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Sugar-Specific Endocytosis of Glycoproteins by Lewis Lung Carcinoma Cells

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Lewis lung carcinoma cells from tumors, metastasis nodules, or from culture bind fluorescent derivatives of neoglycoproteins containing α-D-glucose residues: This binding is competitively inhibited by neoglycoproteins containing α-D-glucose, by mannan, and by several other neoglycoproteins. Cell binding and uptake of the fluorescent derivatives of the neoglycoproteins was quantified by lysing the cells with an alkylpolyol (MAC 19 or MAC 18) and measuring the fluorescence intensity of the supernatant. The amount of cell-associated neoglycoprotein was higher at 37°C than at 4°C with LLC from tumor. The binding and uptake were inhibited by glycoconjugates containing α-D-glucose. These results suggest the presence of sugar specific receptors in Lewis lung carcinoma cells which are involved in a sugar-specific binding and endocytosis phenomenon. The implication of the existence of a carbohydrate-binding protein on the surface of Lewis lung carcinoma cells are discussed with regard to the in vivo behaviour of these cells, especially in relation to their metastatic properties and to the possibility of using neoglycoproteins as specific carriers of cytotoxic drugs. Hybrid molecules of gelonin and a neoglycoprotein containing α-D-glucose were used as targetted toxin: The targetted toxin was found to bind to and to enter the intact cells and was 100 times more toxic than free drug.

Key words: cancerous cells, endocytosis, glycoconjugates, membrane lectins, neoglycoproteins

Abbreviations used: BSA, bovine serum albumin; F-BSA, fluoresceinyl-thiocarbamyl bovine serum albumin; α-Glc-BSA, α-Man-BSA, α-Gal-BSA, α-Rham-BSA, α-Fuc-BSA, β-Lac-BSA, β-GlcNAc-BSA, (α-D-gluosido-, α-D-mannosido-, α-D-galactosido-, α-L-rhamnosido-, α-L-fucosido-, β-lactosido-, *N*-acetyl-β- D-glucosaminido- phenylthiocarbamyl)-BSA, respectively; LLC, Lewis lung carcinoma; MEM, minimum essential medium; PBS, phosphate-buffered saline, pH 7.4; EDTA, ethylenediaminetetraacetate.

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Carbohydrate-specific uptake of glycoproteins is known to occur in many normal cells. These include hepatocytes [1–3], Kupffer cells [4], macrophages [5,6], endothelial cells [7], and fibroblasts [8]. This uptake is known to be mediated by lectins present in membranes of these cells. In this paper, we show that malignant cells also possess the ability to bind and internalize glycoproteins and neoglycoproteins. This is demonstrated in the case of Lewis lung carcinoma [LLC] cells, grown either in vivo or in vitro.

MATERIALS AND METHODS

Bovine serum albumin (BSA), porcine thyroglobulin, and trypsin (2× crystallized) were from Reactif IBF Pointet-Girard (Villeneuve-La-Garenne, France); *p*-nitrophenylglycosides and Triton ×100 from Sigma (St. Louis, Mo, USA), fluorescein isothiocyanate from Molecular Probes (Texas, USA) and Baker's yeast mannan from Koch-Light. *N*-Succinimidyl-3-(2-pyridyldithio)-propionate (SPDP) was from Pharmacia. MAC 13, MAC 14, MAC 16, MAC 18, and MAC 19 surfactants were prepared according to [9]. Seeds of Gelonium multiflorum (Euphorbiaceae) were purchased from F.G. Celo, (Zweibrüchen, West Germany). Hexosamine content of neoglycoproteins was determined in acid hydrolysates (4N HCl, 4 hr, 100°C) according to Elson and Morgan [10]. Neutral sugar content was determined by using a resorcinol sulfuric acid micromethod (to be published). Briefly, to 100 μl neoglycoprotein solution (100 μg/ml) was added 100 μl resorcinol (6 mg/ml) and 500 μl sulfuric acid (75% v/v). The solution was homogenized by shaking with a vortex and heated at 90°C for 20 min. After cooling by immersion in tapwater for 30 min, the absorbance was read at 495 nm or at 540 nm, or alternatively the fluorescence emission was read at 512 nm upon excitation at 495 nm.

Fluoresceinylthiocarbamyl Neoglycoproteins

Glycoproteins and BSA were fluoresceinylated by reaction with fluoresceinyl-isothiocyanate as previously described [11]. *p*-Aminophenylglycosides obtained by catalytic hydrogenation of *p*-nitrophenylglycosides [12] were converted to glycosido-phenylisothiocyanates by reaction with thiophosgene, and phenylisothiocyanate derivatives of sugars were then coupled to BSA according to McBroom et al [13]. The fluoresceinyl substituted markers (F-derivatives) were purified by gel filtration in a column of Ultrogel AcA 202 (Reactifs-IBF) in butanol:water (5:95, v/v) to remove all fluorescent molecules adsorbed on BSA. The markers were lyophilysed after dialysis.

Gelonin-Neoglycoprotein Conjugates

Gelonin was extracted from seeds of Gelonium multiflorum as described by Stirpe et al [14]. In order to conjugate gelonin to BSA or α-Glc_{29}-BSA, each of the proteins was reacted with a heterobifunctional cross-linking reagent (SPDP) according to Carlsson et al [15] under conditions designed to obtain 1.1–1.2 activated disulfide groups per gelonin molecule, and of three to five per BSA or a α-Glc_{29}-BSA molecule. After reduction the gelonin SH-residues reacted with the activated disulfide groups of α-Glc_{29}-BSA or BSA to form conjugates which were separated from free gelonin by filtration on Sephacryl S200 (Pharmacia) in phosphate-buffered saline (pH 7.4).

As estimated by measuring free 2-thiopyridone absorbance at 343 nm according to Carlsson et al [15], two gelonin molecules were coupled per BSA or α-Glc_{29}-BSA; the conjugates are designated as $gelonin_2$-BSA and $gelonin_2$-α-Glc_{29}-BSA.

Tumor Cells

Lewis lung carcinoma cells (LLC), a malignant metastasizing tumor that developed spontaneously in C57 BL/6 mice were kindly provided by Dr. F. Lavelle (Rhône Poulenc Recherche, Vitry sur Seine, France). For growth in vivo, the cells were injected intramuscularly (10^5–10^6 cells/animal) into syngeneic mice (male or female, 8 wk old). Inoculation with 10^6 tumor cells produced local detectable tumors within 2 wk.

Tumor cell suspensions were prepared by mincing the tumor tissue, treating it with PBS containing 0.1% glucose, 0.02% EDTA, and 3 μg/ml trypsin (Type XII, Sigma). After filtration through a nylon mesh and washing, viable cells were separated from dead cells by centrifugation for 10 min at 500 g on Biotrisacryl (Reactifs-IBF); viable cells were at the interface.

Cell Culture and Harvesting Conditions

LLC cells (2×10^4 cells/ml) were grown in tissue culture dishes in minimal essential medium (MEM) with Hank's salts containing 20% heat inactivated fetal bovine serum (FCS) and 0.2% glutamine (complete medium). The cells were maintained at 37°C in a humidified atmosphere of 5% CO_2–95% air. Cells were grown in monolayer up to confluency (3–4 days) and were harvested after a brief (2 min) incubation with 0.02% EDTA in PBS. The cells were washed in MEM and resuspended in the complete culture medium (viability > 95%). Culture of LLC cells from tumor or from spontaneous metastases were used after four or more passages.

Detection of Bound F-Glycoconjugates by Fluorescence Microscopy

Cells were harvested as described above and incubated 30 min at 4°C in PBS containing 1% BSA with F-derivatives (20, 50, 100, or 200 μg/ml) in the absence or in the presence of either 5 mg/ml neoglycoproteins, 0.1 M sugar, 5 mg/ml thyroglobulin or mannan. Cells were washed twice with PBS containing 0.5% BSA. In some of the experiments, the cells were plated on cover slips in Petri dishes and were grown 24 hr in complete medium. Cells adherent to cover slips were treated with F-derivatives as described above.

Binding and Uptake

Cultured LLC cells (5×10^4 cells) suspended in complete medium (2.5 ml) were plated in 35-mm diameter Petri dishes or (2×10^4 cells, 1 ml) in multiwell tissue culture plates (16-mm diameter) and incubated for 2 or 3 days. Nonadherent cells were removed, and the adherent cells were incubated at 4°C or 37°C either in PBS or in MEM containing 1% BSA and F-derivatives, in the presence or in the absence of inhibitors. Supernatants were then removed, and cells were washed twice with PBS containing 0.5% BSA. The cells were lysed in a detergent (1 ml) (see Results). The fluorescence intensity of the supernatant related to a known number of cells was determined with a Fica MK 55 spectrofluorimeter equipped with a Houston 2000 recorder (excitation wavelength, 495 nm; emission wavelength, 520 nm). The number of cells was determined by counting under a microscope or estimated by

measuring the absorbance of the lysate at 260 nm. Tumor cells, harvested as described above, were suspended in MEM containing 1% BSA and were treated as described for adherent cultured cells.

Effect of Gelonin, Gelonin$_2$-BSA, and Gelonin$_2$-α-Glc$_{29}$-BSA Hybrid Molecules on Protein Synthesis in LLC Cells

LLC cells seeded into multiwell tissue culture plates (2.10^4 cells/well) were maintained 2 days in complete medium (20% FCS). Then the medium was removed and replaced by serum-free medium supplemented with 20% Ultroser-G, serum substitute (Reactifs IBF) containing various amounts of gelonin or hybrid molecules (concentration range 10^{-8} to 10^{-5} M, based on gelonin content). After 20 hr the medium was removed and a L-leucine-free serum-free medium (Gibco) was added together with ^{3}H-leucine (3.7 KBq; 20–40 Ci/mmol or 0.7–1.4 TBq/mmol, Commissariar a l'Energie Atomique (CEA), Saclay). Two hours later, cells were collected and washed on a Whatman glass fiber filter with a cell harvester. The radioactivity of cells was measured in aqueous counting scintillant (ACS, Amersham).

RESULTS

Specificity of the Cell Surface Sugar-Binding Components of Lewis Lung Carcinoma

Neoglycoprotein derivatives obtained by reaction of glycosyl phenylisothiocyanate with fluorescein-substituted bovine serum albumin (FBSA) had very close fluorescent emission intensities whatever the number of bound glycosylphenylthiocarbamyl groups (10–50 sugars/molecule). When LLC cells, whether grown in vivo or in vitro, were incubated in the presence of the fluorescein-substituted bovine serum albumin derivatives (up to 200 μg/ml) α-Gal-FBSA, β-Lac-FBSA, or β-GlcNAc-FBSA (15 ± 5 saccharide units per neoglycoprotein molecule), the labeling viewed under a fluorescence microscope was weak. In contrast, α-Glc-FBSA, α-L-Rha-FBSA, α-L-Fuc-FBSA, or α-Man-FBSA (50 μg/ml) labeled all LLC cells significantly. Cells collected in the presence of 0.02% disodium ethylenediaminetetraacetate washed in PBS and immediately incubated in α-Glc-FBSA (200 μg/ml) were slightly labeled. The fluorescence intensity was maximal when cells were incubated in α-Glc-FBSA 1 or 2 days after plating in culture medium. The fluorescence labeling was abolished when cells were incubated with 50 μg/ml α-Glc-FBSA in the presence of 0.1 M maltose or of thyroglobulin, mannan, or α-Glc-BSA (5 mg/ml each) but was not abolished when cells were incubated in 0.1 M D-glucose, D-galactose, D-mannose, *N*-acetyl- D-glucosamine, or *N*-acetyl-D-galactosamine. When cells were preincubated in the presence of inhibitors (5 mg/ml mannan or 0.1 M maltose), then washed twice in PBS-BSA and incubated with α-Glc-FBSA, the labeling was similar to that of cells which were not pretreated; therefore, sugar-binding proteins of LLC cells are not dissociated from the cell surface by washing with a buffer or with a buffer containing a lectin-specific ligand.

Quantitative Measurements of Cell Associated F-Neoglycoproteins

In order to quantitate the amount of fluoresceinyl markers bound to and/or endocytosed by LLC cells, labeled cells were treated with detergents in 0.05 M NaCl, 0.10 M sodium borate, pH 8.5; at this pH, the fluorescence intensity of fluorescein is

maximal. The following detergents were used: Nonidet P40 and Triton X100, which are polyoxyethylene derivatives of alkylphenol, quenched the fluorescence of fluorescein; MAC 13, MAC 14, MAC 16, MAC 18, and MAC 19, which are alkylpolyols, did not quench the fluorescein fluorescence. The maximal intensity was obtained by using 0.25% MAC 19 in borate buffer (Table I).

Binding and endocytosis of the neoglycoproteins by the receptors of LLC cells were measured after incubation at 4°C or at 37°C (2 hr). As shown in Table II, the

TABLE I. Amount of F Derivatives in the Supernatant of Cells Treated With Detergents*

Detergents	mg/ml	α-Glc-FBSA (pg/cell)
Triton X100	10	0.2
MAC 13	5	0.14
MAC 14	5	0.14
MAC 16	5	0.54
MAC 18	5	0.65
MAC 19	1	0.64
MAC 19	2.5	0.70
MAC 19	5	0.68
MAC 19	10	0.48

*Plated cells (10^6) were incubated in MEM (1 ml) containing 1% BSA and 100 μg of α-Glc-FBSA for 2 hr at 37°C. Cells were washed twice in 0.5% BSA-PBS and disrupted in 0.05 M NaCl, 0.1 M sodium borate (pH 8.5) containing surfactants. The supernatant fluorescence was measured, and the fluorescence intensity was used to calculate the amount of cell-associated derivatives. Triplicate experiments; deviation < 10%.

TABLE II. Cell-Associated FBSA and F-Neoglycoproteins by LLC Cells

	Cell associated F-derivatives (pg/cell)		
F-derivatives	4°C	37°C	
FBSA	0.07[a]	0.07[a]	0.09[b]
α-Glc-FBSA	0.26	0.65	0.49
α-Man-FBSA	0.20	0.65	0.27
α-Gal-FBSA	0.31	0.65	0.16
α-L-Fuc-FBSA	0.31	0.73	nt[c]
α-L-Rham-FBSA	0.31	0.88	nt
β-Lac-FBSA	0.18	0.54	0.13
β-GlcNAc-FBSA	0.28	0.65	0.16

[a]The number of saccharide units per molecule of neoglycoproteins was 25 to 35.
[b]The number of saccharide units per molecule of neoglycoproteins was 15 ± 2. Cells were incubated in the presence of 100 μg/ml of F-derivatives for 2 hr at 4°C or at 37°C and treated as described in Materials and Methods. Triplicate experiments, deviation < 10%.
[c]Not tested.

binding of F-BSA to LLC cells was low at both 4°C and 37°C. The amount of F-neoglycoproteins associated with the cells at 37°C was considerably higher than at 4°C and was related to the number of sugar residues. The association of neoglycoproteins with a low sugar content ($\simeq$ 15 residues per molecule) was significantly lower than that of neoglycoproteins with a high sugar content (25 or more residues per molecule). However, neoglycoproteins with a low sugar content exhibited higher specificities than those with a high sugar content. On this basis all further experiments were conducted with α-Glc_{29}-FBSA (29 sugar units per molecule).

The amount of cell associated α-Glc_{29}-FBSA is a function of the concentration of F-derivative, both at 4°C and 37°C (Fig. 1). After 2 hr, the amount of α-Glc-FBSA associated with tumor LLC cells was found to be higher at 37°C than at 4°C; similar results were obtained with neoglycoproteins containing 15 or 29 sugar residues, both with cells grown in vitro and with cells from the primary tumor (data not shown). However, LLC cells obtained from metastatic cells are less effective than primary tumor cells in binding and endocytosing neoglycoprotein specifically (to be published).

To ascertain that tumor or culture cells did internalize at least part of the cell-associated neoglycoproteins, we have examined the effect of trypsin (2 mg/ml, 30 min, 25°C) on the fluorescence associated with cells (5×10^5) preincubated in the presence of α-Glc_{29}-FBSA (6 hr, 37°C). The fluorescence of trypsin-treated cells was 80% of that of untreated ones, suggesting that a large proportion of the associated ligand was internalized.

The involvement of the sugar moiety in the binding and endocytosis process was evidenced by showing that the cell association of α-Glc_{29}-FBSA at both low (4°C) and high (37°C) temperatures was significantly inhibited by neoglycoproteins, glycoproteins devoid of fluorescein, or by glucose or maltose (Table III): Other

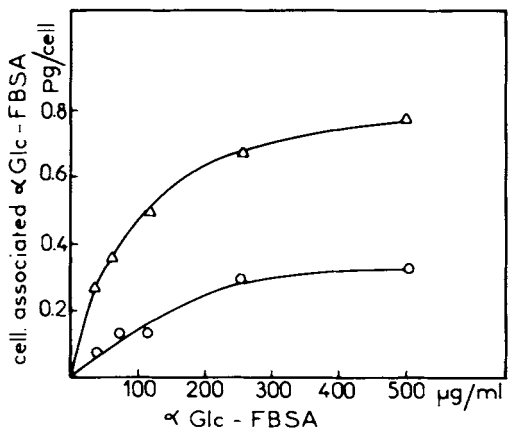

Fig. 1. Cell associated α-Glc_{29}-FBSA. Cells were incubated 2 hr at 4°C and 37°C in PBS 1% BSA containing various concentrations of FBSA and α-Glc_{29}-FBSA. The cells were then washed and disrupted in 0.05 M NaCl, 0.1 M sodium borate (pH 8.5) containing 2.5 mg/ml MAC 19. The amount of α-Glc_{29}-FBSA bound (4°C) or bound and endocytosed (37°C) was calculated by subtracting the amount of FBSA bound in the same range of concentration. Number of cells were evaluated for each value. Values are the mean of three replicate cultures; deviation < 10%. △—△, α-Glc-FBSA minus FBSA, 37°C; ○—○ α-Glc-FBSA minus FBSA, 4°C.

simple sugars—galactose, L-fucose, *N*-acetylgalactosamine, *N*-acetylglucosamine, L-rhamnose or lactose—were ineffective.

Toxicity of Gelonin$_2$-α-Glc$_{29}$-BSA

To test the ability α-Glc$_{29}$-BSA to enter the cells, the toxicity of gelonin$_2$-α-Glc$_{29}$-BSA and gelonin$_{-2}$-BSA hybrid molecules was determined: Protein synthesis was 50% inhibited by 8×10^{-8} M, 8×10^{-7} M of the conjugates, respectively, and by 6×10^{-6} M of free gelonin (Fig. 2). The gelonin$_2$-α-Glc$_{29}$-BSA hybrid molecule

TABLE III. Inhibition of α-Glc$_{29}$-FBSA Binding and Uptake by Various Fluorescein-Free Glycoconjugates*

		Cell-associated F-derivatives (pg/cell)	
Inhibitors	mg/ml	4°C	37°C
None	—	0.23 (0)	0.51 (0)
α-Glc-BSA	5	0.05 (78)	0.15 (71)
α-Man-BSA	5	0.04 (83)	0.34 (34)
α-Gal-BSA	5	0.01 (96)	0.10 (80)
α-L-Fuc-BSA	5	0 (100)	0 (100)
α-L-Rha-BSA	5	0 (100)	0.04 (92)
β-Lac-BSA	5	0.05 (78)	0.26 (53)
β-GlcNAc-BSA	5	0.15 (35)	0.21 (54)
Glucose	18	0.18 (22)	0.45 (12)
Maltose	34	0.05 (78)	0.34 (34)
Mannan	5	0.10 (57)	0.27 (90)
Thyroglobulin	5	0.10 (57)	0.31 (40)

*LLC cells were preincubated 30 min at room temperature in the presence of high-sugar-content neoglycoproteins and other inhibitors. After adding α-Glc$_{29}$-FBSA (50 μg/ml, final concentration) cells were incubated for 2 hr at 4°C or at 37°C. The values in parentheses are the inhibition percents calculated on the basis of the sugar-specific cell association. Cell-associated FBSA was assumed to be due to unspecific adsorption.

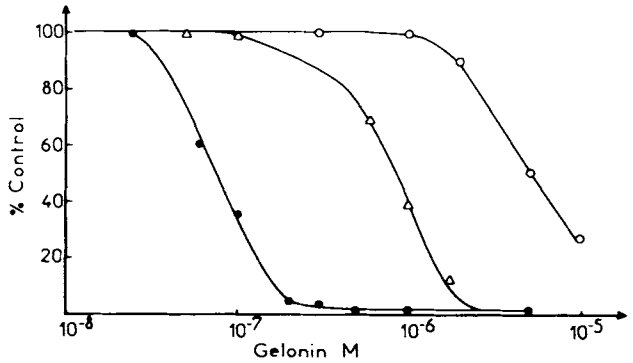

Fig. 2. Effect of gelonin, gelonin$_2$-BSA, and gelonin$_2$-α-Glc$_{29}$-BSA on protein synthesis in LLC cells. Gelonin and hybrid molecules were added to LLC cells growing in multiwell plates in medium supplemented with serum substitutes. Incorporation of ^{3}H-leucine was measured after 24 hr incubation at 37°C. Values are the mean of two replicates cultures; deviation < 10%. ○—○, gelonin; Δ--Δ, gelonin$_2$-BSA; ●—●, gelonin$_2$-α-Glc$_{29}$-BSA. Control experiments were carried out in the presence of α-Glc$_{29}$-BSA.

is thus 100 times more active than free gelonin, showing that this hybrid molecule is endocytised via a glucose-specific receptor.

DISCUSSION

Direct visualization under the fluorescence microscope of sugar-specific binding to cell surfaces by fluorescein-substituted neoglycoproteins is a convenient method of demonstrating the presence of sugar-binding sites on cells. However, the concentration of fluorescent derivatives is critical: At high concentrations, cells are also labeled by nonglycosylated fluorescent derivatives, whereas at low concentrations, cells incubated in the presence of specific glycosylated fluorescent derivatives are poorly labeled. In order to ascertain a specific labeling (labeling that is competitively inhibited by sugar derivatives) we found that it was convenient to use a concentration of 50 μg/ml of glycosylated fluorescent derivatives. Simple sugars did not usually inhibit the binding of F-neoglycoproteins, whereas fluorescein-free neoglycoproteins, glycoproteins, or polysaccharides were found to be quite effective. This observation is related to the high apparent affinity of macromolecule-bound sugars to lectins, which is usually 10^2–10^3 times higher than that of simple sugars [16,17]. LLC cells from tumor (ie, grown in vivo) or from culture (in vitro) were specifically labeled by fluorescein-substituted neoglycoproteins containing 15 residues per mole of either α-D-glucose or α-D-mannose units. This labeling was inhibited by glycoconjugates containing either of these two sugars.

The quantitative spectrofluorimetric determination of cell associated neoglycoprotein required the lysis of cells by a surfactant in order to avoid scattering phenomena induced by intact cells. Alkylphenyl polyoxyethylenes (Triton $\times$100 and Nonidet P40) were found to quench the fluorescein fluorescence (results not shown) and so to lower the sensitivity of the method. Conversely, alkylpolyols did not quench the fluorescein fluorescence. The highest sensitivity was obtained by using MAC 18 or MAC 19, which contain a dodecyl moiety and are more effective in solubilizing cell components than MAC 13, MAC 14, and MAC 16 which contain heptyl, octyl, and decyl moieties, respectively.

On the basis of quantitative analysis, it was found that the association of neoglycoproteins to cells was related to both the concentration and the sugar content of neoglycoproteins. The amount of cell-associated neoglycoproteins was significantly higher with neoglycoproteins that are highly substituted by sugars than with relatively low substituted neoglycoproteins. This result is consistent with the findings of Lee and colleagues [18], who showed that the binding of hepatocytes to immobilized neoglycoproteins was strikingly dependent on the sugar content of the neoglycoproteins.

The enhanced cell association of high-sugar-content neoglycoproteins seemed to impair the selectivity of the binding, since neoglycoproteins containing a high number of α-Glc, α-Man, α-Gal, α-L-Rha, and α-L-Fuc were found to be associated to LLC cells to a similar extent.

The association of neoglycoproteins to tumor or cultured LLC cells was consistently higher at 37°C than at 4°C, suggesting that an endocytosis phenomenon was occurring. The cell association of F-neoglycoprotein containing α-glucose at both

temperatures was significantly inhibited by fluorescein-free α-Glc-BSA, maltose, and mannan as well as by several fluorescein-free neoglycoproteins containing other sugars. These findings are consistent with the low binding selectivity of neoglycoproteins with a high sugar content because of their high apparent affinity and may also indicate that the binding site of the putative surface lectin is not restricted to a simple sugar. Many mammalian lectins [see 2,3] bind complex oligosaccharides, and so it is not surprising that their binding sites may accommodate several types of monosaccharides. The very effective inhibition of the cell association of F-neoglycoproteins by fluorescein-free neoglycoproteins at both 4°C and 37°C clearly indicates that the binding and uptake is related to cell surface sugar receptors and that the high level of cell-associated F-neoglycoproteins at 37°C was not due to a simple pinocytic phenomenon. The sugar-specific endocytosis of neoglycoproteins by tumor LLC cells was further established by showing that trypsin was not able to release more than 20% of cell associated α-Glc-FBSA after a 2-hr incubation at 37°C. The resistance of cell-associated ligands to exogenous proteolysis is taken as evidence for internalization [19]. Finally, the ability of sugar receptors of LLC cells to internalize neoglycoproteins was further demonstrated by the high cytotoxicity of gelonin$_2$-α-Glc$_{29}$-BSA hybrid molecule. Gelonin, a plant toxin [14], strongly inhibits protein synthesis in a cell-free system; it is a monomeric protein devoid of sugar-binding sites and does not inhibit protein synthesis when added at a concentration up to 10^{-5} M on intact cells such as HeLa cells [14]. However, because gelonin is a glycoprotein, sugar moiety of which binds concanavalin A, an α-Glc/α-Man-specific lectin, the protein synthesis by LLC cells was slightly inhibited by gelonin. The inhibition power increased ten times when two molecules of gelonin were linked on one BSA molecule; the increased activity of gelonin$_2$-BSA hybrid molecule may be interpreted as an enhanced binding capacity due either to the "dimerisation" of gelonin as in the case of (Fab')2 versus Fab or to a positive effect of BSA, the reasons for which are not clear. Nevertheless, the fact that the gelonin$_2$-α-Glc-BSA hybrid is ten times more toxic than the gelonin$_2$-BSA hybrid is clearly due to the presence of the α-glucoside moieties on the BSA. This interpretation is in agreement with the findings of Forbes et al [20], who showed that a gelonin-monophosphopentamannose conjugate was toxic toward any cell type that has a functional Man-6-P receptor.

The presence of lectin at the surface of tumor cells has already been reported. In human melanomas, adenocarcinoma, and murine melanoma and fibrosarcoma, β-galactoside-specific lectins were recently detected [21] on the basis of binding or agglutinating experiments. Such lectins could be involved in cell-cell adhesion, as shown with teratocarcinoma cells [22] or lymphocyte adhesion on endothelial cells of microveinules in lymph nodes [23]. Since LLC cells inoculated in a leg muscle rapidly metastasize in the lung, it is tempting to speculate that α-glucose-specific lectin is involved in the arrest and implantation of LLC cells in the lung. It is noteworthy that the lymphoma cells, such as L1210, that have no metastatic potential are devoid of any detectable surfaces lectins (unpublished results). The experiments reported above clearly demonstrate that the surface lectins of LLC cells mediate endocytosis of neoglycoproteins. This process could be the basis of therapeutic treatment of such cancer. Preliminary results of in vitro experiments [24] using a peptidyl drug-substituted α-Glc-BSA similar to the drug arm carrier previously

described [25] support this suggestion. However, such a therapy will require the use of neoglycoproteins containing carbohydrate moieties much more selective than simple sugars in order to specifically target tumor cells.

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