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Evidence for receptor-mediated binding of glycoproteins, glycoconjugates, and lysosomal glycosidases by alveolar macrophages

(mannosyl-glucosyl receptor/mammalian lectin/pinocytosis)

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ABSTRACT **Alveolar macrophages have been shown to bind glycoproteins and synthetic glycoconjugates (neoglycoproteins) that have mannose, *N*-acetylglucosamine, or glucose in the exposed, nonreducing position. Galactose-terminal glycoproteins are not bound. Binding of radiolabeled ligands to cells is nearly completely impaired by the presence of an excess of yeast mannan. Binding is temperature sensitive and proceeds optimally at pH 7.0. Prior treatment of the cells with trypsin severely decreases their capacity to bind ligands. An inhibition assay has been developed, using radioiodinated glucose–albumin conjugate, agalacto-orosomucoid, β-glucuronidase, and RNase B as ligands. Various glycoproteins have been shown to be effective inhibitors of ligand binding, including horseradish peroxidase, agalacto-orosomucoid, β-glucuronidase, ovalbumin, agalacto-fetuin, and RNase B. RNase A and asialo-fetuin are ineffective as antagonists. The results suggest the presence of a cell surface receptor on alveolar macrophages that binds glycoproteins having terminal sugars with the mannose or glucose configuration.**

The survival of modified glycoproteins, glycoconjugates, and lysosomal glycosidases in mammalian plasma is known to be determined largely by the nature of the exposed, or terminal, sugar residues associated with the carbohydrate chains. Rapid *in vivo* clearance of glycosylated macromolecules is sugar-specific, displays saturability, and has all the characteristics of a receptor-mediated process. Recent evidence from several laboratories suggests that rapid *in vivo* clearance of glycoproteins is mediated by at least two distinct, anatomically separate, recognition systems. The innovative work of Ashwell and colleagues (1) has revealed in detail the hepatocyte-dependent receptor-mediated plasma clearance of galactose-terminal (i.e., asialo-) glycoproteins. Based on work from several laboratories (2–10), including our own, evidence for a second pathway has emerged. The latter accomodates many lysosomal glycosidases and glycoproteins having mannose and/or *N*-acetylglucosamine as their respective terminal sugars. This newly described clearance pathway appears to recognize both mannose and *N*-acetylglucosamine (GlcNAc) because, as shown by Achord *et al.* (7), glycoproteins having either of the latter two sugars in the terminal position compete with one another for *in vivo* clearance. An important clue that the two clearance systems (viz., galactose-specific vs. mannose/GlcNAc-specific) are mutually exclusive and mediated by different liver cell types was the observation that, after clearance of lysosomal glycosidases, the bulk of the activity was identified histochemically and by fractionation in liver sinusoidal cells (ref. 11; D. Achord, F. Brot, C. E. Bell, and W. Sly, personal communication; P. Schlesinger and P. Stahl, unpublished observations).

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The conclusion that elements of the reticuloendothelial system recognize and bind mannose/GlcNAc-terminal glycoproteins led us to investigate isolated macrophage cells. The present report describes our results with alveolar macrophages which have been shown to bind glycoconjugates having mannose, GlcNAc, or glucose in the terminal position. An assay system has been developed that allows the quantitative measurement of binding by cells. Galactose-terminal glycoproteins are not recognized by these cells.

MATERIALS AND METHODS

Material. Yeast mannan, [bis(1-hydroxyethyl)amino]-tris(hydroxymethyl)methane (Bistris), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), 2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid (Bes), 1,4-piperazinediethanesulfonic acid (Pipes), trypsin, horseradish peroxidase (type VI), ovalbumin and RNases A and B were obtained from Sigma. RNase B was further purified by concanavalin A-Sepharose chromatography as described by Baynes and Wold (9). Fetuin was obtained from GIBCO, and agalacto-fetuin was prepared chemically by the method of Spiro (12). Agalacto-orosomucoid was a generous gift from Gilbert Ashwell; mutant mannans (MNN) were kindly provided by Clinton Ballou. Neoglycoproteins were prepared (kindly donated by Y. C. Lee) by the method of Lee *et al.* (13) and contained approximately 20 mol of sugar per mol of albumin. β-Glucuronidase was prepared from rat preputial glands and had a specific activity (see ref. 14) of 2200 units/mg.

Isolation of Alveolar Macrophages. Alveolar macrophages were isolated by lung lavage from 200-g female Wistar rats (Harlan Industries, Cumberland, IN) essentially as described by Brain and Frank (15). Briefly, the animal was anesthetized with Nembutal (30 mg/kg), the pleural cavity was opened, and the trachea was cannulated. Isotonic saline was allowed to enter the lungs by gravity. The pooled lavage fluid from these separate washings (50 ml) was centrifuged at low speed followed by resuspension of the cells in standard incubation medium (see below). The cells were counted by using a standard hemocytometer and viability was checked by using 0.01% trypan blue. The cells were always used within 1 hr of their isolation, and viability was always >90%. The isolated cells were shown to be >85% macrophages by their histochemical characteristics (acid phosphatase, β-glucuronidase) and by their ability to take up latex beads.

Abbreviations: GlcNAc, *N*-acetylglucosamine; Bistris, [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Bes, 2-[bis(2-hydroxymethyl)amino]ethanesulfonic acid; Pipes, 1,4-piperazinediethanesulfonic acid; Glc–BSA, GlcNAc–BSA, Man–BSA, and Gal-BSA, conjugates of glucose, GlcNAc, mannose, and galactose with bovine serum albumin; MNN, mutant mannan.

Standard Assay Conditions. Cells were dispensed into 0.4-ml Microfuge tubes. Routinely, 5×10^5 cells in minimal essential medium containing 20% fetal calf serum and buffered to pH 7.0 with 10 mM Hepes, 10 mM Bes, and 15 mM Pipes were incubated with radiolabeled ligand ($\sim 2 \times 10^5$ cpm) in a total volume of 0.1 ml. After incubation at the appropriate temperature, the reaction was terminated by the addition of 0.1 ml of cold ligand-free medium. The tubes were placed on ice for 1 min and then were centrifuged in a Beckman microfuge for 20 sec. The pellets were washed twice with 0.2 ml of cold medium. The washed cell pellets and combined supernatants were assayed for radioactivity.

The inhibition assay used the same conditions as the standard assay above except that the concentration of ligand was constant and the concentration of inhibitor was varied over as large a range as possible. The inhibitor and ligand were presented to the cells simultaneously in a final volume of 0.2 ml, and the same incubation and washing protocol as used in the standard assay above was followed.

Radiolabeling. Glycoproteins and neoglycoproteins were radiolabeled with ^{125}I by the chloramine-T method (16). Specific radioactivities of 0.4–1.5 μCi/μg were achieved, and the radiolabeled ligand was diluted with unlabeled ligand to a known specific radioactivity for use in the assay. β-Glucuronidase was iodinated by the method of Schlesinger *et al.* (5) to a specific radioactivity of 0.05 μCi/μg.

RESULTS

Binding of ^{125}I-Labeled Neoglycoproteins, β-Glucuronidase, Agalacto-orosomucoid, and RNase B to Alveolar Macrophages. The *in vivo* recognition and clearance of glycosylated macromolecules suggests that a surface receptor occurs on macrophage-like cells that binds and internalizes mannose/GlcNAc-terminal glycoproteins. To test this possibility *in vitro*, freshly isolated cells (5×10^5) suspended in 0.1 ml of standard incubation medium together with the appropriate ligand were incubated at 37° for 30 min. Binding of ligand to cells showed specificity and saturability with increasing amounts of ligand added to the incubation mixture (Fig. 1). Mannose-bovine serum albumin conjugate (Man–BSA), GlcNAc–albumin conjugate (GlcNAc–BSA), and glucose–albumin conjugate (Glc–BSA) all displayed apparent specific binding whereas galactose–albumin conjugate (Gal–BSA) and albumin showed a linear relationship between binding and ligand concentration (Fig. 1*A*). The specificity of binding of the first three ligands was confirmed by the observation that the addition of yeast mannan (Sigma) to the incubation mixture fully prevented binding (not shown). These observations are consistent with the *in vivo* studies in which mannan is a most effective inhibitor of clearance of mannose- and GlcNAc-terminal glycoproteins. When three other glycoprotein ligands whose *in vivo* behavior has already been documented [i.e., β-glucuronidase (3), agalacto-orosomucoid (8), and RNase B (9)] were tested for their ability to bind to alveolar macrophages in the same assay system, a saturation binding curve could be constructed for all three ligands (Fig. 1 *B–D*). Moreover, the binding of all the ligands was abolished by the addition of yeast mannan to the incubation mixture. RNase A, a protein without covalently bound carbohydrate, was not bound when added to cells.

Effect of Incubation Conditions on Ligand Binding. The effects of time, temperature, pH, and cell concentration on the binding assay were studied in the assay system just described, with ^{125}I-labeled Glc–BSA as ligand. The results (Fig. 2*A*) showed that binding is temperature sensitive with negligible binding at 4°. Under standard assay conditions at 37°, near-maximal binding was achieved by 30 min.

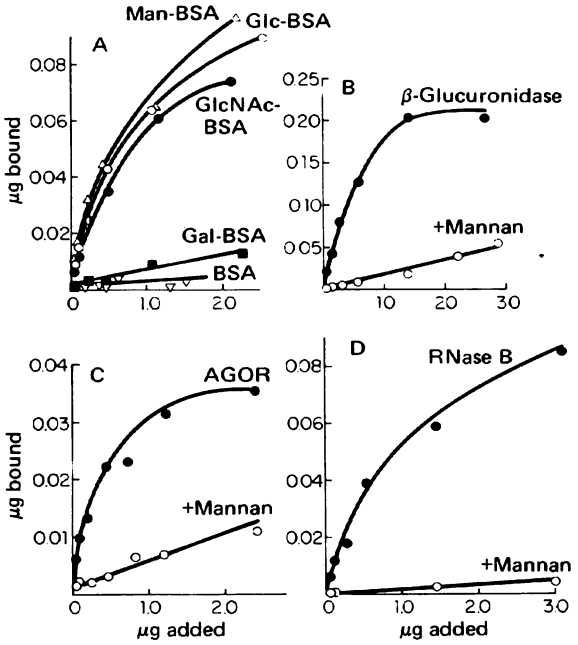

FIG. 1. Concentration-dependence of binding of ^{125}I-labeled neoglycoproteins, β-glucuronidase, agalacto-orosomucoid, and RNase B to alveolar macrophages. Alveolar cells were suspended in standard incubation medium at 10^7 cells per ml. In plastic Microfuge tubes, 50 μl of cells (5×10^5) and 50 μl of standard medium to which radiolabeled ligand (adjusted to a specific radioactivity of approximately 1×10^5 cpm/μg of protein) had been added were mixed and the reaction was allowed to proceed for 30 min at 37°. The reaction was terminated by the addition of 100 μl of cold medium, placed on ice for 1 min, and centrifuged for 20 sec in a Beckman model B Microfuge. The supernatant was aspirated off and the cells were washed twice with 200 μl of cold medium. The supernatants were pooled, and the cells and supernatants were assayed for radioactivity. (*A*) Iodinated Glc–BSA, Man–BSA, GlcNAc–BSA, Gal–BSA, and bovine serum albumin (BSA). (*B*) Iodinated β-glucuronidase in the absence and presence of 125 μg of yeast mannan. (*C*) Same as *B* with iodinated agalacto-orosomucoid (AGOR). (*D*) Same as *B* with iodinated RNase B.

The effect of pH on ligand binding was studied by using different nonvolatile buffers in the incubation medium. Greater binding was observed between pH 6.6 and 7.0, with much less binding above pH 7.4 (Fig. 2*B*).

In the 30-min assay with an optimal ligand concentration (1.25 μg of Glc–BSA per 0.1 ml), the binding of ligand (Fig. 2*C*) varied directly with cell concentration. On the basis of these results, a cell concentration of 5×10^5 cells per 0.1 ml was used in most of the binding assays.

The effect of fetal calf serum on the binding was tested by varying serum concentration from 0 to 30% and by replacing serum with an equivalent amount of bovine serum albumin. The results (not shown) indicated that serum was not required for binding but that, in the absence of serum, nonspecific binding was increased greatly. The serum could be replaced by serum albumin, which produced results similar to those of the standard assay conditions except that the total binding was slightly decreased.

Effect of Protease Treatment on Ligand Binding. Because it is most likely that binding of radiolabeled ligand to alveolar macrophages is mediated by a cell surface receptor, the effect of protease treatment was of interest. To test the effects of

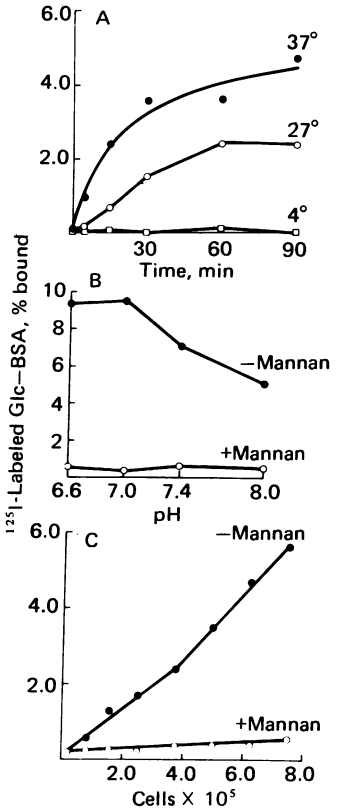

FIG. 2. Effect of time, temperature, pH, and cell concentration on the binding of ^{125}I-labeled Glc–BSA to alveolar macrophages. (*A*) Effect of time and temperature with standard assay conditions. The reaction was initiated by addition of 1.25 μg of ^{125}I-labeled Glc–BSA (1.4×10^5 cpm/μg) to medium containing 5×10^5 cells, followed by incubation for 30 min at 4° (ice), 27°, or 37°. The percentage of ligand bound was calculated as (cellular cpm/total cpm added) × 100. (*B*) Effect of pH on binding, measured by assaying Glc–BSA binding under standard conditions except that the ligand concentration was decreased to 0.5 μg per tube and the medium was adjusted to pH 6.6, 7.0, 7.4, or 8 with the following buffers: pH 6.6, 10 mM Bistris, Pipes, and Bes; pH 7.0, 10 mM Bes and Hepes, 15 mM Pipes; pH 7.4, 10 mM Bes, Tes, Hepes; pH 8.0, 10 mM Bes, Tes, Hepes. The percentage of ligand bound was estimated as before. (*C*) Effect of cell number on ligand binding under standard incubation conditions except that cell number was varied from 2×10^5 to 8×10^5 cells per 0.1 ml. The percentage of ligand bound was calculated as before.

protease, cells (5×10^5) were suspended in 50 μl of serum-free medium with 5 μg of trypsin (0.01%) and were incubated for varying times up to 15 min. The trypsin activity was quenched by the addition of 20% fetal calf serum. Binding assays were initiated immediately after trypsin quenching, under standard assay conditions. The specific binding of Glc–BSA was decreased by 48% in 5 min and by 71% in 15 min (Table 1). Similar results were observed with chymotrypsin and Pronase. Trypsinization had no effect on viability as measured by the trypan blue exclusion test.

Inhibition of Ligand Binding by Purified Mutant Mannans. The inhibitory effect of mannan on binding of glycoprotein ligands to the alveolar macrophage presents an opportunity to analyze the concentration-dependence of mannan inhibition as well as the effect of inhibitor structure on inhibition. These experiments used a series of different mannans that varied in the types of mannose linkages in the terminal sugars. The standard binding assay was used except that varying amounts of the appropriate antagonist were added to the incubation mixture. The inhibitory effects of mannan are particularly interesting because it is among the most potent inhibitors of *in vivo* clearance of mannose- and GlcNAc-terminal glycoproteins (7). [These structures have been reviewed by Ballou and Raschke (17).] The mutant mannans used were MNN 2 (1,6 linkages), MNN 1 (1,2 linkages), MNN 4 (1,3 linkages), and MNN 1,4 (1,2 linkages). MNN 1,4 differs from MNN 1 in that the former contains no phosphomannose residues. The results, presented in Fig. 3, show nearly complete inhibition of ligand binding to alveolar macrophages. The most potent antagonist was MNN 2, a result consistent with the findings of Achord *et al.* (7) in which MNN 2 was shown to be the most potent inhibitor of human β-glucuronidase clearance *in vivo* among those tested.

Table 1. Effect of trypsin treatment on binding of ^{125}I-labeled Glc–BSA to alveolar macrophages

Time, min	+ Trypsin cpm/5 × 10^5 cells	+ Trypsin % bound	− Trypsin cpm/5 × 10^5 cells	− Trypsin % bound	% control
0	7050	8.8	7490	9.4	94
2.5	3480	4.4	6730	8.4	52
5.0	2140	2.7	7280	9.1	30
15.0	1630	2.0	5588	7.0	29

Cells (5×10^5) were incubated in 50 μl of minimal essential medium plus 5 μl of medium containing 0.1% trypsin (0.01% final concentration) or 5 μl of medium without trypsin. After appropriate incubation times, 50 μl of medium containing 20% fetal calf serum and 1.25 μg of ^{125}I-labeled Glc–BSA (8×10^4 cpm) were added. Incubation in the presence of ligand was allowed to proceed for 30 min and then the cells were sedimented and washed as described in Fig. 1. Percentage binding was calculated as in Fig. 2.

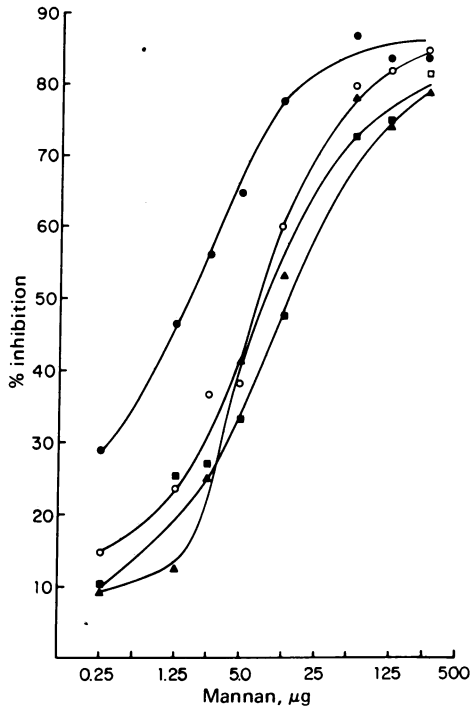

FIG. 3. Effect of mutant mannans on the binding of ^{125}I-labeled Man–BSA to alveolar macrophages. The standard assay was used with 1.25 μg of ^{125}I-labeled Man–BSA. The concentration of mutant mannans was varied in the incubation mixture, and the percentage inhibition of ligand binding was estimated by comparing incubations containing mannan to a control containing ligand but no mannan. The mutant mannans used have a preponderance of specific mannose linkages: ●, MNN 2 (1,6 specific); ▲, MNN 1,4 (1,2 specific); ■, MNN 1 (1,2 specific); ○, MNN 4 (1,3 specific).

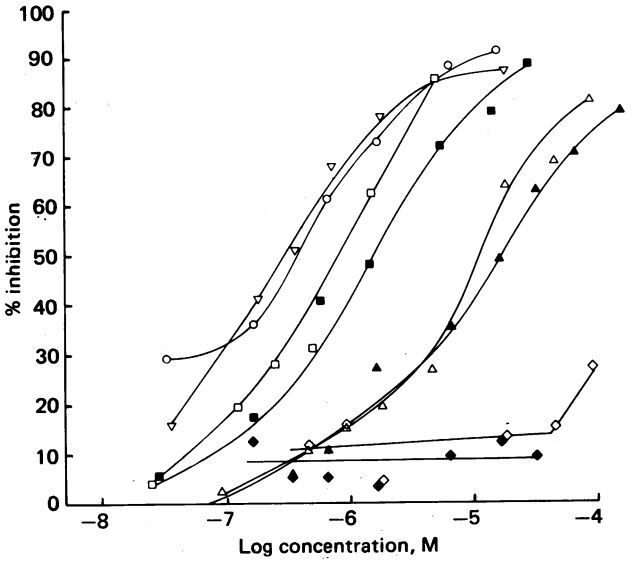

FIG. 4. Inhibition of ^{125}I-labeled Glc–BSA binding by glycoproteins. The standard assay was used with ^{125}I-labeled Glc–BSA. The percentage of inhibition was estimated as described in Fig. 3. ○, Agalacto-orosomucoid; ▽, horseradish peroxidase; □, β-glucuronidase; ■, ovalbumin; △, RNase B; ▲, agalacto-fetuin; ◇, RNase A; ◆, asialo-fetuin.

Inhibition of Ligand Binding to Alveolar Macrophages by Glycoproteins. An inhibition assay was used to analyze the relative inhibitory potency of a series of glycoproteins known to display rapid clearance *in vivo*. The ligands employed were Glc–BSA, β-glucuronidase, agalacto-orosomucoid, and RNase B. The antagonists were horseradish peroxidase, β-glucuronidase, agalacto-orosomucoid, ovalbumin, RNase B, agalacto-fetuin, asialo-fetuin, and RNase A. Horseradish peroxidase is a mannose-rich glycoprotein (18). β-Glucuronidase has mannose and GlcNAc, but the sugar sequence is as yet unresolved (19). Agalacto-orosomucoid is prepared by the sequential enzymatic removal of sialic acid and galactose, respectively, from orosomucoid and bears terminal GlcNAc residues. Ovalbumin has one carbohydrate cluster but displays microheterogeneity (20). Most preparations probably have mannose and GlcNAc in the terminal position. RNase B is a glycoprotein having mannose terminals whereas RNase A is a simple protein (21). Asialo- (i.e., galactose terminal) and agalacto- (i.e., GlcNAc terminal) fetuin presumably differ by one sugar, galactose. The results for inhibition of Glc–BSA binding to alveolar macrophages are displayed graphically in Fig. 4. Nearly complete inhibition of binding was achieved by glycoproteins having terminal mannose or GlcNAc residues whereas a glycoprotein with galactose as the terminal sugar (asialo-fetuin) and a nonglycoprotein, RNase A, were ineffective. Qualitatively similar results were observed with inhibition assays using β-glucuronidase, agalacto-orosomucoid, or RNase B as ligands. Table 2 summarizes the results for inhibition of binding of the four ligands by showing the molar concentration of inhibitor necessary to give 50% inhibition. In all cases, the potency of inhibitors was in the order, horseradish peroxidase > agalacto-orosomucoid > β-glucuronidase ≥ ovalbumin > RNase B > agalacto-fetuin.

Table 2. Inhibition of ligand binding to alveolar macrophages by glycoproteins

Inhibitor	Concentration (μM) of inhibitor required to produce 50% inhibition			
	Glc–BSA	β-Glucuronidase	Agalacto-orosomucoid	RNase B
HRP	0.3	0.09	0.17	0.04
AGOR	0.39	—	—	—
β-Gluc	0.83	—	0.45	0.45
Ovalbumin	1.58	0.56	0.64	0.29
RNase B	10.9	3.92	5.23	—
AGF	16.9	—	—	1.04
RNase A	>50	>50	>50	—
ASF	>50	—	—	—

The inhibition assay was performed as in Fig. 4. Ligand and inhibitor were presented to the cells simultaneously under standard assay conditions (30 min, 37°). The concentration of inhibitor required to produce 50% inhibition of ligand binding was evaluated graphically. The following molecular weight assignments have been used in the calculations: BSA, 67,000; β-glucuronidase (β-Gluc), 280,000; agalacto-orosomucoid (AGOR), 35,000; RNase B, 13,500; ovalbumin, 43,500; agalacto-fetuin (AGF), 40,000; horseradish peroxidase (HRP), 40,000; RNase A, 13,500; asialo-fetuin (ASF), 40,000. Ligand concentration was taken from the results in Fig. 1. Concentrations were as follows: Glc–BSA, 92 nM; β-glucuronidase, 80 nM; agalacto-orsomucoid, 160 nM; RNase B, 460 nM.

DISCUSSION

The results demonstrate the presence of a recognition system associated with alveolar macrophages that mediates the binding of glycoproteins and glycoconjugates having mannose, glucose, or GlcNAc as the terminal nonreducing sugar. This system appears to be qualitatively analogous to the *in vivo* clearance of mannose- and GlcNAc-terminal glycoproteins (2–10) by mammalian liver sinusoidal cells as previously described (11). In contrast, an avian liver recognition system has been described (22, 23) in which the isolated receptor (24) shows strict specificity for GlcNAc-terminal glycoproteins and does not recognize mannose in the terminal position.

The delineation of binding processes as receptor-mediated requires, as minimal criteria, that they be saturable and that they have a definable ligand specificity. The binding and inhibition studies reported here satisfy both of these criteria. The term "binding" is used because no attempt was made to distinguish between adsorption and internalization. The saturability of binding as increasing amounts of ligand are presented to the cells demonstrates a limited number of binding sites per cell, with the binding or inhibitory strength varying with the chemical structure of the ligand.

With regard to specificity, an inhibition assay has been developed that allows quantitative comparison of inhibitory ligands. This has made it possible to reveal the apparent strict specificity of the system for the mannose and glucose configuration at the nonreducing terminal position of the carbohydrate portion of the ligand. Proteins or glycoconjugates with galactose in the terminal position do not bind to alveolar macrophages and are ineffective as inhibitors. Similarly, the protein structure of the ligand appears to contribute little to the binding strength because the nonglycoproteins albumin and RNase A display no inhibitory effects at high concentrations. These observations are consistent with the view that the chemical specificity resides in the carbohydrate moiety of the glycoprotein where the macromolecular configuration is necessary for high-affinity binding to the receptor. Furthermore, it seems likely that variations in receptor affinity for the ligands is due to differences in the carbohydrate structures that they contain. This conclusion is supported by the mannan inhibition experiment which demonstrates that mannose in the α-1,6 linkage is a more potent inhibitor than the same sugar in the α-1,3 or α-1,2 position. Achord *et al.* (7) have shown the same order of

inhibitory potency where MNNs were tested for their ability to antagonize the rapid *in vivo* clearance of human β-glucuronidase in the rat.

In addition, in experiments not reported here, monosaccharides are poor antagonists, suggesting that more than one sugar may be involved in the binding to the receptor. This notion is supported in part by the *in vivo* observations by Baynes and Wold (9) that rapid clearance of RNase B is abolished by α-mannosidase digestion. Because RNase B has one oligosaccharide chain per molecule, a single terminal β-mannose moiety would remain after α-mannosidase digestion. These observations highlight the similarities between the alveolar macrophage binding system and clearance of mannose/glucose-terminal glycoproteins *in vivo*.

The observation that the relative potency of the glycoprotein inhibitors is independent of the identity of the test ligand used in the inhibition study implies that a single species of cell surface receptor is responsible for the binding of all the ligands studied. Because Glc–BSA (glucose-terminal), agalacto-orosomucoid (GlcNAc-terminal), and RNase B (mannose-terminal) all gave the same ranking order of inhibitory potency with a wide variety of unrelated glycoproteins as inhibitors, it is likely that a single receptor molecule is involved. A similar order for the inhibition of β-glucuronidase binding suggests that lysosomal glycosidases are included in the class of glycoproteins that interact with the same alveolar macrophage receptor. This conclusion is partially based upon previously reported observations that agalacto-orosomucoid (2) and mannan (D. Achord, F. Brot, C. E. Bell, and W. Sly, personal communication) block recognition and clearance of a wide variety of lysosomal glycosidases *in vivo*.

The recognition of glycoproteins by alveolar macrophages appears to be distinct from other previously identified macrophage binding functions [viz., F_c, third component of complement, and nonspecific uptake]. The binding of ligand is serum-independent and proceeds efficiently when serum is replaced with heat-inactivated serum or an equivalent amount of bovine serum albumin. Binding is not affected by large doses of colloidal silver or latex beads. Preliminary experiments with human cells suggest that recognition is restricted to macrophages and is apparently not expressed on polymorphonuclear leukocytes or lymphocytes. These results, together with the observation that the receptor is trypsin-sensitive, rules out both F_c and complement third-component receptors as candidates because the former is trypsin-insensitive and both occur on cells other than macrophages. Recently, a trypsin-sensitive F_c receptor on macrophages that binds monomeric IgG has been reported (25). However, this is an unlikely candidate because the binding assays reported here are performed in the presence of 20% fetal calf serum containing an excess of IgG.

The finding that glucose-terminal glycoconjugates are bound by alveolar macrophages is of interest because Glc–BSA has been shown to possess binding activity toward the hepatocyte receptor for galactose (26). Moreover, glucose is a regular component of collagen and has been found covalently bound to various glycoproteins (18, 19, 27). Glucose, in these instances, could serve as a recognition component for uptake into cells or for the binding of cells to some extracellular structure.

The assistance of Dr. Richard White in the techniques for isolating alveolar macrophages and histochemical analysis of the isolated cells is greatly appreciated. We thank Drs. William Sly, Oscar Touster, and Alan Blumberg for critically reading the manuscript. Ron Townsend contributed to the studies on RNase B. The work was supported by Department of Health, Education, and Welfare Grants CA 12858 and GM 21096. P.H.S. holds a Young Investigator Award from the National Heart and Lung Institute (HL 56343).

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