Genetic Characterization of Parainfluenza Virus 3 Derived from Guinea Pigs

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ABSTRACT. To understand the relationship between novel parainfluenza virus 3 (PIV-3), which has recently been isolated from the lungs of guinea pigs, and other PIV-3 strains, we determined the complete nucleotide sequence of the novel PIV-3 (GPv) genome. A comparison of the nucleotide sequence among PIV-3s, including bovine PIV-3, revealed that GPv is closely related to human PIV-3. The results of the phylogenetic analysis clearly showed that GPv is a lineage of human PIV-3, suggesting that GPv has probably been introduced into guinea pig colonies via infected humans.—KEY WORDS: guinea pig, human parainfluenza virus 3, nucleotide sequence.

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Parainfluenza virus 3 (PIV-3), including human PIV-3 (HPIV-3) and bovine PIV-3 (BPIV-3), belongs to the *Paramyxovirus* genus of the family *Paramyxoviridae*. The genome of HPIV-3 consists of a negative-sense, unsegmented RNA approximately 15.4 kb in length [5, 6]. We have recently isolated novel PIV-3 from lung homogenates of sentinel guinea pigs placed together with seropositive asymptomatic individuals, and have provisionally called the agent guinea-pig PIV-3 (GPv). The seropositive guinea pigs were introduced to our laboratory from a closed breeding colony without the entrance of new animals. Since the isolation of PIV-3 from hosts other than human [4], cattle [14], or sheep [10] has not yet been reported, we were interested in the phylogenetic relatedness of GPv to other PIV-3s. In this study we therefore attempted to determine the complete nucleotide sequence of the genome of GPv.

MATERIALS AND METHODS

Cells and virus: Vero cells grown in Eagle's minimum essential medium containing 5% calf serum were infected with 1.5×10^8 PFU/m*l* of GPv, and the supernatant fluid was obtained and concentrated by ultracentrifugation at 10,000 × g for 20 hr at 4°C.

Isolation of viral RNA: The single-step isolation method was adopted for the isolation of viral RNA [13]. In brief, 500 μl of denaturing solution (containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.1 M 2-mercaptoethanol, and 0.5% N-lauroylsarcosine), 50 μl of 2 M sodium acetate (pH 4.0), 500 μl of water-saturated phenol, and 100 μl of chloroform were added to 200 μl of the concentrated virus suspension, vortexed for 20 sec, and then left on ice for 15 min. The lysate was centrifuged at 10,000 × g for 10 min at 4°C. The upper aqueous phase was then removed, and the viral RNA was recovered by precipitation with isopropanol and dissolved in 15 μl of RNase free water.

Reverse transcription (RT) and PCR: The RT-PCR was carried out as described previously using 104 different oligonucleotide primers synthesized based upon reported sequences of the relatively conserved regions of PIV-3s [6, 15–17].

Sequencing: The PCR products separated by agarose gel electrophoresis were directly sequenced with an automated DNA sequencer (ABI model 373A) using the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (ABI, CA., U.S.A.). The RNA ligation methods with oligoribonucleotide (5'-AGGUACCAAGCUUU AAUACGACUCACUAUA), genome and complementary RNA, and T4 RNA ligase (Takara Co., Ltd., Japan), RT-PCR, and TA cloning kit (Invitrogen, CA., U.S.A.) were used for sequencing the 3' leader and 5' trailer of genome RNA.

Computer analysis: Nucleotide and amino acid sequence data were analyzed by DNASIS Mac (Hitachi Software Engineering Co., Ltd., Japan). Phylogenic analyses were performed using the PHYLIP version 3.5c program (Univ. of Washington).

RESULTS

Nucleotide sequence and deduced amino acid sequence: The complete nucleotide sequence of the GPv was determined by sequencing the 29 overlapping PCR products. The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB012132.

Table 1 shows the nucleotide identity found in the genes encoding the major proteins among GPv, WA/47885/57, JS, and BPIV-3 (910N strain). The nucleotides were numbered according to the nucleotide sequence of HPIV-3 strains. The nucleotide identity found between the coding regions of the six major proteins of GPv and HPIV-3 (WA/47885/

Table 1. The nucleotide identities (%) of individual genes between the GPv and two HPIV-3s (WA/47885/57 and JS strains) and BPIV-3 (910N strain)

	HPIV-3s				BPIV-3
	WA/47885/57		JS		910N
	nt	aa	nt	aa	nt
Nucleoprotein (Np)					
3'-untranslated region	100.0		100.0		63.0
coding region	95.3	97.7	97.0	97.9	77.9
5'-untranslated region	88.4		93.0		55.8
Phosphoprotein (P)					
3'-untranslated region	94.9		94.9		48.1
coding region	95.1	92.9	97.1	96.7	71.0
C cistron		95.5		96.5	
D cistron		84.9		88.5	
V cistron		84.6		97.4	
5'-untranslated region	91.0		97.5		48.2
Matrix protein (M)					
3'-untranslated region	96.9		96.9		65.6
coding region	94.8	97.7	96.3	98.9	79.6
5'-untranslated region	85.2		86.9		42.6
Fusion protein (F)					
3'-untranslated region	82.9		87.0		44.6
coding region	93.5	96.3	95.6	97.6	73.9
5'-untranslated region	92.1		92.1		57.9
Hemagglutinin-neuraminidase (HN)					
3'-untranslated region	94.5		95.9		45.2
coding region	94.1	94.1	96.2	95.8	72.6
5'-untranslated region	95.4		95.6		38.9
Polymerase (L)					
3'-untranslated region	90.9		95.5		——
coding region	96.2	98.0	97.9	99.2	——
5'-untranslated region	88.7		90.4		——

57) was 93.5%–96.2%, while that between GPv and HPIV-3 (JS) was 95.6%–97.9%. Homologies in the nucleotide sequence (71.0%–79.6%) between GPv and BPIV-3 indicated that GPv was more closely related to HPIV-3s than BPIV-3. In contrast, the nucleotide sequences of the untranslated regions were highly conserved among PIV-3s, with the exception of the 5' terminus of the WA P gene (nt 3580–3624), where 11 nucleotide changes, including one deletion, were found. It was noteworthy that the D protein of GPv showed the least degree of homology with those of the HPIV-3s, although the function of the D protein is not yet clear. The amino acid sequences of the other viral proteins were well conserved between GPv and two HPIV-3s.

Phylogenic analyses: To determine where GPv came from, a phylogenetic analysis was carried out using the N-J algorithm. Figure 1 illustrates the representative results based on the nucleotide sequences of the HN and NP genes of HPIV-3s reported so far, along with that of GPv. The same drawings were shown on the other proteins. It was evident from the result that GPv is more closely related to HPIV-3 than to BPIV-3.

DISCUSSION

A comparison of the complete nucleotide and deduced amino acid sequences of the novel PIV-3 isolated from a guinea pig (GPv) with those of other PIV-3s showed that GPv is more closely related to human PIV-3s than to a virus of bovine origin. A nucleotide sequence homology of about 95% between GPv and HPIV-3 was apparently higher than the 85% [2, 11, 18, 19] observed for Sendai virus strains, suggesting that GPv belongs to the same genetic group as HPIV-3s. Phylogenic analysis further demonstrated the close relationship between GPv and HPIV-3. Analysis of the HN genes showed that HPIV-3 is most distantly related to BPIV-3 and that HPIV-3 consists of two different lineages, one of which includes GPv.

In our previous study, it was shown that most biological properties of HPIV-3 such as syncytium formation, hemagglutination, and antigenicity are shared by GPv (data not shown). The sequence analysis of F and HN

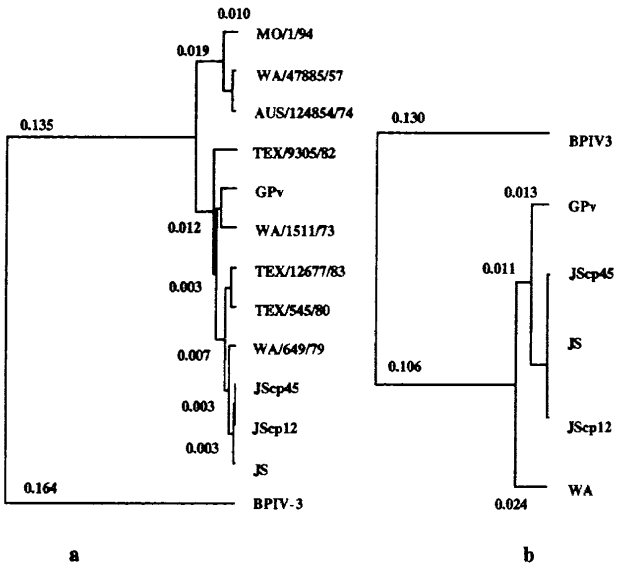

Fig. 1. Phylogenic relationship by the Neighbor-Joining method of GPv and other PIV-3 strains (HPIV-3s and BPIV-3) in the coding regions of HN glycoprotein (a) and NP (b). The lengths of the branches, the arabic figures on the lines, are proportional to the nucleotide-sequence differences. Accession numbers of DDBJ/EMBL/GenBank for the viral sequences are WA/47885/57 (M21649, M21848), JS (Z11575), JScp12 (Z11575), JScp45 (Z11575), MO/1/94 (M21649), AUS/124854/74 (M17641), TEX/9305/82 (M18763), TEX/12677/83 (M18764), TEX/545/80 (M18762), WA/649/79 (M18761), WA/1511/73 (M18759), and BPIV-3 (910N strain: Y00114).

glycoproteins in this study supports these findings, since potential sites for the cleavage of precursor F_0 [8] and asparagine-linked glycosylation [1] as well as amino acids important in keeping the integrity of the tertiary structure of glycoproteins are well maintained in GPv. Critical motifs such as putative phosphorylation sites [7, 9] are also maintained in the other proteins of GPv. We call the new isolated virus GP strain of HPIV-3.

The combination of both guinea pigs and parainfluenza viruses 2 and 3 has been used for an animal model of airway hyperreactivity, indicating that HPIV-3 has the ability to propagate within guinea pigs [3, 12]. The GP strain of HPIV-3 was isolated from the lungs of sentinel animals together with seropositive guinea pigs introduced from a closed breeding colony without the entrance of new animals. These conditions strongly suggest that the GP strain was introduced into a guinea pig colony by a person suffering from HPIV-3 infection. Alternatively, it seems possible that the HPIV-3 originated from a virus circulating in the guinea pig population across the species barrier, although this seems unlikely.

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