Macropodid herpesviruses 1 and 2 occupy unexpected molecular phylogenetic positions within the Alphaherpesvirinae

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The molecular phylogeny of macropodid herpesviruses 1 and 2 (MaHV-1 and -2) has been investigated by cloning and sequencing the genes encoding glycoprotein B from both viruses. Phylogenetic reconstructions based on the putative amino acid sequences of glycoprotein B indicate that MaHV-1 and -2 are most closely related to the subfamily Alphaherpesvirinae. Within the Alphaherpesvirinae, MaHV-1 and -2 are closely associated with those herpesviruses that infect primates. This phylogenetic relationship does not fit the constraints of the proposed co-evolution theory described for other members of the Alphaherpesvirinae which have mammalian hosts.

Macropodid herpesviruses (MaHVs) have been implicated in fatal disease outbreaks amongst the captive marsupial populations of Australia (Finnie et al., 1976; Dickson et al., 1980). These outbreaks have resulted in the isolation of nine MaHVs which have been classified into two species called macropodid herpesvirus 1 and 2 (MaHV-1 and MaHV-2) (Johnson & Whalley, 1990). Serological evidence indicates that these viruses are widespread among Australian kangaroos and wallabies (family Macropodidae) (Webber & Whalley, 1978; Wilks et al., 1981). MaHV-1 and -2 are represented by isolates from the parma wallaby (Macropus parma) and the dorcopsis wallaby (Dorcopsis meulleri luctuosa), respectively (Finnie et al., 1976; Wilks et al., 1981).

The family Herpesviridae is a large group of viruses that have double-stranded DNA genomes. Biological characteristics such as host symptoms, site of replication and site of latency have been used to describe three major subfamilies, Alpha-, Beta- and Gammaherpesvirinae, within the family Herpesviridae. Biological characteristics have been used to place MaHV-1 and -2 within the subfamily Alphaherpesvirinae.

The Herpesviridae is the best characterized at the molecular level of all the large DNA virus families. As a result the molecular phylogeny of the Herpesviridae has been well studied (McGeoch et al., 1995). Current models of mammalian Alphaherpesvirinae evolution indicate that members of this subfamily have co-evolved with their respective hosts (McGeoch et al., 1995). Consequently, this hypothesis predicts that the molecular evolutionary pattern of the Alphaherpesvirinae reflects that of their hosts. Importantly, the molecular datum used to elucidate these relationships has been derived from Alphaherpesvirinae which infect eutherian mammals. Absent from this literature is the molecular phylogenetic position of alphaherpesviruses which infect metatherian mammals (marsupials). It has been estimated that eutherian mammals and marsupials diverged from a common ancestor approximately 130 million years before present (MYBP) (Janke et al., 1997) whereas the divergence of the Alphaherpesvirinae which infect eutherian mammals from a common ancestor in to the currently recognized species has been estimated at approximately 80 MYBP (McGeoch & Cook, 1994; McGeoch et al., 1995). As a result co-evolution theory predicts that MaHV-1 and -2 should form a phylogenetic cluster divergent from alphaherpesviruses of eutherian mammals.

In this paper, we report the complete nucleotide sequences for the glycoprotein B (gB) genes from MaHV-1 and -2. We also report that molecular phylogenetic reconstructions based on the deduced gB amino acid sequences indicate an unexpected phylogenetic, though informative, position for MaHV-1 and -2 within the Alphaherpesvirinae.

Macropodid herpesviruses-1 and -2 were propagated in Potoroo kidney cells. Cells were cultured in Dulbecco’s modified Eagle’s medium containing 5–10% foetal calf serum at 37 °C in a 5% CO2 atmosphere.

All DNA sequencing was performed using dideoxy sequencing chemistry utilizing the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit, with AmpliTaq DNA
polymerase FS according to the manufacturer’s instructions (Applied Biosystems). After recovery, sequencing products were resolved on an ABI automated A373 sequencer according to the manufacturer’s instructions. Database searches were performed using either blastN or blastX search routines. The resultant DNA sequence data were aligned using AssemblyLIGN version 1.0.7 (Kodak).

Viral DNA was purified as previously described (Dorman et al., 1985). An ordered EcoRI genomic library of the MaHV-2 genome was constructed. The 5' and 3' termini of the resultant clones were sequenced and open reading frames putatively identified using the blastX search routine (Altschul et al., 1990). Due to the collinear arrangement of the herpesviral genomes, MaHV-2 clones could be arranged in a putative genomic order. Because of the location of an EcoRI site within UL27, a larger KpnI genomic fragment was then cloned. The genomic KpnI fragment was subcloned following digestion with SacI and a 3·5 kb fragment identified which encoded UL26 at the 5' end and UL28 at the 3' end and hence was deemed to contain the entire UL27 open reading frame. The complete nucleotide sequence of this fragment was then determined as described below.

The UL27 gene from the MaHV-2 was used to probe a Southern blot of restriction enzyme-digested DNA from MaHV-1 (data not shown). A 13 kb BamHI restriction fragment was putatively identified as containing the UL27 homologue of MaHV-1. After cloning, blastX search analysis indicated that this fragment encoded the herpesviral gene homologues UL26 at the 5' end and hence was deemed to contain the entire UL27 open reading frame. The complete nucleotide sequence of this fragment was then determined as described below.

The gB gene of MaHV-1 is 2661 bp in length encoding 887 amino acid residues, while the MaHV-2 gB gene is 2682 bp in length encoding 894 amino acid residues. The properties of the two gB genes, including nucleotide similarity and amino acid identity, are summarized in Table 1.

It has previously been reported that amino acid sequence data give more robust and informative phylogenetic relationships (McGeoch & Cook, 1994). As a result the phylogenetic inferences presented here were determined using putative amino acid sequence data. The amino acid sequences of gB from MaHV-1 and -2 were aligned to the homologous gene products from selected alphaherpesviruses using ClustalW version 1.9 (Thompson et al., 1994). Human herpesvirus 5 and human herpesvirus 4 were included as representatives of the gammaherpesvirus and betaherpesvirus subfamilies respect-
Molecular phylogeny of MaHV-1 and -2

Fig. 2. Neighbour-joining distance tree for glycoprotein B from selected herpesviruses. Divergences between pairs of aligned amino acid sequences were computed and distance trees derived by the neighbour-joining method. The tree is shown in unrooted form with a divergence scale. Virus abbreviations are as in the legend to Fig. 1.
**Simplexvirus** and **Varicellovirus**, contain more than one herpesvirus for some host species. The grouping of BoHV-2 in the lineage with the primate and marsupial viruses also appears to contrast with the co-evolution theory. Alphaherpesviruses with mice and rats as a primary host have not been reported despite the close association of these groups with other eutherian mammals, providing ample opportunity for horizontal transfer, such has been proposed for the avian alphaherpesviruses.

The constructed phylogenies for the two alphaherpesvirus genera indicate that the two groups have possibly radiated separately from a common ancestor. Following divergence from a common ancestor the progenitor for each genus could have co-evolved with the respective mammalian hosts.

There are many putative species within the subfamily alphaherpesviruses for which no molecular data are currently available; obtaining and including these sequences in the data from a wider variety of species will be essential in establishing the evolutionary trends within alphaherpesviruses. A monotreme-specific alphaherpesvirus would also provide an interesting insight into alphaherpesvirus evolution, though none has been recorded.

Here we have reported the first attempt to construct the molecular phylogenies of marsupial alphaherpesviruses. These reconstructions indicate that the proposed co-evolution theory for placental mammal alphaherpesviruses is inconsistent with the contemporary position of marsupial alphaherpesviruses. On currently available data this proposed theory is incongruent with calculated phylogenies. The reconstructed phylogeny presented here, which includes MaHV-1 and -2, supports the previously reported lineages within the alphaherpesviruses (McGeoch et al., 1995). These lineages correspond to the two currently recognized genera of the alphaherpesviruses. More molecular data are required from members representing both genera to establish if the virus-host co-evolution theory can be applied individually to these taxonomic groupings rather than the alphaherpesviruses as a whole. Clearly further studies are required to resolve these issues and should include viral genes with cellular homologues, such as the uracil glycosylase gene, for which rooted molecular phylogenies can be determined. This would allow for a direct comparison of viral phylogeny to mammalian phylogeny to be done.

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**References**


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