Relationships and host range of human, canine, simian and porcine isolates of simian virus 5 (parainfluenza virus 5)

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Sequence comparison of the V/P and F genes of 13 human, canine, porcine and simian isolates of simian virus 5 (SV5) revealed a surprising lack of sequence variation at both the nucleotide and amino acid levels (0–3 %), even though the viruses were isolated over 30 years and originated from countries around the world. Furthermore, there were no clear distinguishing amino acid or nucleotide differences among the isolates that correlated completely with the species from which they were isolated. In addition, there was no evidence that the ability of the viruses to block interferon signalling by targeting STAT1 for degradation was confined to the species from which they were isolated. All isolates had an extended cytoplasmic tail in the F protein, compared with the original W3A and WR monkey isolates. Sequence analysis of viruses that were derived from human bone-marrow cells isolated in London in the 1980s revealed that, whilst they were related more closely to one another than to the other isolates, they all had identifying differences, suggesting that they were independent isolates. These results therefore support previous data suggesting that SV5 can infect humans persistently, although the relationship of SV5 to any human disease remains highly contentious. Given that SV5 has been isolated on multiple occasions from different species, it is proposed that the term simian virus 5 is inappropriate and suggested that the virus should be renamed parainfluenza virus 5.

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INTRODUCTION

Simian virus type 5 (SV5) is in the genus *Rubulavirus* of the subfamily *Paramyxovirinae* of the family *Paramyxoviridae*. SV5 was first isolated from rhesus and cynomolgus monkey kidney-cell cultures and two of the original monkey isolates are referred to as WR and W3A (Hull *et al.*, 1956; Choppin, 1964). It was thus thought initially that monkeys were the natural host for SV5, but epidemiological studies in the 1960s showed that wild monkeys do not have antibodies against the virus. However, these animals seroconvert in captivity and, on this basis, it was suggested that infection of monkeys occurs either in transit or shortly after contact with humans (Tribe, 1966; Atoynatan & Hsiung, 1969; Hsiung, 1972). Indeed, Tribe (1966) suggested that monkeys that were brought into captivity should be immunized immediately against SV5 to prevent them being infected with the virus. There is also experimental evidence supporting the contention that SV5 naturally infects humans (Hsiung, 1972; Goswami *et al.*, 1984). For example, SV5 has been isolated on numerous occasions from a variety of human tissues, including bone-marrow cells (Goswami *et al.*, 1984). Despite this, infection of humans with SV5 has remained a subject of some debate and controversy, fuelled by the fact that SV5 can contaminate primary monkey kidney-cell cultures (and other cell lines), which are commonly used to isolate viruses from clinical samples (Chanock *et al.*, 1961; Hsiung, 1972; Huddlestone *et al.*, 1979; Wallen *et al.*, 1979; Choppin, 1981). Furthermore, antigenic cross-reactions occur between SV5 and known human paramyxoviruses, including human parainfluenza virus type 2 (Randall & Young, 1988; Tsurudome *et al.*, 1989), making interpretation of the earlier seroepidemiological studies difficult (Hsiung, 1972). No acute human disease has been linked reproducibly to infection with SV5, although it has been suggested that SV5 may be a possible cause of some cases of multiple sclerosis (Goswami *et al.*, 1987; Russell *et al.*, 1989). However, this contention has largely been dismissed, as its findings have not been supported by subsequent studies (McLean & Thompson, 1989; Vandvik & Norrby, 1989). It is accepted that SV5 (or canine parainfluenza virus, as it is

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more often referred to in a veterinary context) is a natural cause of the respiratory illness kennel cough in dogs (Binn *et al.*, 1967; Rosenberg *et al.*, 1971; Cornwell *et al.*, 1976; McCandlish *et al.*, 1978; Azetaka & Konishi, 1988). SV5 has also been isolated from a dog with temporary posterior paralysis (Evermann *et al.*, 1980) and this isolate, termed CPI+, caused acute encephalitis when injected intracranially into gnotobiotic dogs (Baumgärtner *et al.*, 1981). From one such experimentally infected dog, a variant, termed CPI−, was isolated that had phenotypic and genotypic differences (see below) from CPI+ (Baumgärtner *et al.*, 1982, 1987a, b, 1991; Southern *et al.*, 1991; Chatziandreou *et al.*, 2002). Several other canine isolates of SV5 have been isolated and studied, including the T1 isolate (Azetaka & Konishi, 1988; Ito *et al.*, 2000). In addition, an isolate of SV5, termed SER, was isolated recently from the lung of a fetus of a breeding sow with porcine respiratory and reproductive syndrome (Heinen *et al.*, 1998; Tong *et al.*, 2002). There is also evidence that cats, hamsters and guinea pigs may naturally be infected with SV5 or a very closely related virus (Hsiung, 1972).

SV5 has a single-stranded, non-segmented, negative-sense RNA genome of 15 246 nt (for isolate W3A), which contains seven genes that encode eight proteins [NP, P and V, M, F, SH, HN and L; for a review of the molecular biology of paramyxoviruses, see Lamb & Kolakofsky (2001)]. The ability to encode eight proteins from seven genes occurs because the V/P gene contains two overlapping open reading frames that give rise to two distinct gene products as a result of RNA editing. These two structural proteins are termed V and P and they have the first 164 aa of the N-terminus in common. However, as a consequence of RNA editing (resulting in the addition of two G residues at the editing site), the reading frames of the mRNAs that encode the two proteins differ past this point and the proteins thus have unique C-terminal domains (Thomas *et al.*, 1988). The P protein forms part of the viral polymerase complex, whilst the V protein is a multifunctional protein that: (i) blocks interferon (IFN) signalling by targeting STAT1 for proteasome-mediated degradation (Didcock *et al.*, 1999a, b; Young *et al.*, 2000; Andrejeva *et al.*, 2002; Parisien *et al.*, 2002; Ulane & Horvath, 2002); (ii) inhibits IFN production by an as yet uncharacterized mechanism (He *et al.*, 2002; Poole *et al.*, 2002; Wansley & Parks, 2002); and (iii) binds to soluble, but not polymeric, NP and may thus have a role in the control of virus replication and encapsidation (Randall & Bermingham, 1996).

The interaction of viruses with the IFN system is one of the critical factors that determine the outcome of acute virus infections (Stark *et al.*, 1998; Goodbourn *et al.*, 2000; Levy & Garcia-Sastre, 2001; Biron & Sen, 2001; Sen, 2001). However, there may be other more subtle consequences of the interaction of viruses with the IFN system. Thus, we have suggested that the ability of paramyxoviruses to establish persistent infections *in vivo* may be linked to their ability, or not, to block the IFN response (Chatziandreou *et al.*, 2002). The ability of viruses to circumvent the IFN response may also be one factor that limits their host range. For example, SV5 fails to block IFN signalling in mouse cells (Didcock *et al.*, 1999a) and is non-pathogenic in normal and severe combined immunodeficient (SCID) mice (Young *et al.*, 1990; Didcock *et al.*, 1999a). However, SV5 is lethal in STAT1-knockout mice, i.e. mice that cannot respond to IFN (He *et al.*, 2001, 2002). Given the suggestion that IFN sensitivity may influence the ability of SV5 to establish persistent infections and that the sensitivity of paramyxoviruses to IFN may limit their host range (Didcock *et al.*, 1999a; Parisien *et al.*, 2002; Park *et al.*, 2003), we undertook this study to compare the ability of different human, simian, canine and porcine isolates of SV5 to block IFN signalling in human and dog cells. Furthermore, we cloned and sequenced both the P/V and F genes of these viruses to see whether there were obvious correlates of sequence diversity with the species from which they were isolated.

METHODS

Cells and viruses. 2fTGH and HEp2 (human), Madin–Darby canine kidney (MDCK) (canine) and Vero (monkey) cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10 % fetal calf serum. Canine variants of SV5 included isolates H221 and 78524 (obtained from O. Jarrett, Faculty of Veterinary Medicine, University of Glasgow, UK) and CPI+ and CPI− (Evermann *et al.*, 1981; Baumgärtner *et al.*, 1981). The human bone-marrow isolates used were MIL, DEN, LN and MEL (Goswami *et al.*, 1984). The porcine SER isolate (Heinen *et al.*, 1998) was obtained from H. D. Klenk (Philipps-Universität Marburg, Germany). Virus stocks were prepared and titrated by using Vero cells.

Cloning and sequencing of the V and F genes. 2fTGH or MDCK cells were infected or mock-infected with the appropriate isolate of SV5 and at 21 h post-infection (p.i.), the medium was replaced with serum-free medium. At 30 h p.i., this medium (containing progeny virus) was harvested, pelleted by ultracentrifugation (30 000 r.p.m. for 90 min in a Beckman SW50 rotor) and viral RNA was isolated by using a Qiagen RNeasy RNA extraction kit. cDNA was prepared from viral RNA by using reverse primers that were specific to the P/V or F gene (outside the coding regions) and Moloney murine leukaemia virus reverse transcriptase (Promega). The products of these reactions were used in PCRs with *Pfu* polymerase (Promega), using forward and reverse primers that were specific to either the P/V or F genes to generate full-length P/V and F gene products. These were then cloned into the pGATA (P/V) or pGEM T-Easy (F) vectors and sequenced by using an automated sequencer and internal oligonucleotides, to ensure that the sequence was covered in both directions.

Phylogenetic analysis. Alignments of the sets of DNA sequences representing the F and V/P genes of isolates were made; for the latter, the two G residues that were introduced during synthesis of the P mRNA were included in each sequence. Phylogenetic analysis was carried out by using the program MrBayes 3 (Ronquist & Huelsenbeck, 2003), which applies Bayesian inference with Markov chain Monte Carlo techniques. From an input starting tree (chosen randomly in our application), the method involves successive perturbations of the current tree by a procedure picked stochastically from a range specifying different alterations in tree topology or branch-lengths, followed by a statistical test that is based on the fit of the input alignment to the new tree to decide whether to accept or reject that tree as the input for the next cycle of the process. The

arrangements for choice of perturbation at each stage and for acceptance of the current tree are constructed in such a way that the output list of accepted trees should converge to represent the posterior probability distribution of trees contingent on the input alignment. A recent review of Bayesian methods in phylogenetic analysis is given by Huelsenbeck *et al.* (2001). By using standard options of the program, the general time-reversible model of nucleotide substitution was employed and rates of change across alignment sites were modelled by a discrete gamma distribution plus an invariant category. The sets of alignment loci that represented the first, second and third codon positions were assigned to separate partitions in the analysis. Each program run included one 'cold' and three 'heated' chains (the latter are a device to aid rapid convergence of the process) and proceeded for 2×10^6 generations, with sampling of trees every 100 generations. Each run started with a uniform prior distribution and a randomly chosen tree and, for each dataset, two runs with different starting trees were carried out to check convergence. The first 1001 trees of each run were discarded to allow the process to become stationary, leaving 19 000 trees for estimation of the probability distribution of trees contingent on the input alignment.

Detection of STAT1 by immunoblot analysis. 2fTGH or MDCK cells were infected with virus (m.o.i. 10) and incubated for 24–29 h prior to lysis in sample buffer [0·05 M Tris/HCl (pH 7·0), 0·2 % SDS, 5 % 2-mercaptoethanol, 5 % glycerol]. Cellular polypeptides were separated by SDS-PAGE. The proteins were then transferred to PVDF membranes and treated sequentially with a polyclonal antibody against STAT1 (catalogue no. G16930; Transduction Laboratories) and a secondary anti-rabbit antibody coupled to horseradish peroxidase (Amersham Biosciences). The membrane was treated with ECL detection reagents (Amersham Biosciences) and exposed to X-ray film. Prior to lysis, the infected cells were examined by immunofluorescence to confirm that >95 % cells were infected.

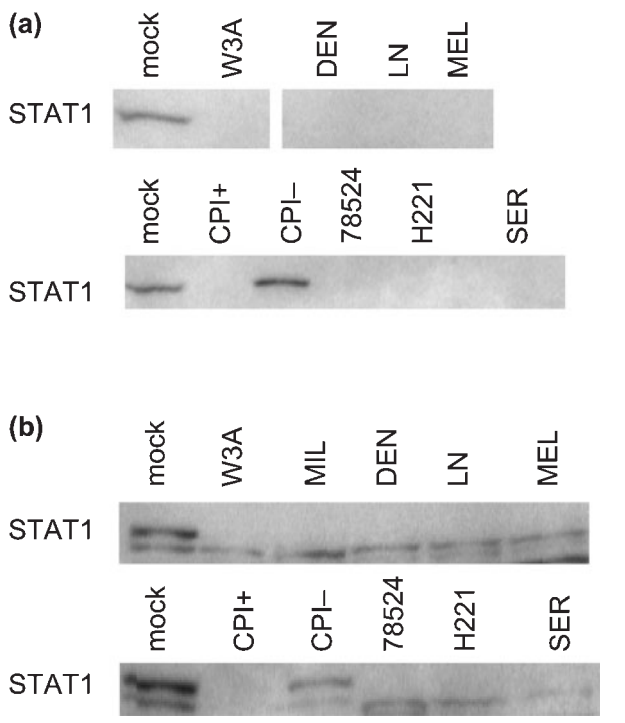

Fig. 1. Immunoblot analysis showing relative levels of STAT1 in (a) human 2fTGH and (b) canine MDCK cells infected with various isolates of SV5, as indicated.

RESULTS

Human, canine, simian and porcine isolates of SV5 promote the degradation of STAT1 in human and canine cells

To determine whether the host range of the SV5 isolates might be restricted by their ability to block IFN signalling only in cells of the species from which they were isolated, their ability to target STAT1 for degradation in human and canine cells was examined. Cells were infected with the various isolates and when >95 % of the cells were positive for virus antigen as judged by immunofluorescence (usually by 24 h p.i.), the relative level of STAT1 in the infected cells was estimated by immunoblot analysis. All of the isolates tested, apart from CPI− (which is known to be sensitive to IFN in both human and canine cells; Chatziandreou *et al.*, 2002; Wansley & Parks, 2002), induced STAT1 degradation in both human and canine cells (Fig. 1).

Sequence variation in the V/P and F genes of human, canine, simian and porcine isolates of SV5

The V/P genes of the human (LN, MEL, MIL and DEN), canine (H221 and 78524) and porcine (SER) isolates were cloned and their nucleotide sequences were determined (see Table 1 for the origin of the various isolates used or reported

Table 1. Origin of the various isolates used, or referred to, in this study

Origin/isolate	Reference/source
Human:	
LN	Goswami *et al.* (1984)
MEL	Goswami *et al.* (1984)
DEN	Goswami *et al.* (1984)
MIL	Goswami *et al.* (1984)
Cryptovirus	S. Robbins, patent no. WO02077211
Canine:	
CPI+	Evermann *et al.* (1980)
CPI−	Baumgärtner *et al.* (1987a)
H221	Obtained from O. Jarrett (Faculty of Veterinary Medicine, University of Glasgow, UK)
78524	Obtained from O. Jarrett
T1	Azetaka & Konishi (1988)
Simian:	
W3A	Choppin (1964)
WR	Hull *et al.* (1956)
Porcine:	
SER	Heinen *et al.* (1998)

Table 2. Amino acid variations in the V/P-common, V-unique and P-unique domains of various strains of SV5 compared with the W3A strain

Strain											aa
W3A	**MIL**	**DEN**	**LN**	**MEL**	**Cryptovirus**	**CPI+**	**CPI−**	**H221**	**78524**	**SER**	
V/P-common domain											
Y							H				26
V						I	I				32
T						I	I				33
G			E								39
L							P				50
T					I						63
S										L	69
A					T						74
K					N						75
P					S						81
D	G								G		90
G					E						95
N											100
L							P				102
S	T										126
T										P	155
S											156
S	F	F	F	F		F	F		F		157
V-unique domain											
E					K						206
P-unique domain											
G					E						166
A					V						169
G						E	E				174
S	L	L	L	L							178
K						R	R				184
S					N						211
V								M	M		226
V					I						258
M			T								277
T					K			K	K	K	293
N								K			306
E					D						366
I								L	L		381

in this study). The nucleotide sequences obtained and derived amino acid sequences (Table 2) of the V and P proteins were compared with the published sequence for the simian isolate W3A and a human isolate, termed cryptovirus, whose sequence has been deposited in GenBank (accession no. AX586923). This analysis established that there was only a low degree of variation within the V/P gene, with the amino acid variation ranging from 0·0 to 2·7 % for the V protein and from 0·5 to 2·8 % for the P protein. In the V protein, nearly all of the differences identified were in the V/P-common N-terminal domain, with only the cryptovirus isolate having a single amino acid difference in the V-unique domain ($E_{206}K$). With regard to the P protein, the amino acid differences between the isolates were distributed throughout the protein.

Given the low sequence variation in the V/P proteins of the different isolates of SV5, it was of interest to ascertain whether this was also reflected in other viral proteins, especially those in which more variation might be expected due to the pressure of immune selection. We therefore determined the sequence of the F genes of the various virus isolates. The deduced amino acid sequences were compared with the published F protein sequences of the W3A, WR, SER, T1 (Paterson *et al.*, 1984; Ito *et al.*, 1997; Tong *et al.*, 2002) and cryptovirus isolates (Table 3). Comparison of the sequences again revealed a surprisingly low degree of variation, similar to that seen in the V/P gene, with amino acid variation ranging from 1·1 to 3·0 %. However, these percentages did not include the extended cytoplasmic tails that all of the isolates had, compared with W3A and WR.

Table 3. Amino acid variation in the F protein of various strains of SV5, compared with the W3A strain

The sequence of the extended cytoplasmic tail from position 530 of the MIL isolate is QAFHHSQSDLSEKNQPATLGTR. Only amino acids that differ from this sequence in the other isolates have been indicated in the table.

Strain													aa
W3A	WR	MIL	DEN	LN	MEL	Cryptovirus	CPI+	CPI−	H221	78524	T1	SER	
G						S							2
T		I	I	I	I								3
I									R	R	R		4
F						S							7
A									S	S	T		17
S		G	G	G	G								19
P	L												22
S							P	P					71
V											M		76
N									Y	Y	Y		92
E							K	K	K	K	K	K	132
A						T							134
A									V	V	V		135
A												T	149
V						I							176
I				M									271
T								A					279
A											V		290
T							K	K					307
M						I	I	I	I	I	I	I	310
M					R								346
L				F									366
V												M	370
Y									F	F			377
M							I	I					407
Y						H	H	H					408
N						D							417
F							L	L					420
V											I		428
S						T	T	T	T	T	T	T	438
S	P	P	P	P	P	P	P	P	P	P	P	P	443
H							N	N					451
I												M	489
L		F	F	F	F								498
L							S	S					500
V							A	A					507
K							R	R					510
V	A	A	A	A	A	A	A	A	A	A	A	T	516
K		N	N	N	N	N	N	N	N	N	N	N	529
Stop	Stop	Q				S	S	S	S	S	S	S	530
		H					Y	Y					533
		S			Stop	P							535
		Q				R	R	R	R	R	R	R	536

This extended tail has already been noted for T1 and SER isolates (Ito *et al.*, 2000; Tong *et al.*, 2002). In all of the isolates apart from MEL, the cytoplasmic tail extension was 22 aa long, whilst in MEL it was only 5 aa long. This is thus in agreement with previously reported data showing that the F protein of MEL (also referred to as MN) migrated faster than those of the other human bone-marrow isolates LN, MIL and DEN on SDS-PAGE (Randall *et al.*, 1987).

Sequence comparisons between the isolates showed sporadic and more defined changes in comparison with W3A (Table 3). Thus, for example, there were three amino acid changes that distinguished the four human bone-marrow isolates from all others: T_3I, $S_{19}G$ and $L_{498}F$. Alignment of the extended cytoplasmic tails also revealed two more amino acid changes that were unique to the human isolates: Q_{530} and Q_{536} (the latter not being present in the shorter extension of MEL). Interestingly, the $M_{310}I$ and $S_{438}T$ changes were common to the canine, porcine and cryptovirus isolates, as were the S_{530} and R_{536} amino acids within the extended cytoplasmic tail. The canine and porcine SER isolates also had the amino acid change $E_{132}K$, relative to the other isolates. As might be expected, the two Glasgow canine isolates (H221 and 78524) appeared to be related more closely to each other than to the other isolates, although the next most closely related virus appeared to be the T1 canine isolate from Japan. Common to all the isolates was the $S_{443}P$ substitution, compared with W3A. In addition, all the isolates had an amino acid change at position 516, with the human and canine isolates having $V_{516}A$, whereas the porcine SER isolate had $V_{516}T$.

Phylogenetic analyses were carried out for the F and V/P genes by using the MrBayes 3 program. Independent runs for each dataset showed excellent convergence. Closely comparable results were obtained for both gene sets and only the results for the F gene are shown here. Fig. 2 shows the consensus tree obtained, in unrooted form. Most features in the tree were assigned maximum credibility values, with two lower values at central loci, indicating that the detail of branching in the core of the tree was not well-resolved. We presume that the root of the tree lies in or close to this central, unresolved region. On this basis, the isolates fell into five clades: (i) monkey and human isolates, except for cryptovirus; (ii) cryptovirus; (iii) German dog isolates CPI+ and CPI−; (iv) pig isolate SER; and (v) Japanese and Scottish dog isolates.

DISCUSSION

All of the isolates examined had an extended cytoplasmic tail in the F protein, compared with isolates W3A and WR, although this was only a 5 aa extension in the case of MEL, compared with a 22 aa extension in the other isolates. Whether the short tail observed in W3A and WR is an adaptation to infection of monkeys or, as seems more likely, the result of laboratory selection of highly fusogenic and thus highly visible variants, remains to be established. Nevertheless, the observation that all isolates had an extended cytoplasmic tail that has been reported to reduce fusion activity (Tong *et al.*, 2002; Seth *et al.*, 2003) suggests that highly fusogenic viruses may be selected against *in vivo*. This contention was supported by the observation that both the W3A and WR isolates had the coding capacity for an extended tail, but that this was interrupted by a single nucleotide change that introduced a stop codon, resulting in truncation of the tail.

A primary reason for initiating these studies was to ascertain whether the host range of SV5 between dogs and humans might be restricted to the species from which they were isolated by their ability to block IFN signalling (Didcock *et al.*, 1999a; Park *et al.*, 2003). However, apart from the previously documented case of CPI−, which fails to block IFN signalling in either canine or human cells (Chatziandreou *et al.*, 2002), all the isolates tested degraded STAT1 in both human and canine cells. Thus, the few amino acid substitutions identified in the N-terminal domain of V did not inhibit the ability of V to block IFN signalling. Also,

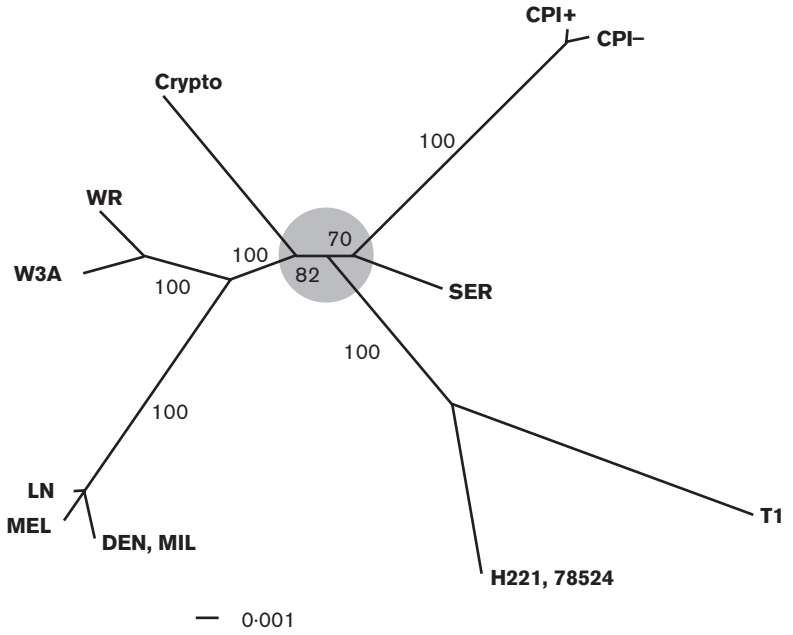

Fig. 2. Gene tree based on F gene sequences of SV5 isolates. The alignment of F gene sequences (1656 nt, 11 distinct sequences) was analysed by using the program MrBayes 3 to produce the consensus tree shown. Credibility values (%) for partitions of the isolates into two groups are indicated at appropriate branches. The two low values in the central part of the tree indicate that branching order in the region marked with a shaded circle could not be inferred with confidence. Bar, 0·001 substitutions per site.

there did not appear to be a clustering of nucleotide or amino acid changes, which might have been indicative of selection pressure on V function in blocking IFN signalling in cells from different species. It was also of note that, apart from cryptovirus with an $E_{206}K$ change, no amino acid substitutions mapped to the C-terminal domain of V, although there were nucleotide changes that altered the amino acid sequence within the P-unique domain that were encoded by the same region of the V/P gene that encodes the unique C-terminal domain of V. This is presumably because few amino acid changes can be tolerated in the highly conserved, cysteine-rich C-terminal domain of V.

Overall, there was a surprising lack of sequence variation at both the nucleotide and amino acid level between the various isolates of SV5, even though they were isolated from different species and geographical regions over a period of approximately 30 years. Indeed, the level of variation was similar to that observed for measles H and mumps and HPIV3 HN proteins (van Wyke Coelingh *et al.*, 1988; Rima *et al.*, 1997; Lim *et al.*, 2003), viruses whose host range is confined to man, and was significantly below that observed for the HN gene of Newcastle disease virus, a virus that infects many types of bird, including chickens and turkeys (Sakaguchi *et al.*, 1989). Within the F protein, in common with the porcine SER isolate, all of the canine isolates had an $E_{132}K$ change and, in common with both the porcine and cryptovirus isolates, also had $M_{310}I$ and $S_{438}T$ as well as S_{530} and R_{536} in their extended cytoplasmic tails. The canine viruses that were isolated in Glasgow (H221 and 78524) and Japan (T1) had I_4R and $A_{135}V$ changes that were not found in the any of the other isolates, including CPI+, which was isolated from a dog in Germany. Interpretation of the SV5 trees based on nucleotide sequence comparisons of the P/V and F genes suggested that there are five lineages that emerge from the unresolved root. Interestingly, the Japanese T1 and Glasgow H221 and 78524 canine isolates appeared on the same branch as each other, but not with the German CPI canine viruses. In the placement of the root, the monkey (W3A and WR) and human bone-marrow viruses (but not cryptovirus) also formed a clade. Also consistent with this analysis, but as yet unproven, is the possibility that there is a division between the dog/pig and monkey/human isolates (Fig. 2). Thus, there is clearly a requirement to sequence more variants of SV5 isolated from different species, in order to look more closely at underlying phylogenetic linkages.

From information published in the patent application relating to cryptovirus (WO02077211/EP1373477), it appears that this virus was a human isolate that may have come from a patient with neurological dysfunction. However, even though in the patent specifications it is claimed that this is a novel virus, this 'cryptovirus' did not show a significantly greater degree of difference from SV5 (2·25 % in F and 2·8 % in P) than any of the other isolates. None of the changes in 'cryptovirus', compared with W3A, were found in any of the human bone-marrow isolates (apart from those at aa 443 and 516 in F, which were common to all isolates, and 529, which was common to all isolates except WR), showing that, at least for the P and F proteins, there are no changes that might help to explain why these viruses were isolated from human tissues. With regard to the viruses that were isolated from human bone-marrow cells, they were clearly related most closely to each other. Thus, they all had the following changes: $S_{178}L$ in the P-unique domain, T_3I, $S_{19}G$, $L_{498}F$ and Q_{530} in the F protein and H_{533} and Q_{536} in the extended cytoplasmic tail. However, there were also several identifying nucleotide and amino acid differences among the various bone-marrow isolates. Thus, the LN isolate had two unique amino acid differences in the V/P proteins and two in the F protein, whilst the MIL isolate had two unique amino acid differences in the V/P proteins and the MEL isolate had a unique amino acid in the F protein, together with an extended cytoplasmic tail of only 5 aa, as opposed to 22 aa for all other isolates. The fact that they are clearly related more closely to each other may not be surprising, as they were isolated at roughly the same time and from the same geographical region (London). Similarly, the two Glasgow canine isolates are also clearly related more closely to each other than to the other isolates. However, the sequence variation between the bone-marrow isolates suggested strongly that the viruses were isolated independently of each other, thereby all but ruling out the possibility that they were laboratory contaminants (Goswami *et al.*, 1984). If, as the weight of evidence now indicates, SV5 has been isolated regularly from bone-marrow cells in which the virus must presumably establish a persistent infection, then the reported isolation of SV5 from, or detection in, patients with a variety of diseases, including multiple sclerosis, subacute sclerosing panencephalitis (Robbins *et al.*, 1981), Creutzfeldt–Jakob disease (Horta-Barbosa *et al.*, 1970), pemphigus (Siegl & Hahn, 1969), atherosclerosis (Behbehani *et al.*, 1965), Paget's disease (Basle *et al.*, 1985), hepatitis (Hsiung, 1972; Liebhaber *et al.*, 1965) and the common cold (Schultz & Habel, 1959), should not be dismissed lightly, especially as in many cases great effort was made to rule out the possibility of contamination (Robbins *et al.*, 1981; Goswami *et al.*, 1984). However, even if SV5 does cause persistent infections in humans, this does not necessarily mean that SV5 has a role to play in any human disease. Indeed, the wide spectrum of diseases from which SV5 has been isolated tends to argue against this. Furthermore, when examined in detail, as in the case of multiple sclerosis, the involvement of SV5 has been largely discounted. Nevertheless, if SV5 establishes persistent infections in a reasonable proportion of individuals, it may be timely to re-evaluate the role of SV5 and other paramyxoviruses in chronic human disease (Randall & Russell, 1991), especially as we now have the tools to perform such studies more incisively. We also now know that SV5 and other paramyxoviruses interfere with cellular processes, including the IFN response and, thus, if there is a loss of cellular function in cells that are persistently infected with paramyxoviruses, the rationale behind any possible link with disease becomes easier to make.

The nomenclature of SV5 has always been problematic, given the repeated isolation of the virus over many years from numerous species. The problem is compounded because of a general assumption that if a virus is termed 'simian', then its natural host must be monkeys. To counter this assumption, new nomenclatures are creeping into use, such as SER virus and cryptovirus. However, these viruses are clearly no more different from the original W3A isolate than any of the other viruses we have examined. Furthermore, the virus is also often referred to as canine parainfluenza virus. Due to this confusion and reluctance by some authors to use the term SV5, we believe that it would be better to rename the virus parainfluenza virus 5 (PIV5), a nomenclature that was attempted in the late 1960s and early 1970s (Hsiung, 1972). At the time, this probably failed because it was suggested that SV5 should be classified as parainfluenza virus 2 (PIV2) of monkeys (Chanock *et al.*, 1961). However, we now know from extensive antigenic and sequence analysis that SV5 and PIV2 are distinct viruses. The advantage of using the term PIV5 is that isolates can be prefixed with nomenclature that refers to the species from which they were isolated, e.g. canine PIV5 or porcine PIV5. However, a potential problem with giving a prefix to an isolate arises where there is doubt as to whether the virus was genuinely isolated from a given tissue or whether it arose as a laboratory contaminant, for example from the use of primary monkey kidney cells in the isolation procedure. Indeed, the knowledge that SV5 can contaminate primary monkey kidney cells is often a reason why diagnostic laboratories discount any isolation of SV5 from human tissues (M. Zambon, Health Protection Agency, Colindale, London, UK; personal communication). However, given the availability of specific reagents to SV5, including mAbs, it should be relatively easy to screen cell lines for the presence of SV5 and thus exclude any possibility of laboratory contamination during the isolation procedure or, if the virus was isolated in primary monkey kidney cells, to go back to the original specimen and try to re-isolate the virus in cell lines that are guaranteed to be free of the virus. There is thus a need to undertake further, well-controlled attempts to isolate SV5 from various human tissues and to determine the incidence of human infection with this virus.

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REFERENCES

Andrejeva, J., Young, D. F., Goodbourn, S. & Randall, R. E. (2002). Degradation of STAT1 and STAT2 by the V proteins of simian virus 5 and human parainfluenza virus type 2, respectively: consequences for virus replication in the presence of alpha/beta and gamma interferons. *J Virol* **76**, 2159–2167.

Atoynatan, T. & Hsiung, G. D. (1969). Epidemiologic studies of latent virus infections in captive monkeys and baboons. II. Serologic evidence of myxovirus infections with special reference to SV5. *Am J Epidemiol* **89**, 472–479.

Azetaka, M. & Konishi, S. (1988). Kennel cough complex: confirmation and analysis of the outbreak in Japan. *Nippon Juigaku Zasshi* **50**, 851–858.

Basle, M. F., Russell, W. C., Goswami, K. K. A., Rebel, A., Giraudon, P., Wild, F. & Filmon, R. (1985). Paramyxovirus antigens in osteoclasts from Paget's bone tissue detected by monoclonal antibodies. *J Gen Virol* **66**, 2103–2110.

Baumgärtner, W. K., Metzler, A. E., Krakowka, S. & Koestner, A. (1981). In vitro identification and characterization of a virus isolated from a dog with neurological dysfunction. *Infect Immun* **31**, 1177–1183.

Baumgärtner, W. K., Krakowka, S., Koestner, A. & Evermann, J. (1982). Acute encephalitis and hydrocephalus in dogs caused by canine parainfluenza virus. *Vet Pathol* **19**, 79–92.

Baumgärtner, W., Krakowka, S. & Blakeslee, J. (1987a). Evolution of in vitro persistence of two strains of canine parainfluenza virus. Brief report. *Arch Virol* **93**, 147–154.

Baumgärtner, W., Krakowka, S. & Blakeslee, J. R. (1987b). Persistent infection of Vero cells by paramyxoviruses. A morphological and immunoelectron microscopic investigation. *Intervirology* **27**, 218–223.

Baumgärtner, W., Krakowka, S. & Durchfeld, B. (1991). In vitro cytopathogenicity and in vivo virulence of two strains of canine parainfluenza virus. *Vet Pathol* **28**, 324–331.

Behbehani, A. M., Melnick, J. L. & DeBakey, M. E. (1965). A paramyxovirus isolated from human atheromatous lesion. *Exp Mol Pathol* **4**, 606–619.

Binn, L. N., Eddy, G. A., Lazar, E. C., Helms, J. & Murnane, T. (1967). Viruses recovered from laboratory dogs with respiratory disease. *Proc Soc Exp Biol Med* **126**, 140–145.

Biron, C. A. & Sen, G. C. (2001). Interferons and other cytokines. In *Fields Virology*, 4th edn, pp. 321–349. Edited by B. N. Fields, D. M. Knipe & P. M. Howley. Philadelphia, PA: Lippincott-Raven.

Chanock, R. M., Johnson, K. M., Cook, M. K., Wong, D. C. & Vargosko, A. (1961). The hemadsorption technique with a special reference to the problem of naturally occurring simian parainfluenza viruses. *Am Rev Respir Dis* **83**, 125–129.

Chatziandreou, N., Young, D., Andrejeva, J., Goodbourn, S. & Randall, R. E. (2002). Differences in interferon sensitivity and biological properties of two related isolates of simian virus 5: a model for virus persistence. *Virology* **293**, 234–242.

Choppin, P. W. (1964). Multiplication of a myxovirus (SV5) with minimal cytopathic effects and without interference. *Virology* **23**, 224–233.

Choppin, P. W. (1981). Isolation of paramyxoviruses from patients with chronic neurologic diseases. *J Infect Dis* **143**, 501–503.

Cornwell, H. J., McCandlish, I. A., Thompson, H., Laird, H. M. & Wright, N. G. (1976). Isolation of parainfluenza virus SV5 from dogs with respiratory disease. *Vet Rec* **98**, 301–302.

Didcock, L., Young, D. F., Goodbourn, S. & Randall, R. E. (1999a). Sendai virus and simian virus 5 block activation of interferon-responsive genes: importance for virus pathogenesis. *J Virol* **73**, 3125–3133.

Didcock, L., Young, D. F., Goodbourn, S. & Randall, R. E. (1999b). The V protein of simian virus 5 inhibits interferon signalling by

targeting STAT1 for proteasome-mediated degradation. *J Virol* **73**, 9928–9933.

Evermann, J. F., Lincoln, J. D. & McKiernan, A. J. (1980). Isolation of a paramyxovirus from the cerebrospinal fluid of a dog with posterior paresis. *J Am Vet Med Assoc* **177**, 1132–1134.

Evermann, J. F., Krakowka, S., McKeirnan, A. J. & Baumgärtner, W. (1981). Properties of an encephalitogenic canine parainfluenza virus. *Arch Virol* **68**, 165–172.

Goodbourn, S., Didcock, L. & Randall, R. E. (2000). Interferons: cell signalling, immune modulation, antiviral responses and virus countermeasures. *J Gen Virol* **81**, 2341–2364.

Goswami, K. K. A., Lange, L. S., Mitchell, D. N., Cameron, K. R. & Russell, W. C. (1984). Does simian virus 5 infect humans? *J Gen Virol* **65**, 1295–1303.

Goswami, K. K. A., Randall, R. E., Lange, L. S. & Russell, W. C. (1987). Antibodies against the paramyxovirus SV5 in the cerebrospinal fluids of some multiple sclerosis patients. *Nature* **327**, 244–247.

He, B., Lin, G. Y., Durbin, J. E., Durbin, R. K. & Lamb, R. A. (2001). The SH integral membrane protein of the paramyxovirus simian virus 5 is required to block apoptosis in MDBK cells. *J Virol* **75**, 4068–4079.

He, B., Paterson, R. G., Stock, N., Durbin, J. E., Durbin, R. K., Goodbourn, S., Randall, R. E. & Lamb, R. A. (2002). Recovery of paramyxovirus simian virus 5 with a V protein lacking the conserved cysteine-rich domain: the multifunctional V protein blocks both interferon-β induction and interferon signaling. *Virology* **303**, 15–32.

Heinen, E., Herbst, W. & Schmeer, N. (1998). Isolation of a cytopathogenic virus from a case of porcine reproductive and respiratory syndrome (PRRS) and its characterization as parainfluenza virus type 2. *Arch Virol* **143**, 2233–2239.

Horta-Barbosa, L., Fuccillo, D. A., Hamilton, R., Traub, R., Ley, A. & Sever, J. L. (1970). Some characteristics of SSPE measles virus. *Proc Soc Exp Biol Med* **134**, 17–21.

Hsiung, G. D. (1972). Parainfluenza-5 virus. Infection of man and animal. *Prog Med Virol* **14**, 241–274.

Huddlestone, J. R., Sipe, J., Braheny, S., Jensen, F. C., McMillan, R., Lampert, P. & Oldstone, M. B. A. (1979). Failure to isolate a transmissible agent from the bone-marrow of patients with multiple sclerosis. *Lancet* **ii**, 415.

Huelsenbeck, J. P., Ronquist, F., Nielsen, R. & Bollback, J. P. (2001). Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**, 2310–2314.

Hull, R. N., Minner, J. R. & Smith, J. W. (1956). New viral agents recovered from tissue cultures of monkey cells. I. Origin and properties of cytopathogenic agents SV1, SV2, SV4, SV5, SV6, SV11, SV12 and SV15. *Am J Hyg* **63**, 204–215.

Ito, M., Nishio, M., Kawano, M., Kusagawa, S., Komada, H., Ito, Y. & Tsurudome, M. (1997). Role of a single amino acid at the amino terminus of the simian virus 5 F2 subunit in syncytium formation. *J Virol* **71**, 9855–9858.

Ito, M., Nishio, M., Komada, H., Ito, Y. & Tsurudome, M. (2000). An amino acid in the heptad repeat 1 domain is important for the haemagglutinin–neuraminidase-independent fusing activity of simian virus 5 fusion protein. *J Gen Virol* **81**, 719–727.

Lamb, R. A. & Kolakofsky, D. (2001). *Paramyxoviridae*: the viruses and their replication. In *Fields Virology*, 4th edn, pp. 1305–1340. Edited by D. M. Knipe & P. M. Howley. Philadelphia, PA: Lippincott-Raven.

Levy, D. E. & García-Sastre, A. (2001). The virus battles: IFN induction of the antiviral state and mechanisms of viral evasion. *Cytokine Growth Factor Rev* **12**, 143–156.

Liebhaber, H., Krugman, S., McGregor, D. & Giles, J. P. (1965). Studies of a myxovirus recovered from patients with infectious hepatitis. I. Isolation and characterization *J Exp Med* **122**, 1135–1150.

Lim, C. S., Chan, K. P., Goh, K. T. & Chow, V. T. K. (2003). Hemagglutinin-neuraminidase sequence and phylogenetic analyses of mumps virus isolates from a vaccinated population in Singapore. *J Med Virol* **70**, 287–292.

McCandlish, I. A., Thompson, H., Cornwell, H. J. & Wright, N. G. (1978). A study of dogs with kennel cough. *Vet Rec* **102**, 293–301.

McLean, B. N. & Thompson, E. J. (1989). Antibodies against the paramyxovirus SV5 are not specific for cerebrospinal fluid from multiple sclerosis patients. *J Neurol Sci* **92**, 261–266.

Parisien, J.-P., Lau, J. F. & Horvath, C. M. (2002). STAT2 acts as a host range determinant for species-specific paramyxovirus interferon antagonism and simian virus 5 replication. *J Virol* **76**, 6435–6441.

Park, M.-S., García-Sastre, A., Cros, J. F., Basler, C. F. & Palese, P. (2003). Newcastle disease virus V protein is a determinant of host range restriction. *J Virol* **77**, 9522–9532.

Paterson, R. G., Harris, T. J. R. & Lamb, R. A. (1984). Fusion protein of the paramyxovirus simian virus 5: nucleotide sequence of mRNA predicts a highly hydrophobic glycoprotein. *Proc Natl Acad Sci U S A* **81**, 6706–6710.

Poole, E., He, B., Lamb, R. A., Randall, R. E. & Goodbourn, S. (2002). The V proteins of simian virus 5 and other paramyxoviruses inhibit induction of interferon-β. *Virology* **303**, 33–46.

Randall, R. E. & Young, D. F. (1988). Comparison between parainfluenza virus type 2 and simian virus 5: monoclonal antibodies reveal major antigenic differences. *J Gen Virol* **69**, 2051–2060.

Randall, R. E. & Russell, W. C. (1991). Paramyxovirus persistence: consequences for host and virus. In *The Paramyxoviruses*, pp. 299–321. Edited by D. W. Kingsbury. New York: Plenum.

Randall, R. E. & Bermingham, A. (1996). NP : P and NP : V interactions of the paramyxovirus simian virus 5 examined using a novel protein : protein capture assay. *Virology* **224**, 121–129.

Randall, R. E., Young, D. F., Goswami, K. K. A. & Russell, W. C. (1987). Isolation and characterization of monoclonal antibodies to simian virus 5 and their use in revealing antigenic differences between human, canine and simian isolates. *J Gen Virol* **68**, 2769–2780.

Rima, B. K., Earle, J. A. P., Baczko, K. & 7 other authors (1997). Sequence divergence of measles virus haemagglutinin during natural evolution and adaptation to cell culture. *J Gen Virol* **78**, 97–106.

Robbins, S. J., Wrzos, H., Kline, A. L., Tenser, R. B. & Rapp, F. (1981). Rescue of a cytopathic paramyxovirus from peripheral blood leukocytes in subacute sclerosing panencephalitis. *J Infect Dis* **143**, 396–403.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Rosenberg, F. J., Lief, F. S., Todd, J. D. & Reif, J. S. (1971). Studies of canine respiratory viruses. I. Experimental infection of dogs with an SV5-like canine parainfluenza agent. *Am J Epidemiol* **94**, 147–165.

Russell, W. C., Randall, R. E. & Goswami, K. K. A. (1989). Multiple sclerosis and paramyxovirus. *Nature* **340**, 104.

Sakaguchi, T., Toyoda, T., Gotoh, B., Inocencio, N. M., Kuma, K., Miyata, T. & Nagai, Y. (1989). Newcastle disease virus evolution. I. Multiple lineages defined by sequence variability of the hemagglutinin-neuraminidase gene. *Virology* **169**, 260–272.

Schultz, E. W. & Habel, K. (1959). SA virus; a new member of the myxovirus group. *J Immunol* **82**, 274–278.

Sen, G. C. (2001). Viruses and interferons. *Annu Rev Microbiol* **55**, 255–281.

Seth, S., Vincent, A. & Compans, R. W. (2003). Mutations in the cytoplasmic domain of a paramyxovirus fusion glycoprotein rescue syncytium formation and eliminate the hemagglutinin-neuraminidase protein requirement for membrane fusion. *J Virol* **77**, 167–178.

Siegl, G. & Hahn, E. E. (1969). A paramyxovirus-like virus isolated from pemphigus-disease in man. *Arch Gesamte Virusforsch* **28**, 41–50.

Southern, J. A., Young, D. F., Heaney, F., Baumgärtner, W. K. & Randall, R. E. (1991). Identification of an epitope on the P and V proteins of simian virus 5 that distinguishes between two isolates with different biological characteristics. *J Gen Virol* **72**, 1551–1557.

Stark, G. R., Kerr, I. M., Williams, B. R. G., Silverman, R. H. & Schreiber, R. D. (1998). How cells respond to interferons. *Annu Rev Biochem* **67**, 227–264.

Thomas, S. M., Lamb, R. A. & Paterson, R. G. (1988). Two mRNAs that differ by two nontemplated nucleotides encode the amino coterminal proteins P and V of the paramyxovirus SV5. *Cell* **54**, 891–902.

Tong, S., Li, M., Vincent, A., Compans, R. W., Fritsch, E., Beier, R., Klenk, C., Ohuchi, M. & Klenk, H.-D. (2002). Regulation of fusion activity by the cytoplasmic domain of a paramyxovirus F protein. *Virology* **301**, 322–333.

Tribe, G. W. (1966). An investigation of the incidence, epidemiology and control of Simian virus 5. *Br J Exp Pathol* **47**, 472–479.

Tsurudome, M., Nishio, M., Komada, H., Bando, H. & Ito, Y. (1989). Extensive antigenic diversity among human parainfluenza type 2 virus isolates and immunological relationships among paramyxoviruses revealed by monoclonal antibodies. *Virology* **171**, 38–48.

Ulane, C. M. & Horvath, C. M. (2002). Paramyxoviruses SV5 and HPIV2 assemble STAT protein ubiquitin ligase complexes from cellular components. *Virology* **304**, 160–166.

Vandvik, B. & Norrby, E. (1989). Paramyxovirus SV5 and multiple sclerosis. *Nature* **338**, 769–771.

van Wyke Coelingh, K. L., Winter, C. C. & Murphy, B. R. (1988). Nucleotide and deduced amino acid sequence of hemagglutinin-neuraminidase genes of human type 3 parainfluenza viruses isolated from 1957 to 1983. *Virology* **162**, 137–143.

Wallen, W. C., Sever, J. L., McFarlin, D. E., McFarland, H. F., Traub, R. G., Rentier, B., Greenstein, J. I. & Moore, P. M. (1979). Attempt to isolate infectious agent from bone-marrow of patients with multiple sclerosis. *Lancet* ii, 414–415.

Wansley, E. K. & Parks, G. D. (2002). Naturally occurring substitutions in the P/V gene convert the noncytopathic paramyxovirus simian virus 5 into a virus that induces alpha/beta interferon synthesis and cell death. *J Virol* **76**, 10109–10121.

Young, D. F., Randall, R. E., Hoyle, J. A. & Souberbielle, B. E. (1990). Clearance of a persistent paramyxovirus infection is mediated by cellular immune responses but not by serum-neutralizing antibody. *J Virol* **64**, 5403–5411.

Young, D. F., Didcock, L., Goodbourn, S. & Randall, R. E. (2000). Paramyxoviridae use distinct virus-specific mechanisms to circumvent the interferon response. *Virology* **269**, 383–390.