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Complete Sequence of the RNA Genome of Human Rhinovirus 16, a Clinically Useful Common Cold Virus Belonging to the ICAM-1 Receptor Group

WAI-MING LEE, WENSHENG WANG, & ROLAND R. RUECKERT
Institute for Molecular Virology, University of Wisconsin, Madison, Wisconsin, USA

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Requests for reprints should be addressed to Wai-Ming Lee, Institute for Molecular Virology, 1525 Linden Drive, University of Wisconsin, Madison, WI 53706, USA.

Abstract. We report here the complete nucleotide sequence and predicted polyprotein sequence of HeLa cell-adapted human rhinovirus 16 (HRV16). This virus is more suitable than human rhinovirus 14 (HRV14) for clinical studies, and its growth and physical properties are favorable for biochemical and crystallographic analysis. The complete message-sense RNA genome of HRV16 is composed of 7124 bases, not including the poly(A) tail. An open reading frame, extending from base 626 to 7084 predicts a polyprotein containing 2152 amino acid residues. Comparison with other rhinovirus sequences shows HRV16 is much more representative of human rhinoviruses than HRV14. No apparent relationship was found between receptor group and amino acid sequence in VP1, the capsid protein bearing the binding site for the intercullular adhesion molecule-1 (ICAM-1) in both HRV14 and HRV16.

Key words: human rhinovirus, sequence, common cold

Introduction

Human rhinoviruses, members of the family Picornaviridae, are probably the single most important causative agents of acute infectious illness of humans; they cause one third to one half of all acute respiratory disease and account for about half of all acute conditions on an annual basis (1). Rhinoviruses have a vast serotype diversity; at least 100 have been identified to date (2). Ninety percent of these serotypes utilize ICAM-1 as a receptor (3–5) and are thus called the "major" receptor group. To date only a few serotypes have been completely sequenced (6–11); this is due in part to their fastidious growth requirements in tissue culture (1).

Serotype 14, a member of the "major" receptor group, has become a popular model for molecular studies on rhinoviruses (2,9,12–20), principally because its crystal structure was the first to be known (21). However, HRV14 has serious deficiencies as a model for clinical studies. First, it produces few colds when inoculated into human volunteers, probably because of its high HID_{50} (50% human infectious dose), which corresponds to 5.7 $TCID_{50}$ (50% tissue culture infectious dose) (1). This low infectivity in humans effectively precludes its use for testing efficacy of antivirals against the common cold. In addition, the sequence of HRV14 indicates that it is a poor representative of human rhinoviruses, distinctly different from other rhinoviruses, which have so far been sequenced: HRV89 (10), a "major" group viruses, and two "minor" group viruses, HRV1b (11) and HRV2 (8).

To cope with this problem we examined three other serotypes (HRV2, HRV16, and HRV39) commonly used for studying colds in human vol-

Genbank accession number: L24917.

unteers (22–24). In our hands HRV2 and HRV39 grown in HeLa cells produced insufficient material for physical studies after purification. HRV16, by contrast, grew as well as HRV14 in HeLa cell (yields about 50–80 μg particles/10^8 cells in suspension culture) (Lee and Rueckert, unpublished data), and its virions proved stable enough for crystallographic study (25). Furthermore, it has a low HID_{50} (0.28 $TCID_{50}$ (1), causes good symptoms in all human volunteers (23,26, 27), and has been used as a model for studying the transmission of colds (28,29) and virus-induced asthma (26,27,30).

Construction, cloning, and sequencing of the cDNA is an important first step in the expression and identification of the viral genes involved in the symptomology of the common cold and in unravelling the connection of HRV16 infection with asthma.

Materials and Methods

HRV16, a gift from E.C. Dick of the Respiratory Virus Research Laboratory, Department of Preventive Medicine, University of Wisconsin, Madison, was grown in a HeLa cell suspension under conditions similar to those for HRV14 (31). After 7 hr of incubation at 35°C, virus particles were harvested and purified under conditions previously described for HRV14 (9). Virion RNA was purified as described (9). The 5′ end sequence of viral RNA was determined by the primer extension method with AMV reverse transcriptase (Life Sciences, St. Petersburg, FL) and a primer complementary to bases 180–199 of the HRV14 genomic sequence.

In order to sequence the rest of the genome, viral RNA was converted to duplex cDNA with AMV reverse transcriptase and *E. coli* DNA polymerase I (9,32). When the product was electrophoresed in 0.8% agarose and stained with ethidium bromide, a dominant band of about 7.2 kb was observed, indicating that it contained a high proportion of full-length duplex cDNA. After several failing attempts to clone the full-length cDNA directly into a plasmid vector, it was cloned as two fragments.

The cDNA was ligated to *Hin*dIII adaptors (New England Biolabs, Beverly, MA), digested with *Bam*H1, which appeared to be the only unique cutter (to give fragments of about 700 bases and 6500 bases in length) among all the restriction enzymes that cleave the polylinker site of the BlueScribe $M13^-$ vector (abbreviated pBS^-; Stratagene Cloning System). The digested cDNA was ligated with the *Hin*dIII and *Bam*H1 double-digested pBS^- vector. The ligation product was transformed into *E. coli*. Colonies were screened by sizing the miniprepared plasmids in 0.8% agarose and restriction analysis. A set of plasmids harboring the small 700-base and the large 6500-base inserts were isolated and characterized by comparing the terminal sequences of the inserts with the corresponding sequences of HRV89 and HRV14. The small insert was found to correspond to the 5′ noncoding region (NCR), while the large insert represented the polyprotein sequence, the 3′ noncoding sequence, and the poly(A) tail.

Representative plasmids, containing the small or large insert, were cesium chloride gradient purified, and for each clone a nest of deleted plasmids (average 150 bases different in size) was generated by exonuclease III digestion (Erase-A-Base System, Promega Corporation, Madison, WI). The deleted plasmids were sequenced by the primer extension method with Sequenase (a modified T7 DNA polymerase from United States Biochemical, Cleveland, OH) and a M13/pUC primer from New England Biolabs. Sequence data were analyzed on computer with the Genetics Computer Group package (33).

Results and Discussion

The final sequence revealed a large open reading frame, extending from base 626 to 7084, which predicts a polyprotein containing 2152 amino acid residues (Fig. 1). Cleavage sites, shown in Fig. 1, defining individual proteins were deduced using published consensus sequences for other picornaviral polyprotein (10,34). The complete genome of HRV16, a messenger sense single-stranded RNA, is composed of 7124 bases, not including the poly(A) tail (Genbank assession no. L24917).

Table 1 compares the predicted amino acid sequence of HRV16 with those of four other human

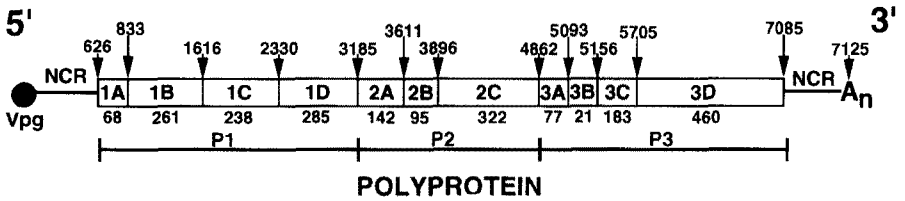

Fig. 1. Organization of RNA genome of human rhinovirus 16. Numbers above the arrows identify the first nucleotide of the indicated protein or the 3′ noncoding region (NCR). Numbers below the gene map indicate amino acid residues predicted for each protein.

rhinoviruses, representing the "major" and "minor" receptor groups, respectively. The sequences are compared by the individual protein elements (1A, 1B, etc.) and the entire polyprotein (P1-P2-P3). Among the protein elements, 1A, also called VP4, is the most conserved; it is an internal protein and is released from the virions during uncoating.

Protein 1D (VP1), on the other hand, is the most variable protein and constitutes a large fraction of the virion surface. In HRV16 VP1 also forms the "canyon," now known to be the acceptor site for the cellular receptor molecule, ICAM-1 (18). X-ray analysis shows that VP1 of HRV14 (21) and HRV1a (35) harbor similar canyons. With the exception of HRV14, however, there was no apparent relationship between receptor group and amino acid sequence in VP1. In fact, the VP1 sequence in HRV16 resembled that of the "minor" group viruses (71% and 72% identity with HRV1b and HRV2, respectively) more than that of the "major" group viruses (65% and 39% identity with HRV89 and HRV14, respectively).

Overall the proteins of HRV16 cover a range of 65–99% identity to HRV89, 71–99% identity to HRV1B, 72–93% identity to HRV2, and 39–63% identity to HRV14. Thus no matter which proteins are compared, HRV14 stands out as distinctly different from the others.

Table 2 compares the noncoding sequence (NCR) of HRV16 with those of HRV89, HRV14, HRV1b, and HRV2. The NCR has an important function in picornaviral RNA replication and

Table 1. Comparison of amino acid sequences encoded by HRV16 with those of two "major" group viruses (type 14 and 89; shown in upper case) and two "minor" group viruses (type 1b and 2; shown in lower case)

	Percent identity[a]						
	HRV16				hrv1b		
Proteins[b]	HRV89	HRV14	hrv1b	hrv2	HRV89	HRV14	hrv2
1A (VP4)	99	54	99	93	100	54	94
1B (VP2)	76	63	87	78	77	62	77
1C (VP3)	76	52	82	72	75	52	74
1D (VP1)	65	39	71	72	66	40	71
2A (pro)	86	40	92	89	85	40	89
2B	66	42	92	85	68	43	88
2C	75	48	90	85	74	49	84
3A	73	43	88	74	75	43	74
3B (Vpg)	81	59	95	90	81	59	90
3C (pro)	78	51	89	84	76	52	83
3D (pol)	73	55	85	81	72	56	83
P1-P2-P3	75	50	85	80	75	50	81

[a]Percent identities were computed with the University of Wisconsin Genetics Computer Group package (33). For comparisons between HRV89, HRV14, and hrv2 see Deuchler (10).
[b]See Figure 1.

Table 2. Sequence comparison of 5′ and 3′ noncoding region (NCR) of HRV16 with four other completely sequenced serotypes (HRV89, HRV14, HRV1b, and HRV2)

	Percent identity[a]						
	HRV16				hrv1b		
NCRs[b]	HRV89	HRV14	hrv1b	hrv2	HRV89	HRV14	hrv2
5′	79	63	79	83	81	63	80
3′	83	48	80	83	68	48	70

[a]Percent identities were computed with the University of Wisconsin Genetics Computer Group package (33). For comparisons between HRV89, HRV14, and hrv2 see Deuchler (10).
[b]See Figure 1.

protein expression (2). In agreement with the relationship found in the polyprotein sequence, both 5′ and 3′ NCRs of HRV16 share more sequence identity with HRV89, HRV1B, and HRV2 (79–83%) than HRV14 (63% for 5′ NCR and 48% for 3′ NCR). Again, HRV16, HRV89, and HRV2 are closely related to each other and HRV14 stands out distinctly (Table 2).

The sequence of HRV16 indicates that it is a better representative than HRV14 for both "major" and "minor" group viruses. Moreover, its ability to grow vigorously in cell culture and to produce reproducible symptoms in human volunteers suggests that this virus will prove more suitable than HRV14 as a model for bridging in vitro and in vivo studies on common cold picornaviruses.

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