cDNA cloning reveals that the major group rhinovirus receptor on HeLa cells is intercellular adhesion molecule 1
(cytokine induction/viral receptor/transfection)

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ABSTRACT A 90-kDa surface glycoprotein was previously isolated and shown to be required for infection by the “major” group of human rhinovirus (HRV) serotypes. In the present work, the amino acid sequence of the receptor protein was obtained from CNBr and trypsin peptides. Using degenerate oligonucleotides predicted from the peptide sequences, we identified four cDNA clones that encode a 3-kilobase mRNA. The clones were ligated, subcloned in a simian virus 40 expression vector, and used to transfect receptor-negative Vero (monkey) cells. Results showed that transfected cells expressed receptor molecules capable of binding HRV and a monoclonal antibody which recognizes the major group HRV receptor. The cloned receptor cDNA encoded a protein with a sequence nearly identical to that of the intercellular adhesion molecule 1 (ICAM-1), indicating that the two surface proteins are one and the same. Both proteins have identical mass, carbohydrate composition, and tissue distribution. In addition, major group receptors on HeLa cells could be induced with various cytokines in a manner similar to the ICAM-1 ligand. A similar induction of the HRV “minor” group receptor was not observed.

Human rhinoviruses (HRVs) are members of the Picornaviridae and are the major causative agent of the common cold in humans (1). Earlier studies have indicated that HRV serotypes can be divided into “major” (78 serotypes) and “minor” (10 serotypes) groups based on receptor specificity (2, 3). An anti-receptor monoclonal antibody (designated antibody 1A6) has been previously isolated which specifically blocks attachment of the major group of HRVs (4). By using this antibody, a 90-kDa glycoprotein was isolated from HeLa cell membranes and shown to be required for attachment of the major group of HRVs to susceptible cells in culture (5). Chimpanzee and human clinical trials testing the efficacy of antibody 1A6 have also demonstrated that this receptor is involved in HRV infection of the nasal cavity in vivo (6, 7). Further biochemical characterization of the purified 90-kDa protein demonstrated that it was an acidic glycoprotein having a pI of 4.2 (8). Carbohydrates accounted for 30% of the molecular mass of the protein and seven N-glycosylation sites were predicted on the basis of partial digestion with N-Glycanase (8).

The intercellular adhesion molecule 1 (ICAM-1) is a cell surface ligand for the lymphocyte function-associated antigen 1 (LFA-1) adhesion receptor (9). ICAM-1 is a single chain 76- to 114-kDa glycoprotein with a polypeptide core of 55 kDa that can be induced by several cytokines (10). The interaction of ICAM-1 and LFA-1 plays an important role in leukocyte adhesion and in the execution of immunological and inflammatory functions (10).

The present study describes the cloning and sequencing of the cDNA that encodes the cell surface receptor required for infection by the major group of HRVs.† Sequence and protein comparisons show that ICAM-1 is the surface glycoprotein utilized by HRVs during infection.

MATERIALS AND METHODS

Peptide Sequence. HRV major group receptor protein was isolated from HeLa R19 cells (2) and purified by immunoaffinity chromatography as previously described (5). Purified protein (40 μg) was reduced in 6 M guanidine hydrochloride/0.1 M Tris-HCl, pH 7.1/0.1% EDTA containing 100 mM dithiothreitol at 50°C for 2.5 hr, then alkylated with 0.5 μM iodoacetic acid in the same buffer at pH 7.8 for 45 min at 23°C. After extensive dialysis against H2O, the protein was lyophilized and treated with 0.4 mM CNBr in 70% (vol/vol) formic acid at 23°C for 20 hr. The sample was chromatographed on a 10% polyacrylamide gel and transferred onto Polybrene-coated glass fiber filters (11). Strips containing peptides were cut out and the N-terminal sequence was determined as described (12).

Alternatively, 80 μg of receptor protein was purified by PAGE and transferred directly onto a nitrocellulose filter. Filter strips were blocked and then digested with 1.2 μg of tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin for 18 hr at 37°C (13). The resulting peptides were separated by reverse-phase HPLC and sequenced as above.

Preparation and Screening of cDNA Libraries. Polysomal mRNA and total RNA were isolated from dividing HeLa R19 cells by using the procedures detailed previously (14, 15). Both RNA preparations were subsequently purified by oligo(dT)-cellulose column chromatography.

Double-stranded cDNA was synthesized (16) by priming poly(A)† RNA with oligo(dT) (Collaborative Research) or degenerate oligonucleotides (21–24 bases in length) predicted from peptide sequences, using cDNA Synthesis Plus reagents (Amersham). Double-stranded cDNA was blunt-end ligated to EcoRI linkers (New England Biolabs) and inserted into λ ZAP dephosphorylated EcoRI arms (Stratagene). The ligated DNA was packaged in Stratagene Gigapack Plus extracts according to directions supplied by the supplier and plated at 35,000 plaque-forming units per 150-mm plate.

Multiple filter lifts (17) of phage DNA were hybridized to 32P-labeled oligonucleotides in 6× SSC/5× Denhardt’s solution/0.5% SDS/50 mM sodium phosphate buffer, pH 6.8, containing 0.2 mg of salmon sperm DNA per ml overnight at 10°C below the calculated Tm of the oligonucleotides used (1× SSC = 0.15 M NaCl/0.015 M sodium citrate; 1× Denhardt’s

Abbreviations: HRV, human rhinovirus; ICAM-1, intercellular adhesion molecule 1; LFA-1, lymphocyte function-associated antigen 1.† To whom reprint requests should be addressed.

† The sequence reported in this paper has been deposited in the GenBank data base (accession no. M24283).
solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin). Filters were then washed in 6× SSC/0.5% SDS for 5 min at 23°C and then for 10 min at the hybridization temperature. Hybridization-positive phage were picked and plaque purified by dilution plating and rescoring.

**Construction of Full-Length cDNA Clone.** A full-length DNA fragment [3048 base pairs (bp)] was obtained by joining the inserts of clones 3–10 and 21 at a common Sac I site at nucleotide 2257 and clones 105 and 20C at a common Bgl I site located at nucleotide 657. The 3-10/21 assembled DNA was then joined to the 105/20C dimer by means of a common Sac I site at nucleotide 1358 to form plasmid pHRVr1. Purified plasmid DNA was sequenced by using the Sequenase Kit (United States Biochemical). The 3'-recessed ends of the full-length cDNA were filled in with the Klenow fragment of DNA polymerase and blunt-end ligated into the simian virus 40 early promoter transfection vector pSVL (18), by insertion into the filled-in Xba I sites of the vector. The construct was used to transform *Escherichia coli* DH5α cells (BRL) to generate plasmid pSVL-HRVr1.

**Transfection of Cells with HRV Receptor cDNA.** CsCl-purified plasmid DNA (pSVL-HRVr1) at 10 μg/ml was transfected into subconfluent monolayers of Vero (monkey) cells by the calcium phosphate precipitation procedure described elsewhere (19). After a 48-hr incubation in medium containing 10% serum at 37°C, cells were subjected to binding studies using [35S]methionine-labeled HRV-15 and 125I-labeled 1A6 antibody as indicated (2).

**Induction of HRV Receptors.** Confluent monolayers of HeLa R19 cells were treated with the indicated concentrations of cytokines for 5 or 24 hr at 37°C. After incubation, medium was removed and replaced with a fresh McCoy’s 5A medium containing [35S]methionine-labeled HRV-2 or HRV-15. Following incubation with virus for 1 hr at 34°C, the percent of virus bound and unbound was determined as previously described (2). Specificity of binding was confirmed by parallel binding assays in the presence of unlabeled 1A6 antibody.

![Fig. 1. Isolation of receptor peptides.](image1)

**Table 1. Peptide sequences of HRV major group receptor**

<table>
<thead>
<tr>
<th>Peptide*</th>
<th>Sequence</th>
<th>Amino acid location</th>
</tr>
</thead>
<tbody>
<tr>
<td>T5</td>
<td>T F L T V</td>
<td>78–82</td>
</tr>
<tr>
<td>C1</td>
<td>Q P V G K X L T L R C Q V E</td>
<td>98–115</td>
</tr>
<tr>
<td>T4</td>
<td>T E L D L R P Q G L E L F E</td>
<td>161–183</td>
</tr>
<tr>
<td>T2</td>
<td>V T L N G V P A Q P L G P</td>
<td>313–325</td>
</tr>
<tr>
<td>T1</td>
<td>V L Y G P R X D E R D</td>
<td>365–375</td>
</tr>
<tr>
<td>T3</td>
<td>D G T F P L I G E S V X</td>
<td>407–421</td>
</tr>
</tbody>
</table>

The standard one-letter code is used; X, undetermined.

**RESULTS**

**Preparation and Sequencing of HRV Receptor Peptides.** Previous studies have demonstrated that purified major group HRV receptor protein can be obtained by antibody 1A6 immunoprecipitation chromatography (5). Sufficient quantities of this protein were obtained by using this procedure to allow chemical and enzymatic cleavage of the protein into peptides for protein sequencing. Initial attempts to sequence the intact protein indicated that the N terminus of the protein was blocked (data not shown). Therefore, fragmentation of the 90-kDa protein was attempted in two ways. First, receptor protein was cleaved with CNBr, the products were separated by PAGE, and then protein fragments were transferred directly onto Polybrene-coated glass fiber filters. Silver staining and Western blot analyses revealed a predominant 80-kDa protein band in addition to uncleaved protein, a minor 73-kDa fragment, and a barely detectable 10-kDa fragment (Fig. 1, Inset). The 80- and 10-kDa fragments were cut out from the corresponding positions on the filter and submitted for N-terminal amino acid sequence analysis. An 18-amino acid stretch (C1) was obtained from the 80-kDa fragment (Table 1). The finding that no sequence could be obtained from the 10-kDa fragment suggested that this small fragment may represent the blocked N-terminal portion of the protein.

A second approach to obtain the amino acid sequence involved partial enzymatic digestion with trypsin. Receptor protein was digested *in situ* on nitrocellulose after transfer from polyacrylamide gels. Released peptides were separated by reverse-phase HPLC (Fig. 1) and several were submitted for sequencing. Only the five peptides having retention times of 27.6, 31.8, 35.5, 39.8, and 41.6 min, 5–23 amino acids in length, had discernible amino acid sequences (Table 1). Antisera prepared against synthesized C1 and T4 peptides coupled to thyroglobulin were found to react with receptor protein in a Western blot assay (data not shown), confirming their derivation from authentic receptor protein.

**Cloning and Sequencing of the Receptor cDNA.** HeLa cell mRNA was isolated and used to synthesize double-stranded cDNA according to the methods outlined in *Materials and Methods.* The standard one-letter code is used; X, undetermined.

![Fig. 2. Cloning of the HRV major group receptor.](image2)
Methods. Because the map locations of peptides were unknown at the time, two strategies were employed to clone the HRV receptor gene. The first method used standard oligo(dT) priming of RNA to synthesize cDNA, while the second used degenerate oligonucleotide primers predicted from peptide sequences T1–4 and C1 utilizing preferred codon frequencies (20). Several λ ZAP libraries were prepared and multiple libraries were screened individually with peptide-derived oligonucleotides other than the one used in priming (i.e., T1-primer libraries would be screened with T2–4 and C1). Of the >5 million phage screened, only 3 hybridization positives were further characterized. Of these, only one clone (20C) was hybridization positive with more than one probe. In fact, clone 20C was positive with probes from peptide sequences T1, T2, and T4. DNA sequencing confirmed that the 788-bp insert of clone 20C encoded the three peptides.

Clones 105 (679 bp) and 3–10 (951 bp) were identified by hybridization with peptides C1 and T3, respectively, and cross-hybridized to the 5′ (105) and 3′ (3–10) ends of clone 20C. Since clone 3–10 did not contain a poly(A) tract, the 3′–10 insert was used to rescreen the oligo(dT)-primed library. Clone 21 (1032 bp) was then identified and shown to contain a poly(A) tract indicative of the 3′ end of the putative receptor gene mRNA.

The four clones were ligated (Fig. 2) as described in Materials and Methods and the complete sequence was determined by dideoxynucleotide sequencing (Fig. 3). The full-length clone (pHRVr1) had a single large open reading frame encoding the HRV major receptor gene. The N-terminal signal and transmembrane sequences were underlined. Amino acids that correspond to sequences derived from isolated peptides are shown by bold underlines.

Fig. 3. Complete amino acid and nucleotide sequences of the HRV major receptor. The N-terminal signal and transmembrane sequences are underlined. Amino acids that correspond to sequences derived from isolated peptides are shown by bold underlines.
coding 532 amino acids if the first ATG was utilized. This would indicate a 71-nucleotide 5' noncoding region and a 1333-nucleotide 3' noncoding region, excluding the poly(A) tail. No additional long open reading frames could be found in the 3' noncoding region, suggesting that the stop codon at position 1668 is the site for termination of protein synthesis. This was verified by in vitro translation of RNA derived from pHRVr1, which resulted in a single polypeptide of 55 kDa (data not shown) that is in close agreement to the 54- to 60-kDa size found for deglycosylated receptor protein (8).

The location of each of the peptide sequences is shown in Fig. 3. Placement of the C1 peptide within the sequence indicated that a methionine does not exist in close proximity to the cleavage site and that cleavage apparently occurred at a tryptophan residue as a result of oxidation during isolation or chemical cleavage procedures. This type of cleavage has been reported previously (21).

**Transfection with HRV Receptor Gene cDNA.** To demonstrate that the cloned HRV receptor sequence did indeed encode the receptor protein of major group HRVs, a DNA insert representing nucleotides 48–2988 was subcloned in a simian virus 40 transfection vector and used to transfect a HRV receptor-minus cell line (Vero). After a 48-h incubation, specific binding of radiolabeled antibody 1A6 and HRV-15 was observed in pSVL-HRVr1-transfected Vero cells (Table 2). No specific binding could be demonstrated with nontransfected cells.

**Homology to ICAM-1 Sequence.** The sequence predicted for the 90-kDa HRV cell receptor from HeLa cells displayed striking homology to the sequence obtained for the ICAM-1 gene obtained from cytokine-induced HL-60 cells (22, 23). Direct comparison of the sequences revealed only six nucleotide differences. The HRV receptor gene had a G-to-A substitution at nucleotide 1476 that resulted in a Glu-to-Lys amino acid change at residue 442, a GG-to-CC substitution at nucleotides 2733–2734, and insertion of a C at nucleotide 2043 and a GT at nucleotide 2743. Review of the protein characteristics of the 90-kDa HRV receptor and the ICAM-1 ligand molecule shows that both are surface proteins of similar molecular weight, are ubiquitous among human-derived cells, and have an equivalent number of glycosylation sites (8, 22, 23).

**Induction of HRV Major Group Receptor.** Previous studies (10) have demonstrated that ICAM-1 receptors can be induced after treatment with different cytokines. This induction was dependent on the cells used and cytokine selected. To confirm that the HRV major group receptor is indeed the ICAM-1 ligand molecule, HeLa R19 cells were treated with several cytokines and assayed for virus attachment. Results (Fig. 4A) clearly demonstrate that treatment with at least two of the cytokines, γ interferon (IFN-γ) and tumor necrosis factor α (TNF), resulted in an enhancement of HRV-15 binding from 17% to 35–38% after a 24-hr incubation. An additive effect could be demonstrated when IFN-γ and TNF were used together. Under these conditions, HRV-15 binding increased 3.3-fold to 57%. The induction obtained in HeLa cells was low compared to that observed in hematopoietic cells but clearly indicates that the HRV receptor is inducible by cytokines.

It has been previously postulated that the receptor molecules used by the major and minor groups of HRVs, polioviruses, and coxsackie B viruses may share some structural homology (24). As a preliminary experiment to ascertain whether the minor group HRV receptor is another adhesion molecule that can also be induced by cytokines, HeLa cells were induced as described above and assayed for their ability to bind the minor group virus HRV-2. As shown in Fig. 4B, no comparable induction of receptors could be observed. Nearly identical results were obtained in experiments involving poliovirus and coxsackie B-1 virus (unpublished data).

**DISCUSSION**

Previous studies have clearly demonstrated that the vast majority of HRVs require the presence of a 90-kDa surface receptor molecule to gain entry into cells (4, 5). Antibody attachment to this surface protein establishes a "receptor blockade" which effectively protects susceptible cells against infection by HRVs. The molecular characterization reported here indicates that the well-characterized HRV receptor protein on HeLa cells is synonymous with the ICAM-1 ligand. In addition to the virtually identical sequences, both the HRV receptor and ICAM-1 proteins have
equivalent mass, tissue distribution, and carbohydrate moieties (5, 8, 10, 23). Amino acids −27 to −1 and 454 to 477 represent putative signal and transmembrane sequences of ICAM-1, respectively (Fig. 3; ref. 23). Several nucleotide differences were found between the two sequences in the 3′ noncoding region and one change occurred in the coding region, replacing a Glu residue by a Lys residue. An additional 14 bases were identified at the 5′ end of the HRV receptor cDNA that were not reported in the ICAM-1 sequence (22, 23). Our clone may not represent the entire 5′ end of the mRNA, since Northern blot analysis indicated that the ICAM-1 mRNA is 3.2–3.3 kb in length (22, 23).

ICAM-1 is a cell surface ligand for LFA-1, and the interaction between these molecules plays an important role in several immunological and inflammatory functions mediated by leukocyte adhesion (10). This cellular adhesion pathway is one of at least three mechanisms utilized (10). The ICAM-1 ligand is a member of the immunoglobulin gene superfamily and is predicted to have five homologous immunoglobulin-like domains defined by amino acids 1–88, 89–185, 186–284, 285–385, and 386–453 (Fig. 2) (23). In addition, ICAM-1 is closely related to two adhesion proteins of the adult nervous system, namely neural cell adhesion molecule (NCAM) and myelin-associated glycoprotein (MAG) (22, 23).

Both the ICAM-1 and HRV receptor molecules have been shown to be ubiquitous on human-derived cells (4, 10) and clearly cannot account for the observed restriction of HRVs to the nasal cavity during a common cold. The significance of HRVs utilizing receptors having immunological and inflammatory functions is unclear. It has been reported that HRV infection involves a very limited number of cells in the nasal epithelium and that the clinical symptomatology that we associate with a cold may instead result from the generation of inflammatory mediators such as kinins (25). It is tempting to speculate that virus interaction with ICAM-1 somehow plays an important role in the production of kinins, other mediators, or both.

Recent mutagenic studies using an infectious DNA clone of HRV-14 have demonstrated that the deepest regions of the virion canyon structure are involved in receptor interaction (24). The finding that this region of the canyon is highly conserved among a number of picornaviruses belonging to different receptor families led to the supposition that the receptor molecules that interact with these different viruses have a structural binding domain in common (24). It was further suggested that the specificity of the receptor protein utilized for attachment was determined by the rim of the canyon, since this region of the capsid was highly variable and thus would define the structural requirements of receptor interaction with the virus.

Support for this hypothesis may be found in the recent cloning and sequencing of the poliovirus receptor gene (26). The poliovirus receptor molecule also appears to be a member of the immunoglobulin gene superfamily, and has three homologous immunoglobulin-like domains and some sequence homology to neural cell adhesion molecule. It is very likely that receptor genes for the HRV minor group and coxsackie B viruses also will be members of the immunoglobulin gene superfamily. It is interesting to note that, unlike the major group receptors, none of the other picornavirus receptors tested could be induced in HeLa cells by cytokine treatment and that the basal level of the minor group receptor on HeLa cells is almost equal to the cytokine-induced level of the major group (Fig. 4 and unpublished data).

Formerly, viral receptor studies were restricted to biochemical and chemical characterization. The availability of receptor gene clones should greatly facilitate our studies of virus–receptor interactions by providing a means to map binding domains via mutagenesis.

Note. Studies identifying the major HRV receptor as ICAM-1 have also been recently reported by two other independent laboratories (27, 28).

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