The nucleoproteins of human parainfluenza virus type 1 and Sendai virus share amino acid sequences and antigenic and structural determinants

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The complete nucleotide sequence of the nucleoprotein (NP) gene of human parainfluenza virus type 1 (hPIV-1) was determined from a cDNA clone of mRNA. The mRNA is 1683 nucleotides long (excluding polyadenylic acid) and encodes a protein of 524 amino acids with a predicted Mr of 57 548. An amino acid identity of 83% was predicted between the NPs of the human pathogen hPIV-1 and the murine paramyxovirus, Sendai virus, compared to 72% similarity at the level of the nucleotide sequence. In contrast, the amino acid sequence identity between the NPs of hPIV-1 and hPIV-3 was 59%, suggesting a more distant evolutionary relationship. The NP amino acid sequences of hPIV-1 and Sendai virus were highly conserved in the amino-terminal half of the molecule, in which 395 of the first 420 amino acids were identical. Of 11 monoclonal antibodies (MAbs) targeted against the Sendai virus NP, five cross-reacted with the hPIV-1 NP. The MAbs that cross-reacted recognize epitopes within regions of high amino acid similarity between the NPs of the two viruses. Also, five of the eight MAbs raised against hPIV-1 NP cross-reacted with Sendai virus NP. Taken together, our observations suggest that the essential amino acid sequence determinants of the NP structures of hPIV-1 and Sendai virus are conserved despite changes in their nucleotide sequences during evolution. This implies that there was a selective pressure to maintain the important functional domains of the protein.

Human parainfluenza virus type 1 (hPIV-1) is a leading cause of respiratory disease in early childhood (Chanock & Parott, 1965; Glezen et al., 1971). The virus is related antigenically to Sendai virus, the murine counterpart to hPIV-1, as indicated by the cross-reactivity of polyclonal antisera from guinea-pigs and humans (Cook et al., 1959). The hPIV-1 genome, like that of Sendai virus, consists of an ssRNA of negative polarity enclosed within a helical nucleocapsid composed mainly of nucleoprotein (NP). Two auxiliary proteins (P and L) are also associated with the nucleocapsid and are believed to function as the viral RNA polymerase. The paramyxovirus nucleocapsid (2600 NP molecules, 300 P molecules and 40 L molecules) plays an important role in viral RNA transcription and replication (Kingsbury, 1990). However, little information is available concerning the structure and function of the nucleocapsid proteins of hPIV-1, although extensive studies have been carried out with Sendai virus and other members of the genus Paramyxoviridae (reviewed by Morgan, 1991).

To study the structure and function of the NP of hPIV-1 and its relationship to the NPs of other members of the Paramyxoviridae genus, we have cloned and sequenced the gene from viral mRNA and compared its sequence with those of the Sendai virus and hPIV-3 NP genes. We also compared the antigenic structure of the NPs of hPIV-1 and Sendai virus, as analysed by the cross-reactivity of monoclonal antibodies (MAbs) directed against the NP of each virus. This report demonstrates conservation of amino acid sequence and antigenic structure between the NPs of hPIV-1, a human pathogen, and Sendai virus, a natural pathogen of mice. To determine the complete nucleotide sequence of the NP gene, we isolated a cDNA clone generated from viral mRNA. Since the nucleotide sequence of the 3' end of the hPIV-1 genome was not known, specific priming could not be used and total mRNA isolated from hPIV-1-infected cells was used as the template (Gorman et al., 1990). Confluent monolayers of LLC-MK2 cells were infected at a multiplicity of 3·0 p.f.u./cell. Actinomycin D (10 µg/ml) was added 24 h after infection to inhibit cellular RNA synthesis and, 16 h later, the cells were washed twice with cold phosphate-buffered saline,
suspended in cold lysis buffer (10 mM-Tris–HCl pH 7.6, 1.5 mM-MgCl₂, 10 mM-KCl, 1% Triton X-100). Dounce-homogenized and the nuclei were removed by centrifugation. The supernatant was adjusted to 0.5% SDS and total RNA was extracted with phenol:chloroform (1:1) and precipitated with ethanol (Gupta et al., 1988); mRNA was purified by oligo(dT) column chromatography (Maniatis et al., 1982). To favour the reverse transcription of viral mRNA templates over cellular mRNA, a virus-specific primer was used. Based on the conserved nature of the last four bases prior to the poly(A) at the 3' termini of paramyxovirus mRNAs, an oligonucleotide complementary to this sequence was found at the 3' end of the mRNAs of the F and HN genes of hPIV-1 (Merson et al., 1988; Gorman et al., 1990) and all the genes of Sendai virus (Gupta & Kingsbury, 1984). The molecular cloning procedure (see Fig. 1) was designed to include a maximal length of sequence from the 3' termini of viral mRNA in the cDNA clone. Recombinant plasmids were identified by differential hybridization using 32P-labelled cDNA probes reverse-transcribed from uninfected and hPIV-1-infected LLC-MK₂ cell mRNA. Nucleotide sequence analysis of the 5' and 3' regions of the insert cDNA of several clones identified two which had a high degree of similarity with the NP gene of Sendai virus. A 32P-labelled DNA probe prepared by nick translation of a PstI insert from the DNA of one of these clones (NP3) was used to identify the other NP-specific clones. One NP clone greater than 1600 nucleotides in length (NP7) was selected for complete DNA sequencing. The cDNA was sequenced by the dideoxynucleotide chain termination method (Sanger et al., 1977) using T7 polymerase (Pharmacia). The 5' and 3' termini of the gene were initially determined using PstI primers of pBR322. The entire cDNA sequence was determined by multiple rounds of sequencing using a series of oligonucleotides obtained from the sequential reactions.

To confirm the nucleotide sequence of a cDNA clone of the entire NP gene of hPIV-1, the sequence is shown in the message sense along with the corresponding amino acid translation. The consensus sequences present at the termini of the gene are underlined. Complementary DNA clones were constructed from viral mRNA using a synthetic oligonucleotide primer (5' ATTCTTTTTTTTTT 3') and reverse transcriptase as described by Gubler (1987). Second-strand synthesis, tailing with dCTP and transformation into E. coli were done as described previously (Gorman et al., 1990). The complete nucleotide sequence of a cDNA clone of the NP gene of hPIV-1 is shown in Fig. 1. The sequence is shown in the message sense along with the corresponding amino acid translation. The consensus sequences present at the termini of the gene are underlined. Complementary DNA clones were constructed from viral mRNA using a synthetic oligonucleotide primer (5' ATTCTTTTTTTTTT 3') and reverse transcriptase as described by Gubler (1987). Second-strand synthesis, tailing with dCTP and transformation into E. coli were done as described previously (Gorman et al., 1990).
amino acid sequences of their NPs. Fig. 2 shows that the NP amino acid sequences of these viruses were highly conserved near the amino-terminal and central regions, as was previously noted for Sendai virus and hPIV-3 (Galski et al., 1986). The NPs of hPIV-1 and Sendai virus were identical in 395 (94%) of the first 420 amino acids. In addition, Morgan (1991) has observed that all the paramyxoviruses, with the exception of respiratory syncytial virus, contain three clusters of conserved amino acids in the central portion of the NP molecule (Fig. 2). Since the conservation of common sequences in two genes presumably signifies functional similarity (Dover et al., 1984), these regions may represent potential binding sites for the L and P proteins or for interaction with other NP molecules or with genomic RNA. It has been shown that the carboxy-terminal region of the Sendai virus NP could be removed by tryptic digestion without altering the structure of the nucleocapsid (Hegeness et al., 1981), whereas the amino half of the protein is believed to interact directly with the virion RNA (Morgan et al., 1984). Another possible consideration is that the conserved regions of the NPs of hPIV-1, Sendai virus and hPIV-3 may play an important role in the encapsidation of the virion RNA.

The overall amino acid identity between the NPs of hPIV-1 and Sendai virus is 83% whereas the identity between those of hPIV-1 and hPIV-3 is 59%. The NP of hPIV-1 was found to be seven amino acids longer than the Sendai virus NP. Little sequence similarity was observed near their carboxyl termini, as was also found among the NPs of other sequenced paramyxoviruses (Kondo et al., 1990; Morgan, 1991). Since the carboxy-terminal half of the Sendai virus NP molecule is probably external in the nucleocapsid structure (Deshpande & Portner, 1984; Gill et al., 1988), these differences may reflect biologically important regions involved in specific interactions during the assembly of each virus. For example, would the NP of hPIV-1 support the nucleocapsid assembly of Sendai virus or assembly of nucleocapsids into virions and vice versa?

All 13 proline and two cysteine residues in the hPIV-1 NP were conserved in Sendai virus, indicating that the overall secondary structure of the NPs may be similar. Moreover, hPIV-3 shares 10 of the 13 proline and both cysteine residues with hPIV-1 and Sendai virus. However, there are an additional four proline residues in Sendai virus (positions 154, 308, 497 and 515) and three in hPIV-3 (positions 309, 426 and 499) which are not shared by the NP of hPIV-1. These common structural features along with the overall amino acid sequence identity of these NPs suggest a close evolutionary relationship between hPIV-1 and Sendai virus, whereas hPIV-3 is somewhat more distantly related. The close sequence identity of these viruses may indicate a recent evolution of the paramyxoviruses.

Alternatively, the difference may be due to the high error rate of reverse transcriptase. The largest open reading frame began with the first ATG (nucleotides 66 to 68) which met the criteria of a favourable translation initiation codon (Kozak, 1986). This similarity with Kozak's consensus sequence and the presence of multiple termination codons in the alternative reading frames indicated that the first ATG was the initiation site for translation of NP mRNA. The open reading frame terminated at TAA (nucleotides 1638 to 1640) and encoded a protein of 524 amino acids with a predicted Mr of 57,548.

To study the evolutionary relationship between hPIV-1, Sendai virus and hPIV-3, we compared the predicted amino acid sequences of their NPs. Fig. 2 shows that the NP amino acid sequences of these viruses were highly conserved near the amino-terminal and central regions, as was previously noted for Sendai virus and hPIV-3 (Galski et al., 1986). The NPs of hPIV-1 and Sendai virus were identical in 395 (94%) of the first 420 amino acids. In addition, Morgan (1991) has observed that all the paramyxoviruses, with the exception of respiratory syncytial virus, contain three clusters of conserved amino acids in the central portion of the NP molecule (Fig. 2). Since the conservation of common sequences in two genes presumably signifies functional similarity (Dover et al., 1984), these regions may represent potential binding sites for the L and P proteins or for interaction with other NP molecules or with genomic RNA. It has been shown that the carboxy-terminal region of the Sendai virus NP could be removed by tryptic digestion without altering the structure of the nucleocapsid (Hegeness et al., 1981), whereas the amino half of the protein is believed to interact directly with the virion RNA (Morgan et al., 1984). Another possible consideration is that the conserved regions of the NPs of hPIV-1, Sendai virus and hPIV-3 may play an important role in the encapsidation of the virion RNA.

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analyses were performed with a panel of MAbs directed against Sendai virus. Table 1 shows the regions in the primary sequence of Sendai virus NP which contain the epitopes recognized by these antibodies. One (MAb M52) of the two antibodies to site I cross-reacted with hPIV-1 NP. The amino acids associated with this epitope-containing region in Sendai virus had a shared identity of 40% with the analogous area of the NP of hPIV-1 (Fig. 2). However, when conservative amino acid replacements were considered to be identical, there was 63% amino acid identity between the two viruses. The identical residues in this region may participate directly in the binding of MAb M52, whereas antibody M8 may bind to residues at the carboxyl terminus of the Sendai virus NP molecule which were different in the hPIV-1 NP. All the MAbs in site III cross-reacted in an ELISA and 92% of the amino acids in this antigenic domain were identical in the two viruses. Antibodies to site II showed no cross-reactivity with hPIV-1 and recognized a region on the Sendai virus NP (residues 426 to 455) with low similarity to hPIV-1 NP (Fig. 2). In general, MAbs that cross-react recognize epitopes within areas of high amino acid similarity between the two proteins, indicating the structural similarity of these regions. It is not known whether these antibodies recognize linear epitopes, or whether the conserved areas in the primary sequence form similar folded structures which contribute to epitope recognition. The possibility that the epitope-containing regions may also include areas near the amino terminus cannot be excluded because one measles virus MAb-binding site is located between amino acids 122 and 150 of the NP (Buckland et al., 1989). However, since the MAbs recognized regions of high primary structure conservation and were characterized using immunoblots from protein-denaturing gels (Gill et al., 1988), it is likely that the epitopes are linear.

We have also isolated hybridomas that produce MAbs directed against the NPs of hPIV-1. Of eight hybridomas, MAbs from five (62%) cross-reacted with Sendai virus, further demonstrating the structural similarity of the NP molecule of these viruses. The binding sites of these MAbs are not known but we predict that the antibodies will probably recognize epitopes restricted to the carboxyl-terminal region of the molecule, as was observed with the Sendai virus NP. Taken together, our observations suggest that there was strong conservation of amino acid sequence and antigenic structure of the NPs of hPIV-1 and Sendai virus, despite differences in host-range specificity. The structural similarities of the NPs may signify an intolerance for amino acid change, implying that common functional domains may be involved in the encapsidation and replication of these viruses. These conserved regions are possible candidates for selection by the host's immune system.

Table 1. Cross-reactivity of MAbs directed against the NP of Sendai virus with hPIV-1

<table>
<thead>
<tr>
<th>Antigenic site</th>
<th>Mapped amino acid epitope positions</th>
<th>MAbs tested</th>
<th>Cross-reactivity with hPIV-1</th>
<th>Identical residues† (%)</th>
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<tbody>
<tr>
<td>I</td>
<td>456 to 517</td>
<td>M8</td>
<td>-</td>
<td>40</td>
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<tr>
<td></td>
<td></td>
<td>M52</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>M13</td>
<td>-</td>
<td>33</td>
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<td></td>
<td></td>
<td>M17</td>
<td>-</td>
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<td></td>
<td></td>
<td>M19</td>
<td>-</td>
<td></td>
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<td></td>
<td></td>
<td>M73</td>
<td>-</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>WSI6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>426 to 455</td>
<td>M4</td>
<td>+</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M6</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>M10</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>M40</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>289 to 425</td>
<td>M40</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* A reciprocal ELISA titre greater than 81000 was scored as + and less than 3000 as -. ELISA was carried out as recommended by van Wyke et al. (1981).
† Analogous regions of the amino acid alignment of the NP shown in Fig. 2 were used to determine the percentage amino acid identity between Sendai virus and hPIV-1.

common ancestor compared to other members of the Paramyxoviridae genus (Morgan, 1991).

The sequence similarity between hPIV-1 and Sendai virus NPs was greater at the amino acid level (83%) than at the nucleotide level (72%), indicating that changes in the nucleotide sequence involved silent mutations. This suggests that there might be a selective evolutionary pressure to conserve the important functional domains of the protein. The NPs of hPIV-1 and Sendai virus were conserved to a greater degree than the HN (Gorman et al., 1990) and F (Merson et al., 1988) proteins of these viruses, which have shared identities of 72% (HN) and 68% (F). However, Matsuoka et al. (1990) reported an amino acid sequence similarity of 83% between the HN proteins of Sendai virus and hPIV-1. The higher value however included a match of identical amino acids of 74%, close to the 72% reported by Gorman et al. (1990), and an additional 9% match of conservative amino acid replacements. The lower conservation of glycoprotein sequences may reflect host immune selection of antigenic variants (neutralization escape mutants) that arise during virus infection of mice or humans.

To compare the antigenic structure of the NPs of hPIV-1 and Sendai virus, MAbs directed against each NP were used as probes to test the cross-reactivity and thus the structural similarity of these two proteins. MAbs to Sendai virus NP were previously shown to recognize three non-overlapping antigenic sites (Deshpande & Portner, 1984) which mapped to the carboxyl-terminal half of the protein (Gill et al., 1988). In the latter study, a series of constructs with truncations at the carboxyl end of NP were expressed in bacteria and immunoblot
for future systems that employ site-directed mutagenesis or internal deletions to dissect the structural and functional requirements of the NP during the replicative process (Luytjes et al., 1989).

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References


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