

# Genome re-arrangements associated with loss of pathogenicity of the $\gamma$ -herpesvirus alcelaphine herpesvirus-1

H. Wright <sup>a</sup>, J.P. Stewart <sup>b</sup>, R.G. Ileri <sup>c</sup>, I. Campbell <sup>a</sup>, I. Pow <sup>a</sup>,  
H.W. Reid <sup>a</sup>, D.M. Haig <sup>a,\*</sup>

<sup>a</sup> *The Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ, UK*

<sup>b</sup> *Centre for Comparative Infectious Disease, Duncan building, Daulby street, University of Liverpool, Liverpool L69 3GA, UK*

<sup>c</sup> *National Veterinary Research Centre, Muguga, P.O. Box 32, Kikuyu, Kenya*

Accepted 10 March 2003

## Abstract

The alcelaphine herpesvirus 1 (AIHV-1) causes malignant catarrhal fever in ruminants. Previous work had shown that serial passage of AIHV-1 in culture resulted in genome alterations that are associated with a loss in pathogenicity. Here we have analysed the re-arrangements that occur in more detail. None of the observed re-arrangements was entirely consistent. However, they did all involve translocation of a similar region of DNA from around the centre of the genome to areas either next to or in between terminal repeat elements at either end of the genome. There was also a concomitant loss of the wild-type locus. These re-arrangements appeared to be associated with the loss of virulence and the appearance of cell-free virus.

© 2003 Elsevier Science Ltd. All rights reserved.

**Keywords:** Malignant catarrhal fever;  $\gamma$ -Herpesvirus; Alcelaphine herpesvirus-1

## 1. Introduction

Malignant catarrhal fever (MCF) is an often fatal, lymphoproliferative disease of susceptible species of cattle, deer (Coulter et al., 2001; Plowright et al., 1960) and pigs (Loken et al., 1998). The causative agents are either of two  $\gamma$ -herpesviruses, the wildebeest-associated alcelaphine herpesvirus-1 (AIHV-1) (Plowright et al., 1960) or the sheep-associated ovine herpesvirus-2 (OvHV-2) (Baxter et al., 1993). Both wildebeest and sheep act as reservoirs of infection but do not succumb to the viruses. Experimentally, both viruses infect rabbits, giving a similar MCF disease to that in the naturally susceptible host (Buxton and Reid, 1980).

Studies of AIHV-1 have focussed on two isolates of the virus. C500 was isolated from an ox with MCF (Plowright et al., 1975), and WC11 was isolated from a blue wildebeest (*Connochaetes taurinus*) (Plowright

et al., 1960). In tissue culture, AIHV-1 recovered from wildebeest or clinically affected animals produces characteristic large multinucleate foci that progress slowly. Virus is cell-associated and can only be passaged to further cultures using intact cells. Following inoculation into cattle or rabbits, MCF is induced. This virus is thus referred to as the cell-associated virulent (CAV) form. However, after prolonged virus passage in culture, the virus-induced cytopathic effect is characterised by swelling and rounding of cells with rapid destruction of the monolayer and production of cell-free virus. This adaptation in culture is accompanied by a loss of pathogenicity for cattle and rabbits (i.e., MCF is not induced). This non-pathogenic form is designated cell-free attenuated (CFA). To determine the genetic basis for the difference between the pathogenic and the non-pathogenic forms of the virus, the genomes of the CAV and CFA forms of C500 were compared by restriction enzyme analysis (Handley et al., 1995). Differences in restriction enzyme profiles of DNA from the C500 CAV and CFA forms were described and three restriction fragments (VIR-1, VIR-2 and ATT-1) associated with

\* Corresponding author. Tel.: +44-131-445-5111; fax: +44-131-445-6111.

E-mail address: [haigd@mri.sari.ac.uk](mailto:haigd@mri.sari.ac.uk) (D.M. Haig).

the changes were cloned, sequenced and designated (Handley et al., 1995). Differences in the nucleotide sequence of regions of these clones were interpreted to be due to deletions and re-assortments of the genome during the transition from the CAV pathogenic form to CFA in tissue culture. Furthermore, the re-arrangements caused the truncation of open-reading frames (ORFs) that were located at the end of the viral genome adjacent to the terminal repeats (Handley et al., 1995). The CAV C500 isolate was subsequently sequenced (Ensser et al., 1997). Comparison of the CFA C500 sequences identified by Handley et al. (1995) with the CAV C500 sequence revealed that the truncated ORFs at the termini of CFA C500 were located in the centre of the CAV C500 genome.

We are interested in defining more closely the genes in AIHV-1 that are associated with pathogenicity and MCF. Previous work had suggested that specific re-arrangements of the AIHV-1 genome in culture were associated with a loss in the ability to cause MCF. We surmised that a detailed knowledge of these re-arrangements might shed light on the genes involved in MCF. In this paper, we present a detailed assessment of the re-arrangements observed after serial passage of AIHV-1 in culture.

## 2. Materials and methods

### 2.1. Viruses

The AIHV-1 C500 strain was isolated and purified from the spleen of an infected rabbit by limiting dilution (Handley et al., 1995). Infectivity was largely cell-associated (CAV). Propagation of C500 virus in bovine turbinate cells (BT) for 30 serial passages resulted in a virus where infectivity was largely cell-free (CFA) and that was non-pathogenic for rabbits. DNA was obtained from virus at low passage levels, after <5, 5 and 10 serial passages (CAV), and from virus that had been passaged more than 500 times (CFA). Viral DNA was prepared as previously described (Handley et al., 1995).

### 2.2. Southern blot analysis

Viral DNA (1 µg) was digested with *SacI*, electrophoresed through agarose gels and transferred to Hybond-N membranes (Amersham International, UK) as described previously (Smith and Summers, 1980). Blotted DNA was hybridised in Rapid Hyb<sup>TM</sup> fluid (Amersham) to double-stranded DNA probes labelled by random priming using [<sup>32</sup>P]dCTP and the Rediprime<sup>TM</sup> system (Amersham). Following a high stringency wash (0.1× SSC, 0.1% SDS at 65°C) the membranes were exposed to X-ray film for 2 h.

### 2.3. Preparation of *SacI* libraries

Viral DNA (1 µg) from four low pass samples (CAV P < 5a, CAV P < 5b, CAV P5 and CAV P10) and one high pass sample (CFA-HP) was digested with *SacI*, ligated to *SacI*-cut, dephosphorylated pBluescript (SK+) (50 ng) and used to transform *Escherichia coli* JM109 using standard techniques (Sambrook et al., 1989). Ampicillin-resistant colonies were screened by colony hybridisation and plasmid DNA was extracted using Wizard mini-prep kits (Promega).

### 2.4. Nucleotide and amino acid sequence analysis

Plasmid DNA was sequenced by the di-deoxy chain termination method using IRD800 labelled oligonucleotides (MWG Biotech) and an automated sequencing machine (LI-COR) at the Department of Veterinary Pathology, University of Edinburgh. The GAP, BEST-FIT and FASTA programs of the University of Wisconsin Genetics Computer Group (UWGCG) (Devereux et al., 1984) were used to compare sequences.

### 2.5. Polymerase chain reaction (PCR)

Amplifications were performed using 2 ng of viral DNA and the Expand Long Template system (Roche). Elongation steps were performed at 68°C.

## 3. Results

### 3.1. Re-assessment of clones containing re-arranged AIHV-1 DNA

Handley et al. (1995) identified regions of the AIHV-1 genome that differed between low-passage, virulent, cell-associated virus (CAV) and higher passage, attenuated, cell-free virus (CFA). DNA segments cloned from these regions of CAV (VIR-1 and VIR-2) and CFA (ATT-1) differed and were found to be derived from near the termini of the unique region of the genome (L-DNA). However, this work was performed before the publication of the complete sequence of the CAV virus (Ensser et al., 1997). Therefore we have re-analysed these clones in the light of this sequence.

The results of this re-analysis are shown in Table 1 and Fig. 1. Clone VIR-2 corresponded completely to the *HindIII* fragment at 73564–77088 bp of the published CAV sequence (Ensser et al., 1997). This clone contained several open-reading frames (ORFs), namely a distal portion of the second exon of ORF50 (ORF50b), A6, and a proximal part of A7. In contrast, the clones VIR-1 and ATT-1 both contained admixtures of a similar part of the sequence from the centre of the genome contained in VIR-2 and a different sequence from

Table 1  
Position of C500 CFA clones relative to AIHV-1 genomic sequence

Clone	Genbank Accession No.	Clone coordinates	Genome coordinates <sup>a</sup>	ORFs present	Orientation <sup>a</sup>
ATT-1	gi1223559	1–3777 3780–3832	72931–76694 881–938 <sup>b</sup>	50b, A6, A7 <sup>c</sup>	+ +
VIR-1	gi1223563	1–1916 1922–3539	73565–75485 129840–128133	50b <sup>c</sup> , A6 <sup>c</sup> A10 <sup>c</sup>	– +
VIR-2	gi1223569	1–3528	73565–77092	50b <sup>c</sup> , A6, A7 <sup>c</sup>	+

<sup>a</sup> Co-ordinates and orientation relative to AIHV-1 C500 CA unique (L-DNA) sequence (gi10140926, [Ensser, 1997 #126]).

<sup>b</sup> Co-ordinates and orientation relative to AIHV-1 C500 CA terminal repeat (H-DNA) sequence (gi2337964, [Ensser, 1997 #126]).

<sup>c</sup> Denotes truncation of ORF in clone.

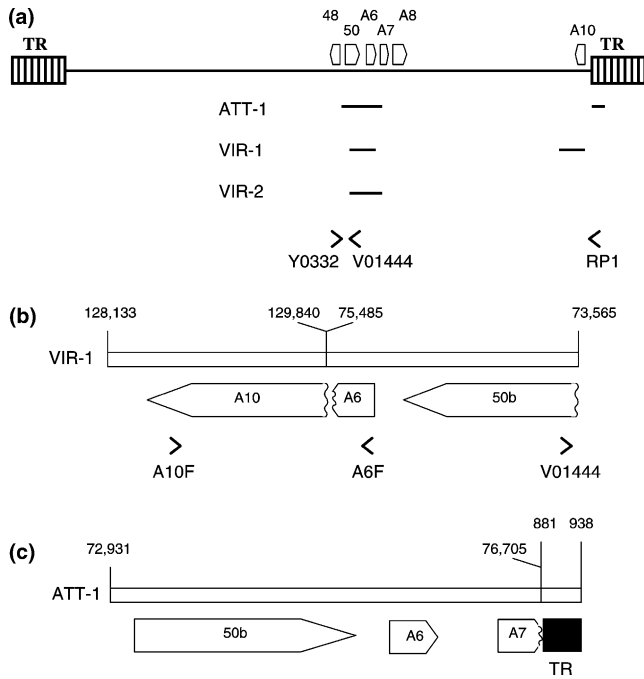


Fig. 1. Diagrammatic representation (not to scale) of the re-arrangements observed by Handley et al. and their relationship to the AIHV-1 genome. (a) The intact AIHV-1 genome is represented as a solid line (unique DNA) bounded by the terminal repetitive elements (TR, vertical lines). Above this are shown the approximate positions of the affected ORFs (open boxes). Below the genome are shown the regions of the genome contained in the three clones and below this the approximate positions of the PCR primers used to determine the genome structure. (b and c) Detailed arrangement of the VIR-1 and ATT-1 clones, respectively. Genome co-ordinates are shown above and the position of the ORFs (open boxes) and TR (solid box) below the line as are the positions of the PCR primers used for diagnostic PCR analysis.

the 3'-end of the genome. Thus, VIR-1 contained a distal part of ORF50b and a proximal part of A6 linked, in the reverse orientation relative to the genome, to a distal part of A10 (Fig. 1b). Clone ATT-1 contained the complete ORF50b, A6 and a proximal portion of A7 linked to part of a terminal repeat (TR) unit (Fig. 1c). Thus, VIR-2 was as predicted and more than likely derived from intact CAV DNA. Likewise ATT-1 contained re-arranged viral DNA that could be associated with attenuation and fits with the hypothesis of Handley

et al. (1995). However, the VIR-1 clone that was derived from CAV contains re-arranged viral DNA. This therefore raised questions about the validity of the clones and hypothesis described by Handley et al., (1995).

### 3.2. VIR-1-like sequences are present in CFA virus

To ascertain whether the VIR-1 clone was an artefact or representative of CAV and CFA DNAs we performed PCR amplifications of DNAs derived from either CAV or CFA virus using primers A6F and A10F located within the A6 and A10 ORFs, respectively (Table 2). These were designed to amplify viral DNA that had been re-arranged in a similar manner to VIR-1 (see Fig. 1b). These primers amplified a product 970 bp in length from the VIR-1 clone that was used as a positive control. When viral DNAs were used in similar amplification reactions only the CFA DNA was positive indicating that this re-arrangement is present in the attenuated virus.

### 3.3. VIR-1-like sequences are present at the 3'-end of CFA viral L-DNA

We wished to confirm whether the re-arranged sequences represented by the clone VIR-1 were present at the 3'-end of the unique portion of the CFA genome. Thus, DNA derived from CFA virus was amplified by PCR using one primer derived from the tandem repeat in conjunction with a primer from within ORF50 (RP1 and V0144 respectively; see Table 2 and Fig. 1a). The resulting 1.25 kbp product indicated that VIR-1-like sequences were indeed present at the 3'-end of the L-DNA. Also, in addition to translocation, the relative orientations of the primers (see Fig. 1a) indicated that the fragment from the centre of the genome had been inverted relative to the AIHV-1 genome.

To obtain the precise junction points the 1.25 bp product was cloned into pGEM-T and sequenced. The sequence is shown in Fig. 2. The positions of the primers are shown in *italics*. The first 288 bp of the sequence, shown in *capitals*, was the sequence of one terminal repeat unit from position 50 back through the *SacI* site,

Table 2  
Oligonucleotides used to characterise re-arranged AIHV-1 DNA

Oligo name	Coordinates <sup>a</sup>	Sequence <sup>c</sup>	Orientation <sup>a</sup>
RP1	50-31 <sup>b</sup>	TCTGTGAGGCTGAGCACCCG	Antisense
V01444	73,630-73,608	ACCTTCTTGGCGGCATTTCATCAT	Antisense
Y0332	72,601-72,614	GCAGGCGCCACTTGGTCTGTTGGTTAGATG	Sense
A6F	75,213-75,242	TCTTTGGGGACTCATGCATAAGCACTCTGC	Sense
A10F	129,004-129,022	GGGCTTGCTGGGTCGTAGT	Sense

<sup>a</sup> Co-ordinates and orientation relative to AIHV-1 C500 CA unique (L-DNA) sequence (gi10140926, [Ensser, 1997 #126]).

<sup>b</sup> Co-ordinates and orientation relative to AIHV-1 C500 CA terminal repeat (H-DNA) sequence (gi2337979, [Ensser, 1997 #126]).

<sup>c</sup> Oligonucleotide sequences are all shown in the 5'–3' orientation.

```

RP1                               SacI
TCTGTGAGGCTGAGCACCCGAGTCAACCCCTAGGGGAGCCGGCTGAGCTCTGTTGGGCCCA 60
GAGACCCCGAGAGAGGGGAAAAAACCAGGGGACGGCCCGGGGGGCTCGGGGGCGG 120
GGGAGGGCCCGGACGACAAAGCCCGGGGCGACCCGGAAAGGGGCGTGGCGCTCCCGC 180
CAGAGAGGTCCCGAGATCTCGGACCTGCCACGCCCTCCGGCAAAAATGGGGCC 240
AGGGGCGGGGAGGGCCCGGAGAAAAAACCTGGGGCCCGGGCGATGCAAGTAAAAA 300
aagtaggtgacaggcttaataaacagccacaggaactgtactcaactaaatctgaccaga 360
ttgtttaagcagccagtgagtaagctcacattttattctctaaagctcatttcacaggg 420
      M S A N N P S C A S R D P
acacaaaatttgactacccaaaatgagtgccaacaacccctcatgtgcaccagagatcct 480
P P K K V R T F F S S I R
cctcctaaaaaggtgaaggactttttttctctatcaggttcatttttaaatggagactta 540
      SmaI
tcttccgggaaacagtaaacacatgtgtggacttaaaagtgtctgtgatctaattact 600
tgattatgtaacagcctttataactaacatattatcttttaggatgattaataaataaca 660
atgactatcctaaaaatgtttttgcatactttaaggtttaaaaatctaaattatatttt 720
      R L S R P I C I D D F I D I
taaatcattttacagaggagactatcaagaccaatgatcatagatgactcatagacatc 780
T A D L G D T I G A A L K S F Q Q N N A
actgctgatcttggatgatacaatagggctgctttaaagtctctccagcaaaaataatgct 840
C T Q E Q S E Q P T R E V Y D V C K N I
tgactcaagaacagagtgagcagtttaccagggagGgtatgatgtctgcaaaaAatatt 900
L Q E N K F R N E M F G F V A D M N L L
Ctcaggagaacaaaatttagaaaatgaaatgtttgattttgtgctgatgaacctgcta 960
N L F A L F R S Y K Q R V R T H F G K Q
AactatltgcccctgttcagaagctataagcagagagtaggaccCactltggaaaaacag 1020
L L C A T A S S Q I I R F F L E R V I R
CTctatgtgcccactgctcatctcaataaattagattctttttgaaagagttataagg 1080
H T D K W F L L A P C N G L I L P Q E L
ctcAkgacaaaatggtttttgttgcccctgcaatggcctcattctcccacaagagctg 1140
A K E M Y V L L L T S E A R G K A L N Q G R
gcGaaggaaaatgatgttctcctgctcaggaagcagggggaaagctttgaaccaaggggag 1200
      HindIII
M F S G G R Q N M M N A A K K V
atgtttagcggaggagacaaaacatgatgaatgccccaagaaggtt 1248
      V0144
    
```

Fig. 2. Sequence of the PCR fragment obtained from CFA virus using oligonucleotide primer RP1 from the terminal repeats and a primer (V01444) from within ORF50. Repeat sequence is in capitals, the positions of the primers are in bold face, and intron/exon boundaries are underlined.

which defines the boundary of the repeat, to position 867 bp of another repeat unit. The rest of the clone was equivalent to the unique L-DNA starting at 72673 bp and included both exons of ORF50. The transcriptional direction of ORF50 in this PCR fragment was away from the sequence of the tandem repeat confirming the inverse orientation of this ORF in this re-arrangement.

Additional PCR amplifications were performed on both CAV and CFA-derived viral DNA using one primer from within and one from without the region translocated in clone VIR-1 (V0144 and Y0332, respectively; see Table 2 and Fig. 1a). A fragment of predicted size (1 kbp) was amplified using DNA derived from CAV virus as template but no product was amplified from CFA viral DNA. This confirms the integrity of the CAV genome used for these experiments and that the DNA was a suitable template for amplification. In addition, it strongly implies that the re-arrangements

observed are associated with translocation and deletion of the parental locus.

Thus the VIR-1 clone is valid, in that the re-arrangement it represents is present in high passage attenuated (CFA) virus. However, contrary to the observation of Handley and colleagues it is not present, at least at a level detectable by this PCR amplification, in virulent virus.

3.4. Clones derived from different levels of passage show similar patterns of re-arrangement

We wished to ascertain whether additional re-arrangements to those identified above were arising upon passage and how rapidly they were occurring. To do this, *SacI* digests of DNA from CAV virus passaged <5, 5 and 10 times was analysed by Southern blotting using a *SmaI/HindIII* fragment from the 5'-end of ORF50 (see Fig. 2) as probe.

As shown in Fig. 3, the CAV DNA samples revealed five different hybridising fragments. The CFA high pass viral DNA *SacI* digest revealed a 4kbp hybridising fragment (HP-4). Approximately three serial passages of CAV are required to generate enough viral DNA for study. During this early passage, fragments of 5.5 and

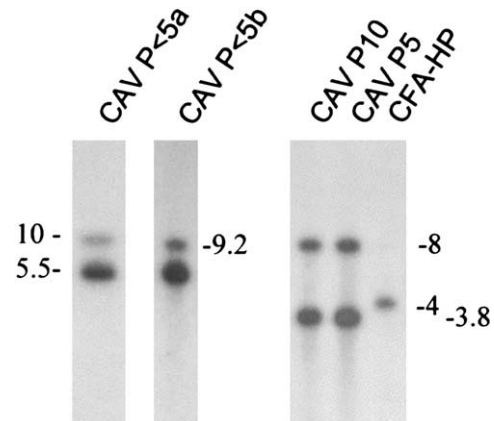


Fig. 3. Southern analysis of AIHV-1 DNAs at sequential stages in passage in culture. Viral DNA was extracted, cut with *SacI* and analysed by Southern blotting using a probe from the 5'-end of the ORF50 gene. The viral DNAs used are shown above each lane and the sizes of the bands (in kbp) are shown next to each band.

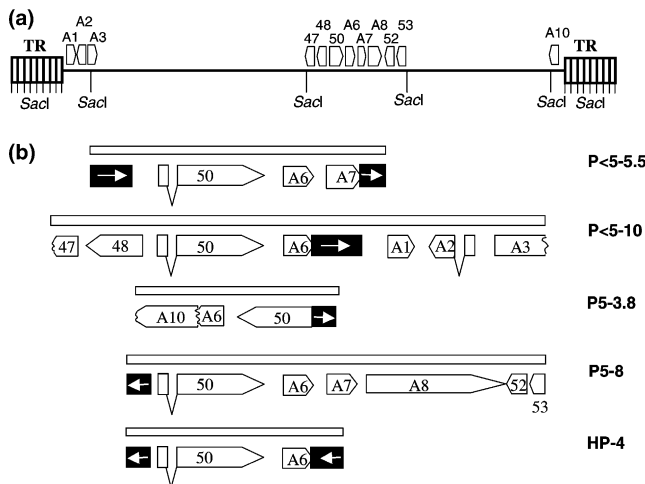


Fig. 4. Diagrammatic representation (not to scale) of the re-arrangements cloned from *SacI*-digested DNA in this work and their relationship to the AIHV-1 genome. (a) The intact AIHV-1 genome is represented as a solid line (unique DNA) bounded by the terminal repetitive elements (TR, vertical lines). Above this are shown the approximate positions of the affected ORFs (open boxes). The positions of relevant *SacI* sites within the unique portion of the genome and the terminal repeats are shown by vertical lines below the genome. NB *SacI* normally cuts at the end of each terminal repetitive element. (b) Detailed arrangement of the *SacI* clones. The position of the affected ORFs (open boxes) and TR (solid box) below the line. The arrows in the TR show the direction of the TR sequences (left to right is genomic).

9.2 kbp or 5.5 and 10 kbp in length hybridised to the probe. At passage levels 5 and 10, an 8 kbp fragment was seen together with one of 3.8 kbp. Each of these *SacI* fragments was cloned into pBluescript and the ends of each clone sequenced to define the boundaries of the clone and to allow orientation of the clones with respect to the intact AIHV-1 genome.

At <5 passages, the 9.2 kbp fragment corresponded exactly to the published, intact CAV sequence. The other fragments contained different re-arrangements but all consisting of regions of the genome around ORF50 adjoined to points at the ends of the L-DNA abutting the terminal repeats. The details of these arrangements are shown schematically in Fig. 4. At <5 passages, the 5.5 kbp fragment (P < 5–5.5) consisted of ORF50, A6 and a truncated A7 inserted in the centre of one terminal repeat unit. The 10 kbp insert at the same passage number (P < 5–10) contained a slightly larger fragment from the centre of the genome consisting of ORF47 through to a truncated A6 adjoining a terminal repeat unit at the left-hand end of the genome. Thus, this clone also contained ORFs A1, A2 and a truncated A3. The intensity of 5.5 kbp band appeared to be stronger than that of large fragments. This was not due to a deficiency of larger fragments transferring since the molecular weight markers transferred equally well (not shown). It is therefore most likely due to more than one copy of the re-arranged fragment being present.

At passages 5 and 10 the 3.8 kbp fragment (p5-3.8) was similar to clone VIR-1 and contained truncated versions of ORF50 and A6 at the right-hand end of the unique portion of the genome in between a truncated A10 and the terminal repeats. The 8 kbp fragment at these passages consisted of a larger portion from the centre of the genome from ORF50 to ORF53 joined to a terminal repeat unit. The CFA high pass 4 kbp clone (HP-4) comprised ORF50 and a truncated A6 in between one terminal repeat unit. It should be noted that the sequences at the left-hand ends of HP-4 and P5-8 corresponded exactly to the sequence of the PCR product described earlier (Fig. 2) derived from CFA virus.

Thus, the re-arrangements observed in the *SacI* clones appear to be consistent in that they all contained ORF50 and A6, sometimes truncated, but always joined to parts of a terminal repeat unit either at the left or right-hand end of the unique portion of the viral genome.

#### 4. Discussion

We have studied genetic re-arrangements in virulent (CAV) versus attenuated (CFA) strains of AIHV-1 by analysing cloned DNA fragments and PCR products derived from these strains. None of the observed re-arrangements was entirely consistent. However, they did all involve translocation of a similar region of DNA from around the centre of the genome to areas either next to or in between terminal repeat elements at either end of the genome. There was also a concomitant loss of the wild-type locus. These re-arrangements appeared to be associated with the loss of virulence and the appearance of cell-free virus.

The area of the genome that was consistently translocated contains two ORFs, ORF50 and A6. The former encodes a protein that in other  $\gamma$ -2 herpesviruses is an immediate-early transcription factor key to productive cycle gene expression and the latent-lytic switch in lymphocytes (Goodwin et al., 2001; Wu et al., 2000). The latter is of unknown function but is predicted to encode a nuclear phospho-protein. Given that the translocation also involves a concomitant deletion, it seems likely that the expression of genes proximal and distal to the locus may also be affected. The two genes that may consistently be affected by this are ORF48 and A7. ORF48 is of unknown function. However, we have shown recently that an A7 homologue in the related OvHV-2 is the upstream exon in a gene including O7 and O8 and encoding a virus glycoprotein (J.P Stewart and J. Rosbottom, unpublished). It seems highly likely that A7 has the same function. Of the above, it is easy to envisage how a transcription factor such as the ORF50 product might have profound effects on both viability in culture and on pathogenicity, i.e., MCF. Likewise, a

glycoprotein such as the A7/A8 product might affect the cell-association of the virus.

Herpesviruses are known to mutate and attenuate during passage. However, the most pertinent example of re-arrangement with respect to this work is the WZhet re-arrangement seen with the related  $\gamma$ -herpesvirus Epstein–Barr virus (EBV). Here, a similar locus at the centre of the EBV genome including the BZLF1 ORF is translocated next to the major internal repeats and latency-associated promoter resulting in the loss of the EBNA2 gene (Cho et al., 1984; Heston et al., 1982). The BZLF1 gene encodes a transcription factor with a similar function to ORF50 and EBNA2 is essential for B cell transformation by EBV. Thus, unlike wild-type, WZhet re-arranged viruses are unable to transform B cells. However, the expression of BZLF1 is not abrogated but dysregulated so that the viruses cannot become latent. These re-arranged EBV mutants are seen not only in vitro but also in vivo, associated with epithelial disease such as oral hairy leukoplakia and idiopathic pulmonary fibrosis (Patton et al., 1990; Sixbey et al., 1991).

It is difficult to predict what effect the observed translocations in the AIHV-1 genome would have on expression of the above ORFs involved in the translocations. In some cases the genes (i.e., ORF50 and A6) have been truncated and it seems highly likely that their functions have therefore been deleted. However, in other cases where intact ORFs are involved, upregulation, downregulation and dysregulation are all equally possible. Furthermore, different re-arrangements were cloned from the same viral stocks. This strongly implies that these stocks are a heterogeneous mixture of viruses. Thus, while our observations reported here provide an interesting and important step in understanding MCF, determination of the transcription pattern from these loci is now required to definitely associate one or more of these target genes with the attenuated phenotype.

### Acknowledgements

The authors acknowledge the Scottish Executive Environment and Rural Affairs Department for financial support. This work was also supported in part by a joint BBSRC/SEERAD Grant (26/S16844). James Stewart is the recipient of a University Research Fellowship from the Royal Society.

### References

- Baxter, S.I., Pow, I., Bridgen, A., Reid, H.W., 1993. PCR detection of the sheep-associated agent of malignant catarrhal fever. *Arch. Virol.* 132, 145–159.
- Buxton, D., Reid, H.W., 1980. Transmission of malignant catarrhal fever to rabbits. *Veterinary Record* 106, 243–245.
- Cho, M.S., Bornkamm, G.W., Zur Hausen, H., 1984. Structure of defective DNA molecules in Epstein–Barr virus preparations from P3HR-1 cells. *J. Virol.* 51, 199–207.
- Coulter, L.J., Wright, H., Reid, H.W., 2001. Molecular genomic characterization of the viruses of malignant catarrhal fever. *J. Comp. Pathol.* 124, 2–19.
- Devereux, J., Haeblerli, P., Smithies, O., 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* 12, 387–395.
- Ensser, A., Pflanz, R., Fleckenstein, B., 1997. Primary structure of the alcelaphine herpesvirus-1 genome. *J. Virol.* 71, 6517–6525.
- Goodwin, D.J., Walters, M.S., Smith, P.G., Thureau, M., Fickenscher, H., Whitehouse, A., 2001. Herpesvirus saimiri open reading frame 50 (Rta) protein reactivates the lytic replication cycle in a persistently infected A549 cell line. *J. Virol.* 75, 4008–4013.
- Handley, J.A., Sargan, D.R., Herring, A.J., Reid, H.W., 1995. Identification of a region of the alcelaphine herpesvirus-1 genome associated with virulence for rabbits. *Vet. Microbiol.* 47, 167–181.
- Heston, L., Rabson, M., Brown, N., Miller, G., 1982. New Epstein–Barr virus variants from cellular subclones of P3J-HR-1 Burkitt lymphoma. *Nature* 295, 160–163.
- Loken, T., Aleksandersen, M., Reid, H., Pow, I., 1998. Malignant catarrhal fever caused by ovine herpesvirus-2 in pigs in Norway. *Vet. Rec.* 143, 464–467.
- Patton, D.F., Shirley, P., Raab-Traub, N., Resnick, L., Sixbey, J.W., 1990. Defective viral DNA in Epstein–Barr virus-associated oral hairy leukoplakia. *J. Infect. Dis.* 64, 397–400.
- Plowright, W., Ferris, R.D., Scott, G.R., 1960. Blue wildebeest and the aetiological agent of bovine malignant catarrhal fever. *Nature* 188, 1167–1169.
- Plowright, W., Herniman, K.A., Jessett, D.M., Kalunda, M., Ramp-ton, C.S., 1975. Immunisation of cattle against the herpesvirus of malignant catarrhal fever: failure of inactivated culture vaccines with adjuvant. *Res. Vet. Sci.* 19, 159–166.
- Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. *Molecular Cloning: A Laboratory Manual*, second ed. Cold Spring Harbor Press, Cold Spring Harbor.
- Sixbey, J.W., Shirley, P., Sloas, M., Raab-Traub, N., Israele, V., 1991. A transformation-incompetent, nuclear antigen 2-deleted Epstein–Barr virus associated with replicative infection. *J. Infect. Dis.* 163, 1008–1015.
- Smith, G.E., Summers, M.D., 1980. The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethyl-paper. *Anal. Biochem.* 109, 123–129.
- Wu, T.T., Usherwood, E.J., Stewart, J.P., Nash, A.A., Sun, R., 2000. Rta of murine  $\gamma$ herpesvirus68 reactivates the complete lytic cycle from latency. *J. Virol.* 74, 3659–3667.