A lectin in rat alveolar macrophage membranes with a high affinity for binding ligands containing L-fucose and N-acetyl-D-glucosamine has been isolated by affinity chromatography on Fuc-BSA-Sepharose (where Fuc is fucosyl and BSA is bovine serum albumin). The lectin was extracted from rat lung homogenates with Triton X-100, adsorbed from the extract onto Fuc-BSA-Sepharose in the presence of Ca\(^{2+}\) and eluted by removal of Ca\(^{2+}\). After a second adsorption to and elution from Fuc-BSA-Sepharose, three protein species were detected electrophoretically in fractions that bind Fuc-BSA. One, which was the mannose/N-acetylglucosamine lectin (\(M = 32,000\)) found earlier in hepatocytes, was removed by adsorption on anti-lectin IgG-Sepharose. Another (\(M = 46,000\)) was removed by adsorption to Fuc-BSA-Sepharose and elution with galactose. The remaining lectin (\(M = 180,000\)) bound fucose and N-acetylglucosamine but not galactose. Binding was maximal between pH 6.5 and 9.0 and dependent on Ca\(^{2+}\). Immunocytological analysis with rabbit anti-lectin IgG and fluorescein-labeled goat anti-rabbit IgG revealed the lectin to be in rat alveolar macrophages and nonparenchymal cells of liver. Thus, the lectin appears to be present in macrophages and is likely involved in receptor-mediated endocytosis. It is distinctly different structurally from the hepatocyte lectin with a similar ligand-binding specificity.

Several animal lectins that form hepatic receptors for specific oligosaccharides have been identified. The first to be discovered in mammals has two types of polypeptide subunits (\(M = 42,000\) and 48,000) (1), binds specifically oligosaccharides with nonreducing terminal galactose or N-acetylgalactosamine (2), and is a transmembrane protein of hepatocytes (3) that contains an asparaginyl-linked oligosaccharide (4). Another lectin with some structural homology to the galactose chain, which contains an asparaginyl-linked oligosaccharide and is on the external surface of the cell. A third type of lectin is found in hepatocytes and has a subunit \(M = 32,000\) and a high affinity for binding N-acetylgalactosamine and mannose (5). Unlike the other hepatocyte lectins it does not appear to be membrane bound (11), and its biological function is unclear.

In contrast to hepatocytes, Kupffer cells of rat liver contain a membrane-bound lectin with a high binding specificity for oligosaccharides containing terminal nonreducing fucose or galactose (12, 13). This protein is found only in Kupffer cells, the resident macrophages of liver, and is not present in macrophages from other tissues or body fluids (14). Alveolar macrophages, however, are known to contain receptors with a binding specificity for oligosaccharides with terminal non-reducing N-acetylgalactosamine, mannose, and fucose (15, 16). In addition, it was found recently (17) that on incubation of alveolar macrophages with mannosylated lactoperoxidase in the presence of Ca\(^{2+}\), Na\(^{125I}\), and \(\text{H}_2\text{O}_2\), a radiolabeled protein with a \(M = 175,000\) was present in detergent extracts of the treated macrophages.

We wish to report here the isolation and partial characterization of a lectin from rat alveolar macrophages that has a \(M = 180,000\) and an oligosaccharide binding specificity very similar to that of receptors on macrophages (15, 16). These studies were undertaken when it was recognized, as described in the preceding paper (14), that the binding and uptake of neoglycoproteins by nonparenchymal cells of liver were not in accord with the binding specificity of the Kupffer cell lectin. Moreover, since liver contained a protein with a high affinity for Fuc-BSA\(^1\) and a molecular weight of about 180,000, a value similar to that found by others for a lectin in alveolar macrophages (17), it was possible that this macrophage lectin may also be present in nonparenchymal cells of liver. The results presented here confirm this view, since antibodies to the alveolar lectin isolated here also react with nonparenchymal cells. A preliminary report of this work has been presented (18).

**EXPERIMENTAL PROCEDURES**

**Materials**—Male Sprague-Dawley (200–400 g) rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. Frozen rat lungs were purchased from Pel-Freeze Biologicals, Rogers, AR. Pansorbin cells were obtained from Calbiochem-Behring. All other materials were obtained as described in the preceding papers (12–14).

**Preparation of Alveolar Macrophage Membranes**—The lungs from anesthetized rats were removed with the trachea still attached and were lavaged as described earlier (15). The cells were collected by

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\(^{1}\) Neoglycoproteins are abbreviated by the standard symbols for monosaccharides and bovine serum albumin. Thus, Fuc-BSA is fucosyl-bovine serum albumin. Unless otherwise designated all monosaccharides used here were of the D-configuration except fucose which was of the L-configuration. The other abbreviation used is: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
Macrophage Lectin

centrifugation at 250 x g for 10 min and freeze-thawed once to rupture any red cells. Membranes were prepared as described (19) and stored in 20 mM HEPES, pH 7.4, containing 0.15 M NaCl, 1 mM EDTA, and 0.02% NaN₃.

Binding of Neoglycoproteins to Alveolar Membranes—Alveolar macrophages were incubated in 10 ng of 125I-Fuc-BSA dissolved in 20 mM HEPES, pH 7.4, containing 0.15 M NaCl, 10 mM CaCl₂, and 1% BSA in a final volume of 0.5 ml for 60 min at 37°C in the absence or presence of neoglycoproteins. The membranes were then collected by centrifugation for 5 min in an Eppendorf microcentrifuge. Unbound ligand was removed, and bound ligand was determined by omitting the omitting the lectin from the assay.

Binding Assay for the Soluble Lectin—Lectin (4–100 ng) was incubated with 10 ng of 125I-Fuc-BSA dissolved in 20 mM Tris-HCl, pH 7.8, containing 0.15 M NaCl, 20 mM CaCl₂, 1% BSA, 0.5% (w/v) Triton X-100, and 0.02% NaN₃. Triton X-100 was added to a final concentration of 2% (w/v) and the suspension stirred vigorously for 2 h. After centrifugation at 16,000 x g for 30 min, the supernatant was discarded, and the residue was resuspended in 1 liter of 20 mM Tris-HCl, pH 7.8, containing 0.2 M NaCl, 20 mM CaCl₂, and 0.02% (w/v) Triton X-100 was added to a final concentration of 2% (w/v) and the suspension stirred vigorously for 2 h. After centrifugation at 16,000 x g for 30 min, the supernatant was made 40 mM in CaCl₂, adjusted to pH 7.8 with 1 M Tris base, and applied to a column (70 ml) containing Fuc-BSA (2 mg/ml)-Sepharose previously equilibrated in Buffer B (20 mM Tris-HCl, pH 7.8 containing 0.2 M NaCl, 0.2% (w/v) Triton X-100, and 0.02% NaN₃). The column was washed with 3 volumes of Buffer B and then eluted with about 300 ml of Buffer C (20 mM Tris-HCl, pH 7.8, containing 0.2 M NaCl, 1 mM EDTA, 0.5% (w/v) Triton X-100, and 0.02% NaN₃). The column was washed with 10 volumes of Buffer B and then eluted with Buffer C (1 ml) and collected and assayed for Fuc-BSA-binding activity. The active fractions were pooled and applied to a column (15 ml) of affinity adsorbents in the presence of calcium and could then be eluted by removing the calcium with EDTA.

Inhibition of binding—Preparation of Antibodies—Alveolar macrophage lectin (56 μg) was precipitated from solution with 4 volumes of ethanol at 0°C, collected by centrifugation, and suspended in 10 mM sodium phosphate, pH 7.4, containing 0.15 M sodium chloride and 0.1% (w/v) Triton X-100. The lectin was cross-linked with Linkulin hecanoycin (56 μg) in 10 mM glutaraldehyde for 1 h at 25°C. The mixture was made 20 mM in glycine and incubated for 1 h at 25°C. Aliquots (1 ml) containing 28 μg of the cross-linked proteins were emulsified with 1 ml of Freund's complete adjuvant (Difco) and 2 ml of the resulting emulsion injected into rabbits to prepare antibodies as described earlier (12). Further antigen (28 μg) was injected 14, 28, and 42 days before collection of antisera on day 49. IgG was prepared from the antisera as described earlier (12).

Preparation of Alveolar Macrophages for Immunocytological Staining—Alveolar macrophages from two rats were washed in Eagle's modified essential medium (14) containing 10% fetal calf serum and 20 mM HEPES, pH 7.4. The cells were diluted into 20 ml of the medium containing 1.8 mM CaCl₂ and plated onto two plastic dishes (15 x 100 mm) containing 6 coverslips each. After 2 h at 37°C, the coverslips were removed, rinsed vigorously with medium, and then incubated overnight at 20°C in ethanol. The cells were stained with fluorescent antibodies as described before (14).

Other Methods—The dodecyl sulfate-polyacrylamide gel electrophoresis (20), silver staining of gels (21), and Western blots (22) were performed as described earlier. Protein concentrations were determined by Amido Black dye binding (23) with BSA as standard. All proteins were iodinated by the chloramine-T method (24), and the iodinated proteins were used for no more than 2 months after preparation. Affinity adsorbs were prepared as described earlier (12).

RESULTS

Binding of Fuc-BSA to Macrophage Membranes—Membranes isolated from alveolar macrophages were incubated with 125I-Fuc-BSA in the presence and absence of a 1000-fold excess of several unlabelled neoglycoproteins. The results listed in Table I show that the membranes bind Fuc-BSA and Man-BSA well, have significant but weaker affinity for GlcNAc-BSA, and bind very little Gal-BSA. This order of binding is the same as that seen with binding of neoglycoproteins to whole cells (16). The binding activity requires Ca²⁺ (data not shown) and can be solubilized in 2% Triton X-100 while retaining a similar binding specificity (Table I). The alveolar macrophage membranes retained essentially all of the Fuc-BSA-binding activity after extraction with 1 M sodium chloride (data not shown).

Purification of the Alveolar Macrophage Lectin—The scheme for purification of the lectin responsible for the binding activity present on alveolar macrophages is shown in Fig. 1. Rat lung was chosen as the starting material because of the difficulty encountered in obtaining enough alveolar macrophages from which a significant quantity of the binding activity could be isolated. The purification is based on adsorption and elution of the binding activity to Fuc-BSA-affinity adsorbents. Fuc-BSA was chosen as the ligand on the affinity matrix because it bound well to whole cells (above). It was reasoned that the soluble lectin would bind to Fuc-BSA-affinity adsorbents in the presence of calcium and could then be eluted by removing the calcium with EDTA.

After obtaining the membrane fraction from the homogenized lung and extracting with 2% Triton X-100, the soluble portion was chromatographed on a column of Fuc-BSA in the presence of calcium. The adsorbed activity was eluted with EDTA and concentrated to a smaller volume on a second smaller Fuc-BSA-affinity adsorbent after recalcification.

The material eluted from the second Fuc-BSA column contained three major protein species with subunit molecular weights of 32,000, 46,000, and 180,000, respectively, as judged by gel electrophoresis (Fig. 2, lane A). The 32,000 molecular weight protein migrated as a single band with an apparent molecular weight of 32,000.

Table I

| Preparation | Inhibition by 1000-fold excess |
|-------------|-----------------------------|
| Fuc-BSA     | 100                         |
| Man-BSA     | 100                         |
| Gal-BSA     | 100                         |
| GlcNAc-BSA  | 99                          |

%  2% Triton X-100 membrane extract

Crude membranes 100 100 12 46

2% Triton X-100 membrane extract 100 99 2 25
The alveolar lectin of rat lung was isolated from whole rat liver and hepatocytes, which is present in serum (11) and binds to Fuc-BSA (25). Although this protein is present in the lung preparations, Western blots of sodium dodecyl sulfate extracts of whole alveolar macrophages with anti-mannose/N-acetylglucosamine lectin IgG show that it is not in these cells (data not shown). Thus, the lectin found in the lung preparations is most likely derived from blood. It can easily be removed by applying this fraction to a column of anti-mannose/N-acetylglucosamine lectin-IgG-Sepharose, which allows the 46,000 molecular weight species (Fig. 2, lane D) and corresponds to about 40% of the binding activity applied. It is not known how much binding activity the 46,000 molecular weight species exhibits in the ammonium sulfate binding assay, since some of the 180,000 molecular weight species (Fig. 2, lane D) also emerges from the column in this step. After removal of the 46,000 molecular weight species, the column is eluted with 30 mM mannose to remove some traces of contaminating proteins seen on gels (Fig. 2). The pure lectin (subunit molecular weight, 200,000) can then be eluted with EDTA (Fig. 2, lane E) in a yield of about 10-20 ng from 100 g of rat lung.

**Effect of Calcium and pH on Binding**—The binding activity of the purified lectin shows a strong Ca\(^{2+}\) dependence, as shown in Fig. 3. The concentration of Ca\(^{2+}\) required for half-maximal stimulation of lectin activity is 0.72 mM, well within physiological Ca\(^{2+}\) concentrations. Dissociation of ligand-lectin complexes as a function of pH is shown in Fig. 4. Maximal stability is observed between pH 6.5 and 9.0, but the complexes dissociate readily below pH 6.5.

**Binding Specificity**—The binding specificity of the lectin was examined by measuring the inhibitory effects of several monosaccharides and neoglycoproteins on the binding of Fuc-BSA (Table II). The order of binding as measured by direct binding of neoglycoproteins or by inhibition of Fuc-BSA binding by monosaccharides is the same, with mannose and fucose being the best ligands. N-Acetylglucosamine and glucose are more weakly bound. Although galactose is weakly bound, N-acetylgalactosamine is not bound.

**Reaction of Alveolar Macrophage Lectin with Specific Antibodies**—Rabbit anti-rat alveolar macrophage lectin IgG did not inhibit the binding of Fuc-BSA to the lectin. As shown in Fig. 5, however, antibody-lectin complexes could be removed from solution by adsorption on immobilized Protein A.

**Immunocytological Localization of Alveolar Lectin**—Since the alveolar macrophage lectin was isolated from whole rat lung preparations, Western blots of sodium dodecyl sulfate extracts of whole alveolar macrophages with anti-mannose/N-acetylglucosamine lectin IgG-Sepharose, which allows the 46,000 and 180,000 molecular weight species to emerge unretracted.

| Weight Species | Western Blot | Antibody-lectin Complexes |
|----------------|--------------|---------------------------|
| Mannose/N-acetylglucosamine lectin | Positive | Removed by adsorption |
| Galactose | Negative | Not removed |
| Glucose | Negative | Not removed |
| N-Acetylglucosamine | Negative | Not removed |
| N-Acetylgalactosamine | Negative | Not removed |

**Immunocytological Localization of Alveolar Lectin**—Since the alveolar macrophage lectin was isolated from whole rat lung preparations, Western blots of sodium dodecyl sulfate extracts of whole alveolar macrophages with anti-mannose/N-acetylglucosamine lectin IgG-Sepharose, which allows the 46,000 and 180,000 molecular weight species to emerge unretracted.

| Weight Species | Western Blot | Antibody-lectin Complexes |
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| Mannose/N-acetylglucosamine lectin | Positive | Removed by adsorption |
| Galactose | Negative | Not removed |
| Glucose | Negative | Not removed |
| N-Acetylglucosamine | Negative | Not removed |
| N-Acetylgalactosamine | Negative | Not removed |
different amounts of rabbit anti-lectin IgG as shown. Pansorbin (50 ng) reacted overnight at 4°C in 20 mM Tris–HCl, pH 7.8, containing 0.2 M NaCl, 1 mM EDTA, 0.5% Triton X-100, and 1 mg/ml BSA with different amounts of rabbit anti-lectin IgG as shown. Pansorbin (50 µl; 10% w/v) in the same medium was added, and after 4 h at 4°C the cells were removed by centrifugation and the lectin-binding activity in the supernatant solution assayed. Preimmune sera did not give significant amounts of lectin-antibody complexes.

**TABLE II**

*Inhibition of lectin-binding activity by neoglycoproteins and monosaccharides*

| Inhibitor       | Inhibition |
|-----------------|------------|
| Fuc-BSA (49)*   | 96.5       |
| Man-BSA (40)    | 95.2       |
| GlcNac-BSA (35) | 46.0       |
| Gal-BSA (42)    | 5.8        |
| Mannose         | 52.6       |
| L-Fucose        | 50.1       |
| N-Acetylgalactosamine | 39.5 |
| Glucose         | 26.2       |
| Galactose       | 9.2        |
| N-Acetylgalactosamine | 0  |

*The numbers in parentheses are the number of residues of monosaccharide/molecule of BSA.

**FIG. 5.** Removal of lectin-antibody complexes from solution by adsorption on immobilized Protein A. Lectin (23 ng) was reacted overnight at 4°C in 20 mM Tris–HCl, pH 7.8, containing 0.2 M NaCl, 1 mM EDTA, 0.5% Triton X-100, and 1 mg/ml BSA with different amounts of rabbit anti-lectin IgG as shown. Pansorbin (50 µl; 10% w/v) in the same medium was added, and after 4 h at 4°C the cells were removed by centrifugation and the lectin-binding activity in the supernatant solution assayed. Preimmune sera did not give significant amounts of lectin-antibody complexes.

**FIG. 4.** Stability of lectin Fuc-BSA complexes as a function of pH. Complexes were formed at 25°C under the same conditions used for assay of lectin binding. After 1 h the reaction mixtures were adjusted to the pH values indicated and after an additional hour, the complexes precipitated and quantitated as in the standard assays. The final buffer concentration at each pH was 0.25 M. Acetate buffer (●); cacodylate buffer (○); HEPES buffer (■); and Tris buffer (□).

**FIG. 6.** Ordinate: percent maximal binding; abscissa: pH.

**DISCUSSION**

The lectin purified to homogeneity from rat lung as described here is most likely derived from alveolar macrophages. It is immunologically unrelated to either the galactose (1), the mannose/N-acetylgalactosamine (10), or the fucose (12) lectins of rat liver as judged by Western blot analysis. The pure lectin has the same carbohydrate-binding specificity as that of intact macrophages, showing a high affinity for mannose and fucose, a weaker affinity for N-acetylgalactosamine, and no binding to galactose. In addition, it has the same apparent molecular weight as that of the receptor on the cell surface of alveolar macrophages that was labeled in vitro by Wileman and Stahl (17).

Two other lectins are isolated along with the macrophage lectin after two affinity chromatographic steps (Fig. 1). One, with a molecular weight = 32,000 is identical to the mannose/N-acetylgalactosamine lectin from hepatocytes as judged by Western blot analysis. Since Western blot analysis also shows that this lectin is not present in alveolar macrophages, it is likely derived from the blood (11) that contaminates the lungs used as starting material. Little is known about the second lectin (molecular weight = 46,000). Although it is eluted from Fuc-BSA adsorbsents with solutions of galactose, attempts to purify it on Gal-BSA or asialoorosomucoid adsorbers failed. It is not related to the hepatocyte galactose lectin, which has a similar molecular weight, as judged by Western blot analysis.

It is not possible to calculate the yield of the macrophage lectin that is obtained by the purification procedure (Fig. 1) described here, since at least three lectins with overlapping binding specificities copurify through the first several steps. Some loss of the lectin occurs in the first affinity chromatography step on Fuc-BSA where small amounts of lectin pass through the column unretarded. The lectin in this fraction can be adsorbed to Fuc-BSA-Sepharose in Ca²⁺ and eluted from the adsorbent with EDTA. Based on the intensity of staining of bands on electrophoretic gels in sodium dodecyl sulfate under reducing conditions, some lectin is lost during elution of the third Fuc-BSA adsorbent with 100 mM galactose. Very little lectin is lost on elution of this same column with 30 mM mannose, even though mannose is a good inhibitor of binding to Fuc-BSA. Once bound to the adsorbent in the presence of Ca²⁺, the lectin appears to dissociate only slowly in the presence of monosaccharides to which it normally binds in solution.

The binding of oligosaccharides to the macrophage lectin is Ca²⁺ dependent, just as for some other lectins (5). The Ca²⁺ concentration (0.72 mM) for half-maximal binding is well within the physiological Ca²⁺ concentration of extracellular fluids. Thus, ligands could easily bind to the cell surface and be released at the lower intracellular Ca²⁺ concentrations. Similarly, the lectin forms complexes with ligands that are stable at the pH values of extracellular fluids, but the complexes dissociate at the lower pH values seen in lysosomes.
(pH 6). In conjunction with the fact that the lectin is probably membrane-bound, as judged by the need of detergent to solubilize the activity, the Ca²⁺ and pH dependence of this lectin make it a very good candidate as a receptor involved in endocytosis of glycoproteins.

The exact distribution of the lectin in rat tissues other than alveolar macrophages is unclear. Antibodies to the lectin react with nonparenchymal cells of liver, in accord with the observation (12,14) that liver contains a lectin with a $M_r$ = 180,000, but it cannot be ascertained as yet what cell type(s) contain the lectin. In an earlier study (26), only endothelial cells in the nonparenchymal fraction of liver were found to bind ligands that also bind the alveolar macrophage lectin described here. Further studies, however, will be required to ascertain whether endothelial cells, Kupffer cells, or both, contain the same lectin as alveolar macrophages.

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