ± 11 | 605 ± 8 | ND | 448 ± 17 | ND |

* Culture medium.
* Mean ± S.D. of 3 values.
* ND, not determined.

Chart 3. Scatchard analysis of anti-galactosyltransferase monoclonal antibodies. The procedure is described in "Materials and Methods." Dilutions of the ascites were as follows: 4C6, 1:500; 6C2, 1:1000; 11D1, 1:250.
Immunoprecipitation of Various Reactions Catalyzed by Galactosyltransferase. The purified galactosyltransferase catalyzed 4 transfer reactions, namely, transfer of galactosyl to Reaction 1, fetuin (-AcNeu, galactose) forming alkali-stable \( \beta \)-galactosidase-sensitive product; Reaction 2, bovine submaxillary gland mucin (-AcNeu) forming alkali-labile \( \beta \)-galactosidase-sensitive product; Reaction 3, to free GlcNAc-forming N-acetylgalactosamine; Reaction 4, to glucose in the presence of \( \alpha \)-lactalbumin-forming lactose (results to be published). Previous studies in milk and rat liver have suggested that Reactions 1, 3, and 4 are probably catalyzed by the same enzyme (4, 5). Berger et al. (1) obtained some data which suggested that Reaction 2 also is catalyzed by the same enzyme in human serum. By titration of these activities in a crude extract by a polyclonal anti-galactosyltransferase antibody raised in rabbits, we found that the titration curves were parallel, suggesting that the reactions are catalyzed by the same protein. Since a monoclonal antibody recognizes a single determinant in a protein, precipitation of these activities will further prove that these reactions are catalyzed by the same protein. Results summarized in Table 3 showed that the distributions of the activities in the monoclonal immune precipitate and supernatants were similar for every acceptor after treatment with 3 different monoclonal antibodies.

Slab Gel Electrophoresis of Galactosyltransferase under Nondenaturing Conditions. Gel electrophoresis of galactosyltransferase in nondenaturing gels produced a diffused protein pattern in which at least 3 distinct bands can be seen (Fig. 3, Lane A). At a higher current, the protein formed a smear. Incorporation of Triton X-100 in the gels did not affect this pattern. After immunoblotting with 3 monoclonal antibodies, the diffused pattern containing 2 bands can be seen (Lanes B, C, and D), suggesting that the extra band on the top of the gel is a contaminant. A well on the slab gel was sliced horizontally into 2-mm pieces and crushed in test tubes with blades. The enzyme from the gel slices was extracted by incubation at 37°C for 1 hr with 0.5 ml of a buffer containing 100 mM cacodylate (pH 5.3), 0.01% Triton X-100 and heated bovine serum albumin (1 mg/ml). Ten \( \mu l \) extract from each tube were then assayed using the 4 acceptors. The activity profiles of the enzyme with these acceptors coincided (Fig. 3, upper curve), and all these activities were present only in the stained area. These data further proved that the same protein molecule catalyzed these reactions.

DISCUSSION

Serum levels of galactosyltransferase in cancer patients (2, 9, 19, 23) and in animals bearing tumors (7) are good indicators of the tumor burden. Occasional false-positive and false-negative values (8, 10, 23) in several follow-up studies may have been caused by specific activators or inhibitors or endogenous acceptors of this enzyme which have sometimes been detectable in these pathological fluids (25). One can eliminate these interferences by assay of the enzyme protein rather than the enzyme activity, by assays such as radioimmuno- or immunoradiometric assays. From this panel of antibodies, we can choose several pairs to set up a 2-site radioimmunometric assay, the most sensitive design for such assays. This is because at least 3 of them detect distinct epitopes on the enzyme molecule (Table 2) and showed high affinity for this enzyme (Charts 2 and 3).

Since this enzyme is weakly immunogenic, it is important to establish that the monoclonal antibodies are indeed against the enzyme and not against any high immunogenic minor contaminant. These antibodies can precipitate about 80% of the enzyme from crude extracts at very dilute concentrations (Table 3, clone 6C2). Had these antibodies been directed against any contaminant, most of this enzyme must be rather tightly bound to this putative contaminant during immunoprecipitation reactions. The immunoblotting experiment described in Fig. 1 showed that the intensity of staining with monoclonal antibodies of the M, 47,000 major protein band, increased with increasing purification of the enzyme. If these antibodies were directed against any contaminant, the M, 47,000 major protein band must be such putative contaminant which have been purified together with the enzyme during various affinity chromatographic steps. Such a tight noncovalent association of a contaminant with the enzyme under such diverse experimental conditions is very unlikely. In the nondenaturing gels, although a diffuse band was obtained (Fig. 3), all of the enzyme activity was associated with the area of filters stained by these antibodies, suggesting that these are indeed directed against the enzyme.

We established the monoclonality of these hybrid cells by 2 cycles of limiting dilution. SDS-polyacrylamide gel electrophoresis of the purified IgG from antibodies showed only one type of heavy chain and one type of light chain (data not shown). Scatchard analysis data (Chart 3) also showed one type of binding, suggesting that these are indeed homogeneous antibodies. Detection of 2 bands at M, 54,000 (Figs. 1 and 2) with clone 4C suggests that these are either precursor proteins or proteins modified by glycosylation or by other mechanisms. The band at M, 44,000 may be a degradation product. It is interesting that the distinctive stained pattern of the SDS gels with clone 4C is no longer present in nondenaturing gels (Figs. 2 and 3).

### Table 3

Various reactions catalyzed by galactosyltransferase in the immune precipitates and supernatants

A dilute solution of purified galactosyltransferase was treated with 1:100 diluted ascites or buffers, followed by precipitation with rabbit anti-mouse immunoglobulin absorbed in IgG sorb as described for screening of the hybridomas in "Materials and Methods." The pellet after washing was suspended in 100 \( \mu l \) PBST. Ten-\( \mu l \) aliquots from supernatant and pellet suspension were assayed for various galactosyltransferase reactions.

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Fetuin (-AcNeu, Gal)</th>
<th>BSM (-AcNeu)</th>
<th>GlcNAc</th>
<th>Glc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppt.</td>
<td>sup.</td>
<td>ppt.</td>
<td>sup.</td>
</tr>
<tr>
<td>4C</td>
<td>2.01 (49) ( ^a )</td>
<td>2.06 (51)</td>
<td>0.20 (56)</td>
<td>0.16 (44)</td>
</tr>
<tr>
<td>6C</td>
<td>3.63 (87)</td>
<td>0.54 (13)</td>
<td>0.30 (72)</td>
<td>0.12 (28)</td>
</tr>
<tr>
<td>1ID</td>
<td>1.94 (51)</td>
<td>1.87 (49)</td>
<td>0.17 (43)</td>
<td>0.18 (51)</td>
</tr>
<tr>
<td>Buffer</td>
<td>0.06 (1)</td>
<td>6.88 (99)</td>
<td>0.03 (7)</td>
<td>0.47 (93)</td>
</tr>
</tbody>
</table>

\( ^a \) Gal, galactose; BSM, bovine submaxillary gland mucin; Glc, glucose; ppt., precipitate; sup., supernatant.

\( ^b \) Numbers in parentheses, percentage of activity distributed between the supernatant and pellet.
This is probably due to the diffused patterns of staining in the nondenaturing gels.

It appears in Fig. 3 that the enzyme activity peak is rather symmetrical, and it coincided more closely with the second immunoband (from the top of the gel). This suggests that this band represents the active enzyme. However, during staining and electroblotting, there were changes in the gel dimensions. Moreover, the manual slicing of the gel may not be very accurate. All these factors make this conclusion uncertain. Since we have demonstrated the presence of 2 active components of galactosyltransferase by chromatography in a concanavalin A-Sepharose 4B column (12), it is more likely that both immunobands represent the active enzyme. However, this remains to be established. By short-time incubation of the gel slices near the peak areas, we could not thus far resolve the enzyme activity peak shown in Fig. 3 into more than one component.

Galactosyltransferases are highly specific enzymes with respect to the donor nucleotide-sugar and the acceptor glycoconjugate (22). Precipitation by the monoclonal antibodies of all 4 reactions catalyzed by the purified galactosyltransferase is therefore surprising, especially the reactions with mucin-type and N-glycosidic-type acceptors. Our results suggest that they are due to the same protein (Table 3). Alternately, they may be catalyzed by different protein, which have these determinants recognizable by the monoclonal antibodies. Since in SDS-polyacrylamide gels single protein bands can be seen except for clone 4C4 (Fig. 2) and in nondenaturing gels all these activities overlapped (Fig. 3), this alternative explanation is unlikely.

Kim et al. (21) observed that, in cancerous colonic mucosa, the specific activity of galactosyltransferase assayed with alkali-stable N-glycosidic acceptors were significantly reduced compared to corresponding normal tissues. The levels of the enzyme assayed with mucin-type acceptors, on the other hand, were comparable in both types of tissues. These results suggest that, in colonic mucosa, 2 different enzymes carry out these 2 transfer reactions. An alternative explanation may be that the ability of the enzyme to use N-glycosidic acceptors is more labile compared to its ability to use mucin type acceptors, and this is preferentially destroyed in malignant tissues.

Although experiments from several laboratories demonstrated that galactosyltransferase resides on cell surface, some questions about the validity of these experiments remain (16, 20). These include the leaking and lysis of cells during incubation, phagocytosis of the external macromolecular acceptor, and the hydrolysis of the donor nucleotide-sugar followed by reincorporation of the free sugar intracellularly. Staining of the cells by these monoclonal antibodies will unequivocally demonstrate the presence of this enzyme on the cell surface. Moreover, any functional role of this enzyme on the cell surface can also be studied by using these antibodies.

After our work was submitted for publication, a paper describing the production and characterization of monoclonal antibodies to serum galactosyltransferase has appeared (26).

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