Short Communication

A novel homologue of *Human herpesvirus 6* in chimpanzees

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Among the *Betaherpesvirinae*, human cytomegalovirus is the only virus to possess simian homologues. Indeed, intriguingly, no close simian homologue of the roseoloviruses *Human herpesvirus 6* (HHV-6) and *Human herpesvirus* 7 (HHV-7), the other two human members of the *Betaherpesvirinae*, has been identified to date. Here, the first simian homologue of HHV-6 is described, which was identified in common chimpanzees and designated PanHV6. By using a degenerate consensus PCR method, three different gene fragments were amplified, corresponding to the DNA polymerase (U38), β-chemokine receptor (U12) and viral transactivator (U42) genes, with 94–96 % (nucleotide) and 95–97 % (amino acid) sequence identity to the corresponding genes of HHV-6B. Analysis of 77 predominantly wild-caught chimpanzees identified a unique PanHV6 strain in 21 animals, with no viral sequence variation between the different chimpanzee subspecies that were found to be infected. Characterization of this virus represents a great potential to gain a better understanding of the diseases associated with HHV-6.

Human herpesvirus 6 (HHV-6), first isolated in 1986 from the peripheral blood of patients with lymphoproliferative disorders, is one of the most recently characterized of the human herpesviruses (Salahuddin *et al.*, 1986). Two distinct variants of HHV-6, HHV-6A and HHV-6B, are recognized (Schirmer *et al.*, 1991). These variants are similar with respect to genomic and genetic organization, but have been reported to be different in epidemiology, tropism and pathogenesis (Ablashi *et al.*, 1991; Dominguez *et al.*, 1999). Nevertheless, both HHV-6A and HHV-6B have been isolated from blood of infected children and, whilst it appears that HHV-6B has greater prevalence in countries where it has been studied the most (i.e. USA, UK and Japan), an equal prevalence, based on PCR from blood DNA, was identified in South Africa (Kasolo *et al.*, 1997). Further, both HHV-6A and HHV-6B have been identified as brain commensals but, where HHV-6B is more prevalent in blood, it is also more prevalent in brain (Tuke *et al.*, 2004). Finally, whilst HHV-6B has been isolated from peripheral blood leukocytes (PBL) and cerebrospinal fluid (CSF) of children with primary infection, HHV-6A, rare in these American studies, was isolated more frequently from CSF than from saliva or PBL, suggesting that HHV-6A has a greater neurotropism than HHV-6B (Hall *et al.*, 1998). This virus is one of the most widespread human herpesviruses, with up to 90 % of the population infected as infants, where primary infection is symptomatic; it causes mainly febrile illness, with a minority of patients developing roseola infantum, a mild skin rash with occasional severe or fatal complications (Hall *et al.*, 1994; Yamanishi *et al.*, 1988; Zerr *et al.*, 2005). Like other herpesviruses, it can establish a latent or persistent infection, which remains for the lifetime of the host and can reactivate during immunosuppression. Viral reactivation can result in the development of severe diseases. Indeed, HHV-6 has been associated with several pathological conditions in immunocompromised patients, such as post-transplantation complications, neurological diseases, lymphadenopathy, infectious mononucleosis-like illness, fulminant hepatitis and autoimmune disorders [reviewed by Clark (2000)]. Further, the virus has been linked to the autoimmune neurological disease multiple sclerosis (Challoner *et al.*, 1995; Soldan *et al.*, 1997). Nevertheless, despite these associations, the search for a causative role for HHV-6 in a particular illness, apart from roseola infantum, has not been conclusive.

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Supplementary tables showing the sequences of oligonucleotide primers and GenBank accession numbers are available in JGV Online.

Soon after the characterization of HHV-6, chimpanzee lymphocytes were shown to be susceptible to experimental HHV-6 infection (Lusso *et al.*, 1990). In addition, testing of captive chimpanzees by an HHV-6 immunofluorescence assay revealed that 75–90 % were seropositive, depending on where they were living, a prevalence similar to that in humans (Higashi *et al.*, 1989). Furthermore, the existence of HHV-6-related viruses in other non-human primates has also been strongly suggested by serological studies (Blewett *et al.*, 2001; Higashi *et al.*, 1989; Jenson *et al.*, 2002). Nevertheless, such viruses have never been characterized. Thus, to confirm the existence of an HHV-6 homologue indigenous to chimpanzees, we examined blood samples obtained from 77 common chimpanzees.

The larger series of chimpanzees comprised 42 wild-born animals (22 *Pan troglodytes vellerosus* and 20 *P. troglodytes troglodytes*) originating from different parts of Cameroon, where they were gathered in wildlife-rescue centres in the south-western province of Cameroon or in Yaoundé, and from the large primate centre of the Centre International de Recherches Médicales de Franceville (CIRMF) in Gabon. The second series (35 chimpanzees, of which 33 were *P. troglodytes verus* and two were *P. troglodytes schweinfurthii*) was obtained from a closed breeding colony of chimps housed at the Biomedical Primate Research Centre (BPRC), Rijswijk, the Netherlands (Table 1). DNA was extracted from buffy coats with a QIAamp DNA Blood mini kit (Qiagen), following the manufacturer's instructions. Then, by using consensus degenerate primers targeting the herpesvirus DNA polymerase gene (Rose *et al.*, 1997), we attempted to amplify herpesvirus sequences from these DNAs as described previously (Lacoste *et al.*, 2001) (see Supplementary Table S1 in JGV Online). Briefly, DNA samples were initially amplified with the primer pools DFASA and GDTD1B, and an aliquot of these amplification products was then used as a template in a subsequent nested PCR (nPCR) with the VYGA and GDTD1B primer pools. nPCR products were examined by agarose-gel electrophoresis, purified by using a QIAquick gel-extraction kit (Qiagen) and then cloned by TA cloning in the pCR2.1 cloning vector (Invitrogen). Cycle sequencing was performed by Eurogentec (Seraing, Belgium) using BigDye Terminator technology. Initial screening was done on the 25 chimpanzees, mainly from Cameroon, from which we previously identified different rhadinoviruses (Lacoste *et al.*, 2000a, 2001). Among these 25 DNAs, 20 scored positive on the ethidium bromide-stained gel. Sequencing of multiple individual clones from each positive chimp allowed us to identify different herpesviruses. Whilst most of the sequences amplified corresponded to PanRHV1a, PanRHV1b, PanRHV2 or PtroLCV1 (Ehlers *et al.*, 2003; Lacoste *et al.*, 2000a, 2001), three of them, from three different chimps, were related closely to HHV-6, as observed by database BLAST searches. To obtain the sequence extending upstream of the VYGA region, a gene-specific, non-degenerate primer (P6as; see Supplementary Table S1 in JGV Online) was derived from the complementary sequence of the 'HHV-6-like' VYGA–GDTD1B fragment and used in an nPCR amplification with the DFASA primer pool. The PCR products (DFASA–GDTD1B) from the initial PCR were used as template DNAs in these subsequent amplification reactions. The nucleotide sequences of the DFASA–GDTD1B fragments yielded 476 bp sequences after exclusion of the primer sequences. Sequence analyses and alignments were performed by using the MacVector 6.0 and AssemblyLIGN software packages (Oxford Molecular Ltd). Phylogenetic analyses of nucleotide and deduced amino acid sequences were performed by using the PHYLIP package (version 3.52c; Felsenstein, 1993), as described previously (Lacoste *et al.*, 2001). The phylogenetic analyses based on this fragment further confirmed that the identified sequence was related closely to HHV-6 (Fig. 1). Lastly, an additional degenerate primer pool (POL6A), upstream of DFASA and derived from a conserved amino acid motif within the DNA polymerase gene of roseoloviruses, as well as two new gene-specific primers (POL6B1 and POL6B2) derived from the complementary sequences of the DFASA–GDTD1B fragments, have been designed to finally obtain an 883 bp sequence (see Supplementary Table S1 in JGV Online). This was 6 and 7 % divergent at the nucleotide level and 3 % at the protein level with respect to HHV-6B and -6A sequences, respectively (Table 2). This novel herpesvirus, related closely to the sixth human herpesvirus, was provisionally named PanHV6 for *Pan troglodytes* herpesvirus 6. However, among the specialists of

Table 1. Common chimpanzees (*Pan troglodytes*) tested for PanHV6 by molecular methods and survey results

Subspecies	Origin	Status	No. animals	No. PanHV6-positive (%)*
P. troglodytes verus	The Netherlands†	Colony	33	3 (9)
P. troglodytes vellerosus	Cameroon/Gabon	Wild-caught	22	11 (50)
P. troglodytes troglodytes	Cameroon/Gabon	Wild-caught	20	7 (35)
P. troglodytes schweinfurthii	The Netherlands	Colony	2	0 (0)

*As determined by PCR amplification with specific PanHV6 primers (Pan6s/Pan6as1 and Pan6s/Pan6as2) followed by Southern blot hybridization with the PanHV6 probe (Pan6p).

†These chimpanzees belong to a closed breeding colony housed at the Biomedical Primate Research Centre, Rijswijk, The Netherlands, whose founders originate from Sierra Leone (Niphuis *et al.*, 2003).

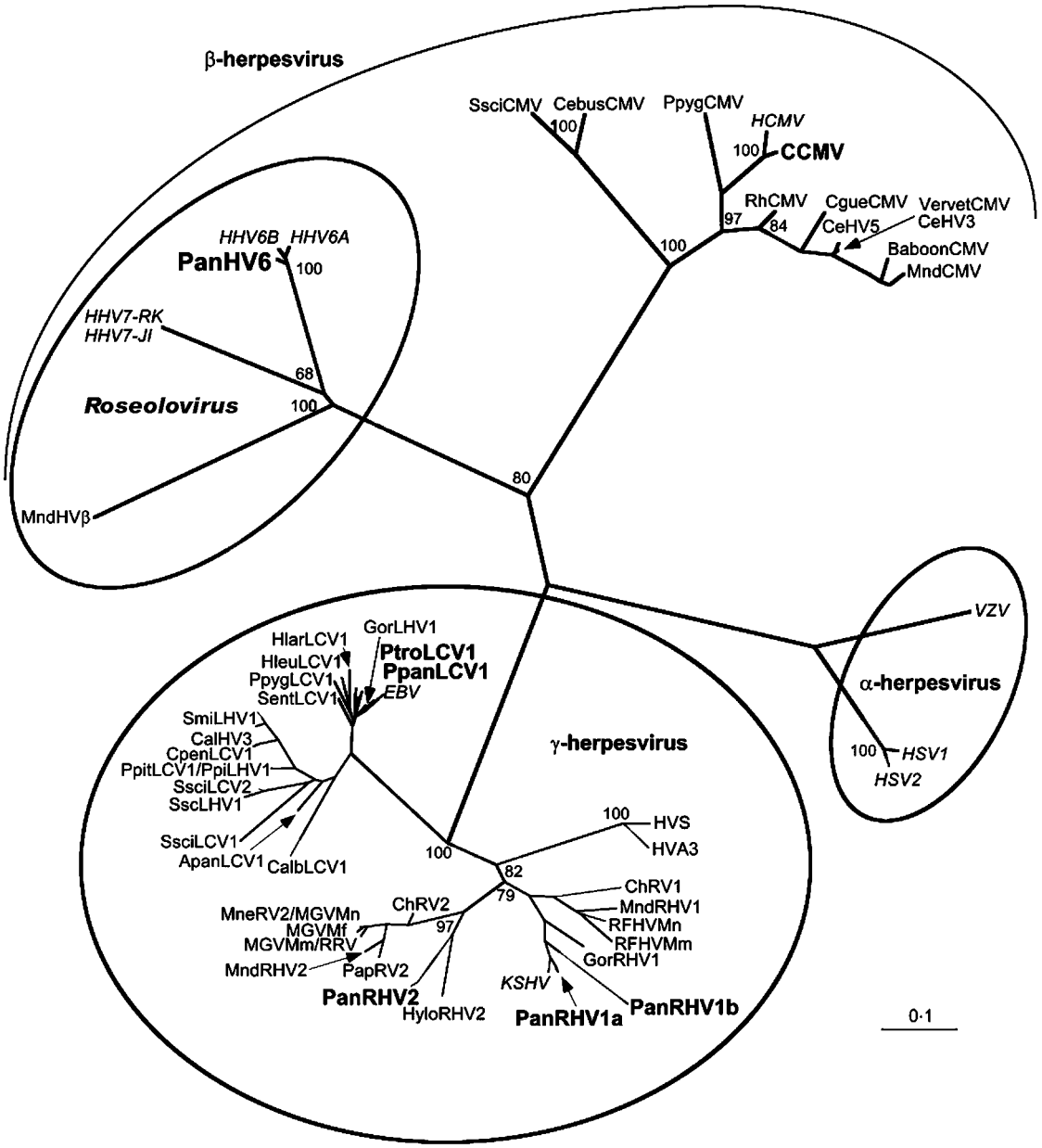

Fig. 1. Neighbour-joining protein distance tree for the 151 amino acid residues encoded by a 454 bp fragment of the DNA polymerase gene available for all these sequences. Sequences were aligned by using CLUSTAL_X and analysed with the PROTDIST and NEIGHBOR programs in PHYLIP. One thousand replica samplings were subjected to bootstrap analysis (SEQBOOT). The branch lengths are proportional to the evolutionary distance (scale bar) between the taxa. Previously published sequences are included and their GenBank accession numbers are given in Supplementary Table S2, available in JGV Online. Sequences in italics correspond to human herpesviruses, whilst sequences in bold correspond to chimpanzee viruses. Due to space constraints, not all EBV-like viruses from Old World monkeys are labelled.

the field, a new proposal will certainly be discussed and debated. When a new name is approved, we will modify its name accordingly.

The PanHV6 DNA polymerase sequence was subsequently used to determine the prevalence of this agent in chimpanzees. The 77 chimpanzee DNA samples from all four recognized subspecies (*P. troglodytes verus*, *P. troglodytes vellerosus*, *P. troglodytes troglodytes* and *P. troglodytes schweinfurthii*), predominantly wild-caught (*P. troglodytes vellerosus* and *P. troglodytes troglodytes*), were subjected to an nPCR using PanHV6-specific primers (Pan6s/Pan6as1 and Pan6s/Pan6as2), and subsequently screened by Southern blot hybridization using a PanHV6-specific probe, Pan6p

Table 2. Nucleotide and amino acid sequence identities between PanHV6 and other primate β-herpesviruses

Virus	PanHV6 sequence identity (%):	
	Nucleotide	Amino acid
U38 (DNA polymerase; GenBank no. AY359407*)	**883 bp**	**294 aa**
HHV-6B strain Z29	94	97
HHV-6A strain U1102	93	97
HHV-7 strain RK	66	72
HHV-7 strain JI	66	72
MndHVβ†	59	62
Human CMV	48	37
Chimpanzee CMV	46	37
Rhesus CMV	50	38
Vervet CMV†	47	39
Mandrillus CMV†	48	39
Baboon CMV†	49	39
Cebus CMV†	50	43
Saimiri CMV†	50	42
U42 (transactivator; GenBank no. AY359408*)	**640 bp**	**213 aa**
HHV-6B strain Z29	96	97
HHV-6A strain U1102	94	94
HHV-7 strain RK	62	59
HHV-7 strain JI	62	59
Human CMV UL69	41	30
Chimpanzee CMV UL69	39	28
Rhesus CMV UL69	37	27
U12 (GCR homologue; GenBank no. AY359406*)	**490 bp**	**163 aa**
HHV-6B strain Z29	95	95
HHV-6A strain U1102	91	87
HHV-7 strain RK	57	51
HHV-7 strain JI	57	51
Human CMV UL33	38	22
Human CMV US28	29	13
Human CMV US27	31	12
Chimpanzee CMV UL33	37	22
Chimpanzee CMV US28	30	14
Chimpanzee CMV US27	33	13
Rhesus CMV UL33	41	27
Rhesus CMV US28	29	14

*GenBank accession numbers of the three PanHV6 gene fragments identified from *P. troglodytes verus*. Due to the quasi-identity of the sequences obtained from the three chimpanzee subspecies, only PanHV6 sequences obtained from *P. troglodytes verus* have been submitted to GenBank.

†Comparison of these viral strains was performed only on the common 454 bp fragments.

(see Supplementary Table S1 in JGV Online). In 21 chimpanzees (27 % prevalence), evidence was found of infection with this new simian β-herpesvirus (Table 1). To be totally confident about the sensitivity of our approach and to address the question of whether distinct PanHV6 genotypes infect the different chimpanzee subspecies, the 883 bp sequence has been generated, by using the same PCR strategy as described above, from one randomly selected chimpanzee from each of the three subspecies infected with PanHV6. Three clones of each PCR product amplified from the selected chimps were sequenced on both strands. The 100 % nucleotide identity of the PanHV6 DNA polymerase sequences that we obtained from the three chimpanzees is consistent with the fact that this gene is highly conserved and confirms that our PanHV6 prevalence study can be interpreted without any ambiguity.

By defining other sets of degenerate primers (see Supplementary Table S1 in JGV Online), we amplified two additional fragments of 490 and 640 bp, corresponding to the U12 and U42 genes of HHV-6, respectively. Whilst U42 is a member of the only family of genes involved in transactivation that is conserved among all of the herpesviruses (Dominguez *et al.*, 1999; Isegawa *et al.*, 1999), the U12 gene of HHV-6 encodes a functional β-chemokine receptor (Isegawa *et al.*, 1998), representative of a group of similar G-coupled receptor (GCR) homologues conserved between human and animal members of the *Betaherpesvirinae* (Murphy, 2001). It has been shown that the individual viral homologues within this GCR group show greater identity to each other than to the closest known cellular homologue (Raftery *et al.*, 2000). Moreover, as the PanHV6 U12 homologue shows the same degree of similarity to its HHV-6 counterpart as the DNA polymerase gene, all of these data suggest that these viruses acquired this gene from their cellular host before viral speciation (Table 2). Sequencing of these two other gene fragments from the same three chimpanzees as mentioned above (each belonging to one subspecies) showed no significant sequence variation (1–2 nt, with no amino acid change). These results further confirm that there is no distinct PanHV6 genotype infecting the different chimpanzee subspecies. Nevertheless, as our sequencing studies focused on highly conserved regions of the viral genome, another HHV-6 genotype might be identified if regions of greater divergence, such as those between HHV-6A and HHV-6B, are scrutinized.

These studies represent a definitive identification of roseolovirus infection in non-human primates. Indeed, although we previously identified a β-herpesvirus from mandrill and drill monkeys that is related to the human roseoloviruses, this virus, named MndHVβ, is only distantly related to them (Lacoste *et al.*, 2000b). Similar to the other chimpanzee herpesviruses, PanHV6 is related very closely to its human counterpart. It is worth noting that the PanHV6 DNA polymerase gene sequence obtained shows the same degree of similarity to its human counterpart compared with the chimpanzee Epstein–Barr virus (EBV), human cytomegalovirus and Kaposi's sarcoma-associated herpesvirus homologues (95–97 %) (Davison *et al.*, 2003; Ehlers

et al., 2003; Lacoste *et al.*, 2000a). We assume that, when more data become available from other simian herpesvirus 6 isolates, they will support the theory of co-evolution of herpesviruses with their host species (McGeoch *et al.*, 1995). Interestingly, the sequences obtained from the buffy-coat DNA of these animals are related more closely to HHV-6B than to HHV-6A. This suggests that, if a PanHV6-A variant exists, it it is more likely to be detected in the central nervous system of chimpanzees, due to the apparently different tropism of the two human viral variants.

Since its discovery in 1986, HHV-6 is increasingly recognized as an important pathogen in immunocompromised patients (especially transplant recipients) and is currently hypothesized as a strong suspect in the origin of multiple sclerosis (Dockrell, 2003; Soldan *et al.*, 1997). Due to the high prevalence of latently infected individuals in the healthy population, its precise role in the formerly mentioned conditions is not well understood (Agut, 1993; Fillet *et al.*, 1998; Le Cleach *et al.*, 1998). Therefore, it will be of interest to determine whether any pathology due to PanHV6 infection occurs in the common chimpanzees. Further characterization of the PanHV6 genome will enable us to make meaningful comparisons between the human and chimpanzee herpesvirus 6 sequences, yielding numerous insights into evolutionary paths within the genus *Roseolovirus*, and to decipher different biological information encoded in these two genomes.

The sequences reported in this paper have been deposited in GenBank under accession numbers AY359406–AY359408. Due to the quasi-identity of the sequences obtained from the three chimpanzee subspecies, only PanHV6 sequences obtained from *P. troglodytes verus* have been submitted to GenBank.

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