Sequence comparison between the haemagglutinin–neuraminidase genes of simian, canine and human isolates of simian virus 5

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The nucleotide sequence of the haemagglutinin–neuraminidase (HN) gene was determined for a simian (W3), human (LN) and two canine (CPI+/CPI−) isolates of simian virus 5 (SV5). A comparison of the predicted amino acid sequences revealed that the human and canine isolates varied from the simian isolate by 1.7% and 2.4% respectively. This lack of significant variation between the HN proteins of the four SV5 isolates suggests that insufficient differences have occurred between isolates to confine them to a specific host.

Simian virus 5 (SV5), a member of the paramyxovirus family (Kingsbury et al., 1978), is closely related to human parainfluenza virus (PIV) types 2 and 4, and mumps virus (Randall et al., 1987; Precious et al., 1990; Southern et al., 1990; Komada et al., 1991; Bando et al., 1990). Like those of all paramyxoviruses the SV5 virion is composed of a helical nucleocapsid, surrounded by a lipid envelope derived from the host cell during virus release. Two viral glycoproteins, the haemagglutinin–neuraminidase (HN) and fusion (F) proteins, are exposed on the surface of the virus membrane and mediate virus attachment and entry respectively (Choppin & Scheid, 1980). Associated with the inner surface of the virus envelope is the matrix (M) protein which is responsible for maintaining the integrity of the virion. The nucleocapsid structure is a complex of the nucleoprotein (NP) and the ssRNA genome. Associated with this structure are the phospho- (P) and large (L) proteins which constitute the viral RNA polymerase activity (Buetti & Choppin, 1977; Hamaguchi et al., 1985). SV5 also encodes two additional proteins, V, which is encoded by the P gene, and a small hydrophobic protein, SH (Paterson et al., 1984, 1989; Heibert et al., 1988). Although V has been identified in purified virus, its function in the replicative cycle of SV5 remains unclear. SH is a non-structural protein, which although expressed on the surface of infected cells, appears to be selectively excluded from virus particles.

SV5 has been isolated from the tissues of a number of animals, including monkeys, humans and dogs (Hsuing, 1972; McCandlish et al., 1978; Mitchell et al., 1978; Robbins et al., 1981; Goswami et al., 1984). Although originally isolated from cultures of monkey kidney cells (Hull et al., 1956), SV5 is most notable as a causative agent of upper respiratory tract infection in dogs, and is therefore often referred to as canine parainfluenza virus (CPI) (McCandlish et al., 1978).

A neurotropic strain of SV5, termed CPI+, has been isolated from the cerebrospinal fluid of a dog suffering from temporary posterior paralysis (Baumgartner et al., 1981). More recently, a second isolate, CPI− was isolated from the brain tissues of a dog which had been experimentally infected with CPI+ (Baumgartner et al., 1987a). Interestingly, it was observed that in comparison with CPI+, CPI− showed a diminished virulence in vivo, and readily established a persistent infection in vitro, without apparent cell fusion (Baumgartner et al., 1987b). A series of 53 monoclonal antibodies (MAbs) generated against the human LN isolate (Goswami et al., 1984) of SV5 (Randall et al., 1987), was used to determine the antigenic differences between the two canine isolates and a simian isolate, W3 (Southern et al., 1991). Seventeen MAbs specific for the HN protein failed to detect any antigenic differences between CPI+ and CPI−. However, the two canine isolates could be distinguished on the basis of the inability of CPI− to bind one of the MAbs specific for the P protein (MAb P-k). Three amino acid differences were observed between the P/V proteins of CPI+ and CPI−. The inability to bind MAb P-k was mapped to one of these differences, a leucine to proline change in CPI− (Southern et al., 1991).

A previous comparison between the P/V genes of the canine isolate CPI+ and the simian isolate W3 revealed about 1% nucleotide and amino acid difference (Southern et al., 1991). Since the sequence of the internal nucleocapsid-associated proteins is generally conserved (Morrison, 1988), it was therefore of interest to deter-
mine the level of variation occurring in the HN proteins of SV5 isolated from different hosts. In this study, the nucleotide sequence of the HN genes of CPI+, CPI−, LN and W3 isolates were compared.

To determine the sequence variation between the four SV5 isolates, each of the HN genes was amplified by the polymerase chain reaction (PCR; reviewed in Bell, 1989), and cloned directly into M13. In this procedure, two overlapping PCR fragments were generated for cloning, since we had observed when trying to clone the nucleotide sequence of the HN genes of CPI+, CPI−, cPI+. SV5 which hybridized to the HN genes at the positions shown two overlapping PCR fragments were generated for mine the level of variation occurring in the HN proteins LDL−. Laboratory W3 and the published sequence (Hiebert et al., 1985) is underlined. Nucleotide differences in LN, CPI+ and CPI− are shown below the W3 sequence. Those differences which result in an amino acid change are shown (●). Also indicated is the position and sequence of the four oligonucleotide primers used in PCR amplification prior to cloning (see text). Message sense primers are shown in superscript, whereas those in the viral sense are shown in subscript; arrows indicate the direction of priming.

An alignment of the HN genes of the simian, human and canine isolates of SV5 are shown in Fig. 1. It should be noted that the nucleotide sequence of the W3 HN gene obtained within our laboratory differed from that previously, using oligo-4 as a primer (Precious et al., 1990). Separate PCR reactions containing primer pairs 1–2 and 3–4 were used to synthesize fragments of 427 and 1392 bp respectively. To account for any Taq polymerase-induced nucleotide misincorporations which may have occurred during amplification, at least four individual M13 clones were sequenced for each insert. It is of interest to note that only four nucleotide misincorporations were detected in over 10000 bases sequenced.

An alignment of the HN genes of the simian, human and canine isolates of SV5 are shown in Fig. 1. It should be noted that the nucleotide sequence of the W3 HN gene obtained within our laboratory differed from that published (Hiebert et al., 1985) by one nucleotide, due to a C to T transition at position 1647. CPI+ showed 37 nucleotide changes. In terms of the derived amino acid sequence (Fig. 2), 10 and 16 changes were identified in the LN and CPI+ respectively. Of these amino acid differences, six were common to both isolates. Surprisingly, CPI+ had one more amino acid difference (at position 42) than CPI−, when compared to both W3 and LN. Given that CPI− was isolated from the brain of a dog previously infected with CPI+, either there must have been a reversion in CPI− or, more likely, this amino acid substitution may have arisen upon subsequent passage of CPI+ in tissue culture cells. In contrast, a previous study identified three amino acid substitutions in the P/V proteins, which were unique to CPI− (Southern et al., 1991), supporting the premise.
that the differences in the biological properties may be attributable to alterations in the P and/or V proteins. However, a precise determination of the effect of these differences on the phenotype and virulence of CPI must await a more detailed molecular analysis.

It has been demonstrated previously that very few antigenic differences exist between the simian, human and canine isolates of SV5 (Randall et al., 1987). These findings are supported by results from this study, which show that on comparison to W3, the HN proteins of LN and CPI vary by 1.7% and 2.4%, respectively. Owing to immune selection pressure, it might be reasonable to expect a greater variation between isolates in the HN proteins than in the nucleocapsid-associated proteins. However, the variation between the HN proteins of the four SV5 isolates was not significantly greater than that observed between the P/V proteins of W3 and CPI+ (Southern et al., 1991). Therefore the variation occurring in both the external envelope proteins and the internal nucleocapsid proteins appears to occur at similar rates and probably reflects the error rate of the virus RNA polymerase. The variation between the four SV5 isolates involved in this study is either similar to or less than that observed between isolates of paramyxoviruses recovered from their natural hosts. For example, the HN proteins of Newcastle disease virus (NDV) samples, isolated over a period of 53 years, were shown to vary by 12.1% (Sakaguchi et al., 1989). Furthermore, two NDV isolates, separated by a single year, varied by 4.4%. However, a similar comparison of the HN genes of human PIV-3 strains isolated over a period of 26 years showed very little sequence variation (0.2 to 1.4%) (van Wyke Coelingh et al., 1988). In contrast, it appears that the human and bovine strains of PIV-3 have evolved sufficiently to be confined to separate species. The overall amino acid sequence identity between the HN proteins of the human and bovine strains is 80% (Suzu et al., 1987). In addition, distinct antigenic differences have been found to exist between the human and bovine PIV-3 strains (van Wyke Coelingh et al., 1986).

Although the SV5 isolates examined in this study were derived from human, canine and simian isolates, the results presented here suggest that the amino acid differences between the four isolates may be insufficient to confine each of the isolates to a particular host. Although the virus was originally isolated from cultures of monkey kidney cells and was therefore presumed to be of simian origin, it has been suggested that infection of monkeys may have occurred in captivity, through contact with infected humans (Tribe, 1966; Hsung, 1972). To date, the only known pathological consequence of natural SV5 infection occurs in dogs. Therefore SV5 may be a canine pathogen which can be spread to monkeys through human transmission.

We are extremely grateful to Dr W. K. Baumgärtner for providing samples of CPI+ and CPI—. David Baty is indebted to the MRC for a research studentship. Jill Southern is supported on a grant from the Cunningham Trust and R. E. Randall is the recipient of a Wellcome Trust University Award.

References


(Received 10 June 1991; Accepted 20 August 1991)