Veterinary Microbiology 173 (2014) 17–26

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Identification of immuno-reactive capsid proteins of malignant catarrhal fever viruses

Kathryn Bartley [a], David Deane [a], Ann Percival [a], Inga R. Dry [a,1], Dawn M. Grant [a], Neil F. Inglis [a], Kevin Mclean [a], Erin D.T. Manson [a], Lisa H.J. Imrie [a], David M. Haig [b], Felix Lankester [c,d], George C. Russell [a,*]

[a] Moredun Research Institute, Pentlands Science Park, Penicuik EH26 0PZ, UK
[b] School of Veterinary Medicine and Science, Nottingham University, Sutton Bonington, Leicestershire LE12 5RD, UK
[c] Institute for Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK
[d] Paul G. Allen School of Global Animal Health, Washington State University, USA

ARTICLE INFO

Article history:
Received 28 April 2014
Received in revised form 2 July 2014
Accepted 7 July 2014

Keywords:
Inflammatory disease
Malignant catarrhal fever
Antibodies
Proteomics
Recombinant expression
Antigen identification

ABSTRACT

Malignant catarrhal fever (MCF) is a fatal disease of cattle and other ungulates caused by certain gamma-herpesviruses including alcelaphine herpesvirus-1 (AlHV-1) and ovine herpesvirus-2 (OvHV-2). An attenuated virus vaccine based on AlHV-1 has been shown to induce virus-neutralising antibodies in plasma and nasal secretions of protected cattle but the targets of virus-specific antibodies are unknown. Proteomic analysis and western blotting of virus extracts allowed the identification of eight candidate AlHV-1 virion antigens. Recombinant expression of selected candidates and their OvHV-2 orthologues confirmed that two polypeptides, the products of the ORF17.5 and ORF65 genes, were antigens recognised by antibodies from natural MCF cases or from AlHV-1 vaccinated cattle. These proteins have potential as diagnostic and/or vaccine antigens.

1. Introduction

Malignant catarrhal fever (MCF) is a fatal lymphoproliferative viral disease caused by several gamma-herpesviruses belonging to the genus *Macavirus* (subfamily *Gammaherpesvirinae*; family *Herpesviridae*; order *Herpesvirales*). The best characterised and economically important of the MCF viruses are ovine herpesvirus 2 (OvHV-2) and alcelaphine herpesvirus 1 (AlHV-1). Both viruses are maintained in their respective reservoir hosts, sheep for OvHV-2 and wildebeest for AlHV-1, without any apparent disease. Transmission of AlHV-1 from the wildebeest to susceptible species appears to be associated with parturition, when virus shedding peaks but the situation for other MCF viruses is less clear (reviewed by Russell et al., 2009). MCF affects a wide range of susceptible species worldwide, which are generally ruminants of the order *artiodactyla* but MCF has also been reported in the pig (Loken et al., 1998) and horse (Costa et al., 2009). MCF can also be induced by experimental infection of a wider range of species, including laboratory animals such as rabbits and hamsters (Buxton et al., 1988; Buxton and Reid, 1980; Li et al., 2012; Liggitt et al., 1980). Bison and deer are

* Corresponding author. Tel.: +44 131 445 6149; fax: +44 1313 445 6111.
E-mail address: george.russell@moredun.ac.uk (G.C. Russell).
[1] Present address: The Roslin Institute, University of Edinburgh, Midlothian EH25 9RG, UK

http://dx.doi.org/10.1016/j.vetmic.2014.07.004
0378-1135/

particularly susceptible, and MCF is considered the most economically important viral disease in commercial farming of these species (Russell et al., 2009). MCF can have severe impacts on subsistence farming of domestic livestock and is a particular problem for sub-Saharan pastoralists and Indonesian cattle farmers (Bedelian et al., 2007; Cleaveland et al., 2001; Wiyono et al., 1994).

AlHV-1 can be propagated in culture and has been used as a model system for the understanding of MCF pathogenesis and for the development of diagnostics and vaccines. Thus AlHV-1 was the first MCF virus to be fully sequenced (Ensser et al., 1997), and has been used to define the proteomic composition of the virus particle (Dry et al., 2008). AlHV-1 that has been propagated in culture for up to five passages is capable of inducing MCF in susceptible species such as cattle and certain laboratory animals but further laboratory passage of the virus leads to the attenuation of pathogenicity and is accompanied by rearrangements of the virus genome (Wright et al., 2003). Attenuated strains of AlHV-1 have been used as the basis for all serological tests for MCF described to date (Fraser et al., 2006; Li et al., 1994; Russell et al., 2012) and the attenuated strain of AlHV-1 C500 at passage >1000 has been used as a candidate vaccine that could protect cattle from intranasal challenge with a fatal dose of pathogenic AlHV-1 in experimental trials (Haig et al., 2008; Russell et al., 2012). In susceptible species, primary, transient viral replication appears to occur within lung epithelium, after which the virus disseminates systemically (Cunha et al., 2012). It has been suggested that establishment of a protective mucosal immune barrier in the naso-pharyngeal region is critical for vaccine protection against MCF infection (Haig et al., 2008; Parameswaran et al., 2014). A recent vaccination study has shown that a high-titre virus-specific antibody response prior to challenge correlates with protection (Russell et al., 2012). The antibody titre in protected animals did not rise significantly after challenge suggesting that virus was unable to penetrate the mucosal barrier and boost the response. Conversely, in vaccinated animals that succumbed to MCF, the antibody titre rose significantly after challenge, indicating successful virus entry and stimulation of an anamnestic response that was not protective (Russell et al., 2012; Parameswaran et al., 2014). This study also showed that in a few animals, protection from MCF was found despite the generation of little or no neutralising antibody, suggesting that immune mechanisms other than virus-neutralising antibody may influence protection from AlHV-1 (Parameswaran et al., 2014). Despite the apparent importance of the antibody response in protection against challenge, little is known regarding the identity of the targets of these antibodies.

The analysis of antibody responses to MCF infection generally has been based on the use of relatively crude extracts of AlHV-1 infected cells as a source of antigen (Fraser et al., 2006; Li et al., 1994; Russell et al., 2012). These assays provide information on the recognition of AlHV-1 antigens by the test serum but do not help identify the specific antigenic components. Prior to the availability of proteomic analysis, immunoblotting of AlHV-1 structural proteins identified polypeptides that were recognised by serum from infected wildebeest and sheep or from cattle with MCF (Herring et al., 1989), but again the identity of the antigens remained unknown. With the advent of proteomic technologies, the structural components of virulent and attenuated AlHV-1 were identified using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) (Dry et al., 2008).

In this paper, we have combined immunoblotting with targeted proteomic analyses to identify putative immunoreactive components of the AlHV-1 virion. Recombinant expression of putative antibody targets was then used to confirm the identity of two antigens recognised by MCF-specific antibodies.

2. Materials and methods

2.1. Viruses and propagation

Attenuated (high passage) and virulent (low passage) AlHV-1 strains of the C500 isolate (Plowright et al., 1975) were propagated in bovine turbinate (BT) cells cultured in Iscove's modification of Dulbecco's medium (IMDM) supplemented with 10% (v/v) foetal bovine serum (FBS), 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin. Attenuated AlHV-1 was harvested at a passage number greater than 1000; while virulent AlHV-1 was used at passage number five or less (Haig et al., 2008; Wright et al., 2003).

2.2. Antibodies

Bovine nasal secretions containing antibodies (NS-Ab) were pooled from cattle vaccinated with attenuated AlHV-1 C500 and challenged with virulent AlHV-1 C500 (Haig et al., 2008). A NS-Ab pool was created from samples taken 28–84 days post challenge which demonstrated high virus neutralizing antibody titres when tested with the AlHV-1 virus neutralization assay (Haig et al., 2008).

Plasma samples from experimental vaccination and/or challenge infection with AlHV-1 were obtained from previous studies (Haig et al., 2008; Russell et al., 2012). Natural MCF case plasma samples (AlHV-1 and OvHV-2) were obtained from the virus surveillance unit (Moredun Research Institute) or from ongoing studies of MCF in east Africa. All samples were defined as MCF positive by real-time PCR testing of parallel buffy coat DNA samples (Hussy et al., 2001) and/or by detection of high antibody titre in the AlHV-1 C500 ELISA (Russell et al., 2012).

2.3. Purification of AlHV-1 virions

AlHV-1 C500 virions were purified through 20% to 50% continuous sucrose gradients as described previously (Dry et al., 2008). The resulting virion preparation was analysed by electron microscopy exactly as described previously (Dry et al., 2008) and was found to be of high titre (4.43×10^{10} particles per ml) and intact (<10% of virions classed as non-enveloped/defective), with minimal cellular contamination.