Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Microbiology 113 (2006) 283–291
veterinary microbiology
www.elsevier.com/locate/vetmic

Biological characterization of bovine herpesvirus 1 recombinants possessing the vaccine glycoprotein E negative phenotype

Benoît Muylkens [a], François Meurens [a], Frédéric Schynts [b], Katalin de Fays [a], Aldo Pourchet [a], Julien Thiry [a], Alain Vanderplasschen [a], Nadine Antoine [c], Etienne Thiry [a,*]

[a] *Department of Infectious and Parasitic Diseases, Virology and Immunology, Faculty of Veterinary Medicine, University of Liège, B-4000 Liège, Belgium*
[b] *Division of Animal Virology, CER, B-6900 Marloie, Belgium*
[c] *Department of Morphology and Pathology, Histology, Faculty of Veterinary Medicine, University of Liège, B-4000 Liège, Belgium*

Abstract

Intramolecular recombination is a frequent event during the replication cycle of bovine herpesvirus 1 (BoHV-1). Recombinant viruses frequently arise and survive in cattle after concomitant nasal infections with two BoHV-1 mutants. The consequences of this process, related to herpesvirus evolution, have to be assessed in the context of large use of live marker vaccines based on glycoprotein E (gE) gene deletion. In natural conditions, double nasal infections by vaccine and wild-type strains are likely to occur. This situation might generate virulent recombinant viruses inducing a serological response indistinguishable from the vaccine one. This question was addressed by generating in vitro BoHV-1 recombinants deleted in the gE gene from seven wild-type BoHV-1 strains and one mutant strain deleted in the genes encoding gC and gE. In vitro growth properties were assessed by virus production, one step growth kinetics and plaque size assay. Heterogeneity in the biological properties was shown among the investigated recombinant viruses. The results demonstrated that some recombinants, in spite of their gE minus phenotype, have biological characteristics close to wild-type BoHV-1.

Keywords: Bovine herpesvirus 1; Recombination; Glycoprotein E; Marker vaccine

1. Introduction

Bovine herpesvirus 1 (BoHV-1), classified as an alphaherpesvirus, is a major pathogen of cattle. Primary infection induces various clinical manifestations such as infectious bovine rhinotracheitis (IBR),

* Corresponding author at: Department of Infectious and Parasitic Diseases, Laboratory of Virology, Faculty of Veterinary Medicine, University of Liège, Boulevard de Colonster, 20, B43b, B-4000 Sart-Tilman, Liège, Belgium. Tel.: +32 4 366 42 50; fax: +32 4 366 42 61.
E-mail address: etienne.thiry@ulg.ac.be (E. Thiry).

0378-1135/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2005.11.038

infectious pustular vulvovaginitis, abortion and generalized systemic infection (Tikoo et al., 1995; Kaashoek et al., 1996). On the basis of differences in restriction endonuclease profiles of genomic DNA, BoHV-1 has been divided into three subtypes: 1.1, 1.2a and 1.2b (Engels et al., 1986; Smith et al., 1995). Subtypes 1.1 and 1.2a have been associated with severe disease including infection of the fetus and abortion. Subtype 1.2b was not associated with abortion and is the only subtype found in Australia. In regards to the important losses due to diseases and trading restrictions, European countries have initiated BoHV-1 control program based on the use of marker vaccines deleted in the glycoprotein E (gE) gene (Van Oirschot et al., 1997). These marker vaccines, either inactivated or live attenuated, used together with a serological detection of gE specific antibody (Ab), allow differentiation to be made between infected and vaccinated animals.

BoHV-1 encodes 10 glycoproteins that are involved in different steps of the viral cycle. Sequence analysis of glycoprotein D (gD), gG, gI and gE encoded by the unique short (U_S) region of the genome suggested that they have evolved by gene duplication (McGeoch, 1990). BoHV-1 gE and gI form a non-covalently linked heterodimer in infected cells and in virion envelope (Whitbeck et al., 1996). In vitro analysis of mutant viruses lacking gE and/or gI has shown that the complex is involved in cell to cell spread (Rebordosa et al., 1996; Mahony et al., 2002; Trapp et al., 2003). The effect of gE deletion can differ according to the viral strain from which the mutant virus originated. A gE minus mutant constructed from a bacterial artificial chromosome (BAC) BoHV-1 obtained from a BoHV-1.2b strain was no more able to form visible plaques in cell culture overlaid by agarose (Mahony et al., 2002). In opposition to this result, gE minus mutants obtained from BoHV-1.1 strains still induced visible plaques under agarose overlay, even if plaques were smaller (Baranowski et al., 1996; Rebordosa et al., 1996; Trapp et al., 2003). Additional effects on plaque size reduction were observed by deleting other genes involved in cell to cell spread like gG or gM (Brack et al., 2000; König et al., 2002; Trapp et al., 2003).

Intramolecular recombination is a mechanism of genetic material exchange related to alphaherpesvirus replication cycle (Thiry et al., 2005). It was shown to occur frequently between strains of herpes simplex virus (HSV-1) (Umene, 1985), feline herpesvirus 1 (FeHV-1) (Fujita et al., 1998), pseudorabies virus (PrV) (Glazenburg et al., 1994) and BoHV-1 (Schynts et al., 2003; Meurens et al., 2004a,b). Several studies have demonstrated that mixed infection with two avirulent strains of the same alphaherpesvirus species can result in synergistic increase in the severity of disease through the generation of recombinant viruses (Javier et al., 1986; Nishiyama et al., 1991; Brandt et al., 2003). Moreover, following co-inoculation with PrV vaccine strains, some isolated recombinant viruses were shown to possess marker deleted phenotype with restored virulence genes (Henderson et al., 1990). Recently, a virulent strain of infectious laryngo-tracheitis virus sharing genetic sequences from wild-type and vaccine strains was isolated from infected chicken (Han and Kim, 2001). In natural conditions, double nasal infection between vaccine and wild-type BoHV-1 strains may occur in cattle. It can be postulated that such co-infection might generate recombinant viruses that combine the gE negative phenotype of the vaccine strain with the virulence of the wild-type strain.

The aim of the present study was to assess the biological properties of BoHV-1 recombinants issued from parental wild-type strains and possessing the vaccine gE negative phenotype. Co-infections experiments were performed in vitro with selected wild-type BoHV-1 strains and a mutant strain carrying complete deletions in gC and gE genes. Recombinants possessing the gE negative phenotype issued from these co-infections were characterized in order to study the acquisition of biological properties from the parental wild-type strains able to compensate for the lack of gE functions.

2. Material and methods

2.1. Viruses and cell culture

Madin Darby Bovine Kidney cells (MDBK; ATCC CCL-22) were grown as previously described (Meurens et al., 2004a). Eight BoHV-1 strains were used in this study (Table 1). The virulence of wild-type strains had been previously assessed in vivo (Kaashoek et al., 1996; Lemaire et al., 1999). Viral stocks were produced by infection of MDBK cells at a multiplicity of infection (m.o.i.) of 0.01. At 72 h after infection,