Isolation and Characterization of a New Member of the Scavenger Receptor Superfamily, Glycoprotein-340 (gp-340), as a Lung Surfactant Protein-D Binding Molecule

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Uffe Holmskov‡§, Peter Lawson†, Børge Teisner‡, Ida Tornøe‡, Antony C. Willist, Cliff Morgan‡, Claus Koch‡*, and Kenneth B. M. Reid‡

From the †Department of Medical Microbiology, Institute of Medical Biology, University of Odense, DK-5000 Odense, Denmark, the §MRC Immunochemistry Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, United Kingdom, the ¶Department of Anaesthesia and Intensive Care, Royal Brompton National Heart & Lung Hospital, London SW3 6NP, United Kingdom, and **Statens Seruminstitut, Copenhagen, DK-2300, Denmark

We have purified a previously unknown glycoprotein (designated gp-340) from human bronchoalveolar lung washings from a patient with alveolar proteinosis. gp-340 was identified by its calcium-dependent binding to lung surfactant protein D (SP-D) and by its molecular mass of 340 kDa in the reduced state on SDS-polyacrylamide gel electrophoresis (PAGE). gp-340 was purified from the 10,000 × g pellet of the lavage fluid by ion exchange and gel permeation chromatography. On SDS-PAGE, gp-340 showed an apparent molecular mass of 290 kDa in the unreduced state. On gel chromatography under non-dissociating conditions, the apparent molecular mass of gp-340 was >1000 kDa. The presence of N-linked glycosylation was shown by digestion with N-glycosidase F, which reduced the apparent molecular mass of gp-340 under reducing condition to about 300 kDa. Partial amino acid sequence data showed the presence of scavenger-receptor type domains. Monoclonal and polyclonal antibodies were raised against gp-340, and their specificities were confirmed by Western blotting. The antibodies were used for immunohistochemical localization of gp-340 in the lung, where it was found on the surface of and within alveolar macrophages. Direct binding between gp-340 and SP-D took place at physiological ionic strength, required the presence of calcium, and was not inhibited by maltose. The binding between SP-D and mannann also required the presence of calcium, but this interaction was completely inhibited by maltose. The same binding pattern was seen between gp-340 and recombinant human SP-D composed of the trimeric neck region and three carbohydrate recognition domains. These findings indicate that the binding between gp-340 and SP-D is a protein-protein interaction rather than a lectin-lectin interaction and that the binding to gp-340 takes place via the carbohydrate recognition domain of SP-D. We conclude that gp-340 is a new member of the scavenger-receptor superfamily and likely to be a truncated form of a receptor for SP-D.

Lung surfactant protein D (SP-D)1 belongs to a group of C-type lectins called collectins (1, 2). Five collectins have been described, mannan-binding lectin (MBL), conglutinin and collectin-43 (CL-43), which are serum proteins produced by the liver; and lung surfactant proteins A and D (SP-A and SP-D), which have until recently been described as surfactant-associated proteins synthesized by alveolar type II cells and by non-iliated bronchial epithelial cells but both have now been found to be present in cells lining the gastrointestinal tract (3, 4). Like the other collectins, SP-D is an oligomeric lectin with subunits composed of three identical polypeptides held together by disulfide bonds and non-covalent interactions at the N-terminal ends of the chains. Each polypeptide chain consists of a short N-terminal segment, followed by a collagen-like sequence, a short α-helical sequence, and the carbohydrate recognition domain (CRD) (5–8). The subunits are linked into oligomers consisting of four to eight subunits (9, 10). SP-D has been shown to bind directly to carbohydrates on the surface of various bacteria as well as to influenza A virus and to Cryptococcus neoformans (11–13). SP-D also interacts with surfactant-associated phospholipids in vitro, suggesting a role in the turnover of pulmonary surfactant in vivo (14, 15). SP-D binds directly to alveolar macrophages (16, 17) and is thereby able to enhance the production of oxygen radicals (18). SP-D can also be internalized by alveolar macrophages in vivo (19). However, the receptor responsible for the SP-D binding remains to be defined.

Two molecules have so far been found to interact with collectins, and both were first described as receptors for the complement protein C1q. One was found to bind MBL, conglutinin, CL-43, and SP-A, and this receptor is now referred to as the collectin receptor (20–22). This molecule, which has a high degree of homology with calreticulin, has been observed on many cell types, including macrophages and alveolar type II cells. The other receptor is referred to as the C1q receptor with a molecular mass of 126 kDa. A monoclonal antibody that recognizes this cell surface molecule inhibited the MBL-mediated enhancement of phagocytosis (23). However, neither of these receptors has been shown to bind SP-D.

In the present paper, we describe the purification and characterization of a 340 kDa glycoprotein obtained from lung la-

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1 The abbreviations used are: SP-D, lung surfactant protein D; SP-A, lung surfactant protein A; CRD, carbohydrate recognition domain; MBL, mannan-binding lectin; CL-43, collectin-43; BAL, bronchoalveolar lavage; SRCR domain, scavenger receptor cystein-rich domain; PAGE, polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; TBS/Tw, TBS-Tween 20; TBS/E, TBS-ethylenglycol; rSP-D, recombinant neck-CRD of human SP-D.

‡ To whom all correspondence should be addressed: Dept. of Medical Microbiology, Institute of Medical Biology, Odense University, DK-5000, Denmark. Tel.: 45 65 57 37 75; Fax: 45 65 91 52 67; E-mail: holmskov@imbmed.ou.dk.

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§ According to all correspondence should be addressed: Dept. of Medical Microbiology, Institute of Medical Biology, Odense University, DK-5000, Denmark. Tel.: 45 65 57 37 75; Fax: 45 65 91 52 67; E-mail: holmskov@imbmed.ou.dk.

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vage from a patient with alveolar proteinosis, and we provide data consistent with this molecule being a secreted form of a putative receptor for SP-D.

MATERIALS AND METHODS

Buffers and Reagents—The buffers and reagents used were Tris-buffered saline (TBS) containing 140 mM NaCl, 10 mM Tris, 7.5 mM Na2PO4, pH 7.2; TBS/Tw consisting of TBS containing 0.05% (v/v) Tween 20 (polyoxyethylene sorbitan monolaurate, Merck-Schuchardt, FRG); TBS/E consisting of TBS containing 0.05% (v/v) emulphogene (polyoxyethylene tridecyl ether, BC 720, from Sigma); coating buffer containing 0.1 M sodium carbonate, pH 9.6; diethanolamine buffer containing 10% (v/v) diethanolamine, 0.5 mM MgCl2, 7.5 mM NaN3, pH 9.8; carbazole emulphogene.

Buffers—20% (polyoxyethylene sorbitan monolaurate, Merck-Schuchardt, FRG); coating buffer containing 0.04% 3-amino-9-ethylcarbazole (Sigma A-5754) in 0.015% H2O2, 50 mM sodium acetate buffer, pH 5.0; and buffer A containing 10 mM Tris, 10 mM EDTA, 0.05% (v/v) emulphogene, pH 7.5. TSK-maltose gel was prepared by combining 100 mg of polyacrylamide of divinylsulfone-activated Fractogel TSK HW-55(F) (1485, Merck, Darmstadt, Germany) (24). Rabbit anti-human Ig (Code A 190, Dakopatts A/S, Copenhagen, Denmark) was coupled to CNBr-activated Sepharose 4B (Pharmacia Biotech Inc.) at a concentration of 2.5 mg of IgG/ml of gel in 0.1 M citrate buffer, pH 7.5, for 2 h at 4 °C.

SDS-PAGE and Western Blotting—Electrophoresis was performed on gels (25) of polyacrylamide gradient gels with discontinuous buffers (25). Samples were reduced by heating at 100 °C for 3 min in 40 mM dithiothreitol, 1.5% (w/v) SDS, 5% (v/v) glycerol, 0.1% Tris, pH 8.0, and were carboxamidated by the addition of iodoacetamide to 90 mM. Unreduced samples were heated in sample buffer with 90 mM iodoacetamide. Protein bands were detected by silver staining (26).

For Western blotting, the FAST system (Pharmacia, Uppsala, Sweden) was used. Proteins were separated on 7.5% polyacrylamide gradient gels with the discontinuous buffer system and blotted onto polyvinylidene difluoride membranes (Innominon B, Millipore, Bedford, MA).

Human Bronchioalveolar Lavage (BAL) —BAL was obtained from a patient suffering from pulmonary alveolar proteinosis. Both lungs were lavaged for therapeutic purposes at an interval of one week, using a heat exchanger and a modified perfusion apparatus to improve the efficiency of the lavage. The result was monitored by analysis of total protein content. BAL was also obtained from four patients undergoing lung washings for diagnostic purposes other than alveolar proteinosis.

Purification of SP-D—One liter of human BAL was centrifuged at 300 × g for 15 min and then at 10,000 × g for 30 min. The 10,000 × g supernatant was made 5 mM in CaCl2 and 10 mM in Tris and applied to a 50-ml maltose-TSK column. After washing with TBS/E containing 5 mM CaCl2, the column was eluted with the same buffer containing 100 mM maltose. The eluate was applied to a column of rabbit anti-human Ig Sepharose (15 ml) to remove contaminating anti-carbohydrate antibodies and then passed through a Superose 6 FPLC column (Pharmacia) equilibrated with TBS containing 10 mM EDTA and 0.05% (v/v) emulphogene.

Purification of gp-340—Purification of gp-340 was performed on a computer-monitored FPLC system (FPLC Director Version 1.3, Pharmacia). One liter of human BAL was centrifuged at 300 × g for 10 min. After separation of the cell pellet, the supernatant was centrifuged at 10,000 × g for 30 min. The precipitate was dissolved in 500 ml of TBS/E containing 10 mM EDTA and incubated for 18 h at 4 °C with gentle stirring. Insoluble material was removed by centrifugation at 10,000 × g for 30 min. The supernatant was dialyzed against buffer A and retained proteins were eluted with a linear gradient from 0 to 1 mM NaCl in buffer A. Fractions containing gp-340 were diluted in buffer A, applied to a Resource Q column (1-l ml packed column, Pharmacia) and eluted with a linear gradient from 0 to 1 mM NaCl in buffer A. The gp-340-containing fractions were further separated by gel permeation chromatography on a Superose 6 prep grade column (1.6 × 57 cm, Pharmacia) with TBS, pH 7.4, containing 10 mM EDTA, at a flow rate of 30 ml/h. The purity of the resulting gp-340 preparation was analyzed by gel permeation chromatography on a Superose 6 column (1 × 30 cm, Pharmacia) and by SDS-PAGE.

The amount of gp-340 was estimated by measurement of absorption at 280 nm assuming that ε 280 of 1 mg/ml for gp-340 was 1.0.

Amino Acid Sequencing and Amino Acid Analysis—The procedures were as described in (27). Amino acid analysis was performed directly on purified gp-340 using an Applied Biosystems 420A amino acid analyzer (Perkin-Elmer, Waltham, MA). All sequences of intact gp-340 were unsuccessful. To obtain internal sequence data, partially pure gp-340 was run on 7.5% SDS-PAGE, and protein bands were detected with Coomassie Brilliant Blue R-250. The two bands of gp-340 (A = upper band and B = lower band) were excised from the SDS-PAGE gel, homogenized in 100 mM Tris, pH 8.0, and digested with trypsin (Modified Trypsin-Sequence Grade, Promega UK Ltd.) at an estimated enzyme:substrate ratio of 1:20 for 16 h at 37 °C. The supernatants were then run on by reverse phase chromatography using a Brownlee BU-300 VC4 column (100 × 1 mm). Peaks were collected and sequenced using an Applied Biosystems 470A protein sequencer (Perkin-Elmer). Purified gp-340 was also digested directly with trypsin digestion and purification of peptides as above. Sequences similar to gp-340 were searched for in GenBankTM/EBI data bank plus DDBI and Protein Data Bank data bases using the BLAST program. Alignment of multiple sequences were carried out using the Clustal method with PAM250 residue weight table.

Expression of Recombinant Neck-CDR of Human SP-D (rSP-D)—A recombinant polypeptide composed of the α-helical neck region and the CDR of human SP-D was expressed in E. coli using the glutathione S-transferase gene fusion vector (Pharmacia) and purified as described in (8).

Preparation of Anti-gp-340 Antiserum—Antisera were raised in rabbits by subcutaneous immunization with an emulsified mixture of equal volumes of gp-340 (approximately 20 μg per dose) and Freund's complete adjuvant (Statens Seruminstitut, Copenhagen, Denmark). The rabbits were boosted at 1 month intervals with gp-340 in Freund's incomplete adjuvant. Irrelevant antibodies were removed by absorption: the antiserum was incubated with 1/10 volume of normal human serum and with 80 μg of SP-A/ml for 18 h at 4 °C followed by a centrifugation at 10,000 × g for 30 min. The IgG fraction of the rabbit antiserum against gp-340 was purified on a 1-ml HitrapTM Protein G column (Pharmacia, Uppsala, Sweden), and the specificity was confirmed by Western blotting.

Production of Monoclonal Antibody Against gp-340—CF1 × BALB/c female mice were immunized with 10 μg of purified gp-340 in 0.25 ml of 0.1 M sodium phosphate, adsorbed onto 1 mg of Al(OH)3, gel (Alhydrogel®, Superfoss, Denmark) per injection. The injections were repeated at intervals of 14 days. Mice with a high antibody titer against gp-340, as determined by direct enzyme-linked immunosorbent assay on gp-340-coated microtiter plates, were selected for fusion of spleen cells. Cells were fused with wells antibodies against gp-340 were recloned through the use of the limiting dilution method (30). The monoclonal antibody selected was of IgG1 subclass, and its specificity was tested by Western blotting.

Deglycosylation of gp-340—The amount of N-linked saccharides in gp-340 was evaluated by enzymatic digestion with N-glycosidase F for 18 h at 37 °C using the PNGase F kit (New England Biolabs, Beverly, MA) followed by SDS-PAGE.

Imunohistochemistry—The indirect immunoperoxidase staining technique was used. Briefly, the procedure was as follows. The dried frozen sections were fixed in acetone for 10 min, washed in TBS, preincubated in 2% (v/v) BSA in TBS for 10 min, incubated for 30 min with the monoclonal or polyclonal antibody against gp-340 diluted in 1% (v/v) BSA in TBS, washed with TBS, incubated for 1/2 h with peroxidase-labeled goat anti-mouse Ig (P 447, Dakopatts) or peroxidase-labeled goat anti-rabbit Ig (P 448, Dakopatts) diluted 1:75 in TBS, washed with TBS and H2O, incubated with carbazole buffer for 20 min, washed, counterstained with Mayer's hematoxylin for 2 min, and mounted in Aquamount (BDH Limited Poole, UK). Staining was also performed with a monoclonal anti-CD68 antibody as primary antibody (C6178, Dakoppats).

Conventional staining controls were performed as well as replacing the primary antibody with an unrelated monoclonal antibody of the same subclass as the anti-gp-340 antibody, or preabsorbing the primary polyclonal antibody with highly purified gp-340.

Analysis of SP-D and rSP-D binding to gp-340—Microtiter plates (Polsor, Nune, Kemstrup, Denmark) were coated with gp-340 (300 ng/ml in coating buffer) by overnight incubation. This incubation and

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RESULTS

During the purification of human SP-D from the 10,000 × g supernatant from BAL of a patient suffering from alveolar proteinosis, a molecule with an apparent molecular mass of 340 kDa was observed by SDS-PAGE in reducing conditions. When the supernatant was applied to a maltose-TSK column in the presence of calcium, the component was bound and could be eluted with maltose together with SP-D and anti-carbohydrate antibodies. The component could then be separated from SP-D and immunoglobulins by gel permeation chromatography in the presence of EDTA (data not shown). Rechromatography of the recovered component on a maltose-TSK column in the presence of calcium showed no binding, indicating that the initial retention of the component on the column must be mediated by one of the other proteins bound to the maltose. Because of low yields, another purification procedure was developed, utilizing the 10,000 × g pellet from BAL as starting material for ion-exchange and gel permeation chromatography. The component was named gp-340 because of its glycoprotein nature and because of its molecular mass of 340 kDa in the reduced state on SDS-PAGE (see below).

Purification of gp-340—gp-340 was eluted from the 10,000 × g pellet in the presence of EDTA and purified by ion-exchange and gel permeation chromatography. Fig. 1 shows the elution profile from ion-exchange on Mono-Q Fast Flow (Fig. 1A), on Resource Q (Fig. 1B), and the gel permeation chromatography on Superose 6 Pregrade (Fig. 1C). Purified gp-340 was analyzed by chromatography on Superose 6 where it emerged close to the void volume, corresponding to an apparent molecular mass >1000 kDa (Fig. 1D). Approximately 500 μg of gp-340 (estimated by E280) was obtained from 1 liter of washings.

Fig. 2 shows a comparison by SDS-PAGE of gp-340 reduced (lane 1), unreduced (lane 2), and reduced after treatment with N-glycosidase F (lane 3). gp-340 showed two closely spaced bands with a mobility corresponding to a molecular mass of approximately 340 kDa when reduced and an apparent molec-
ular mass of 290 kDa in its unreduced state. When glycosidase-
digested gp-340 was examined on SDS-PAGE in its reduced
state, the two closely spaced bands appeared at a position
corresponding to a molecular mass of approximately 300 kDa.

Amino Acid Sequencing—Amino acid sequences of different
fragments obtained by tryptic digestion of gp-340 are shown in
Fig. 3. Peptide 5 obtained from the upper band of gp-340 (band
A) was found to have an identical N-terminal sequence to that
of peptide 6 obtained from the lower band of gp-340 (band B).

Amino Acid Composition—The amino acid composition of
gp-340 is shown in Table I. The presence of 0.3% hydroxypro-
line indicated that gp-340 might contain collagen-like se-
quenences. However, gp-340 was not digested by collagenase
(data not shown), and the appearance of hydroxyproline in the
amino acid composition of gp-340 may be due to small amounts
of SP-A or SP-D in the gp-340 preparation.

Characterization of Antibodies—The specificities of the se-
lected monoclonal and polyclonal antibodies against gp-340
was analyzed by Western blots of both purified gp-340 and
crude lung lavage separated on SDS-PAGE in the reduced and
unreduced state (Fig. 4). The monoclonal antibody directed
against gp-340 reacted with both bands of 340 kDa equally well
in the reduced and unreduced state, and it also reacted with
deglycosylated gp-340 (Fig. 4A). The rabbit anti-gp-340 anti-
body reacted with both bands of 340 kDa, but, in contrast to the
monoclonal antibody, it displayed less binding after reduction
of the antigen and no binding when the antigen was reduced
and deglycosylated (Fig. 4B).

Western blot analysis revealed that the monoclonal antibody
also reacted with gp-340 in lung washings from four patients
undergoing diagnostic bronchoalveolar lavage for other rea-
sons than alveolar proteinosis (data not shown).

Immunohistochemistry—Staining of normal human lung
revealed gp-340 immunoreactivity on and within the alveolar
macrophages (Fig. 5). The staining was distinctly granular
with both types of antibody. However, staining with the mon-
oclonal antibody was distinctly weaker than that obtained with
the polyclonal antibody. Staining of serial sections with mono-

![FIG. 4. Specificities of monoclonal (A) and polyclonal (B) anti-
gp-340 antibodies tested by Western blotting. Lane 1, reduced
purified gp-340; lane 2, unreduced purified gp-340; lane 3, reduced
purified gp-340 after N-glycosidase F treatment; lane 4, reduced crude
lung washing; lane 5, unreduced crude lung washing. The blots were
incubated either with monoclonal anti-gp-340 antibody (A) (50 ng/ml),
or polyclonal anti-gp-340 antiserum diluted 1:1000 (B). Bound antibody
was visualized by means of alkaline-phosphatase-coupled rabbit anti-
mouse Ig (A) or alkaline-phosphatase-coupled goat anti-rabbit Ig (B)
and substrate.](http://www.jbc.org/content/182/17/4166/F4)

![FIG. 5. Immunohistochemical localization of gp-340 in normal
human lung. The sections were counterstained with Mayer’s hema-
toxylin. A, section of human lung stained with the monoclonal antibody
against gp-340 in which a macrophage shows distinct staining corre-
sponding to the membrane region. B, section of human lung stained
with the polyclonal antibody against gp-340. C, Human lung stained
with control monoclonal antibody.](http://www.jbc.org/content/182/17/4166/F5)
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The present report describes the identification and purification of an SP-D-binding protein from BAL obtained from a patient with alveolar proteinosis. The SP-D-binding molecule has provisionally been termed gp-340 since it is a glycoprotein...
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Fig. 9. Bovine gallbladder mucin is a densely glycosylated molecule which is the primary secretory product of the gallbladder epithelium (33). Four SRCR domains are found in bovine gallbladder mucin along with short tandem repeating sequences found in mucins. Ebnerin, which was cloned from a rat taste bud gland cDNA library (34), is also a mosaic protein containing four SRCR, two bone morphogenic domains, a zona pellucida domain, a transmembrane region, and a short cytoplasmic tail. CRP-ductin-alpha (35) has an overall domain organization similar to Ebnerin, but it has eight SRCR domains instead of four and five bone morphogenic domains instead of two. The cDNA of CRP-ductin-alpha, which is the largest of the three molecules, predicts a protein of 2083 amino acid residues with a molecular mass of 227 kDa and shows an apparent molecular mass of 260 kDa in the reduced state on SDS-PAGE. The molecular mass of reduced and deglycosylated gp-340 was estimated to be 300 kDa using reduced and unreduced fibronectin (molecular mass = 220 kDa and 440 kDa, respectively), purified α-2 macroglobulin-receptor (molecular mass = 500 kDa, a gift from Søren Moestrup, Aarhus University, Denmark), together with standard molecular weight markers (data not shown). A very high degree of homology was found both between the SRCR domains of mouse CRP-ductin-alpha and bovine gallbladder mucin and peptides 1–7 of gp-340 (100% amino acid sequence identity between SRCR 1, 3, and 4 of bovine gallbladder mucin, and with SRCR 3 of CRP-ductin-alpha and the 19 amino acid residues in peptide 6 of gp-340) (Fig. 9). However, as the same high degree of homology is seen on comparison of the different proteins of SRCRs and between species, and, in view of the size difference between gp-340 and the gall-bladder mucin, Ebnerin, and the CRP-ductin-alpha proteins, it seems unlikely that gp-340 is the human analogue of these proteins. Moreover, CRP-ductin-alpha mRNA was only expressed in cells lining the small and large intestine, the pancreatic duct system, and the larger hepatic ducts, and CRP-ductin-alpha mRNA was detected in the lung (35).

Purified gp-340 was used to immunize mice and rabbits, and the resulting monoclonal and polyclonal antibodies were used for the immunohistochemical localization of gp-340. In the lung, both antibodies revealed a distinct granular staining of alveolar macrophages, with the most intense staining at the cell membrane, but in some cells, organelles considered to be phagolysosomes were also stained. This staining pattern is in agreement with what would be expected for a receptor for SP-D. It has earlier been shown that SP-D is found in the endocytic compartment of rat and human alveolar macrophages, and a receptor for SP-D will probably be internalized together with SP-D and material bound via the CRD of SP-D (19, 36). It is also worth noting that, to date, all the proteins containing SRCRs of type B are known to be membrane bound, and both CRP-ductin and Ebnerin are known to have both a membrane-bound form and a free form due to either cleavage of the molecules or alternative splicing of the molecules (34, 35).

Direct binding of SP-D to purified gp-340 was observed at physiological ionic strength. The binding was saturable, and dependent on the presence of calcium ions. No inhibition was observed on the addition of the disaccharide maltose. In contrast, binding of mannose to SP-D was completely inhibited by maltose. The same binding pattern was seen between rSP-D (consisting of the neck-region and CRD of SP-D) and gp-340. Maltose would be expected to inhibit any binding of the CRD of SP-D to carbohydrates on gp-340. The result thus suggests that the binding between SP-D and gp-340 is a protein-protein interaction rather than a lectin-carbohydrate interaction, and the fact that the rSP-D is also bound shows that the binding between SP-D and gp-340 is a protein-protein interaction.

Fig. 8. Binding of rSP-D to gp-340 and mannan. A, mannan-coated microtiter plates were incubated with serial dilutions of rSP-D in the presence of calcium (●●●), calcium and 100 mM maltose (▲▲▲), or with 10 mM EDTA (■■■). B, gp-340-coated microtiter plates were incubated with serial dilutions of SP-D in the presence of calcium (●●●), calcium and 100 mM maltose (▲▲▲), or with 10 mM EDTA (■■■). Bound SP-D was detected by means of rabbit anti-human SP-D, followed by alkaline-phosphatase-coupled goat anti-rabbit Ig and substrate.

Fig. 9. Alignment of peptides 1–4, 6, and 7 from gp-340 with SRCR domains of members of group B of the scavenger-receptor superfamily. The domains are aligned by the Crustal method with the PAM250 residue weight table. The sequences shown are bovine gallbladder mucin domains 1–4 (33); CRP-ductin-alpha domains 1–8 (35); and Ebnerin domains 1–4 (34).
site is located within the neck-CRD structure.

The crystal structure of the CRD of rat MBL-A complexed with an oligosaccharide revealed two calcium binding sites (37, 38). The calcium ion in site 2 forms coordination bonds with the carbohydrate ligand while no such bonds were found for the calcium ion in site 1. All the amino acid residues involved in calcium and carbohydrate binding in rat MBL-A are present in human SP-D. No function of the first calcium binding site has so far been suggested for any CRD. We, therefore, suggest that this calcium binding site may be important for recognizing ligands other than carbohydrates. It is interesting to note that CD72, which has one extracellular CRD, is the ligand for CD5 which has three extracellular SRCRs (39).

It has earlier been shown that the direct binding of SP-D to alveolar macrophages (and neutrophil granulocytes) can be completely abolished by maltose, indicating that SP-D in these experiments bound to the cells via a carbohydrate-lectin interaction (19). SP-D also enhances the respiratory burst in rat alveolar macrophages (18), a reaction which was also inhibited by carbohydrates. These observations suggest that more than one ligand or receptor for SP-D is present on alveolar macrophages.

We conclude that gp-340 is the soluble form of a molecule that binds SP-D in a calcium-dependent manner. The gp-340 molecule is thus a potential receptor for SP-D that has been shed or cleaved from the surface of the alveolar macrophages.

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REFERENCES