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Gene for an extracellular matrix receptor protein from *Pneumocystis carinii*

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ABSTRACT **An initial and crucial step in the establishment of many microbial infections is the attachment of the pathogen to the host cells. Thus, adherence of *Pneumocystis carinii* (Pc) to type I pneumocytes is believed to be important in the induction of Pc pneumonia. Little is known about the nature of the attachment of Pc to type I cells, although extracellular matrix (ECM) proteins, such as fibronectin and laminin, have been implicated in the process. We report here the isolation of a Pc gene encoding a receptor protein that binds both fibronectin and laminin *in vitro*. A cDNA clone encoding the Pc ECM receptor was isolated from a Pc cDNA library and identified on the basis of sequence homology to the human colon carcinoma laminin receptor. Southern blot analysis of Pc genomic DNA confirmed that the cDNA was of Pc origin. Northern blot analysis of Pc total RNA showed a predominant mRNA of ≈1400 nucleotides that hybridized to the ECM receptor gene. The ECM receptor predicted from the cDNA sequence is 295 amino acid residues long, with a molecular mass of 32.8 kDa. The C-terminal third of the polypeptide is highly negatively charged, whereas the N-terminal two-thirds contains hydrophobic segments that may play a role in membrane association. Sequence analysis and alignment of the N terminus with the laminin receptor cDNA sequence of human colon carcinoma support the conclusion that the Pc ECM receptor cDNA clone is a full-length clone. A Western blot of the overexpressed ECM receptor protein bound both laminin and fibronectin *in vitro*. Antibodies raised to the overexpressed receptor protein interacted with a 33-kDa protein in total Pc cell lysates. These findings raise the possibility that the Pc ECM receptor protein may mediate the organism's attachment to type I pneumocytes and, thus, may play a crucial role in Pc pathogenesis.**

Pneumocystis carinii (Pc) is an opportunistic pathogen found widely distributed among mammalian species (1). In humans, it may give rise to fatal pneumonia when the patient is immunosuppressed. Electron microscopic studies have shown that when the Pc trophozoite is in contact with type I alveolar pneumocytes, it attaches to the luminal surface, often becoming surrounded by the cell without, however, evidence of membrane fusion (2). During this process, the type I pneumocyte becomes thicker, and there is reduplication of the laminin-containing basement membrane (2). Recently, the extracellular matrix (ECM) proteins, fibronectin and laminin, associated with the type I pneumocytes have been shown to be involved in the initial attachment of Pc to type I pneumocytes (3). This suggests that a specific receptor may exist on the surface of Pc to mediate such a selective attachment. In this paper, we report the isolation and characterization of a cDNA clone for a 33-kDa receptor protein from Pc of rat origin. The product of the receptor gene has sequence similarity to the nonintegrin laminin receptor family (4). The receptor gene has been cloned into an expression vector.** The expressed 33-kDa protein product of the Pc receptor gene binds both laminin and fibronectin *in vitro*; hence, it is an ECM receptor protein. On a Western blot, polyclonal antibodies raised to the 33-kDa overexpressed protein recognize a 33-kDa protein in total Pc cell lysates.

MATERIALS AND METHODS

Screening Pc cDNA Libraries. Two Pc cDNA libraries (Pc-3 and Pc-4) were prepared from rat Pc RNA inserted into λ phage YE1 (J.C.E., unpublished data). This vector permits excision *in vitro* of inserts into a phagemid vector that contains the necessary elements for propagation in *Escherichia coli* and for expression and replication in yeast (Fig. 1). *E. coli* XL1 blue and DH5α were used as phagemid hosts and were grown on LB medium supplemented with tetracycline (12.5 μg/ml) and/or ampicillin (60 μg/ml). An oligonucleotide (45-mer) was synthesized (Keck Biotechnology Resource Laboratory, Yale University) corresponding to a conserved region of the GSY-1 gene of *Saccharomyces cerevisiae* (5): GSY-1-B, 5′-CGCGGCTATCACTTGGGTGTTTTTCCATCCTATTAC-TACGAACCATGG-3′. The oligonucleotide was 5′-end-labeled with [γ-^{32}P]ATP (6) and used as a probe to screen the Pc cDNA library. To select full-length clones, the 560-bp insert from clone 5 was ^{32}P-labeled by hexamer random-chain extension and used as a probe. Approximately 10^3 colonies were screened by filter hybridization, and potentially positive colonies were picked and rescreened with the same probe, under stringent conditions (6).

Southern and Northern Blot Analyses. Pc genomic DNA and total RNA were isolated from 5×10^8 cultured Pc organisms isolated from rats by the method of Radding *et al.* (7). For Southern blot analysis, 5–10 μg of Pc genomic DNA was digested with *Hin*dIII and *Bam*HI. In addition, control *Hin*dIII digests of HeLa cell DNA, *Saccharomyces cerevisiae* DNA, and rat and mouse liver DNA were prepared. All samples were electrophoresed on a 0.7% agarose gel, blotted onto a Zeta-Probe membrane (Bio-Rad), baked (6), and then probed with the 560-bp clone 5 fragment randomly labeled with [α-^{32}P]dATP (Boehringer Mannheim random labeling kit). For a Northern blot analysis, Pc total RNA was subjected to electrophoresis on a 2.2 M formaldehyde/1.5% agarose gel (6) and RNA bands were then transferred to a Zeta-Probe membrane (Bio-Rad). The 560-bp fragment from clone 5 was ^{32}P-labeled by hexamer random labeling and used as a probe in filter hybridization.

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Abbreviations: Pc, *Pneumocystis carinii*; ECM, extracellular matrix; IPTG, isopropyl β-D-thiogalactoside; ORF, open reading frame.

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**The sequence reported in this paper has been deposited in the GenBank data base (accession no. U09451).

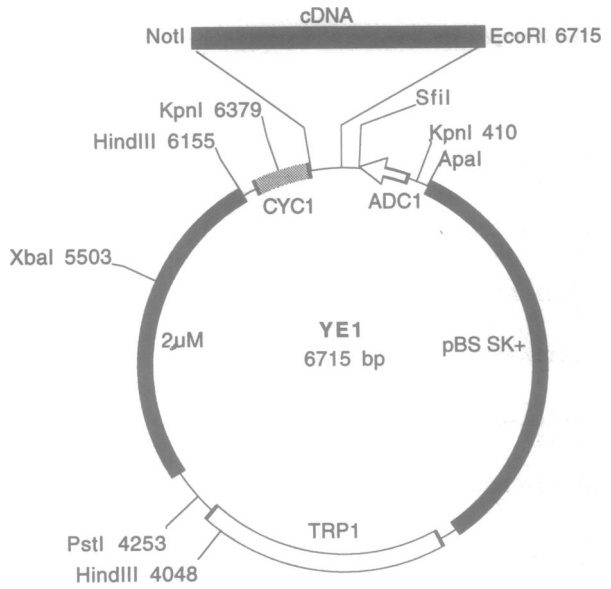

FIG. 1. Restriction map of phagemids excised from YE1. cDNAs are directionally cloned as *Eco*RI–*Not* I (5′ → 3′) fragments into the vector. ADC, yeast constitutive alcohol dehydrogenase promoter; CYC, yeast cytochrome *c* terminator; 2 μm, yeast 2-μm replication origin; TRP1, yeast tryptophan gene, selectable marker.

Nucleotide Sequencing. Oligonucleotide primers YEI-1 and YEI-2, complementary to portions of the constitutive *ADC* promoter and *CYC1* terminator regions of the YE-1 vector flanking the insert, were used in DNA sequencing reactions. Sequence alignment and homology searches were carried out using either the program FASTA (8) or the GCG comparison programs.

Expression Plasmid Construction. The full-length 1.0-kb Pc receptor (*ecmR*) gene was excised from the YE1-1 vector by digestion with *Eco*RI and *Not* I and, subsequently, cloned into the *Eco*RI–*Not* I fusion cloning site of the pET22b vector (Novagen). The recombinant pET22b vector was then digested with *Nco* I, which cuts at the initiation codon of the Pc gene (CCATGG), and *Xho* I to release a 0.9-kb insert containing the entire coding region of the Pc *ecmR* gene. For protein expression, the restriction fragment was subcloned into the *Nco* I/*Xho* I-digested pET15b vector (Novagen). This in-frame construct (pET15b-ecmR) allows efficient translation from the initiation ATG codon of the Pc *ecmR* gene, resulting in a full-length 33-kDa protein product. For protein production, the recombinant plasmid was introduced into the host strain BL21 (9), which is inducible by isopropyl β-D-thiogalactoside (IPTG; 1 mM). Pc *ecmR* gene expression was induced for 5 h with IPTG (1 mM), and the soluble and insoluble fractions were separated as described by the manufacturer (pET System Manual, Novagen). The expressed protein product remained in the insoluble fraction and was dissolved in 6 M urea/50 mM Tris·HCl, pH 8.0, containing 1 mM EDTA, 2 mM dithiothreitol, and 0.1 M NaCl. After centrifugation at 10,000 × *g* for 10 min, the supernatant was dialyzed for 48 hr against six changes of this buffer containing decreasing concentrations of urea (6–0 M).

N-Terminal Sequence Analysis. The urea-solubilized fraction of the induced BL21/pET15b-ecmR cell lysate was electrophoresed on a Laemmli SDS/10% polyacrylamide gel (10) and electrotransferred to Immobilon membrane according to the manufacturer's protocol (Millipore). The membrane was stained with Coomassie blue, and the protein band corresponding to the expressed protein was excised and microsequenced (11) at the W.M. Keck Foundation Biotechnology Facility.

Western Blot Analysis of the Expressed Protein. The total urea-solubilized protein fraction from BL21/pET15b-ecmR gene cells (10 μg) was electrophoresed on a Laemmli SDS/10% polyacrylamide gel (10). The proteins were electrotransferred to Immobilon P membranes and a sandwich Western blot analysis was performed (12). Binding to the ligand laminin (Sigma) or fibronectin (Sigma) was analyzed using anti-laminin or anti-fibronectin (Sigma) polyclonal antibodies as the primary antibody and anti-rabbit horseradish peroxidase-conjugated secondary antibody (13).

Extraction of Pc Proteins and Western Blot Analysis. Polyclonal antibodies were raised in rabbits to the gel-purified expressed Pc receptor protein in complete Freund's adjuvant. Pc was purified and total protein was extracted by the method of Williams *et al.* (14). The protein fraction was concentrated 10-fold to 100–200 ng of protein per μl and applied to a Laemmli 10% polyacrylamide gel (10), electrophoresed, and electrotransferred to an Immobilon P membrane. The Western blot was probed with anti-Pc receptor protein polyclonal antibodies.

RESULTS

A Full-Length cDNA Clone for a Pc ECM Receptor Gene Is Identified. During an earlier study on Pc glucan synthases, a probe based on the glucan synthase *GSY1* gene of *Saccharomyces cerevisiae* (5) was used to screen two Pc cDNA phagemid libraries and after three rounds of stringent hybridization, five potentially positive clones were selected. Digestion of phagemid DNA from three clones with *Eco*RI and *Not* I yielded a 560-bp insert. Pc DNA from each of these clones was amplified by PCR, using oligonucleotide primers complementary to the YE1 vector sequences that flank the *Eco*RI and *Not* I cloning sites. When the insert DNAs were probed with the *GSY1* oligonucleotide by Southern blot analysis, all three PCR-amplified 560-bp fragments hybridized to the *GSY1* probe and were identical on sequence analysis. A single open reading frame (ORF) of 474 bp was present, predicting a protein fragment of 158 amino acid residues. At the 3′ end of the nucleotide sequence was a translation termination codon, followed by 78 nucleotides of presumably untranslated sequence and a poly(A) tail (Fig. 2). Comparison of the predicted protein to Swiss-Prot and GenBank data bases using the FASTA program (8) revealed significant similarity to the derived amino acid sequence of the 33-kDa human colon carcinoma laminin receptor protein (15) but no obvious homology to previously characterized glucan synthases. It is not clear why the *GSY1* gene-based probe hybridized to the three clones since it exhibited only a maximum of 78% complementarity to the Pc *ecmR* gene fragment over a stretch of 14 bases.

The same Pc cDNA libraries were rescreened using the 560-bp partial PC *ecmR* fragment as a hybridization probe, and six positive clones were obtained. The clone with the largest cDNA fragment, P-23, contained a 1.0-kb insert whose nucleotide sequence (Fig. 2) overlapped with the entire 560-nucleotide sequence of the original clones and contained an additional 419 nucleotides of upstream sequence. The 979-bp cDNA sequence contains a single ORF of 885 bases. This ORF begins with a methionine and is preceded by an in-frame stop codon. A near-consensus polyadenylylation signal sequence (AAUAA) and a small poly(A) region are present at the 3′ end of the insert. These findings establish the orientation of the clone with respect to mRNA transcription and suggest that the entire coding sequence is contained within clone P-23. The ORF in clone P-23 encodes a protein of 295 amino acids with a predicted molecular mass of 32.8 kDa. The derived amino acid sequence was compared with that of laminin receptors from other sources and the detected similarities are shown in Fig. 3. Members of this family of laminin receptors share considerable amino acid sequence similarity in the region

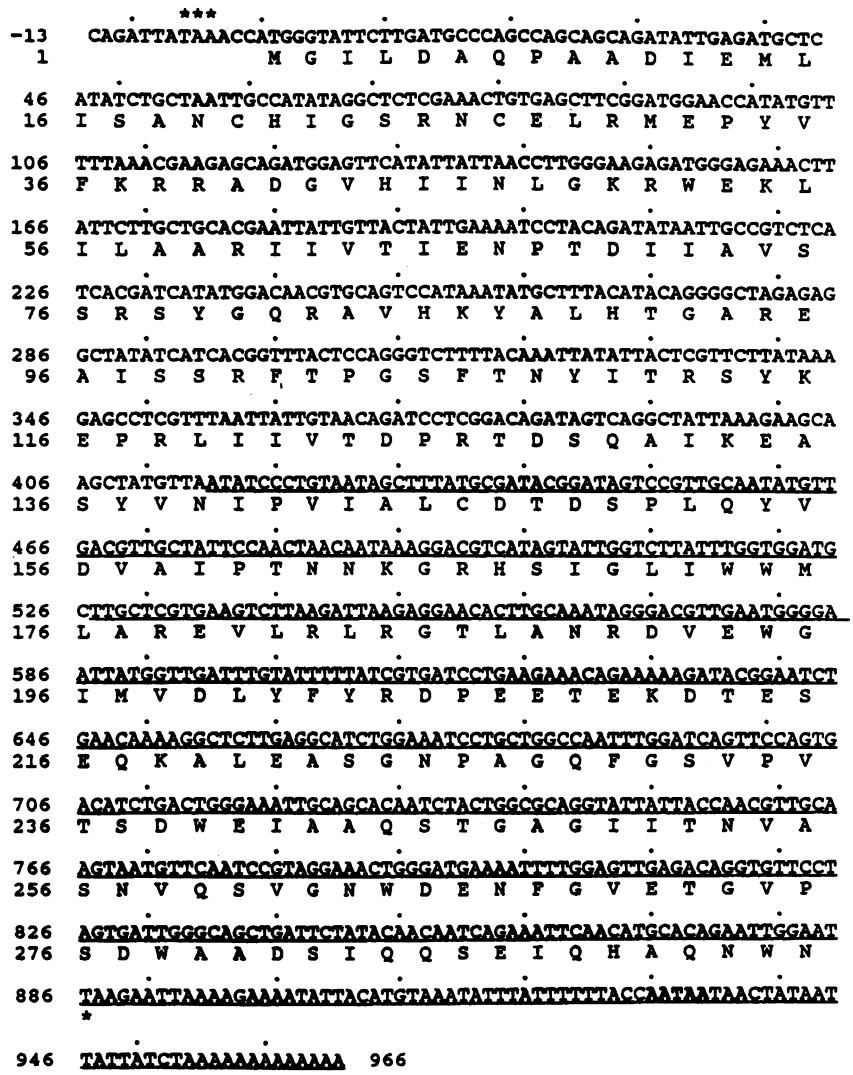

FIG. 2. Nucleotide sequence of the cDNA insert of clone P-23 and the derived amino acid sequence. The deduced amino acid sequence is shown below the nucleotide sequence of the DNA coding strand. Nucleotides and amino acids are numbered on the left margin (nucleotide 1 is the first nucleotide of the initiation codon). The in-frame stop codon in the 5′ untranslated region of the mRNA is indicated above the DNA sequence by three asterisks. The near-consensus polyadenylylation signal in the 3′ untranslated region is in boldface type. The underlined sequence from nucleotides 416 to 966 designates the overlapping sequence of the 560-bp partial *ecmR* gene fragment.

between residues 1 and 211. In this region there is ≈68% similarity (60% identical residues and 8% conservative substitutions) between the Pc ECM receptor protein and other proteins of this class. The C-terminal one-third is hydrophilic and the N-terminal two-thirds has four or five hydrophobic regions that are potential transmembrane segments. This, again, is similar to what is predicted for mammalian laminin receptors (15, 16).

The lack of amino acid (and nucleotide sequence) similarity between the C terminus of the Pc ECM receptor and mammalian laminin receptors suggests that the cDNA clones come from Pc mRNA and not from rat mRNA. Also, the sequence of P-23 cDNA is A+T-rich (62% A+T in the coding region) and the codon usage is biased toward adenosine or thymidine in the third position (80% A/T) as opposed to a 68% G/C bias in the murine laminin receptor gene (16). Similar A+T-rich biases are seen in other Pc genes (17–19).

The ECM Receptor Gene Is of Pc Origin. Fig. 4 shows a Southern blot hybridization of genomic Pc DNA digested with two restriction enzymes. Both digests gave a single fragment that hybridized with the 560-bp Pc *ecmR* probe. Similar analysis of restriction fragments of rat liver DNA (data not shown), human HeLa cell DNA, mouse liver DNA, and *Saccharomyces cerevisiae* DNA showed no bands that hybridized to the Pc *ecmR* probe (Fig. 4). Thus, cDNA clone P-23 appears to be of Pc origin.

The Pc ECM Receptor Gene Is Transcribed. A predominant band of ≈1.4 kb hybridized with the 560-bp *ecmR* gene fragment, as shown in Fig. 5. The estimated size of the band is slightly larger than the 979-bp long cDNA obtained. This may be due to the presence of a long poly(A) tail, although the few Pc mRNAs characterized to date have short 5′ and 3′ untranslated regions (20). It should be noted that murine (16) and human (15, 18) laminin receptor mRNAs appeared by Northern blot analysis to be 1.4–1.7 kb. However, primer-extension assays and cDNA sequencing indicated that the mRNAs were only 1086 and 977 nucleotides long, respectively. Thus, the discrepancy in the size of the Pc mRNA between agarose gel analysis (Northern blot) and cDNA sequence analysis has experimental precedent.

Overexpression and the N-Terminal Sequence of the Pc ECM Receptor. The 1.0-kb full-length coding region of the Pc *ecmR* gene was cloned into the pET15b expression vector and expression of the gene in BL21 cells was induced by IPTG. After cell lysis, the induced protein remained in the insoluble fraction and was dissolved in 6 M urea. The urea-soluble fraction was electrophoresed on a Laemmli SDS/10% polyacrylamide gel, electrotransferred to an Immobilon membrane, and microsequenced. The protein band was found to be >95% pure and 16 cycles of amino acid sequencing showed identity with the predicted amino acid sequence of the Pc *ecmR* gene.

The Pc ECM Receptor Protein Binds Laminin and Fibronectin. A sandwich Western blot analysis of the overexpressed protein product was carried out to examine its ligand specificity. Laminin and fibronectin were tested as potential ligands and their binding was assayed. The 33-kDa protein band representing the protein product of the Pc *ecmR* gene

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Pc  #1    M·GILDA·QP AA·DIEMLIS ·ANC·HIGSR NCELRMEPYV FKRRADGVHI INLGKR·WEK LILAARIIVT IENPTDIIAV
Dro #1                    --VA ·T··T-L-SE -VNFQ--Q-- Y--RA--VN- L-----·T--- -Q----A--A -D--S--FVI
Hum #1    -S-A--VL-M KEE-V···KF L-AGT-L-GT -LDFQ--Q-I Y--KS--IY- ---·--T--- -L----A--A ----A-VSVI
Mur #1    -S-A--VL-M KEE-V···KL L-AGT-L-GT -LDFQ--Q-I Y--KS--IY- ---·--T--- -L----A--A ----A-VSVI

Pc  #75   SSRSYGQRAV HKYALHTGAR EAISSRFTPG SFTNYITRSY KEPRLIIVTD PRTDSQAIKE ASYVNIPVIA LCDTDSPLQY
Dro #61   ---PI----- L-F-KY-DTT ·P-AG----- A---Q-QPAF R----LV--- -NT-H-PIM- ---------- FTN-----R-
Hum #78   ---NT----- L-F-AA---T ·P-AG----- T---Q-QAAF R----LV--- --A-H-PLT- -----L-T-- --N-----R-
Mur #78   ---NT----- L-F-AA---T ·P-AG----- T---Q-QAAF R----LV--- --A-H-PLT- -----L-T-- --N-----A-

Pc  #155  VDVAIPTNNK GRHSIGLIWW MLAREVLRLR GTLANRDVEW GIMVDLYFYR DPEETEKDTE SE·QKALEAS GNPAGQFGSV
Dro #140  I·I---C--- SA-----M-- L--------- --IS··S--- PVV---F--- ----A--EE· AAA··E-LPP PKIEEAVDH.
Hum #157  -·T---C--- -A--V--M-- --------M- --IS··EHP- EV-P------ -------EEQ AAAE--VTKE EFQ··-EWTAP
Mur #157  -·T---C--- -A--V--M-- --------M- --IS··EHP- EV-P------ -------EEQ AAAE--VTKE EFQ··-EWTAP
Rat                                                                                   EFQ.-EWTAP

Pc  #234  ·PVTSDWEIA  AQSTGAGIIT  NVASNVQSVG  NWDENFGVET  GVPSDWAADS  IQQSEIQHAQ  NWN*    #295
Dro #216  ·--EETTNWA  DFVAAETVGG  VEDW-EDT-K  TSWGSDGQF*                                  #254
Hum #235  A-EFTATQPE  VADWSE-VQV  PSVPIQ-FPT  EDWSAQPATE  DW·-AAPTAQ  ATEWVGATT.  E-S*    #295
Mur #235  A-EFTAAQPE  VADWSE-VQV  PSVPIQ-FPT  EDWSAQPATE  DW·-AAPTAQ  ATEWVGATT.  E-S*    #295
Rat       A-EFTAAQPE  VADWSE-VQV  PSVPIQ-FPT  EDWSAQPATE  DW·-AAPTAQ  ATEWVGATT.  E-S*
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FIG. 3. Comparison of Pc ECM receptor to sequences of known laminin binding proteins. Protein sources for laminin receptor primary structures are designated as follows: *Drosophila melanogaster* (Dro; GenBank accession no. M77133), human colon carcinoma (Hum) (15), murine (Mur) (16), and rat (Rat) (GenBank accession no. M27798). Sequence comparisons were performed using the programs FASTA (14) and BESTFIT (GCG). Identities with Pc ECM receptor are denoted by dashes; gaps are shown as dots; termination sites are designated by an asterisk. The reported laminin receptor from rat is a partial sequence that is aligned by homology to the murine laminin receptor and does not include the initiator methionine.

(Fig. 6) binds both laminin and fibronectin (Fig. 7 *A* and *B*). Little or no binding was observed in the control lanes. A competition binding assay showed that binding of one did not inhibit the binding of the other.

Pc Protein Extracts Contain Protein Bands with the Same Properties as the Overexpressed ECM Receptor Protein. Total protein from Pc was analyzed by electrophoresis, on an SDS/10% polyacrylamide slab gel (10), a portion of which was stained with Coomassie blue. Several polypeptides ranging in size from 20 to 100 kDa were seen. After transfer of the proteins to Immobilon P, the membrane was probed with polyclonal antibodies raised against overexpressed Pc ECM receptor protein. A band corresponding to 33 KDa was identified in Pc total cell lysate (Fig. 7*C*). Urea-solubilized expressed protein fractions also showed a single cross-reacting band at 33 kDa. No bands were observed in the control lane.

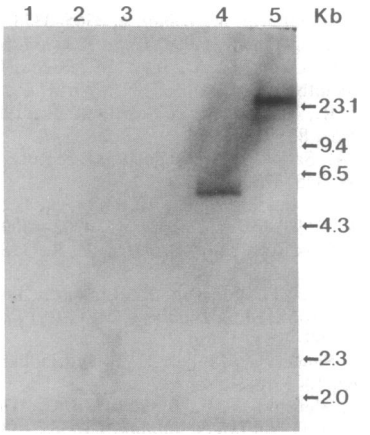

FIG. 4. Southern blot analysis of Pc genomic DNA. DNAs from Pc, HeLa cells, yeast, and mouse were digested with restriction enzymes and analyzed. After electrophoresis and blotting, the membrane was hybridized with the ^{32}P-labeled 560-bp *Eco*RI–*Not* I fragment containing ≈50% of the coding region of the *ecmR* gene. Lanes: 1, *Hin*dIII digest of HeLa cell DNA; 2, *Hin*dIII digest of mouse liver DNA; 3, *Hin*dIII digest of *Saccharomyces cerevisiae* DNA; 4, *Hin*dIII digest of Pc DNA; 5, *Bam*HI digest of Pc DNA.

DISCUSSION

We have shown that Pc of rat origin contains a gene that codes for a 33-kDa ECM receptor protein. This gene is expressed in cultured Pc as a 1.4-kb mRNA. Polyclonal antibodies raised to the overexpressed Pc ECM receptor protein recognize a 33-kDa protein in Pc total cell lysates. The recombinant protein product of this *ecmR* gene binds both fibronectin and laminin and a competition binding assay demonstrated that the binding of the two ECM proteins to the Pc ECM receptor is not competitive.

Little is known about the attachment of Pc to the type I pneumocyte although this is thought to be a crucial step in establishing infection. There is some evidence that ECM molecules such as laminin and fibronectin may be involved in the binding of Pc to lung cells (3, 22). Fishman *et al.* (22) showed with a number of different lung cell lines that the effects of laminin and fibronectin on Pc attachment were variable and appeared to depend on the quantity of host ECM proteins adsorbed onto the surface of Pc. More recently, vitronectin has also been shown to be involved in the binding of Pc to the type I cells (23). Laminin and fibronectin receptors have been described in Pc and in other organisms such as *Candida albicans* (12), *Staphylococcus aureus* (24–

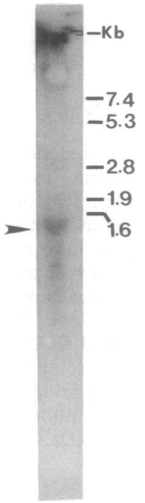

FIG. 5. Northern blot analysis of total Pc RNA. RNA was isolated from 1×10^8 cultured Pc cells (7). Total RNA (20 μg) was subjected to electrophoresis on a formaldehyde/1.5% agarose gel and transferred to a Zeta-Probe membrane. The filter was hybridized to the ^{32}P-labeled 560-bp *ecmR* gene fragment. The length of the hybridized RNA species was determined by comparison with the mobilities of RNA molecular mass marker II (Boehringer Mannheim).

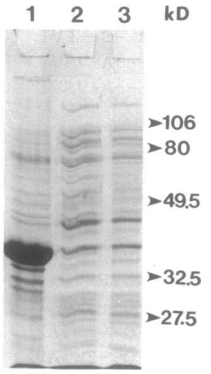

FIG. 6. Expression of the Pc ECM receptor protein in *E. coli*. Total protein extracts from *E. coli* BL21 carrying either pET15b-ecmR or pET15b were analyzed by SDS/PAGE in 10% gels. Lanes: 1, pET15b-ecmR induced with IPTG; 2, pET15b-ecmR uninduced; 3, pET15b induced with IPTG. kD, kDa.

26), *Staphylococcus pyogenes* (27), and Mycobacteria (28) and are part of their pathogenic mechanism.

Laminin (ECM) receptors have been classified structurally into two families, integrins and nonintegrins. The Pc ECM receptor protein described here shows considerable similarity to the nonintegrin laminin receptor protein of human (15, 18), mouse (16), rat (GenBank accession no. M27798), and *Drosophila* (GenBank accession no. M77133) origin. While human, mouse, and rat laminin receptors have sequence homology throughout the entire polypeptide, the Pc and *Drosophila* proteins have little sequence similarity to each other or to the human, mouse, and rat proteins in their C-terminal region. Nevertheless, the C-terminal one-third of Pc laminin binding protein resembles human, mouse, and rat proteins in that it is highly negatively charged (18% glutamic and aspartic acid residues) and many of the acidic residues are clustered between residues 206 and 221, as they are in the human laminin receptor. In addition, between residues 202 and 238, the Pc ECM receptor protein exhibits a short amino acid sequence with fibronectin binding repeat units of the *Staphylococcus aureus* fibronectin binding proteins FnBPA (25) and FnBPB (26).

It is interesting to note that in some systems, ligation to the laminin/elastin receptor results in remodeling of the host cell (29), and it is possible that the structural changes that occur in type I pneumocytes secondary to Pc attachment may involve the Pc laminin receptor.

In lung macrophages, the mannose receptor is involved in Pc uptake (30). Since one cannot be sure that a single receptor

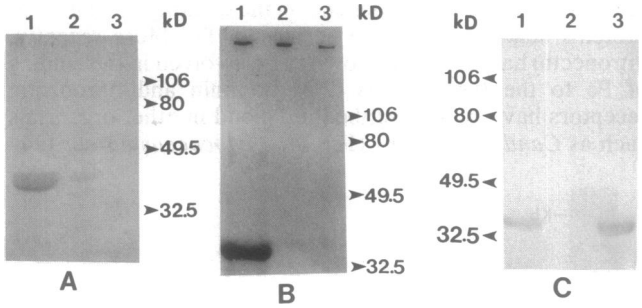

FIG. 7. Western blot analysis of overexpressed ECM receptor protein and Pc total protein extracts. Proteins were electrophoresed on SDS/10% polyacrylamide gels, transferred to Immobilon P membranes, and probed with antibodies as follows: (*A*) Laminin–anti-laminin sandwich Western blot of total cell protein from induced (lane 1), uninduced BL21/pET15b-ecmR cells (lane 2), and induced BL21/pET15b cells (lane 3). (*B*) Fibronectin–anti-fibronectin sandwich Western blot of total cell protein from induced (lane 1), uninduced BL21/pET15b-ecmR cells (lane 2), and induced BL21/pET15b cells (lane 3). (*C*) Western blot of proteins from total Pc lysates (lane 1), induced BL21/pET15b cells (lane 2), and induced BL21/pET15b-ecmR cells (lane 3), probed with polyclonal antibodies raised to the expressed Pc ECM receptor protein. kD, kDa.

is both necessary and sufficient by itself for Pc attachment and uptake, the mannose receptor gene was transfected into a nonphagocytic cell and it was shown that by itself it mediated Pc attachment to and uptake by lung macrophages (30). Analogous experiments will be needed to determine whether the ECM receptor is both necessary and sufficient for the interaction of Pc with type I pneumocytes.

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