

# Proteomic Analysis of Pathogenic and Attenuated Alcelaphine Herpesvirus-1

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## Introduction

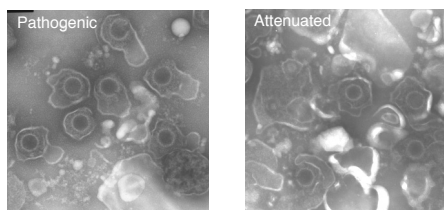
The gamma-herpesvirus Alcelaphine herpesvirus-1 (AIHV-1) is one of the causative agents of malignant catarrhal fever (MCF) in cattle and other ungulates. This fatal lymphoproliferative disease is characterized by lymphocyte accumulation and necrosis in multiple organs. This pathogenic virus can be grown in tissue culture but it becomes attenuated following extended culture [1]. We have recently shown that the attenuated AIHV-1 can be used to protect cattle from fatal MCF challenge with the pathogenic form of AIHV-1 (see poster by Haig et al. in this meeting).

As part of our studies to identify the protective antigens encoded by MCF viruses, we have purified and analyzed both the pathogenic and the attenuated forms of AIHV-1 using a 'shotgun proteomics' approach [2,3]. Purified virus preparations were fractionated by SDS-PAGE and each gel lane was cut into 20 segments that were subjected to electrospray ionization tandem mass spectrometry (ESI-MS/MS) to identify the proteins in each gel slice.

In parallel, purified and fractionated virus preparations were blotted with pooled immunoglobulin fractions from the nasal secretions of animals that were protected from MCF by immunisation with attenuated AIHV-1. This approach should allow us to identify antigens in fractionated virus preparations that may be useful diagnostic reagents or potential vaccine components.

## Results

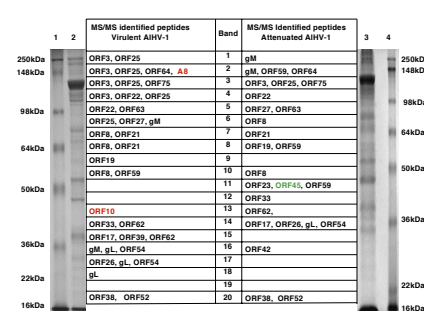
### 1. Gradient-purified AIHV-1 virus particles



Virulent and attenuated AIHV-1 particles were obtained by freeze-thaw lysis of infected cells and were purified by sucrose density gradient centrifugation. The virus preparations were analysed by electron microscopy of negatively stained particles (above) and by western blotting to detect uncleaved glycoprotein B in contaminating cellular membrane fragments (panel 3, below).

The viruses were found to be at  $8 \times 10^9$  and  $4 \times 10^{10}$  particles/mL, respectively, with less than 10% unenveloped particles

### 2. Proteomic analysis of AIHV-1 virus particles

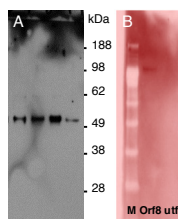


Virulent (lane 2) and attenuated (lane 3) AIHV-1 virus particles were analysed by proteomics after fractionation by SDS-PAGE. Each protein listed was identified by at least one unambiguous peptide in two independent virus preparations.

Glycoprotein B peptides were found in two bands: an 80kDa band with peptides that were N-terminal of a predicted Furin cleavage site; and a 50kDa band containing only C-terminal peptides. This suggests that AIHV-1 gB is cleaved by Furin in the mature virus.

Only two polypeptides were found in the virulent virus that were not detected in the attenuated virus preparation – ORF10 and A8 – while one protein was detected only in the attenuated virus – ORF45. ORF10 is a dUTPase-related protein while A8 is an AIHV-1-specific virus glycoprotein. The lack of A8 in the attenuated virus may be significant, as rearrangement of this region of the virus genome is associated with attenuation.

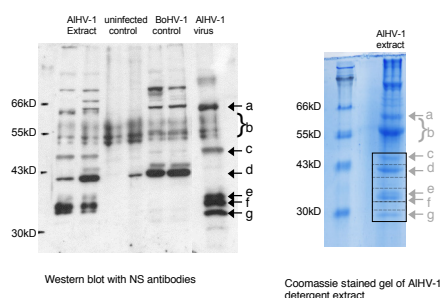
### 3. gB is Furin-cleaved in AIHV-1 virus particles



Western blotting with mAb 12B5 [4] that recognizes a 50kDa band in gradient-purified AIHV-1 virus fractions (A) and a 100kDa band in lysates of HEK293T cells transfected with the AIHV-1 gB gene (B: orf8) but does not detect any bands in lysates of untransfected cells (B: utf).

This supports the view that gB in the mature AIHV-1 virion was cleaved by Furin. mAb 12B5 recognized the C-terminal fragment of gB, while the recombinant protein expressed in a human cell line was not cleaved, running at about 100kDa. The lack of uncleaved gB in the purified virus fractions (A) suggests they were free of contaminating cellular membrane fragments.

### 4. Nasal secretions from immunised cattle recognise AIHV-1-specific antigens



Left panel: Detergent extracts of AIHV-1 infected, BoHV-1 infected, and uninfected bovine fibroblasts were blotted with pooled nasal secretion (NS) antibodies from cattle protected from challenge with virulent AIHV-1 (see poster by Haig et al). The results are from a single blot with one irrelevant lane removed.

A range of non-specific bands are seen around the 55kDa marker (b); virus-specific bands at about 60kDa (a) and 50kDa (c) appear to cross react slightly with BoHV-1; a strongly antigenic band at about 40kDa (d) appears to be conserved in BoHV-1; while a doublet at about 35kDa (e,f) appears to be both AIHV-1-specific and the most intensely blotting bands in the AIHV-1 extract. Gradient-purified virulent AIHV-1 virus lacks band (d) and has one extra antigenic band (g) that was not detected in any of the detergent extracts.

Right panel: Coomassie-stained protein gel of AIHV-1 detergent-soluble extract used for antigen analysis. The boxed areas were cut from the gel and proteins in the gel slices will be identified as described for the AIHV-1 virus particle proteomics. Potential assignment of stained bands is given in grey on the right of the gel.

## Conclusions

- Sucrose-gradient purification yielded high quality virus preparations for proteomic studies
- AIHV-1 glycoprotein B is Furin-cleaved in the purified pathogenic virus.
- One protein was detected only in the attenuated virus preparations - orf45
- Two proteins were detected in only the virulent virus preparations - A8 and ORF10
- Nasal secretion antibodies from cattle immune to AIHV-1 challenge recognise a number of AIHV-1-specific antigens
- Work is under way to identify these antigens

## References

- 1 Wright et al. 2003. Genome rearrangements associated with loss of pathogenicity of the gamma-herpesvirus alcelaphine herpesvirus-1. Res. Vet. Sci. 75:163–168.
- 2 Dry et al. 2008. Proteomic Analysis of Pathogenic and Attenuated Alcelaphine herpesvirus-1. J.Virol. 82: 5390–5397.
- 3 Batorycka, M., et al. 2006. Ultra-fast tandem mass spectrometry scanning combined with monolithic column liquid chromatography increases throughput in proteomic analysis. Rapid Commun. Mass Spectrom. 20: 2074–2080.
- 4 Adams, S.W., Hutt-Fletcher, L.M., 1990. Characterization of envelope proteins of alcelaphine herpesvirus 1. J.Virol. 64: 3382–3390.

## Acknowledgements

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