LECTIN AND CHOLERA TOXIN BINDING TO GUINEA PIG TUMOR (104C1) CELL SURFACES BEFORE AND AFTER GLYCOSPHINGOLIPID INCORPORATION

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SUMMARY- ^{125}I-Labeled Dolichos biflorus lectin and cholera toxin were used as probes for identification of Forssman- and GM1-type receptor sites on guinea pig tumor (104C1) cell surfaces. Increased binding of ^{125}I-labeled lectin and toxin to 104C1 cell surfaces was observed after the cells were treated with exogenous Forssman glycosphingolipid and GM1 ganglioside, respectively. Biosynthesis in vitro of these two glycosphingolipids from their precursor molecules was established using a membrane preparation isolated from confluent cultures of guinea pig tumor 104C1 cells.

Lectins, the cell agglutinating proteins, are widely distributed in plants, eel serum and snails (1-4). They specifically combine with terminal sugars and can be used as molecular probes for the detection of glycoproteins and glycosphingolipids present on cell surfaces. Some lectins have been used to quantitate the agglutinability of transformed and nontransformed cells (5-8). Several hypotheses have been proposed to explain the agglutinability of tumor and transformed cells by plant lectins. It could be due to unmasking of cryptic agglutinin receptor sites, or to increased synthesis de novo of the receptor sites in the transformed cells. It appears (9, 10) that the number of lectin receptor sites on a cell bears no relationship to its agglutinability by different lectins. The agglutination process is dependent not only on the content and the nature of the lectin bound, but also on the mobility of the receptor sites on cell surfaces (11-13). These receptors are thought to be glycoproteins (14) and glycosphingolipids (15). Until now, very little has been published regarding the incorporation of an exogenous receptor into cell surface

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membranes with a concomitant increase in specific lectin binding activity. Previously we reported (15-16) the differential binding of α-fucose-binding ^{125}I-labeled lectins (Ulex europeus and Lotus tetragonolobus) to eukaryotic primate cell surfaces (monkey kidney fibroblasts, Vero). Nonprimate cell surfaces, such as mouse neuroblastoma (adrenergic N1E-115 and NS-20) contain predominantly B-active glycosphingolipids, quantitated by binding to ^{125}I-labeled Bandeiraea simplicifolia lectin (17). We now report the results of studies on the binding of ^{125}I-labeled Dolichos biflorus and cholera toxin to 104C1 (guinea pig tumor (18, 19)) cell surfaces after addition of exogenous Forssman glycosphingolipid and GM1 ganglioside, respectively. The Forssman antigen has lately been implicated in density dependent inhibition and tumorigenicity in BHK (20) and NIL hamster cells (21-24). Studies on the binding of cholera toxin to isolated rat fat cells (25) and normal intestinal cells (26) of different species (human, porcine, bovine and rabbit) have been reported in recent years.

MATERIALS AND METHODS

Cell culture- A clone of guinea pig tumor cells (104C1) was maintained on RPMI-1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco). Cultures were grown in Falcon T-flasks (75 cm^2) containing 15 ml of medium and incubated in a water-saturated air /5% CO_2 atmosphere at 37°C. The medium was changed once before harvesting, and cells were subcultured when they reached a population density of 3 to 5 x 10^6 cells/T-flask. For lectin binding studies 6 to 12 x 10^6 cells per T-flask were used. Cell counts were obtained from the experimental flasks after harvesting with PBS: phosphate buffered saline containing 7.0 mM KPO_4, 0.14 M NaCl and 0.1% ethylenediaminetetraacetate (EDTA), pH 7.2. Cells were processed according to our previously published method (15, 16) and membrane fractions for glycolipid transferase activities were isolated at the junction of 0.32 and 1.2 M discontinuous sucrose gradient. We would like to thank Mrs. Montha Falk for technical help in the cell culture work.

^{125}I-labeled lectin and cholera toxin - Purified cholera toxin was purchased from Schwarz/Mann and labeled with $Na^{125}I$ in the presence of Chloramine-T according to the method of Cutrecasas (27). The Dolichos biflorus lectin was a gift from Dr. Marilyn Etzler (28). The lectin was labeled with $Na^{125}I$ in the presence of Sepharose 4B-bound lactoperoxidase according to the method of David and Reisfeld (29).

Glycosphingolipids - Forssman glycosphingolipid (GalNAcα1-3GalNAcβ1-3Galα1-4-Galβ1-4Glc-cer) was isolated from sheep erythrocytes according to the method employed for B-lacPent-Cer(Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-cer) from rabbit erythrocytes (30). Both globoTri-Cer (Galα1-4Galβ1-4Glc-cer) and globoTet-Cer (GalNAcβ1-3Galα1-4Galβ1-4Glc-cer) were isolated from porcine erythrocytes and hearts according to our previously published methods (31). The purified glycosphin-

golipids were analyzed by gas-liquid chromatography (30) and glc-mass spectrometry (32).

Binding of glycosphingolipids and ^{125}I-labeled proteins to cells - Falcon T-flasks (75 cm^2) containing confluent population of 104Cl guinea pig sarcoma cells were washed with 1X PBS (2 x 10 ml.) at 15-20°C and allowed to react with a specific glycolipid in 3 ml of serum free medium, RPMI-1640(Gibco) and incubated at 37°C for 30 min.The medium was removed, and the cell layer was washed gently with two 10-ml portions of PBS at 15-20°C. ^{125}I-Labeled cholera toxin or the lectin was added in 3 ml of RPMI-1640 medium (without serum) and allowed to incubate for 25 to 30 min at 37°C. At the end of the incubation period the medium was removed and the cell layer was washed twice with PBS at 15-20°C, and then 5 ml of 0.1% EDTA in PBS (pH 7.2) was added to the flask and kept at 37°C in an incubator for 10-15 min. At this stage cells had come loose from the plastic surface and were finally dispersed by transferring them to a 15-ml graduated centrifuge tube with a Pasteur pipet. An aliquot (0.5 to 1 ml) was taken and filtered through borosilicate fiber discs (Whatman GF/A, porosity, 1.0 μm; diameter, 2.4 cm) in a Millipore apparatus. The discs were washed with cold 5% trichloroacetic acid, dried at 100°C for 15 min and ^{125}I content was quantitatively determined in a toluene scintillation system in the presence and absence of PCS (Amersham/Searle) with a Beckman scintillation counter (LS 3133 T).

RESULTS AND DISCUSSION

The chemical structure of Forssman antigen has been proved recently to be GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-Cer (33). Except for erythrocytes (34,35), tissue homogenates from almost all organs of the guinea pig cross-react with anti-Forssman antiserum (36,37). Recently Forssman antigen has been recognized on NIL hamster cell surfaces (24,38), using labeled antibody against Forssman glycosphingolipid. The presence of Forssman glycosphingolipid on guinea pig tumor cell surfaces was not known until now. Using mono- and oligosaccharides, it was shown that the Dolichos biflorus lectin (28,39) combined specifically with terminal nonreducing α-linked N-acetylgalactosamine residues. For our present studies binding of ^{125}I-labeled Dolichos biflorus lectin to surface glycoproteins and glycosphingolipids was determined at various concentrations of lectin (Table IA) using the guinea pig sarcoma 104Cl cells (18). As shown in Table IB, there was a 3-fold increase in binding of the lectin after the cells had been incubated in the presence of pure Forssman glycosphingolipids (0.13 mg/ml). Uptake of GM1 ganglioside was observed by Cuatrecasas (25,27) in fat cell plasma membranes and by Holmgren

Table I

Binding of Dolichos biflorus [^{125}I]lectin to 104Cl tumor cell surfaces

A		B	
Dolichos biflorus [^{125}I]lectin		Forssman glycolipid	[^{125}I]lectin bound
added	bound	added	
μg/ml	cpm/10^6 cells	μg/ml	cpm/10^6 cells
8	204	-	507
12	692	33	776
24	1580	66	828
36	2379	99	1005
48	2651	132	1357

A. Effect of increasing concentration of Dolichos biflorus [^{125}I]lectin (specific activity, 3.6×10^7 cpm/mg) on lectin binding to untreated guinea pig sarcoma (104Cl) cells. Experimental conditions are given in the text.

B. Effect of increasing concentration of Forssman-active glycosphingolipid (isolated from sheep erythrocytes) on binding of Dolichos biflorus [^{125}I]lectin (12μg/ml) to treated 104Cl cells. Experimental conditions are given in the text.

et al.(26) in intestinal cells, using ^{125}I-labeled cholera toxin, which specifically combines with GM1 ganglioside (40). We quantitated the [^{125}I]cholera toxin binding sites (Table IIA) on guinea pig tumor(104Cl) cell surfaces. As shown in Table IIB, a 10-fold increase in the binding of [^{125}I]cholera toxin was observed after the cells had been treated with GM1 ganglioside (6.6 μg/ml; isolated from human brain). Distinct morphological changes were observed in 104Cl cells after treatment of the control flask with GM1 ganglioside (0.03 mg/3 ml RPMI-1640 medium) and cholera toxin (0.02 mg/3 ml RPMI-1640 medium). A significant change was also observed after the cells were treated with GM1 ganglioside or toxin in separate flasks.

In a membrane preparation isolated from confluent cultures of 104Cl cells,

Table II

Binding of [^{125}I] cholera toxin to 104Cl tumor cell surfaces

A		B	
[^{125}I]cholera toxin		GM1 ganglioside	[^{125}I]toxin bound
added	bound	added	
μg/ml	cpm/10^6 cells	μg/ml	cpm/10^6 cells
		-	397
3.3	390	3	1102
6.6	392	12	4383
13.2	712	20	4735
26.4	932	40	5424

A. Effect of increasing concentration of [^{125}I]cholera toxin (specific activity, 1.9 x 10^6 cpm/mg protein) on toxin binding to untreated guinea pig sarcoma 104Cl cells. Experimental conditions are given in the text.

B. Effect of increasing concentration of GM1 ganglioside on binding of [^{125}I]cholera toxin (6.6 μg/ml) to treated 104Cl cells. Experimental conditions are given in the text. Each value is the average of three separate experiments.

the highest activity was observed with UDP-Gal:lacTri-Cer β-galactosyltransferase (41) (EC 2.4.1.86), whereas activities of UDP-Gal:GM1 β-galactosyltransferase (42) (EC 2.4.1.62) and UDP-Gal:lac-nTet-Cer α-galactosyltransferase (30) (EC 2.4.1.87) were 5- to 10-fold lower than that of EC 2.4.1.86 (Table III). Both UDP-GalNAc:globoTri-Cer β-N-acetylgalactosaminyltransferase (31) (EC 2.4.1.79) and UDP-GalNAc:globoTet-Cer α-N-acetylgalactosaminyltransferase (43,44) (EC 2.4.1.88) were (Table IV) in the membrane preparation isolated from 104Cl cells. However, lac-nTet-Cer (Galβ1-4GlcNAcβ1-3Galβ1-4Glc-cer) was a poor acceptor for producing a blood group A-type glycosphingolipid with this membrane.

From the above results we conclude that 104Cl guinea pig tumor cell surfaces do contain Forssman glycosphingolipid and GM1 receptor sites and that the number

Table III

Glycosphingolipid: galactosyltransferase activities

Acceptor	[^{14}C]Galactose incorporated
	pmoles/mg protein/ 2 hr
Lactosylceramide	37
lacTri-Cer	6604
lac-nTet-Cer	761
lacPent-Cer (H)	51
GM2 ganglioside	1259

Complete incubation mixtures contained the following components (in micromoles) in a final volume of 0.07 ml : acceptor lipids, 0.05; Triton CF-54, 50 μg; cacodylate-HCl buffer, pH 7.3, 10; $MnCl_2$, 0.25; UDP-[^{14}C]galactose (1.9 x 10^6 cpm /μmole), 0.026; and enzyme fraction, 0.3 to 0.6 mg of protein. The mixtures were incubated for 2 hr at 37° and the reactions were stopped by adding 2.5 μmoles of EDTA (pH 7.0). The incorporation of radioactive galactose units into acceptor glycolipids was assayed by double chromatographic methods as described previously (30). Under these conditions the rate of reaction remained constant up to 2 hr and was proportional to protein concentration between 0.1 to 0.6 mg per incubation volume. Values were corrected for endogenous incorporation.

of receptor sites increases as the amount of exogenously added glycosphingolipids is increased. These cells also contain enzymes for the biosynthesis of these substances. The presence of high β-galactosyltransferase activity (41) (EC 2.4.1.86) is of great importance so far as tumorigenicity is concerned (17). Recently Gahmberg and Hakomori (23) isolated lac-nTet-Cer from polyoma-transformed NIL cells, whereas this glycosphingolipid was practically absent from untransformed NIL cells. We have also detected a high level of β-galactosyltransferase (EC 2.4.1.86), which catalyzes the synthesis of lac-nTet-Cer, also in adrenergic N1E-115 (45) and cholinergic NS-20 (17) neuroblastoma cells. Whether the levels of this enzyme change during the cell cycle in synchronized cultures (104Cl and neuro-

Table IV

Glycosphingolipid: N-acetylgalactosaminyltransferase activities

Acceptor	[^{14}C]N-Acetylgalactosamine incorporated
	pmoles/mg protein/ 2 hr
Lactosylceramide	39
globoTri-Cer	829
globoTet-Cer	104
lac-nTet-Cer	57

Complete incubation mixtures contained the following components (in micromoles) in a final volume of 0.07 ml; acceptor lipids, 0.05; the detergent sodium taurocholate, 100 μg; MES (2-(N-morpholino)ethanesulfonic acid) buffer, pH 6.4, 10; $MnCl_2$, 0.25; UDP-[^{14}C]GalNAc (1.88×10^6 cpm/μmole), 0.017, and enzyme protein 0.3 to 0.6 mg. The mixtures were incubated for 2 hr at 37°C, and the reactions were stopped by adding 2.5 μmoles of EDTA, pH 7.0. The incorporation of radioactive N-acetylgalactosamine into glycosphingolipids was assayed by double chromatographic method as described previously (31). Under these conditions the rate of reaction remained constant up to 2 hr and was proportional to protein concentration between 0.1 to 0.6 mg per incubation volume. Values were corrected for endogenous incorporation.

blastoma) and various growth conditions is under investigation. The relationship of lac-nTet-Cer to the tumorigenicity of these cells of nonprimate origin has yet to be determined. It is interesting to note that, unlike cells of primate origin, such as African green monkey kidney, Vero (15,16), these cell surfaces had very few receptor sites for Ulex europeus [^{125}I]lectin. Whether binding will increase after incubation with H-active glycosphingolipid is under investigation.

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