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Genetic fusion of peste des petits ruminants virus haemagglutinin and fusion protein domains to the amino terminal subunit of glycoprotein B of bovine herpesvirus 1 interferes with transport and function of gB for BHV-1 infectious replication

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ABSTRACT

Peste des petits ruminants is an emerging, often fatal viral disease of domestic and wild small ruminants caused by peste des petits ruminants virus. The haemagglutinin and the fusion protein are viral envelope glycoproteins and essential for the infection process and both induce a protective immune response in infected or vaccinated animals. Attempts to generate pseudotyped bovine herpesvirus 1 recombinants firstly by integration of expression cassettes for PPRV-H and PPRV-F into the herpesviral genome or secondly to generate pseudotyped BHV-1 by genetically fusing relevant parts of both PPRV glycoproteins to the amino-terminal subunit of glycoprotein B, approaches that had been successful for heterologous viral membrane glycoproteins in the past, failed repeatedly. We therefore analyzed at which intracellular stage generation of viable BHV-1 hybrid-gB recombinants might be inhibited. Results obtained from transient protein expression experiments revealed that, dependent on the fusion protein, transport of the hybrid glycoproteins beyond the endoplasmic reticulum is impeded. Thus, expression of heterologous glycoproteins using BHV-1 interferes more than expected from published experience with BHV-1 gB transport and consequently with virus replication.

1. Introduction

Peste des petits ruminants (PPR), is one of the most destructive and economically important diseases of domestic and wild small ruminants and is caused by peste des petits ruminants virus (PPRV), a member of the *Morbillivirus* genus in the *Paramyxoviridae* family (Gibbs et al., 1979). It is one of the animal diseases which have to be notified to the World Organization for Animal Health (OIE) due to its highly contagious nature and capacity for rapid spread (OIE, 2013). After its initial appearance in the Ivory-coast (Cote D'Ivore) in West-Africa (Gargadennec and Lalanne, 1942), the disease spread aggressively into new countries until it became prevalent in nearly most of Africa and Asia, the Middle East, Turkey, with the recent occurrence in Georgia and Mongolia (Banyard et al., 2010; Albina et al., 2013; Libeau et al., 2014; Parida et al., 2015; Baron et al., 2017). PPRV is known to infect mainly sheep and goats however outbreaks of the disease were reported in some wild small ruminants as well as in camels (Kinne et al., 2010; Parida et al., 2015; Marashi et al., 2017).

The linear, non-segmented, single-stranded negative sense RNA genome of PPRV is 15.948 nucleotides (nt) in length and contains six nonoverlapping transcription units encoding six structural proteins namely: the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the large protein or polymerase (L), the fusion protein (F) and the haemagglutinin (H), the last two being virion membrane glycoproteins (Bailey et al., 2005; Chard et al., 2008). In addition, the P protein transcription unit encodes the C and V nonstructural proteins (Mahapatra et al., 2003). Up to now only one serotype is recognized. However, four genetic lineages (I, II, III and IV) are present, defined by differences either in partial sequences of F- (Forsyth and Barrett, 1995; Shaila et al., 1996; Dhar et al., 2002; Ozkul et al., 2002), N- (Couacy-Hymann et al., 2002; Kwiatek et al., 2007) or H- open reading frames (Kaul, 2004; Balamurugan et al., 2010).

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The H protein, a type II membrane glycoprotein, promotes binding and attachment of the virus to the host cell receptor. It exhibits neuraminidase and haemadsorption activity (Seth and Shaila, 2001a, 2001b). The F protein, a type I membrane glycoprotein, mediates fusion of the viral envelope with the host cell membrane leading to virus penetration into the host cell and induces cell-cell fusion enabling viral spread within the host (Seth and Shaila, 2001a, 2001b). Owing to their location in virions, surface glycoproteins of PPRV are the main target of the host immune response thus inducing both cell mediated and humoral immunity. Neutralizing antibodies in response to PPRV infection are directed predominantly against H but also F-specific neutralizing antibodies are raised (Diallo et al., 2007; Chen et al., 2010). In contrast to H, F is highly conserved among morbilliviruses. H might play a significant role in determining the host range whereas F is believed to be important in providing cross protection between members of the genus especially between PPRV and rinderpest virus and vice versa (Chard et al., 2008).

The open reading frame (ORF) coding for H of PPRV is 1830 nt long and encode a protein of 609 amino acids (aa) with a predicted molecular mass of 68 KDa (Bailey et al., 2005; Dhar et al., 2006). The most characteristic features of the H-ORF is the presence of a long hydrophobic domain, the membrane anchor from aa 35 to aa 58, the signal peptide from aa 1 to aa 34 within the N-region of H, and the conserved C-terminal segment of H consists of 204 aa (aa 392 – aa 595) which is used for PPRV lineage identification (Fig. 1A) (Barrett et al., 2006). The ORF coding for F of PPRV is 1641 nt long and encode a protein of 546 aa with a predicted molecular weight for the primary translation products of 59.137 KDa (Bailey et al., 2005; Dhar et al., 2006). F0, an inactive precursor, is cleaved by the cellular protease furin at RRTRR cleavage site (aa 104 - aa 108) into two subunits F_2 (89 aa) and F_1 (438 aa) that remain linked by two disulphide bonds. The deduced apparent molecular masses are 60 kDa for F_0, 48 kDa for F_1 and 9.8 kDa for F_2 (Fig. 1B) (Scheid and Choppin, 1977; Meyer and Diallo, 1995). F_0 has two variable hydrophobic domains, the signal peptide (aa 1 - aa 19) and the variable hydrophobic domain (aa 485 - aa 517) near the C-terminus contains the membrane anchor (aa 485 - aa 502) of the F and the conserved leucine zipper or zinc finger domain (aa 459 – aa 480) exists just before the membrane anchor (Fig. 1B) (Dhar et al., 2006; Barrett et al., 2006).

Bovine herpesvirus-1 (BHV-1), a member of the Varicellovirus genus of the *Alphaherpesvirinae* subfamily in the *Herpesviridae* family has a double-stranded DNA genome of 136 kbp and is a common vector for the expression of heterologous proteins (reviewed in Brun et al., 2008). A major component of the envelope of BHV-1 virions is glycoprotein B (gB) which is essential for virus penetration into the host cell and direct cell-to-cell spread and thus for infectious virus replication. Virions lacking gB are non-infectious (Schröder and Keil, 1999). After removal of the signal peptide during translocation into the endoplasmic reticulum, mannose-rich N-glycans are added to yield the 117 kDa apparent molecular mass gB precursor. In the Golgi apparatus the N-glycans are converted to the complex form and O-glycans are added resulting in the 130 kDa gB which, after transport into the *trans*-Golgi network, is cleaved by furin (Fig. 2) into the amino- and carboxy terminal subunits with 72 kDa and 55 kDa apparent molecular masses (Lawrence et al., 1986; van Drunen Littel-van den Hurk and Babiuk, 1986). The subunits remain covalently linked by disulfide bonds (Fig. 2). Cleavage, which occurs after the consensus sequence for furin cleavage $RARR_{504}$ (Misra et al., 1988; Whitbeck et al., 1988), however, is not required for gB-function (Kopp et al., 1994). Several applications using gB of BHV-1 as transporter to display heterologous (glyco) polypeptides have been described. Even large polypeptides like GFP (Keil et al., 2005; Keil, 2009) or the avian influenza HA1 (H5) subunit (Keil et al., 2010) can be genetically fused to the NH_2-subunit of gB without destroying gB function. Recombinant virions containing the HA1-gB fusion protein in their envelopes are susceptible to neutralization by H5 specific antibodies (Keil et al., 2010).

An eradication program for globally eliminating PPR from endemic countries by the end of 2030 was recently launched (OIE, 2014; OIE-FAO, 2015). This requires development of diagnostic techniques based on recombinant viruses expressing PPRV H and F glycoproteins to eliminate the necessity of handling the live virus that will interferes with the eradication campaign. Also raised the necessity for establishment of DIVA vaccines, to differentiate vaccinated from PPR naturally infected animals, based on the fact that PPR naturally infected animals elicit an anti-nucleoprotein immune response (Baron et al., 2017). To avoid the need to handle highly pathogenic viruses in enhanced biosafety level 3 or 4 (BSL3 or BSL4) containment areas which are, when available, rather expensive to operate, different viruses were engineered to provide sensitive, specific and safe pseudoparticle neutralization assays for the reliable detection of neutralizing antibodies against such hard to handle viruses. This approach, has been successfully established for detection of neutralizing antibodies against PPRV using a pseudotyped vesicular stomatitis virus (VSVΔG) expressing surface glycoproteins of PPRV (Logan et al., 2016).

To pseudotype BHV-1 with PPRV H and F epitopes displayed on the envelope of recombinant virions, domains of different lengths of PPRV-H and -F were genetically fused to the NH_2-subunit of gB. However, attempts to rescue infectious virus expressing the respective fusion proteins were not successful. Here we report experiments to spot reasons for the apparent interference of the PPRV domains with gB function and provide evidence that intracellular transport and processing of the hybrid glycoproteins is inhibited.

2. Materials and methods

2.1. Cells and viruses

Madin-Darby bovine kidney (MDBK) cell line, originally established from the epithelial renal tissue of an adult steer (*Bos primigenius taurus*) was kindly provided by Alfred Metzler (Zürich, Swizerland). BunD cells are transgenic MDBK cells that express BHV-1 gD and gB (Günther Keil, unpublished). Bovine pharyngeal cell line Kop-R (CCLV-RIE 244), a permanent cell line generated from pharyngeal tissue of a newborn calf, human embryonic kidney 293T (HEK293T) cells (Graham et al., 1977) and rabbit kidney cell line 13 (RK13), originated from kidneys of a five-week-old rabbit (*Oryctolagus cuniculus*) were kindly provided by the Collection of Cell Lines in Veterinary Medicine, FLI, Insel Riems, Germany. Cells were maintained in Eagle's minimum essential medium (MEM), supplemented with 10% fetal calf serum (FCS), 2.4 mM $_L$-glutamine, 100 U/ml of penicillin, 100 µg/ml of streptomycin and grown at 37°C in a humidified atmosphere in a cell culture incubator containing 5% CO_2.

The gB-negative mutant gB^--GKD (BHV-1) was described previously (Keil et al., 2005). The gB^--mutant N569 (BHV-5) with BHV-5 N569, as parent strain, was prepared accordingly (Günther Keil, unpublished). The Organelle Lights™ baculoviruses were obtained from Invitrogen (Darmstadt, Germany).

2.2. Construction of plasmids

Plasmids pSP73-PPRV-H and pSP73-PPRV-F contain the full length ORFs coding for H and F of PPRV Nigeria 75/1 vaccine strain (Diallo et al., 1989; GenBank accession no. X74443.2) were used for PCR amplification of segments from the ORFs of PPRV-H or PPRV-F. Respective primer pairs (Eurofins, Ebersbach, Germany) are given in Table 1. From the H ORF 3 segments were amplified encoding PPRV-H amino acids 56 to 196 (primers 02FuPPRV-H56+ and 02FuPPRV-H196-), amino acids 56 to 389 (primers 02FuPPRV-H56+ and 02FuPPRV-H389-) and amino acids 56 to 609 (primers 02FuPPRV-H56+ and 02FuPPRV-H609-) (Fig. 1). From the F ORF 2 segments were amplified encoding PPRV-F amino acids 35 to 112 (primers 02FuPPRV-F35+ and 02FuPPRV-F112-) and amino acids 35 to 485 (primers