Short Communication

The UL24 consensus regions of bovine herpesvirus 2 isolate 554 and clinical isolates of herpes simplex viruses 1 and 2

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The herpesvirus UL24 gene is a core herpesvirus gene, with the alphaherpesviruses having five amino acid conserved domains within this gene sequence (Jacobson et al., 1998; Shimojima et al., 1997). This gene has not been extensively investigated and the product of this gene has only recently been reported. The protein product of UL24 of human herpes simplex virus type 2 (HSV-2) is a minor virus capsid protein, possibly a tegument protein (Hong-Yan et al., 2001). The UL24 gene of herpes simplex virus type 1 (HSV-1) was found to code for a late protein that associates with nuclei (Pearson and Coen, 2002). Previous studies have indicated that introduced alterations within the gene may or may not affect the plaque size and virus yield of laboratory strains of HSV-1 (Jacobson et al., 1989). Further, UL24 has been reported to be important for the productive infection of mouse sensory ganglia (Jacobson et al., 1998).

The herpesvirus UL24 gene sequence overlaps the UL23 thymidine kinase (TK) gene in a head to head arrangement in most herpesviruses (including HSV-1 and HSV-2), but not all herpesviruses. The overlap of UL24 into UL23 can be as little as 2 nucleotides for bovine herpesvirus 1 to 111 nucleotides for infectious

laryngotracheitis virus, with the overlap in HSV-1 and HSV-2 being 66 and 99 nucleotides respectively (Griffin and Bournsnel, 1990). For those herpesviruses that do not have an overlap, the separation between these two genes can be as little as 11 nucleotides for canine herpesvirus to 183 nucleotides for Marek's disease virus, with 74 nucleotides separating the bovine herpesvirus 2 (BHV-2) genes (May, 2002).

We previously studied six field isolates of BHV-2 (a member of the genus *Simplexvirus*, subfamily *Alpha-herpesvirinae*) in which the first half of the UL24 gene was sequenced using polymerase chain reaction (PCR)-generated DNA products (May, 2002). One BHV-2 isolate designated 554 contained defective UL24 genes with a 4 nucleotide insertion in half of its UL24 genes. This alteration of the UL24 gene occurred within consensus region III of the UL24 (see Fig. 1). We report here the isolation of 554 clones containing either normal or defective UL24 genes, indicating the original field isolated was a mixture with respect to the status of the UL24 gene. Moreover, as UL24 is present in all alphaherpesviruses and the gene has five conserved regions in the first half of the gene, we extended this study to include HSV-1 and HSV-2, using clinical isolates, to determine if defective UL24 genes can also be generated in these closely related viruses. The clinical isolates used were isolated from 1976 to 1984, prior to the use of acyclovir for treatment of HSV infections. This drug has been re-

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ported to alter nucleotides within the overlap region of UL23 (TK and target of the drug) and UL24 of a clinical isolates of HSV-1 (Morfin et al., 2000a). One of these alterations would have resulted in histidine replacing arginine at amino acid 6 of the UL24 protein. Moreover, HSV-1 and HSV-2 both have five different nucleotides within this overlap region, which if appropriately altered by acyclovir, could chain terminate the UL24 gene product. The region of the UL24 covered in this study contains the consensus areas of the genes in which the predicted amino acids of the protein product of BHV-2 has 70% identity with HSV-2 and 67% identity with HSV-1 (Fig. 1).

BHV-2 isolate 554 was obtained from Tallygaroopna in November 1973 during an outbreak of bovine mamillitis in dairy cattle during 1973 to 1974, in Victoria, southern Australia (Turner et al., 1976). The original stock used in this study was obtained in 1985 from Attwood Veterinary Research Institute, Melbourne, Victoria. BHV-2 infection of cattle also occurs in the UK, some European countries, Africa, USA and has recently been detected in Japan (Imai et al., 2002). Original clinical HSV-1 isolates were obtained from eye swabs and clinical HSV-2 isolates from genital swabs from Fairfield Infectious Disease Hospital, Melbourne, Victoria. All viruses were stored frozen within our department until resurrected for this study. BHV-2 isolate 554 was grown and plaque purified on MDBK (Madin-Darby bovine kidney cells) cells (Handley et al., 1996; Orders et al., 1995). Virus plaques were carefully selected from tissue culture dishes containing one or two plaques, as described previously (Orders et al., 1995). BHV-2 can also grow to a lesser extent in BHK-21 (baby hamster kidney) cells. All HSV isolates were grown on BHK-21 cells.

The DNA was extracted from infected cells using a Nucleospin Tissue Kit (Clontech) and used in the PCR essentially as described earlier (Handley et al., 1996; May, 2002), using the AmpliTaq Gold PCR Master Mixture (Applied Biosystems). Each PCR was performed in duplicate. After an initial denaturation and enzyme activation at 95°C for 5 min, 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min were performed in a thermal cycler (Bresatec). For BHV-2 the forward primer was 5'-CGCCGTGGTCTTACCCAGTCC-3' starting within the thymidine kinase (TK) gene (nucleotides 60–40) and the reverse primer 5'-GAGGATGGGACACAAATACAC-3' starting within the middle of the UL24 gene (nucleotides 447–427) generated

a product of 581 bp. Under identical PCR conditions the HSV-1 forward primer was 5'-GATGGCAGGGGTACGAAGCC-3' starting within consensus sequence I of UL24 (nucleotides 45–64) and the reverse primer was 5'-GATATTACCGGAGACCTTCTGC-3' from consensus sequence V of UL24 (nucleotides 501–522) yielding a product of 478 bp. Similarly, for HSV-2 the primers were from similar positions in UL24 to HSV-1 with the forward primer being 5'-GTTGGCCGGCGTGAGAAGCC-3' (nucleotides 78–97) and the reverse 5'-CCAAAATAGGACACAGGTAGAC-3' (nucleotides 460–481) which yielded a product of 404 bp. All PCR DNA products were sequenced at the Monash University Sequencing Facility, Melbourne, Victoria (May, 2002), using the above reverse primers for both BHV-2 and HSV-2, and the forward primer for HSV-1.

Five clones of 554 were extensively studied. Four of the clones represented by 554 clone 4 (cl 4; Fig. 1) were found to contain exclusively the altered UL24 with 4 nucleotides (AGGG) inserted at base 280 which is predicted to result in chain termination within consensus region III (as given in Genbank Acc. No. AY098932). One clone which grew extremely slowly in dense cells, 554 clone 9 (cl 9), was found to have a normal UL24 sequence (as given in Genbank Acc. No. AY046323). Passage of both these clones (cl 4 and cl 9) through five and ten passages of growth in MDBK cells or one in BHK-21 cells did not result in any change in their UL24 sequences. This suggested that the UL24 was a non-essential gene for in vitro growth. The variation in the UL24 sequences had no effect of the level of virus produced nor did it alter the virus plaque size. It would appear that the 554 isolate actually consisted of a mixture of two viruses with respect to their UL24 status. Of the 6 original field isolates of BHV-2 previously tested, only 554 contained some defective UL24 genes (May, 2002). No other variations within the nucleotide sequence were detected in these strains.

Given the close similarity of the UL24 genes within the alphaherpesviruses we also investigated if similar alterations could be detected in original clinical isolates of HSV-1 and HSV-2. All previous studies of the HSV UL24 gene have been restricted to established laboratory stains (Jacobson et al., 1998). None of the 22 HSV-2 and 20 HSV-1 isolates we tested contained any altered UL24 with respect to nucleotide insertions or deletions. Even when passaged at high multiplicity of infection, no variation was detected unlike with BHV-2

Region I	
HSV-2	MARTGRRAAVGRPARTSSLTERRRVLLAGVRSHTRFYKAFAREVREFNAT
HSV-1	MAARTSLVERRRVLMAGVRSHTRFYKALAEVREFHAT
	K
BHV-2 554c19	MASRRMRSVNRRSILLAGMRSHVKFYKVFADSVRVFNSD
BHV-2 554c14	MASRRMRSVNRRSILLAGMRSHVKFYKVFADSVRVFNSD
Region II	
HSV-2	RICGTLTLLMSGSLQGRSLFEATRVTLLICEVDLGPRRPDCICVFEFANDK
	A
HSV-1	KICGTLTLLSGSLQGRSVFEATRVTLLICEVDLGPRRPDCICVFEFANDK
BHV-2 554c19	RICGPVLQMMERSLQGRSLFDATHVSLICEVDLGARRPDCICVLEFENDA
BHV-2 554c14	RICGPVLQMMERSLQGRSLFDATHVSLICEVDLGARRPDCICVLEFENDA
Region III	
Region IV	
HSV-2	TLGGVCVILELKTCKSISSGDTASKREQRTTGMMQLRHSLKLLQSLAPPG
HSV-1	TLGGVCVILELKTCKYISSGDTASKREORATGMMQLRHSLKLLQSLAPPG
	C
BHV-2 554c19	TIGGVCVIVELKTCRYISGVETARKREQRATGLSGLRDSVRLRLGLVAPG
BHV-2 554c14	TIGGRGVCD
Region V	
HSV-2	DKVVYLCPILVFVAQRTLRSRV
HSV-1	DKIVYLCPIVLVFVAQRTLRSRV
BHV-2 554c19	DRQVYLCPILVFVAQETLRVSRT

Fig. 1. Predicted amino acid sequences covering the UL24 consensus gene regions of BHV-2, HSV-1 and HSV-2.

The five consensus sequences of HSV-1 proposed by Jacobson et al. (1998) are indicated. The italicized first 22 and 33 amino acids of HSV-1 and HSV-2 respectively, indicate the overlap regions with UL23. The BHV-2 strain 544 clone 4 (with the 4 nucleotide insertion at UL24 nucleotide 280) amino acid composition is given along with the 554 clone 9 UL24 sequence. The alterations found within the clinical isolates of HSV-1 and HSV-2 are indicated below the normal sequences.

strain BHM-1 clone 3 (May, 2002; Genbank Acc. No. AY064482). The only alterations detected in these gene sequences, resulted in limited nucleotide changes within the sequence investigated, with rare amino acid alterations occurring. In HSV-1 (sequence from Genbank Acc. No. NC001806) nucleotide 91 A substituted for G (in 10% of isolates) resulting in glutamic acid changing to lysine (this has been found within laboratory strains also; Jacobson et al., 1989). Nucleotide 174 G substituted for A (in 85% of isolates) and nucleotide 320 G substituted for C (in one isolate only) resulting in serine changing to cysteine. Only single variations of bases were found in the HSV-1 isolates tested. HSV-2 (sequence from Genbank Acc. No. NC001798) was found to have more nucleotide variations and multiple combinations of nucleotide changes could occur, which differed from that found with HSV-1. Nucleotide 120 T substituted for C (23% of isolates), nucleotide 159 C substituted for T (18%), nucleotide 162 C substituted for A (27%), nucleotide 278 C substituted for T (18%) resulting in valine

changing to arginine and nucleotide 282 T substituted for C (32%). While nucleotide variability within the studied UL24 region was found, no chain termination was detected in either HSV-1 or HSV-2 isolates.

Chain termination has been reported to occur in non-essential genes of clinical isolates of both HSV-1 and HSV-2. These include glycoprotein G in which 5/2,400 of HSV-2 (Liljeqvist et al., 1999) and 1/180 of HSV-1 (Rekadbar et al., 2002) isolates were found to have a chain termination site within the gene. One HSV-1 clinical isolate had chain termination within the glycoprotein C gene (Toh et al., 1993). Furthermore, clinical herpesvirus isolates can have the TK gene terminated when acyclovir has been used during clinical infection of patients (Fillet et al., 1998; Morfin et al., 2000b). While chain termination of non-essential gene products does occasionally occur, any termination within the essential genes of herpesviruses stops the virus from growing. Although, selective deletions within the essential glycoprotein B gene of clinical isolates of HSV-2 have been reported; the nucleotide sequence

CGGCGGCCC (coding for amino acids AAP) from a 2 or 3 repeat nucleotide sequence can be removed without impairing virus growth (Terhune et al., 1998).

Thus of six field isolates of BHV-2 previously tested (May, 2002), one (554) was found in this current study to be a mixture of two variants with respect to the UL24 gene, one of which had a chain termination alteration within the gene. Studies described here using a limited number of HSV clinical isolates (a total of 42), did not detect any chain termination occurring within the UL24 gene. Minor variations within the HSV nucleotide sequences were the only changes detected, all outside the overlap region with UL23. Thus chain-terminating alterations within the UL24 gene within the consensus regions may be a phenomenon associated with BHV-2 alone; the loss of this gene product does not appear to affect virus growth in vitro. Whether the loss of the UL24 gene product in BHV-2 can stop the initiation of latency by this virus, as has been suggested in one study with HSV-1 (Jacobson et al., 1998), remains to be determined. If UL24 is indeed only a non-essential gene of HSV-1 or HSV-2, which has also been suggested previously (Jacobson et al., 1989; Tognon et al., 1990), then much larger numbers of HSV isolates may have to be investigated to detect any chain terminations within the gene (as was the case with the glycoprotein G gene; Liljeqvist et al., 1999).

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