Antigenic and genomic identity between simian herpesvirus aotus type 2 and bovine herpesvirus type 4

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Herpesvirus aotus type 2 (HVA-2) was isolated from a culture of kidney cells from a healthy owl monkey (Aotus trivirgatus). Bovine herpesvirus type 4 (BHV-4) is frequently isolated from diseased and even healthy cattle and occasionally from sheep, wild ruminants and cats. The two viruses are related antigenically, as was revealed by an indirect fluorescent antibody test using polyclonal antisera from experimentally infected rabbits or monoclonal antibodies raised against six BHV-4 proteins, three of which were glycosylated. The genome structures of the two viruses consist of a unique central sequence flanked at both ends by G+C-rich tandem repeats. Restriction maps (produced using EcoRI, BamHI and HindIII) of these two viruses were nearly identical but the unique sequence of the HVA-2 genome possessed two additional BamHI sites. Four genomic regions of variable size were detected, two located in the unique part, one in the repetitive part and one in the left junction between the unique and the repeated part of the genome; these slight variations were similar to those observed between various BHV-4 isolates. These results suggest that HVA-2 and BHV-4 belong to the same virus species; HVA-2 could be either a BHV-4 contaminant of owl monkey kidney cell cultures or an isolate from an owl monkey accidentally infected with BHV-4.

Herpesvirus aotus type 2 (HVA-2) was isolated from an uninoculated kidney cell culture obtained from an apparently normal owl monkey (Aotus trivirgatus) (Barahona et al., 1973); bovine herpesvirus type 4 (BHV-4) has been isolated from a variety of diseased or healthy cattle (reviewed by Thiry et al., 1989), sheep (Van Opdenbosch et al., 1986), American bison (Bison bison) (Todd & Storz, 1983), African buffalo (Syncerus caffer) (Rossiter et al., 1989) and cats (Kit et al., 1986; Kruger et al., 1989). Published restriction patterns of HVA-2 (Fuchs et al., 1985) and BHV-4 (Bublot et al., 1990) are very similar but the two viruses have been shown to possess distinct epidemiological features.

Antibodies against HVA-2 were detected in the serum of 42% of owl monkeys, suggesting that this species is the most probable reservoir in nature (Barahona et al., 1973); furthermore, HVA-2 does not appear to be pathogenic or oncogenic (Rüger et al., 1980). BHV-4 infection is distributed worldwide and its prevalence can be as high as 50%, depending on the country; its pathogenic role remains unclear and only a few experiments have successfully reproduced the disease (Thiry et al., 1989).

The genome structure of the two viruses is similar to that of herpesvirus saimiri (Bornkamm et al., 1976) and is typical of the group B herpesviruses according to the classification of Roizman (1990); it contains a unique 110 kbp central part (L-DNA, low G + C content) flanked at both ends by tandem repeats (H-DNA, high G + C content), also called polyrepetitive DNA (prDNA) (Fuchs et al., 1985; Ehlers et al., 1985). Reassociation kinetics have not detected any sequence similarity between the genome of HVA-2 and those of herpesvirus saimiri and herpesvirus atelites (Fuchs et al., 1985). This study was therefore undertaken to analyse the relationships between HVA-2 and BHV-4.

HVA-2 (isolate OMKI 68-69; Barahona et al., 1973; kindly provided by M. D. Daniel from the New England Regional Primate Research Centre, Harvard Medical School, Southborough, Mass., U.S.A.) and BHV-4 (V. Test strain; Thiry et al., 1981) were grown on Georgia bovine kidney (GBK) cells. Non-plaque-purified HVA-2 was designated NP; two viral populations (C1 and C2) of HVA-2 were obtained after three successive plaque purifications. Total c.p.e. appeared 5 to 6 days post-infection (p.i.) for HVA-2 NP and C2 and 2 to 3 days p.i. for HVA-2 C1 and BHV-4 (V. Test).

Polyclonal sera against HVA-2 C1 and BHV-4 (V. Test) were produced in experimentally infected rabbits as described by Osorio et al. (1982); these sera and a
panel of monoclonal antibodies (MAbs) raised against proteins of BHV-4 (V. Test) were used in an indirect fluorescent antibody test, as previously described (Dubuisson et al., 1988). Thirteen MAbs (VT-1, -8, -10, -12, -15, -24, -26, -27, -28, -30, -35, -36 and -38) recognized glycoprotein gp6/gp10/gp17 (150K/120K/51K), 11 MAbs (VT-2, -6, -7, -9, -16, -18, -19, -20, -23, -29 and -33) glycoprotein gp11/VP24 (120K/16.5K) (Dubuisson et al., 1989a, 1990), three MAbs (VT-107, -113 and -114) glycoprotein gp8 (135K) (J. Dubuisson & I. Koromyslov, unpublished data) and MAbs VT-34, -39 and -21 recognized non-glycosylated proteins VP7 (140K), VP14 (78K) and VP25 (16K), respectively (J. Dubuisson, unpublished data).

Purification of DNA, restriction analysis and Southern blot hybridization of HVA-2 NP, C1 and C2 (fifth passage on GBK cells for HVA-2 NP and fifth passage on GBK cells after plaque purification for HVA-2 C1 and C2) and BHV-4 (V. Test) were performed as previously described (Bublot et al., 1990). DNA from recombinant phages, covering the entire length of the BHV-4 (V. Test) genome (Bublot et al., 1990), was used as the probe in Southern blot experiments to establish the EcoRI, BamHI and HindIII restriction maps of HVA-2 DNA. Variable regions were confirmed by hybridization with shorter specific probes from a BHV-4 (V. Test) DNA plasmid library (Bublot et al., 1990).

The highest dilutions of anti-BHV-4 (1/128) and anti-HVA-2 (1/1024) polyclonal sera exhibiting specific fluorescence were identical when HVA-2 C1 and BHV-4 (V. Test) were used as antigens; moreover, HVA-2 C1 was recognized by all but one (VT-26) anti-BHV-4 MAb raised against the gp6/gp10/gp17 glycoprotein, by all MAbs raised against the gp11/VP24 glycoprotein (VT-7 reacted with a weaker fluorescent intensity) and all the other MAbs. These results show a close antigenic relationship between the two viruses.

HindIII, EcoRI and BamHI restriction patterns of BHV-4 (V. Test) and HVA-2 NP, C1 and C2 DNAs were very similar (Fig. 1a, b and 2a); these patterns were typical of the Movar 33/63-like (Movar-like) group of BHV-4 described previously (Thiry et al., 1989; Bublot et al., 1990). A restriction map of HVA-2 was constructed using the above-mentioned method; one genome unit was defined as the length of the L-DNA flanked at both ends by one prDNA; the size of one unit of HVA-2 (113.1 kbp) was estimated to be only 300 bp shorter than that of BHV-4 (V. Test) (113.4 kbp) (Fig. 3).

The prDNAs of BHV-4 (V. Test) and HVA-2 possessed one EcoRI site and one BamHI site but no HindIII site (see the hypermolar bands in Fig. 1b and 2a). The EcoRI and BamHI patterns of the BHV-4 (V. Test) DNA showed a strong hypermolar band having a size of 2.65 kbp (major prDNA) and a weaker hypermolar band having a size of 2.85 kbp (minor prDNA). HVA-2 NP DNA restriction patterns clearly showed two major prDNAs, one of 2.25 kbp which was present in greater amounts than one of 2.05 kbp. The intensity of the 2.05 kbp prDNA band decreased in the HVA-2 C2 viral DNA pattern and only one prDNA fragment, of 2.25 kbp, was visible in the HVA-2 C1 pattern (see Fig. 1b and 2a). These prDNA fragments were detected by Southern blot hybridization using cloned prDNA used as the probe (probe pr); this probe also detected the terminal fragments and fragments from the junction between L-DNA and H-DNA of the BHV-4 genome in EcoRI (data not shown) and BamHI (Fig. 2b) patterns. The left terminal fragments (T1) had a constant size of 700 bp in the BamHI pattern and the right terminal fragments (Tr) were 700 bp shorter than the corresponding prDNA fragments (Fig. 2a and 2b). This observation indicates that the BamHI site is located 700 bp from the left end of the prDNA and that the region responsible for the prDNA size variation is located to the right of this BamHI site. The prDNA size variation is probably of the same order as that already observed among BHV-4 strains and is due to the presence of a variable number of a 200 bp fragment inside the prDNA.
(Ehlers et al., 1985). The right junction fragment (Jr) between the H-DNA and L-DNA of these two viruses was identical in size; however, variation occurred in the size of the left junction fragment (J1), which could be specifically detected by Southern blot hybridization of BamHI and EcoRI patterns with probes J11 (Fig. 2c) and J12 (data not shown) respectively (see Fig. 3 for localization of probes). The left junction region of HVA-2 DNA was proportionally larger than that of BHV-4 (V. Test) and possessed one additional BamHI site located near the left terminus of the L-DNA at position 0-026 (Fig. 3).

The presence of a second additional BamHI site at position 0-130 in the HVA-2 L-DNA sequence was confirmed by Southern blot hybridization with probes P and U (see Fig. 3 for localization of probes and the second additional BamHI site); this additional restriction site is responsible for the shift of fragment A and the presence of an additional fragment named J in the BamHI pattern of HVA-2 DNA (Fig. 2a).

Probes Q, E1 and E2 were used in Southern blot experiments (data not shown) to confirm the size variation present at the left and right ends of L-DNA (see Fig. 3 for localization of probes and variable fragments). These size variations could be observed in the BamHI patterns between fragments having a size ranging from 4-8 to 5-7 kbp (Fig. 2a). The size variation present at the left end of L-DNA could be localized to HindIII fragment Q (positions 0-063 to 0-082) (Fig. 1a and 3); HindIII fragment U, which is known to vary in size between BHV-4 strains (Bublot et al., 1990, 1991), was the same size in the two strains analysed in this study (Fig. 1a).

This study has clearly shown a close relationship between BHV-4 and HVA-2. The two viruses share many common epitopes as revealed by analysis with polyclonal sera and MAbs raised against BHV-4 (V. Test); BHV-4 strains are also antigenically closely related (Ludwig, 1983; Osorio et al., 1985; Dubuisson et al., 1989b). A quantitative test, such as ELISA, and glycoprotein analysis by immunoprecipitation will provide more information about the antigenic relationship existing between BHV-4 and HVA-2.

The genomes of HVA-2 and BHV-4 (V. Test) shared many similarities; indeed, the central part (from position 0-130 to position 0-831) of their L-DNA was indistinguishable by restriction analysis using EcoRI, BamHI and HindIII. The two additional BamHI sites present at the left end of HVA-2 L-DNA and the size variation of the corresponding BHV-4 (V. Test) BamHI fragment K have not previously been reported; all the other size variations detected in this study (HindIII fragment Q, BamHI fragment I, J1 and the prDNA fragment) have been observed in DNA from different BHV-4 strains (Ehlers et al., 1985; Bublot et al., 1990, 1991). Furthermore, the presence of a variable amount of prDNA of one size in different DNA preparations, also observed for HVA-2 by Fuchs et al. (1985), has been described for BHV-4 (Bublot et al., 1990). Variations observed between BHV-4 (V. Test) and HVA-2 DNAs were therefore mainly similar to those detected between BHV-4 strains or isolates. Furthermore, HVA-2 had a
Fig. 3. Restriction maps of BHV-4 (V. Test) and HVA-2 genomes for enzymes EcoRI, BamHI and HindIII. Only three prDNAs were arbitrarily represented at each side of the unique sequence; this schematic structure does not represent the BHV-4 genome as it appears in the virion (see text). Only one prDNA size has been chosen for this figure [2.65 and 2.25 kbp for BHV-4 (V. Test) and HVA-2 DNA respectively]. The numbers noted above the fragments correspond to their sizes in kbp; only the size of the variable fragments is indicated. Arrows show the two additional BamHI sites detected in the HVA-2 genome. The localization and the name of the probes are noted under the maps. One genome unit was defined as the length of the unique central part, flanked at both ends by one prDNA.

restriction pattern similar to the Movar-like group of BHV-4 strains and therefore constitutes the first American Movar-like strain identified so far (Thiry et al., 1990).

The antigenic and genomic analyses are consistent with the hypothesis that HVA-2 and BHV-4 belong to the same virus species and suggest that HVA-2 is a BHV-4 strain; it could be either a BHV-4 contaminant of an owl monkey kidney cell culture or an isolate from an owl monkey accidentally infected with BHV-4. Indeed, the in vivo and in vitro host ranges of BHV-4 are relatively wide (Thiry et al., 1990) and, furthermore, contaminant BHV-4 has been found in foetal calf serum (C. Whetstone, personal communication) and bovine vaccines (F. Osorio, personal communication); contaminant BHV-4 DNA has been detected by the polymerase chain reaction in a BHV-1 DNA preparation (M. Bublot & E. Thiry, unpublished results). BHV-4 contamination of OMK cell culture before the appearance of a c.p.e., which began only at the ninth transfer (day 80) (Barahona et al., 1973), therefore cannot be excluded. Alternatively, an accidental infection of owl monkeys in a research centre by contact with a BHV-4-infected animal of another species, or by experimental inoculation of BHV-4-contaminated material, is also possible.

The latter hypothesis could explain the presence of specific antibodies in the sera of owl monkeys (42%, 44/106) and spider monkeys (25%, 2/8) in two primate or medical research centres (Barahona et al., 1973). These seropositive animals could also harbour a natural virus antigenically related to BHV-4 but not yet identified. Antigenically related herpesviruses can be present in unrelated species, for instance bovine herpesvirus type 2, human herpes simplex virus and simian herpesvirus B (Ludwig, 1983). Further serological studies and viral isolations therefore need to be done on owl monkeys living in zoos, natural reserves and their natural habitat to confirm the suggestion (Barahona et al., 1973) that the owl monkey is a natural host for HVA-2.

A similar situation occurs with BHV-4 and feline cell-associated herpesvirus (FeCAHV). FeCAHV was isolated from diseased and healthy kittens (Fabricant et al., 1971) and shares antigenic and genomic similarities with BHV-4 (Kit et al., 1986; Kruger et al., 1989). A serological survey in the U.S.A. showed specific antibodies in 30% of cats (J. M. Kruger, personal communication), suggesting that this virus or a related one is prevalent in the American cat population. BHV-4 infection of cats could occur after a contact with BHV-4-infected cattle or by inoculation of BHV-4-contaminated
biological material, such as an attenuated vaccine. Infection with BHV-4 could be therefore widely distributed and other species could also be candidates for harbouring this virus; they should be studied to determine their role in the dissemination of this virus among cattle. Furthermore, biological materials should be tested for BHV-4 contamination before inoculation.

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