Proteomic Analysis of Pathogenic and Attenuated Alcelaphine Herpesvirus-1

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Introduction

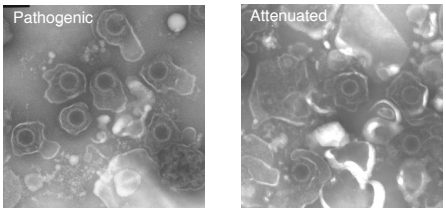
The gamma-herpesvirus Alcelaphine herpesvirus-1 (AlHV-1) is one of the causative agents of malignant catarrhal fever (MCF) in cattle and other ungulates. This fatal lymphoproliferative disease is characterized by lymphocyte accumulation and necrosis in multiple organs. This pathogenic virus can be grown in tissue culture but it becomes attenuated following extended culture [1]. We have recently shown that the attenuated AlHV-1 can be used to protect cattle from fatal MCF challenge with the pathogenic form of AlHV-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 AlHV-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 AlHV-1. This approach should allow us to identify antigens in fractionated virus preparations that may be useful diagnostic reagents or potential vaccine components.

Results

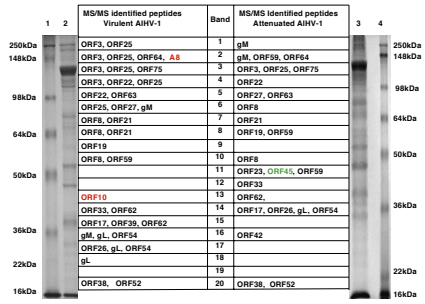
1. Gradient-purified AlHV-1 virus particles



Virulent and attenuated AlHV-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 $8x10^9$ and $4x10^{10}$ particles/mL, respectively, with less than 10% unenveloped particles

2. Proteomic analysis of AlHV-1 virus particles



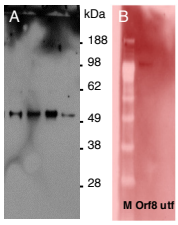
MS/MS identified peptides Virulent AlHV-1	Band	MS/MS identified peptides Attenuated AlHV-1
ORF3, ORF25	1	gM
ORF3, ORF25, ORF64, A8	2	gM, ORF59, ORF64
ORF3, ORF25, ORF75	3	ORF3, ORF25, ORF75
ORF3, ORF22, ORF25	4	ORF22
ORF22, ORF53	5	ORF27, ORF63
ORF25, ORF27, gM	6	ORF8
ORF8, ORF21	7	ORF21
ORF8, ORF21	8	ORF19, ORF59
ORF19	9	
ORF8, ORF59	10	ORF8
	11	ORF23, ORF45, ORF59
	12	ORF33
ORF10	13	ORF62,
ORF33, ORF62	14	ORF17, ORF26, gL, ORF54
ORF17, ORF39, ORF62	15	
gM, gL, ORF54	16	ORF42
ORF26, gL, ORF54	17	
gL	18	
	19	
ORF38, ORF52	20	ORF38, ORF52

Virulent (lane 2) and attenuated (lane 3) AlHV-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 AlHV-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 AlHV-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.

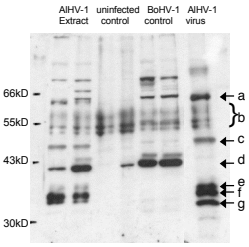
3. gB is Furin-cleaved in AlHV-1 virus particles



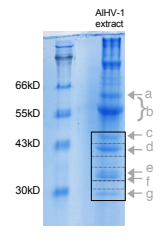
Western blotting with mAb 12B5 [4] that recognizes a 50kDa band in gradient-purified AlHV-1 virus fractions (A) and a 100kDa band in lysates of HEK293T cells transfected with the AlHV-1 gB gene (B: orf8) but does not detect any bands in lysates of untransfected cells (B: utf).

This supports the view that gB in the mature AlHV-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 AlHV-1-specific antigens



Western blot with NS antibodies



Coomassie stained gel of AlHV-1 detergent extract

Left panel: Detergent extracts of AlHV-1 infected, BoHV-1 infected, and uninfected bovine fibroblasts were blotted with pooled nasal secretion (NS) antibodies from cattle protected from challenge with virulent AlHV-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 AlHV-1-specific and the most intensely blotting bands in the AlHV-1 extract. Gradient-purified virulent AlHV-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 AlHV-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 AlHV-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
- AlHV-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 AlHV-1 challenge recognise a number of AlHV-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.

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4 Adams, S.W., Hutt-Fletcher, L.M., 1990. Characterization of envelope proteins of alcelaphine herpesvirus 1. J.Virol. 64: 3382-3390.

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