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MONOCLONAL ANTIBODIES TO SOLUBLE, HUMAN MILK GALACTOSYLTRANSFERASE (LACTOSE SYNTHASE A PROTEIN)*

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ABSTRACT

Monoclonal antibodies have been produced against soluble human milk galactosyltransferase of a blood group O donor. After initial screening by radioimmunoassay, fourteen hybridomas were further characterized by enzyme-linked immunosorbent assay, immunoblotting of purified enzyme following sodium dodecyl sulfate–polyacrylamide gel electrophoresis, enzyme activity modification, and enzyme localization in HeLa cells by immunofluorescence. Of these fourteen clones, seven had titers between 1500 and 7800 as estimated by ELISA. In general, the titer correlated with staining intensity on immunoblots and in immunofluorescence. In the presence of monoclonal antibody, enzyme activity was usually slightly enhanced or stabilized. Subcloning yielded four monoclonal antibody preparations designated as GT2/24/108, GT2/36/118, GT2/61/14, and GT2/77/22, which belong to Ig class G2b, G3, M, and G1, respectively. They all recognized the enzyme in purified form or in defatted milk as a single, broad band on electrophoresis–immunoblotting and produced a concise juxtanuclear fluorescence typical for the Golgi apparatus in HeLa cells.

INTRODUCTION

Monoclonal antibodies are useful reagents in biochemistry and clinical chemistry and have found widespread application for immunoaffinity purification of antigens and as serological reagents for immunocytochemistry and molecular biology (for reviews, see refs. 1–3). Thus, any compound of biological interest such as enzymes, hormones, toxins, etc. is amenable to new fields of investigation if these reagents are available.

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Glycosyltransferases are still poorly understood despite their importance in glycan biosynthesis[4]. We report herein the production and characterization of monoclonal antibodies to soluble human milk D-galactosyltransferase, an enzyme involved in chain elongation of *N*-glycoproteins or lactose biosynthesis in the lactating mammary gland[5,6] (EC 2.4.1.22 and 2.4.1.38). These antibodies have been elicited with several specific objectives: (a) They will prove useful in designing a "sandwich" ELISA procedure[7] for the determination of galactosyltransferase-immunoreactive protein in clinical chemistry[8], (b) these antibodies will be of use in double-labeled immunofluorescence for comparative localization of galactosyltransferase with other antigens, (c) they may prove useful in determining structural differences between charged isoforms of galactosyltransferase[9], and (d) they may be of help in the investigation of possible functions of ecto-galactosyltransferase in cell adhesion and recognition[10].

EXPERIMENTAL

Materials. — Human milk galactosyltransferase was purified from a single donor having blood group O as described previously[6,9]. HeLa cells were grown in minimal essential medium (Gibco) on glass cover slips. Human α-lactalbumin was purified from human whey by gel filtration on Sephadex G-100 (height of column 180 cm, 9000 mL) as described[11]. Ovalbumin, bovine serum albumin (BSA), and UDP-D-galactose were from Sigma, and antisera to mouse immunoglobulins from Nordic. Chemicals: UDP-D-[6-^{3}H]galactose was from Amersham; and hypoxanthine, aminopterine, and thymidine (for HAT medium) were from Sigma. All chemicals were of analytical grade.

Production of monoclonal antibodies. — A protocol described earlier[12] was adapted as follows. Balb/c mice were immunized intradermally or intraperitoneally with galactosyltransferase (50 μg) adsorbed to $Al(OH)_3$ in complete Freund's adjuvant. Three weeks later, a booster injection of galactosyltransferase (50 μg) in incomplete Freund's adjuvant was given intraperitoneally. A week later, mice were tail bled and antibody titers were measured in the sera by the solid-phase radioimmunoassay as described later. The route of administration did not significantly influence the immune response. After another 4 to 12 weeks, a final booster injection of galactosyltransferase (25 μg) in phosphate-buffered saline (PBS) intravenously and galactosyltransferase (25 μg) in PBS intraperitoneally was applied, followed by cell fusion after three days. PAI myeloma cells[13] (10^8) were fused with spleen cells[14] (2×10^8), and the fusion mixture was distributed into ten 96-well microtiter plates. Hybridomas were selected in HAT medium[15] and screened for antibody production by solid-phase radioimmunoassay[16] (RIA). Briefly, galactosyltransferase (1 μg/mL in PBS) was adsorbed to flexible PVC multiwell plates overnight. Wells were treated sequentially with blocking solution (2% bovine serum albumin in PBS, w/v) and rabbit anti-mouse immunoglobulin (50 μg/mL of PBS supplemented with 0.1% BSA). Radioactivity in individual wells

was measured and positive cultures were expanded to 100-mm plates at which stage the cells were frozen. Selected hybridomas were thawed and cloned by limited dilution. The resulting positive clones were further expanded and frozen; respective culture supernatants were concentrated by $(NH_4)_2SO_4$ precipitation at 50% saturation. The monoclonal antibodies were classified by double immunodiffusion[17] using class-specific antisera to mouse immunoglobulins.

Determination of galactosyltransferase activity. — UDP-galactose: ovalbumin galactosyltransferase activity was measured in an assay volume of 100 μL containing enzyme (20 μL), $MnCl_2$ (1 μmol), Na cacodylate (10 μmol) (adjusted to a pH of 7.35), ovalbumin (1 mg), UDP-D-[6-^{3}H]galactose (specific activity 5.0 Ci · mol^{-1}) (20 nmol); incubation was for 15 min at 37° and was terminated by precipitating ovalbumin with ~1 mL of ice-cold $H_3[P(W_3O_{10})_4]$ · 14 H_2O (phosphotungstic acid) (5%, w/v) dissolved in 2M HCl; the precipitate was filtered through glass-fiber filters (Whatman GF/A), washed with ice-cold ethanol, and the radioactivity determined by toluene-based scintillation counting (counting efficiency ~40%).

UDP-D-galactose:D-glucose galactosyltransferase (lactose synthase) activity was measured in an assay volume of 50 μL containing enzyme (10 μL), Na cacodylate (2.5 nmol) (pH 7.35), $MnCl_2$ (0.5 μmol), human α-lactalbumin (50 μg) (or, for control, 50 μg of bovine serum albumin), D-glucose (0.5 μmol), and UDP-D-[6-^{3}H]galactose (specific activity 5 Ci · mol^{-1}) (20 nmol); incubation was for 30 min at 37° and was terminated by adding exactly 20 μL of 0.1M EDTA; 35 μL of the reaction mixture was deposited on Whatman No. 1 paper and subjected to high-voltage electrophoresis in 1% (w/v) $Na_2B_4O_7$ for 60 min at 42 V · cm^{-1}. Standards included lactose (25 μg), D-glucose (25 μg), and UDP-D-[6-^{3}H]galactose (55 000 d.p.m.), and were developed according to Trevelyan *et al.*[18] or by cutting out 2-cm slices of the corresponding paper lane to measure radioactivity by liquid-scintillation counting.

Electrophoresis. — Sodium dodecylsulfate–polyacrylamide gel electrophoresis was carried out in 12.5% (w/v) polyacrylamide gels according to Laemmli[19] as modified by Maniatis *et al.*[20].

Immunoblotting. — Slab gels obtained by sodium dodecyl sulfate($NaDodSO_4$)–polyacrylamide gel electrophoresis were subjected to electroblotting onto nitrocellulose BA85 sheets (Schleicher & Schuell) according to Towbin *et al.*[21]; transfer buffer was 20mM tris(hydroxymethyl)aminomethane (Tris), 150mM glycine, and 20% (v/v) methanol; transfer was overnight at 36 V and cooled by running tap water.

After blotting, nitrocellulose sheets were treated with 3% BSA in Tris-buffered saline solution (TBS) overnight, washed with TBS, and then incubated with undiluted hybridoma supernatant or ascites for 30 min at room temperature, washed (5 times) during 5 min with TBS, and incubated with rabbit anti-mouse Ig-peroxidase (Nordic), diluted 1:1000 in TBS containing 3% BSA. After a washing as just described, the chromogenic-substrate solution [3,3′-diaminobenzidine (25 mg) in 0.1M Tris (50 mL), pH 7.6, and 0.01% H_2O_2, v/v] was added.

Immunofluorescence. — HeLa cells were grown on glass cover slips which were placed in small Petri dishes containing minimal essential medium supplemented with 5% fetal calf serum. After aspiration of the medium, the cells were washed with prewarmed PBS containing 0.1M Ca^{2+} and 0.1M Mg^{2+}. To fix and permeabilize the cells, the cover slips were treated with ice-cold acetone for 15 min, and then briefly dipped in PBS, followed by water, and air-dried. The primary antibody (25 μL) was spotted onto parafilm, and the cover slip inverted and placed on the antibody drop; capillarity insured an even distribution of antibody solution over the whole cover slip. The cover slips were returned to the Petri dish and washed with PBS. The whole procedure was repeated for incubation with the second antibody, either using rabbit anti-mouse Ig-FITC or swine anti-rabbit Ig-FITC (Sevac). The cover slips were then washed again and mounted cell-face down on glass slides in a medium described by Heimer and Taylor[22]. Fluorescence was observed in a Leitz–Diavert microscope equipped for epifluorescence; photographs were taken on an Ilford HP5 film (400 ASA).

ELISA. — Microtiter plates (Immulon, Nunc) were coated overnight at 4° with purified galactosyltransferase (110 μL, 1 μg/mL) or, for control, α-lactalbumin (1 μg/mL) in 0.1M carbonate buffer, pH 9.6. All subsequent steps were carried out at room temperature. The plates were washed three times with 0.9% (w/v) NaCl containing 0.05% (v/v) Tween 20, and then blocked with BSA (0.5% w/v) in PBS for 60 min, washed, and incubated with serially diluted antibody solution for 4 h. After 3 washes with NaCl–Tween, the second antibody (100 μL), *i.e.*, rabbit anti-mouse Ig-peroxidase diluted 1:1000 with PBS–Tween, was added, left for 1 h, and then removed by washing three times with NaCl–Tween. Peroxidase was measured with 0.04% (w/v) *o*-phenylenediamine (1,2-diaminobenzene) (200 μL) in a buffer containing 0.1M citric acid, 0.2M Na_2HPO_4, and 0.01% H_2O_2 (v/v). After 5–15 min incubation, the reaction was stopped by adding 1.25M H_2SO_4 (40 μL). The optical density of wells were assessed in an automatic ELISA plate spectrophotometer (Titertek Multiskan, Flow) at 492 nm.

RESULTS

The antibodies described herein were obtained in a single-fusion experiment with one mouse spleen. Out of 960 tested culture supernatants, 41 produced antibodies to galactosyltransferase as measured by solid-phase radioimmunoassay (RIA). After expanding the positive hybridomas to 100-mm plates, 14 cultures continued to secrete antibodies to galactosyltransferase. These 14 hybridomas were further characterized by titer determination as shown in Fig. 1. Only half of the 14 supernatants reacted against galactosyltransferase in solid-phase, enzyme-linked immunosorbent assay (ELISA), and reached different saturation levels. Under identical conditions, polyclonal, affinity-purified antibodies always reached the highest saturation level (not shown). One of the antibodies reacted more strongly with α-lactalbumin (used for control) than with galactosyltransferase, whereas all

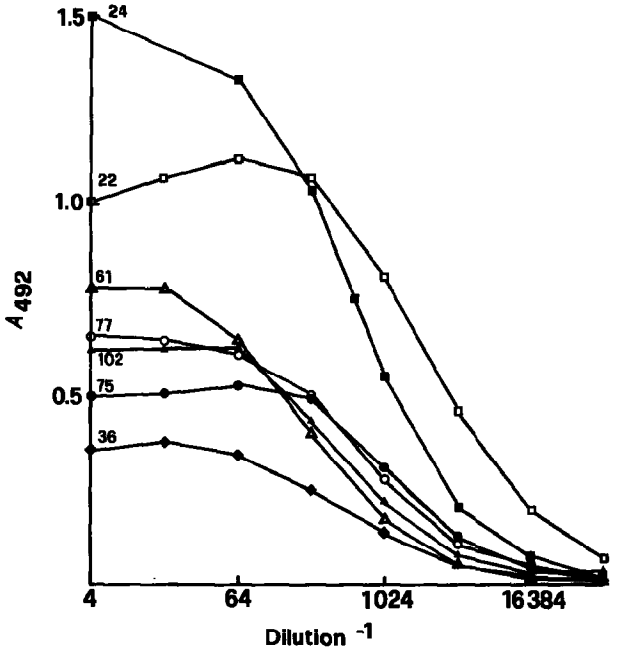

Fig. 1. Antibody titer determination by ELISA of hybridoma supernatants after primary screening. The ELISA procedure is described in the Experimental section. Supernatants were incubated in serial, four-fold dilutions from saturation (left) to background readings.

the others were negative. Titers, as estimated by half the absorbance reached at saturation, ranged between 1:1500 and 1:7800 (*cf.* Table I). The effect of these 14 supernatants on galactosyltransferase activity using ovalbumin as acceptor substrate was then investigated. For this purpose, all supernatants were heat treated for 60 min at 56° in order to inactivate low but substantial, endogenous galactosyltransferase activity released from hybridoma cells. The result is shown in Fig. 2 and indicates an activating effect of supernatants GT2/36 and GT2/24, and slight inhibition by supernatant GT2/10, which remained stable for at least one week. In some instances (*e.g.*, GT2/36), activation was found to be a slow process. Interestingly, activation by GT2/36 was also noticed in the case of lactose synthase activity, whereas GT2/10 inhibited weakly and polyclonal antibody strongly. In absence of α-lactalbumin, galactosyltransferase incubated with GT2/36 was still able to catalyze the formation of lactose to an extent of 20% of the value recorded in the presence of α-lactalbumin, whereas all other supernatants did not exert this effect.

The supernatants were further characterized by electroblotting of purified galactosyltransferase onto nitrocellulose sheets and immunostaining. A qualitative correlation between reaction in solid-phase ELISA and immunoblotting was observed. The supernatants that reacted positively in ELISA (*cf.* Fig. 1) also

TABLE I

REACTIVITY OF HYBRIDOMA SUPERNATANTS WITH GALACTOSYLTRANSFERASE

Supernatant GT2/[a]	*Titer*[b]	*Maximum effect on galactosyltransferase activity*[c]	*Immunoblotting*[d]	*Immunofluorescence*[e]
10	0	−19		CA
36	500	+25	S	G
62	0	+11		Nucleoli
75	2000	+10	S	G, C
37	0	+13		(G)
102	2600	+4	D	Sk
24	2700	+17	D	G, C
28	0	0		
22	7800	0	S	G
87	0	+13	S	G, C
86	0	0	S	(G), C
77	3300	+12	S	G, (C)
106	0	0		
61	1500	−1	D	G, (C)
PA[f]		−80	D	G

[a]Hybridoma supernatants. [b]As measured by the ELISA method (*cf.* Fig. 1). [c]Percent of nonimmune hybridoma supernatant. [d]D, diffuse; S, sharp. [e]G, Golgi; C, contact sites; Sk, cytoskeletal elements; and CA, punctate cytoplasmic appearance. [f]PA, polyclonal rabbit antiserum.

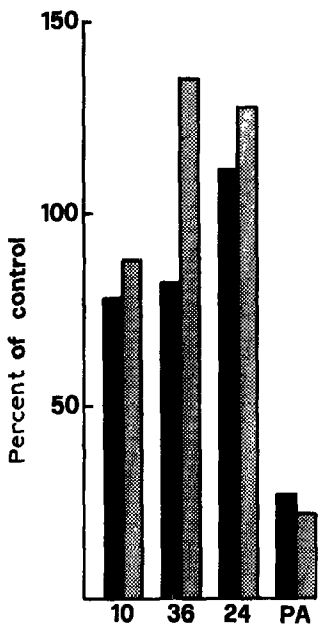

Fig. 2. Effect of antibody on galactosyltransferase activity. Purified enzyme from human milk (3 μg) was diluted with 10mM Tris buffer, pH 7.4, containing BSA 0.1% (w/v) (1 mL). Equal volumes of undiluted hybridoma supernatant or rabbit antiserum, all treated for 1 h at 56° to inactivate endogenous galactosyltransferase activity, were added to the enzyme solution to obtain a final volume of 100 μL. After 2 (dark bars) or 9 (light bars) days, galactosyltransferase activity was measured in aliquots of antigen–antibody mixture.

reacted with blotted enzyme; the intensity of immunostaining, however, did not closely correlate with the titer (not shown).

Finally, one of the most reliable ways to specifically identify an antigen is its detection by immunofluorescence *in situ*, provided that a specific localization is known. Such studies were carried out in HeLa cells that were fixed and permeabilized by acetone as previously described[23]. These studies demonstrated that, in HeLa cells, galactosyltransferase was always detected by use of polyclonal antisera exclusively in the Golgi apparatus[23,24]. This characteristic localization, therefore, can be applied to further define the specificity of monoclonal antibodies. A clear Golgi picture, as shown on Fig. 3B, was produced with all supernatants that were positive in ELISA and by immunoblotting. However, a few unexpected stainings were observed also with supernatants having apparent anti-galactosyltransferase specificity, as indicated in Table I. GT2/102 yielded a picture on which some cytoskeletal elements were visualized and, in addition, a clear punctate pattern confined to nuclei was found in single cells that were not surrounded by others (Fig. 3D). GT2/24, GT2/87, and GT2/86 produced clear Golgi stainings but, in addition, a fluorescence delimited to contact sites of the cell surface (Fig. 3C). The supernatants which were negative in ELISA and by blotting did not react with the Golgi apparatus; GT2/62 was able to make visible nucleoli, and GT2/10

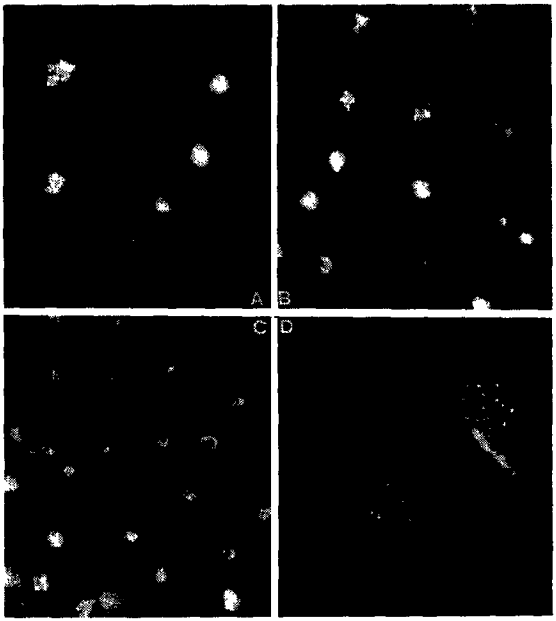

Fig. 3. Immunofluorescent staining of galactosyltransferase in HeLa cells using mono- and poly-clonal antibodies. The procedures are described in the Experimental section: (A) Affinity-purified polyclonal rabbit antiserum. The brillant spot corresponds to the Golgi apparatus in a compact juxtanuclear location; (B) GT2/61; (C) GT2/24; and (D) GT2/102.

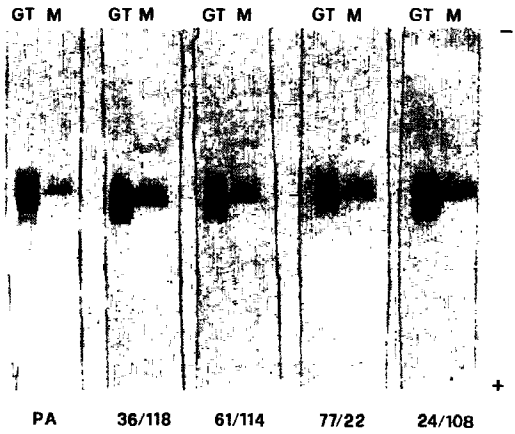

Fig. 4. Immunoblotting of purified and crude milk galactosyltransferase by subcloned and polyclonal antibodies. The procedures are described in the Experimental section. Defatted, fresh human milk of a blood group O donor (M) and purified galactosyltransferase (GT) (10 μL and 2 μg, respectively, per slot) were subjected to $NaDodSO_4$–PAGE–immunoblotting. The numbers indicate the hybridoma subclone. Usually the enzyme moves as a broad band which indicates microheterogeneity[9] and corresponds to an apparent molecular size of 55 kDa (refs. 9 and 27).

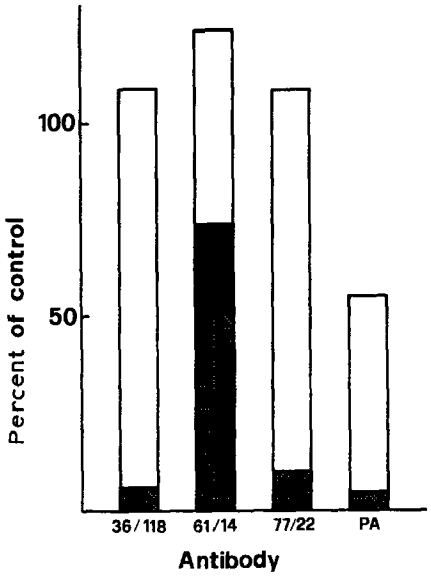

Fig. 5. Immunoprecipitation of galactosyltransferase activity by mono- and poly-clonal antibodies. The enzyme solution (100 μL), as described in the legend to Fig. 2, was incubated with hybridoma supernatant, affinity-purified rabbit antibody, or control supernatant (20 μL each) for 2 h at room temperature. Addition of a 10% suspension of *Staphylococcus aureus* (Cowan strain) did not precipitate activity at this time (open bars). The second antibody was then added (goat anti-rabbit Ig for the polyclonal antiserum and rabbit anti-mouse Ig for the hybridoma supernatants), followed by overnight incubation at 4° and addition of *S. aureus* A and centrifugation for 5 min at 12 000 r.p.m. Galactosyltransferase activity was then measured in the supernatant (closed bars).

produced a diffuse punctate pattern spread over the whole cytoplasm (not shown). A summary of these experiments is given in Table I.

We decided to subclone those hybridomas that exhibited the best defined anti-galactosyltransferase specificity. They included GT2/36/118, GT2/24/108, GT2/77/22, and GT2/61/14. These antibodies were reevaluated by ELISA, immunoblotting, effect on enzyme activity, and immunofluorescence in HeLa cells. Essentially identical results were obtained, except that contact sites in HeLa cells were no longer visible. Their immunoglobulin class was determined by double immunodiffusion and was G2b for GT2/24/108, G3 for GT2/36/118, M for GT2/61/14, and G1 for GT2/77/22. In order to test the potential usefulness of these monoclonal antibodies for clinical chemistry, their possible crossreactivity with other milk components (as source of antigen) was evaluated by $NaDodSO_4$–PAGE–immunoblotting. Monospecificity and lack of visible crossreactivity of affinity-purified polyclonal antiserum were observed (Fig. 4), but all four monoclonal antibodies appeared to cross-react weakly with other milk components the nature of which is at present unknown.

Since binding to solid-phase antigen, as measured by ELISA, appeared to be different among monoclonal antibodies, we compared also their reactivity with enzyme in soluble phase by an immunoprecipitation of immune complexes that uses a second antibody and protein A–Sepharose, followed by enzyme measurements in the supernatant. The enzyme activity could be removed from the solution by all the monoclonal antibodies including the polyclonal antiserum, except by GT2/61/14 (Fig. 5). This latter antibody preparation, in contrast, bound avidly to solid-phase antigen (not shown), which indicated that GT2/61/14 binds to an epitope that has become more accessible after binding to a solid phase.

DISCUSSION

Monoclonal antibodies to human serum galactosyltransferase have been described and characterized by solid-phase binding assay[25], by immunoprecipitation from soluble phase and by immunoblotting[26]. We report herein the production of monoclonal antibodies to soluble, human milk galactosyltransferase and their characterization. This included binding to solid-phase antigen after coating to wells of microtiter plates (Fig. 1) and after electroblotting on nitrocellulose following $NaDodSO_4$–PAGE (Table I, Fig. 4), *in situ* immunostaining in HeLa cell Golgi apparatus (Fig. 3), and comparison with well characterized, monospecific polyclonal antibodies[23,24,27]. In addition, binding of monoclonal antibodies was also investigated in solution by assessing their effect on enzymes by use of a second antibody (Fig. 5). Some striking differences between mono- and polyclonal antibodies were noted, *i.e.*, monoclonal antibodies usually did not inhibit the enzyme activity, whereas polyclonal antisera were always strongly inhibitory[27]. In one instance, monoclonal antibodies mimicked the modifying effect of α-lactalbumin on galactosyltransferase to an extent of 20%; usually they were slightly

activating or, at least, stabilized the enzyme activity. An elegant way to demonstrate enzyme activation by monoclonal antibodies was described by Frackelton *et al.*[28] who could demonstrate activation of a genetically defective β-galactosidase of *E. coli* by monoclonal antibodies. This approach may prove useful to probe for defective galactosyltransferase variants in lectin-resistant cell lines as described by Stanley[29]. One of the most reliable ways to determine specificity of monoclonal antibodies is by immunocytochemical methods, provided that the specific localization of an antigen is known. In the case of galactosyltransferase, a specific Golgi staining in HeLa cells has been described at the light[23] and electron microscopic level[24]. The Golgi staining observed with most of the monoclonal antibodies provided strong evidence for specificity. In this context, it is worth mentioning that Golgi-specific reagents for immunocytochemical determination have only been available since recently and are, except for the antibodies described herein, directed against poorly characterized antigens[30–32]. In some of our hybridomas, specificities other than for Golgi components were detected. In particular, one of the supernatants contained antibodies specific for "contact sites" of confluent HeLa cells that had a cobble-stone appearance on immunofluorescence (Fig. 3C). Whether this cell-surface reactivity relates to ecto-galactosyltransferase, as for instance in intestinal cells[33], needs further evaluation. A similar picture has been produced by use of a polyclonal antiserum against soluble, bovine milk galactosyltransferase applied to fixed, but not permeabilized F9 embryonic carcinoma cells[34]. The antibodies presently available are specific only for the Golgi apparatus as judged by immunofluorescence in HeLa cells.

One of the immediate objectives in the production of monoclonal antibodies was the development of a "sandwich"-ELISA procedure[7] for which two antisera with different specificities are advantageous. Thus, only hybridomas that demonstrated unequivocal specificity for galactosyltransferase as determined by the above criteria were further cloned. To our surprise, additional bands were made visible by immunoblotting–NaDodSO$_4$–PAGE of human milk which remained unstained by affinity-purified polyclonal antisera. Whether this finding indicates true crossreactivity to unrelated proteins remains to be investigated. Since the monoclonal antibodies will eventually serve to adsorb the soluble enzyme from serum, we determined the ability of our subcloned antibodies to bind the enzyme from the soluble phase. Although the polyclonal antiserum was able to immunoprecipitate the enzyme by use of the second antibody, the monoclonal antibody that showed highest affinity towards solid-phase enzyme was not able to bind to the soluble enzyme (Fig. 5). Adolf *et al.*[35] made a similar observation for yeast catalase T. One may conclude that, upon adsorption to solid-phase, galactosyltransferase unmasks an epitope that is not accessible in the soluble phase.

In conclusion, several murine monoclonal antibodies to human milk galactosyltransferase were characterized, and those that were shown to react by all criteria were subcloned and will be used for *in vitro* mass production. This procedure appears preferable for Golgi antigens, as autoantibodies directed against Golgi

components have been observed in ascites[36]. The antibodies recovered from mass-cultured hybridoma supernatants will be useful as reagents for Golgi membranes in immunocytochemical methods and may, after the possibility of crossreactivity has been eliminated, serve in the development of a "sandwich"-ELISA for the serum enzyme.

REFERENCES

1 C. MILSTEIN, *Sci. Am.*, 243 (1980) 66–123.
2 G. S. EISENBARTH, *Anal. Biochem.*, 111 (1981) 1–16.
3 P. A. W. EDWARDS, *Biochem. J.*, 200 (1981) 1–10.
4 H. SCHACHTER AND S. ROSEMAN, in W. J. LENNARZ (Ed.), *The Biochemistry of Glycoproteins and Proteoglycans*, Plenum Press, New York, 1980, pp. 85–160.
5 R. L. HILL AND K. BREW, *Adv. Enzymol.*, 43 (1975) 411–490.
6 E. G. BERGER, J. ROTH, AND G. J. A. M. STROUS, *Gann Monogr.*, 29 (1983) 207–220.
7 A. VOLLER, in E. T. MAGGIO (Ed.), *Enzyme-immunoassays*, CRC Press, Boca Raton, 1980, pp. 191–196.
8 B. VERDON, E. G. BERGER, S. SALCHLI, A. GOLDHIRSCH, AND A. GERBER, *Clin. Chem.*, 29 (1983) 1928–1933.
9 A. C. GERBER, I. KOZDROWSKI, S. R. WYSS, AND E. G. BERGER, *Eur. J. Biochem.*, 93 (1979) 453–460.
10 M. PIERCE, E. A. TURLEY, AND S. ROTH, *Int. Rev. Cytol.*, 65 (1980) 1–47.
11 F. J. CASTELLINO AND R. L. HILL, *J. Biol. Chem.*, 245 (1970) 417–424.
12 G. GALFRE, S. C. HOWE, C. MILSTEIN, G. W. BUTCHER, AND J. D. HOWARD, *Nature (London)*, 266 (1977) 550–552.
13 J. W. STOCKER, H. K. FORSTER, V. MIGGIANO, C. STAEHLI, G. STAIGER, B. TAKACS, AND T. STAEHELIN, *Res. Dis.*, (1982) 155–157.
14 S. FAZEKAS DE ST, GROTH AND D. SCHEIDEGGER, *J. Immunol. Methods*, 35 (1980) 1–21.
15 J. W. LITTLEFIELD, *Science*, 145 (1964) 709–710.
16 H. P. HAURI, E. STERCHI, D. BIENZ, J. A. M. FRANSEN, AND A. MARXER, *J. Cell. Biol.*, 101 (1985) 838–851.
17 O. OUCHTERLONY, *Prog. Allergy*, 5 (1958) 1.
18 W. E. TREVELYAN, D. P. PROCTER, AND J. S. HARRISON, *Nature (London)*, 166 (1950) 444–445.
19 U. K. LAEMMLI, *Nature (London)*, 227 (1970) 680–685.
20 T. MANIATIS, E. F. FRITSCH, AND J. SAMBROOK, *Molecular Cloning*, Cold Spring Harbor Laboratory, 1982.
21 H. TOWBIN, T. STAEHELIN, AND J. GORDON, *Proc. Natl. Acad. Sci. U.S.A.*, 76 (1979) 4350–4354.
22 G. V. HEIMER AND C. E. TAYLOR, *J. Clin. Pathol.*, 27 (1974) 254–256.
23 E. G. BERGER, T. MANDEL, AND U. SCHILT, *J. Histochem. Cytochem.*, 29 (1981) 364–370.
24 J. ROTH AND E. G. BERGER, *J. Cell Biol.*, 93 (1982) 223–229.
25 D. K. PODOLSKY AND K. J. ISSELBACHER, *Proc. Natl. Acad. Sci. U.S.A.*, 81 (1984) 2529–2533.
26 S. K. CHATTERJEE, M. BHATTACHARYA, AND J. J. BARLOW, *Cancer Res.*, 44 (1984) 5725–5732.
27 E. G. BERGER, B. VERDON, T. MANDEL, H. FEY, AND G. STROUS, *Enzyme*, 29 (1983) 175–182.
28 A. R. FRACKELTON, J. ROTMAN, AND B. ROTMAN, *J. Biol. Chem.*, 255 (1980) 5286–5290.
29 P. STANLEY, in W. J. LENNARZ (Ed.), *The Biochemistry of Glycoproteins and Proteoglycans*, Plenum Press, New York, 1980, pp. 161–189.
30 J. J.-C. LIN AND S. A. QUEALLY, *J. Cell. Biol.*, 92 (1982) 108–112.
31 B. BURKE, G. GRIFFITHS, H. REGGIO, D. LOUVARD, AND G. WARREN, *EMBO J.*, 1 (1982) 1621–1628.
32 Y. CHICHEPORTICHE, P. VASSALLI, AND A. M. TARTAKOFF, *J. Cell Biol.*, 99 (1984) 2200–2210.
33 J. ROTH, M. LENTZE, AND E. G. BERGER, *J. Cell Biol.*, 100 (1985) 118–125.
34 D. B. SHUR, *Mol. Cell Biochem.*, 61 (1984) 143–158.
35 G. R. ADOLF, E. HARTER, H. RUIS, AND P. SWETLY, *Biochem. Biophys. Res. Commun.*, 95 (1980) 350–356.
36 Z. D. J. SMITH, F. D'EUGENIO-GUMKOWSKI, K. YANAGISAWA, AND J. D. JAMIESON, *J. Cell Biol.*, 98 (1984) 2035–2046.