Available online at www.sciencedirect.com
ScienceDirect
Virology 352 (2006) 345–356
VIROLOGY
www.elsevier.com/locate/yviro

Modulation of virion incorporation of Ebolavirus glycoprotein: Effects on attachment, cellular entry and neutralization

Andrea Marzi, Anja Wegele, Stefan Pöhlmann*

Institute for Clinical and Molecular Virology, University Erlangen-Nürnberg, 91054 Erlangen, Germany
Nikolaus-Fiebiger-Center for Molecular Medicine, University Erlangen-Nürnberg, 91054 Erlangen, Germany

Received 24 January 2006; returned to author for revision 23 February 2006; accepted 27 April 2006
Available online 13 June 2006

Abstract

The filoviruses Ebolavirus (EBOV) and Marburgvirus (MARV) cause severe hemorrhagic fever in humans and are potential agents of biological warfare. The envelope glycoprotein (GP) of filoviruses mediates viral entry into cells and is an attractive target for therapeutic intervention and vaccine design. Here, we asked if the efficiency of virion incorporation of EBOV-GP impacts attachment and entry into target cells and modulates susceptibility to neutralizing antibodies. In order to control the level of EBOV-GP expression, we generated cell lines expressing the GPs of the four known EBOV subspecies in an inducible fashion. Regulated expression of GP on the cell surface allowed production of reporter viruses harboring different amounts of GP. A pronounced reduction of virion incorporation of EBOV-GP had relatively little effect on virion infectivity, suggesting that only a few copies of GP might be sufficient for efficient engagement of cellular receptors. In contrast, optimal interactions with cellular attachment factors like the DC-SIGN protein required incorporation of high amounts of GP. Antibody-mediated neutralization of virions bearing high amounts of GP was slightly more efficient than neutralization of virions harboring low amounts of GP, suggesting that the efficiency of GP incorporation into virions might modulate susceptibility to neutralizing antibodies. Finally, regulated expression of GP in permissive 293 cells did not reduce EBOV-GP-driven infection but diminished vesicular stomatitis virus GP (VSV-G) and amphotropic murine leukemia virus (A-MLV) GP mediated entry in a dose-dependent manner. Therefore, intracellular GP does not seem to downmodulate expression of its receptor(s) but might alter expression and/or function of molecules involved in VSV-G and A-MLV-GP-dependent entry. Our results suggest that the efficiency of virion incorporation of GP could impact EBOV attachment to target cells and might modulate control of viral spread by the humoral immune response.

Keywords: Ebolavirus; Glycoprotein; Receptor; Attachment factor; Expression level; DC-SIGN

Introduction

The filoviruses Ebolavirus (EBOV) and Marburgvirus (MARV) are negative stranded RNA viruses that cause severe hemorrhagic fever in humans (Feldmann et al., 2003; Geisbert and Hensley, 2004). The genus EBOV contains four subspecies, *Zaire ebolavirus* (ZEBO), *Sudan ebolavirus* (SEBOV), *Ivory coast ebolavirus* (ICEBOV) and *Reston ebolavirus* (REBOV), while Lake Victoria MARV is the only member of the MARV genus. The EBOV subtypes exhibit differential pathogenicity in humans. ZEBOV infection is lethal in up to 90% of the cases, while REBOV is considered nonpathogenic (Feldmann et al., 2003; Geisbert and Hensley, 2004). However, the underlying pathogenicity determinants are incompletely understood. The first step of the viral life cycle, entry into target cells, is mediated by the envelope glycoprotein (GP) (Kawaoka, 2005). EBOV- and MARV-GP is encoded by the fourth gene of the viral genome (Sanchez et al., 1993; Volchkov et al., 1992; Will et al., 1993). The EBOV- but not the MARV-GP gene contains two open reading frames. The major product synthesized from the EBOV-GP gene is a secreted form of GP, termed small GP (sGP) (Sanchez et al., 1996; Volchkov et al., 1995), which is not produced in MARV infected cells. The role of sGP in EBOV infection is incompletely defined (Kindzelskii et al., 2000; Maruyama et al., 98 A.D.; Yang et al., 1998). About 20% of the EBOV-GP message is transcriptionally edited by the viral

* Corresponding author. Nikolaus-Fiebiger-Center for Molecular Medicine, Glückstraße 6, 91054 Erlangen, Germany. Fax: +49 9131 8529111.
E-mail address: snpoehlm@viro.med.uni-erlangen.de (S. Pöhlmann).

0042-6822/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2006.04.038

polymerase to encode a membrane bound from of GP (Volchkov et al., 1995). The membrane bound EBOV- and MARV-GP is incorporated into budding virions and mediates entry into target cells (Kawaoka, 2005).

An N-terminal signal peptide targets the nascent GP into the endoplasmatic reticulum, where the polypeptide chain is modified with high mannose carbohydrates (Feldmann et al., 1991, 1994; Will et al., 1993). Subsequently, posttranslational modification of GP continues in the Golgi apparatus, and upon transport into the trans-Golgi, GP is cleaved by the cellular endoprotease furin into the subunits GP_1 and GP_2 (Volchkov et al., 1998). GP_1 and GP_2 remain covalently associated via disulfide bonds (Volchkov et al., 1998) and form trimeric heterodimers in the cellular membrane (Malashkevich et al., 1999), where they are incorporated into budding virions (Jasenosky and Kawaoka, 2004). Expression of GP of the highly pathogenic ZEBOV subspecies downmodulates cellular surface proteins, triggers detachment of adherent cells in culture and disrupts the integrity of the vascular endothelium (Chan et al., 2000; Simmons et al., 2002; Takada et al., 2000; Yang et al., 2000). Its has therefore been proposed that GP is an important determinant of viral pathogenicity and might be responsible for development of hemorrhages (Yang et al., 2000). While the latter concept has been challenged (Geisbert et al., 2003), the cytopathic effect of GP is undisputed. In fact, a major purpose of sGP synthesis might be to limit expression of GP in infected cells (Volchkov et al., 2001).

The functional organization of EBOV-GP is similar to that of various other viral glycoproteins, termed class I fusion proteins (Kawaoka, 2005). Thus, the GP_1 subunit is believed to interact with cellular receptors, while the GP_2 subunit, which is oriented perpendicular to the viral membrane, drives fusion of the viral and a cellular membrane, thereby allowing delivery of the viral genome into the cellular cytoplasm (Kawaoka, 2005). The fusion machinery in GP_2 has been defined on a functional and structural basis (Ito et al., 1999; Malashkevich et al., 1999; Watanabe et al., 2000; Weissenhorn et al., 1998a, 1998b). However, the elements in GP_1 required for recognition of cellular receptors are less clear. Recent mutagenic analysis suggested that the N-terminal 150 amino acids might be involved in receptor binding (Manicassamy et al., 2005). In contrast, a C-terminal mucin-like domain, which exhibits high variability between the EBOV subspecies and constitutes a determinant of cytopathicity (Simmons et al., 2002; Yang et al., 2000), is dispensable for GP-driven infection (Yang et al., 2000). Engagement of cellular receptors by GP_1 is believed to introduce EBOV into endosomal vesicles, where cathepsin mediated proteolysis of GP_1 triggers the membrane fusion activity of GP_2 (Chandran et al., 2005; Kawaoka, 2005; Schornberg et al., 2006). While the cellular receptor(s) for filoviruses remain elusive, binding of GP to several cellular lectins was shown to profoundly augment filovirus infection (Alvarez et al., 2002; Becker et al., 1995; Gramberg et al., 2005; Lin et al., 2003; Simmons et al., 2003; Takada et al., 2004).

GP is a target for neutralizing antibodies, and such antibodies can provide protection against EBOV infection in small animal models (Wilson et al., 2000). However, the generation of neutralizing antibodies is inefficient in infected individuals (Peters and LeDuc, 1999). Nevertheless, GP-specific neutralizing antibodies have been detected in convalescent donors (Maruyama et al., 1999). Because of its indispensable function in the viral life cycle and its susceptibility to antibody inhibition GP is a key target for therapeutic intervention and vaccine development. Thus, immunization with GP encoding adenoviruses (Sullivan et al., 2000, 2003) or vesicular stomatitis viruses (Jones et al., 2005) protected macaques from filovirus infection, and these vaccines are attractive candidates for evaluation in humans.

It is appreciated that expression levels of GP in infected cells impact cellular viability and virus production (Simmons et al., 2002; Volchkov et al., 2001). However, it is unclear if the efficiency of GP incorporation into budding virions affects the interaction with cellular factors and the recognition by the humoral immune response. For simian immunodeficiency virus (SIV), it has been reported that the efficiency of envelope protein (Env) incorporation into virions can profoundly modulate infectivity and neutralization sensitivity (Yuste et al., 2004, 2005), and a correlation between Env content in virions and virion infectivity has also been observed for human immunodeficiency virus type-1 (HIV-1) (Bachrach et al., 2005). By employing cell lines that express EBOV-GP of the four subspecies upon induction, we investigated how the level of EBOV-GP incorporation into virions affects the interaction with cellular factors and the susceptibility to antibody neutralization. We provide evidence that relative few copies of GP are sufficient to allow robust infection, while only high amounts of GP allow optimal engagement of cellular lectins. We also show that the density of GP on virions can impact neutralization efficiency and that the expression of high levels of GP by target cells does not interfere with EBOV-GP mediated infection.

Results

Inducible expression of EBOV-GPs by 293 T-REx cells

We sought to investigate if the efficiency of virion incorporation of EBOV-GP affects the interaction of virions with cellular factors and their recognition by neutralizing antibodies. In order to generate virions harboring different copy numbers of GP, we employed a tetracycline inducible system to express GP. Thus, expression constructs for the GPs of the four EBOV subspecies were stably introduced into 293 T-REx cells, which express transgenes upon induction with tetracycline. The 293 T-REx cell line, a derivative of the 293 cell line, was chosen because these cells are readily transfectable and should allow efficient production of reporter viruses bearing EBOV-GPs. Western blot analysis of 293 T-REx cell lines stably transfected with EBOV-GP expression constructs revealed that all cell lines expressed the EBOV-GPs in a doxycycline (a tetracycline derivative)-dependent fashion, while no GP signal was detected in parental 293 control cells (Fig. 1A). The rabbit serum used for detection of EBOV-GP expression was raised against ZEBOV-GP and preferentially recognizes this protein (data not shown); therefore, differences in the signal intensities between