Available online at www.sciencedirect.com
ScienceDirect
Virus Research 123 (2007) 40–49
Virus Research
www.elsevier.com/locate/virusres

Characterization of herpes simplex virus type 1 recombinants that express and incorporate high levels of HCV E2-gC chimeric proteins

V. Kouvatsis [a], R. Argnani [b], E. Tsitoura [a], M. Arsenakis [c], U. Georgopoulou [a], P. Mavromara [a,*], R. Manservigi [b]

[a] *Molecular Virology Laboratory, Hellenic Pasteur Institute, Athens 11521, Greece*
[b] *Department of Experimental and Diagnostic Medicine, University of Ferrara, I-44100 Ferrara, Italy*
[c] *Department of Biology, Aristotle University of Thessaloniki, Greece*

Received 4 April 2006; received in revised form 21 July 2006; accepted 27 July 2006
Available online 20 September 2006

Abstract

We report the construction of two HSV-1 recombinants encoding chimeric forms of the E2 glycoprotein of HCV-1a composed of the ectodomain of E2 (aa384–611 or 384–711) fused to different parts of the transmembrane and cytoplasmic domain of the HSV-1 gC glycoprotein (gC). The parental HSV-1, known as KgBpK^{-}gC^{-}, is deleted for gC and the main heparan sulphate (HS) binding domain of gB, and it exhibits impaired binding (ca. 80%) to HS compared to the wild type virus KOS [Laquerre, S., Argnani, R., Anderson, D.B., Zucchini, S., Manservigi, R., Glorioso, J.C., 1998. Heparan sulphate proteoglycan binding by herpes simplex virus type 1 glycoproteins B and C, which differ in their contributions to virus attachment, penetration, and cell-to-cell spread. J. Virol. 72, 6119–6130]. We show that gC:E2 proteins are efficiently expressed and transported to the cell surface. We also demonstrate that HSV-1 can incorporate both gC:E2 chimeric proteins into particles and show that incorporation of both chimeric molecules in the viral envelope partially restored binding (ca. 20%) of the HSV-1 recombinants to heparan sulphate. Finally, we showed that the gC:E2ScaI chimeric glycoprotein was able to bind a recombinant form of hCD81 and virion-expressed gC:E2ScaI permitted the binding of the HSV-1 recombinant virus to the hCD81 molecule.

Keywords: HCV; gC:E2 chimeric proteins; HSV-1; Heparan sulphate; HCD81

1. Introduction

Hepatitis C virus (HCV) is a major cause of liver diseases, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Major et al., 2001). HCV is a small enveloped virus that belongs to the *Hepacivirus* genus within the *Flaviviridae* family (Lindenbach et al., 2005). The positive single stranded RNA genome encodes a single polyprotein precursor of ∼3010 amino acid (aa) residues, which is cleaved co- and post-translationally by cellular and viral proteases to yield at least 10 mature products (Lindenbach et al., 2005; Penin et al., 2001). Furthermore, a novel protein is produced from an alternative ORF present at the N-terminal region of the polyprotein (Varaklioti et al., 2002; Walewski et al., 2001; Xu et al., 2001).

The two envelope glycoproteins (gps), E1 and E2, are type I trans-membrane proteins with large N-terminal ectodomains carrying multiple N-linked glycosylation sites and C-terminal hydrophobic regions containing ER retention signals (Goffard and Dubuisson, 2003; Op et al., 2001). Most of the glycosylation sites (5 and 11 potential glycosylation sites for E1 and E2, respectively) are well conserved and play an essential role in the functional properties of HCV envelope gps, which are currently being investigated for their role in mediating HCV entry into host cells (Goffard et al., 2005; Zhang et al., 2004). After their synthesis, E1 and E2 assemble into non-covalent heterodimers through their trans-membrane domains (TMDs). The E1/E2 complexes are mainly retained in the ER, a proposed site for HCV assembly and budding (Op and Dubuisson, 2003). Because suitable cell culture systems for *in vitro* virus propagation and efficient production of infectious particles have been limited until recently (Cai et al., 2005; Lindenbach et al., 2005; Wakita et al., 2005; Zhong et al., 2005), a number of surrogate

* Corresponding author.
E-mail address: penelopm@hol.gr (P. Mavromara).

0168-1702/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2006.07.017

systems have been employed to study the functional properties of HCV glycoproteins including E1/E2 liposomes (Lambot et al., 2002), vesicular stomatitis virus (VSV)-HCV pseudotypes (Basu et al., 2004; Buonocore et al., 2002; Matsuura et al., 2001; Meyer et al., 2000), virus-like particles (VLPs) expressed in insect cell systems (Baumert et al., 1998; Triyatni et al., 2002; Welnitz et al., 2002), and retro- or lentiviral pseudotypes harbouring HCV envelope gps (Bartosch et al., 2003a,b; Zhang et al., 2004). These surrogate assays have led to the identification of several candidate receptors for HCV, including human CD81 (hCD81) (Flint et al., 2004; Pileri et al., 1998).

Human CD81, a member of the tetraspanin membrane protein superfamily (Levy et al., 1998), interacts with E2 and appears to function as an essential entry co-receptor in infection studies with retroviral particles harbouring the HCV gps (Cormier et al., 2004; Lavillette et al., 2005; Zhang et al., 2004). The hCD81 binding site for E2 has been localized within the second extracellular loop (EC2) of CD81 (Higginbottom et al., 2000) whereas the binding site for E2 is less well defined. Nevertheless, it appears that aa 613–618 of E2 are critical for binding to hCD81 (Drummer et al., 2002; Roccasecca et al., 2003).

Cell surface heparan sulphate proteoglycans (HSPG) play an important role in the initial cell attachment for several viruses, including herpes simplex virus 1 (HSV-1) (Shieh et al., 1992; Shukla and Spear, 2001) and members of the *Flaviviridae* family, such as the Dengue (Chen et al., 1997; Germi et al., 2002; Hilgard and Stockert, 2000), classical swine fever (CSF) (Hulst et al., 2001), and tick-borne encephalitis viruses (TBE) (Mandl et al., 2001). Recent studies have shown that VSV/HCV pseudoparticles or VLPs require HS for binding to the target cells, and the first hypervariable region (HVR1) of E2 gp (residues 384–410) is responsible for this interaction (Barth et al., 2003; Basu et al., 2004). HVR1 exhibits strong amino acid sequence variability among the different HCV strains, but its overall physicochemical properties and conformation are highly conserved, due to the conservation of basic residues at specific positions (Penin et al., 2001). These amino acid residues are critical for interaction with the negatively charged HSPGs (Barth et al., 2003; Basu et al., 2004).

HSV-1 is a large neurotropic double-stranded DNA virus that represents a common human pathogen (Cai et al., 2005; Roizman and Whitley, 2001; Whitley and Roizman, 2001). Entry of HSV-1 is a complex process of several events, including an initial interaction of the virus to heparan sulphate glycosaminoglycan moieties of the host cell surface mediated mainly by the envelope glycoproteins C (gC) and B (gB), followed by binding of the glycoprotein D (gD) to specific cellular receptors and subsequent penetration of the cell membrane via fusion of the viral envelope with the plasma membrane (Shukla and Spear, 2001; Spear and Longnecker, 2003). Cumulative evidence indicates that HSV-1-based vectors are valuable tools to study biological properties of heterologous viral proteins, including protein/receptor interactions (Anderson et al., 2000; Grandi et al., 2004; Laquerre et al., 1998; Zhou and Roizman, 2005). Interestingly, we have recently shown that HSV-1 recombinants expressing the HBV preS1 peptide are able to bind preferentially to hepatocytes through the recognition of a specific receptor (Argnani et al., 2004).

In the present study, we investigate the potential use of HSV-1 as a surrogate system for expression of the HCV E2 glycoprotein. We chose an HSV-1 double mutant virus, known as KgBpK$^-$gC$^-$, which is deleted for the glycoprotein C (gC) as well as the lysine rich main heparan sulphate binding domain of gB (residues 68–76). As a result, the double mutated virus exhibits impaired binding (ca. 80%) to HS compared to the wild type virus KOS (Laquerre et al., 1998). We show that two different chimeric glycoproteins bearing parts of the E2 gp ectodomain fused to C-terminal parts of HSV-1 glycoprotein C are efficiently expressed in the context of the HSV-1 virus genome and are incorporated into the virus envelope. The incorporation of the chimeric protein in the viral envelope partially repaired binding (ca. 20%) of the recombinant viruses to HS and permitted the binding of the recombinant HSV viruses to the hCD81 molecule.

2. Materials and methods

2.1. Cells and viruses

Huh-7 (kindly provided by Dr Bartenschlanger) and Vero cells (ATCC) were maintained at 37 °C in Dulbecco's modified minimal essential medium (DMEM) supplemented with 10% fetal bovine serum. Vero cells were used to propagate all the HSV-1 viral strains. All HSV-1 recombinant viruses were derivatives of HSV-1 strain KOS. The HS-binding-defective virus KgBpK$^-$gC$^-$ was described previously (Laquerre et al., 1998).

2.2. Construction of plasmids

Plasmid pGEM-gC was created by inserting the HSV-1 glycoprotein C (gC) coding sequence (UL44) from stain KOS into the cloning vector pGEM-5Zf(+) (Promega). An *Hpa*I (93730)-*Hind*III (98823) HSV-1 fragment containing the gC coding sequence was modified to have *Xho*I recognition sites at the extremities, and was inserted into the *Sal*I site of the pGEM-5Zf(+) plasmid. After digestion of the pGEM-gC plasmid with *Pst*I and *Xba*I, a fragment of 1867 bp, containing upstream and coding sequences of gC (*Pst*I-95802; *Xba*I-97669), was excised and inserted into the respective recognition sites of the pUC19 plasmid (New England Biolabs), yielding the pUC19-gC plasmid.

The plasmid pHPI698, having a substitution of the signal sequence of the E2 HCV protein replaced by the signal sequence of the HSV-1 glycoprotein E (gE) (nt 141183–141309) corresponding to wild type HSV-1 (strain F), was described previously (Tsitoura et al., 2002). After digestion of this plasmid with *Xba*I-*Sca*I and *Xba*I-*Hinc*II, two fragments containing the gE signal sequence and different portions of the E2 ectodomain (gEE2ScaI (1089 bp) and gEE2HincII (815 bp), respectively) were excised. Both fragments were inserted into the *Nhe*I (96296)–*Eco*RV (97649) and *Nhe*I (96296)–*Nru*I (97387)