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ScienceDirect
Virology 368 (2007) 376–387
VIROLOGY
www.elsevier.com/locate/yviro

Characterization of retrovirus-based reporter viruses pseudotyped with the precursor membrane and envelope glycoproteins of four serotypes of dengue viruses

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Received 25 April 2007; returned to author for revision 14 May 2007; accepted 19 June 2007
Available online 26 July 2007

Abstract

In this study, we successfully established retrovirus-based reporter viruses pseudotyped with the precursor membrane and envelope (PrM/E) proteins of each of the four serotypes of dengue viruses, which caused the most important arboviral diseases in this century. Co-sedimentation of the dengue E protein and HIV-1 core proteins by sucrose gradient analysis of the pseudotype reporter virus of dengue virus type 2, D2(HIVluc), and detection of HIV-1 core proteins by immunoprecipitation with anti-E monoclonal antibody suggested that dengue viral proteins were incorporated into the pseudotype viral particles. The infectivity in target cells, as assessed by the luciferase activity, can be inhibited by the lysosomotropic agents, suggesting a pH-dependent mechanism of entry. Amino acid substitutions of the leucine at position 107, a critical residue at the fusion loop of E protein, with lysine resulted in severe impairment in infectivity, suggesting that entry of the pseudotype reporter virus is mediated through the fusogenic properties of E protein. With more and more dengue viral sequences available from different outbreaks worldwide, this sensitive and convenient tool has the potential to facilitate molecular characterization of the PrM/E proteins of dengue field isolates.

Keywords: Dengue virus; Pseudotype reporter virus

Introduction

Among the 70 or so arthropod-borne flaviviurses, epidemics of the four serotypes of dengue virus (DENV1, DENV2, DENV3, and DENV4) continue to be a major public health problem in the tropical and subtropical areas (Gubler, 2002; Guzman and Kouri, 2002). It has been estimated that more than 2.5 billion people in over 100 countries are at risk of infection, and approximately 100 million dengue infections occur annually worldwide (Gubler, 2002; Guzman and Kouri, 2002). The clinical presentations of dengue virus infection range from asymptomatic infection, to a relatively mild disease, dengue fever, and severe and potentially life-threatening diseases, dengue hemorrhagic fever/dengue shock syndrome (Gubler, 2002; Guzman and Kouri, 2002).

Dengue viruses are members of the genus *Flavivirus* in the family *Flaviviridae*. It contains a positive-sense, single-stranded RNA genome of approximately 10,600 bases in length. Flanked by the 5′ and 3′ non-translated regions, the single open reading frame of the genome encodes a polyprotein precursor, which is subsequently cleaved by cellular and viral protease into three structural proteins, the capsid (C), precursor membrane (PrM), and envelope (E), as well as seven non-structural (NS) proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (Lindenbach and Rice, 2001). After the interaction of E protein with the cellular receptor, dengue virus is believed to enter the cell through receptor mediated endocytosis (Guirakhoo et al., 1993; Lindenbach and Rice, 2001; Mukhopadhyay et al., 2005; Randolph and Stollar, 1990). The acidic environment in the endosome triggers a series of conformational changes of E protein, which result in the fusion of viral and endosomal

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0042-6822/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2007.06.026

membranes and release of the nucleocapsid into cytoplasm (Lindenbach and Rice, 2001; Modis et al., 2004; Mukhopadhyay et al., 2005). Following translation and genome replication, assembly occurs in the membrane structures derived from ER and the virions are released through the secretary pathway (Lindenbach and Rice, 2001; Mukhopadhyay et al., 2005).

The PrM/E proteins of dengue virus, present as a heterodimer on the surface of the virion, are the major determinants of cellular tropism of dengue virus (Bray et al., 1998; Chen et al., 1995; Gualano et al., 1998; Kawano et al., 1993; Lindenbach and Rice, 2001; Modis et al., 2004; Mukhopadhyay et al., 2005). In addition, PrM/E proteins are the major targets of neutralizing and enhancing antibodies, which are thought to play important roles in the pathogenesis of dengue (Green and Rothman, 2006; Guzman and Kouri, 2002; Halstead, 1988; Lindenbach and Rice, 2001). With the establishment of full-length infectious cDNA clones of dengue viruses and generation of chimeric clones, the functions of PrM/E proteins in the context of viral particles can be investigated (Blaney et al., 2004; Bray and Lai, 1991; Chen et al., 1995; Gualano et al., 1998; Kapoor et al., 1995; Kinney et al., 1997; Lai et al., 1991; Puri et al., 2000; Sriburi et al., 2001; Whitehead et al., 2003a,b). However, the requirement of multiple steps including in vitro transcription, RNA transfection and infectivity assay, which is laborious and time-consuming, has restricted its wide use. A convenient system is needed to facilitate molecular characterization of the PrM/E proteins of dengue viruses.

Pseudotype reporter viruses that contain the E protein of one virus and the core and genome with reporter gene of another virus have been shown to be a convenient molecular tool to study the functions of E protein, such as tropism, mechanism of entry, sensitivity to neutralization, enhancement and entry inhibitors (Bartosch et al., 2003; Buonocore et al., 2002; Chan et al., 2001; Cronin et al., 2005; Deng et al., 1997; Fukushi et al., 2005; Giroglou et al., 2004; Hanika et al., 2005; Hsu et al., 2003; Ma et al., 1999; Matsuura et al., 2001; Moore et al., 2004; Nie et al., 2004; Simmons et al., 2004; Wool-Lewis and Bates, 1998). Retrovirus-based reporter viruses pseudotyped with the E proteins of different families of RNA viruses, including simian immunodeficiency virus, hepatitis C virus (HCV), Ebola virus, La Crosse virus, Hantaan virus, and SARS-CoV, have been successfully established (Bartosch et al., 2003; Chan et al., 2001; Deng et al., 1997; Giroglou et al., 2004; Hsu et al., 2003; Ma et al., 1999; Moore et al., 2004; Nie et al., 2004; Simmons et al., 2004; Wool-Lewis and Bates, 1998). In this study, we developed a lentivirus-based pseudotype reporter virus for dengue virus, which contains the core and defective genome of human immunodeficiency virus type 1 (HIV-1), the luciferase reporter gene, and the PrM/E proteins of one of the four serotypes of dengue viruses. We characterized the biochemical properties of the pseudotype reporter viral particles and demonstrated that they enter target cells through a pH-dependent mechanism. Moreover, mutations introduced to a critical residue at the fusion loop of E protein resulted in impairment in infectivity of the pseudotype reporter virus.

Results

Generation of pseudotype reporter viruses containing PrM/E protein of DENV2

We first generated a pseudotype reporter virus, D2(HIVluc), by co-transfection of the PrM/E-expressing construct of DENV2, pCB-D2, and the lentivirus-based luciferase reporter construct, pNL4-3.Luc.R-E− (Fig. 1A). Since particle formation of lentivirus and dengue virus is known to occur at different subcellular locations, i.e. plasma membrane for lentivirus and ER for dengue virus (Freed and Martin, 2001; Lindenbach and Rice, 2001), we next constructed a chimeric PrM/E-expressing plasmid, pCB-D2VSV, by replacing the TM domain of E protein with the TM and CY domains of VSV G protein, which contained the membrane-targeting signal (Rose and Whitt, 2001), to enhance the surface expression of PrM/E protein and generated the pseudotype reporter virus, D2VSV(HIVluc) (Fig. 1A). Western blot analysis of the cell lysates revealed compatible amounts of PrM and E proteins, indicating that the expression of PrM/E protein was intact in the constructs pCB-D2 and pCB-D2VSV (Fig. 1B). To detect proteins present in the pseudotype reporter viral particles, pellets derived from the

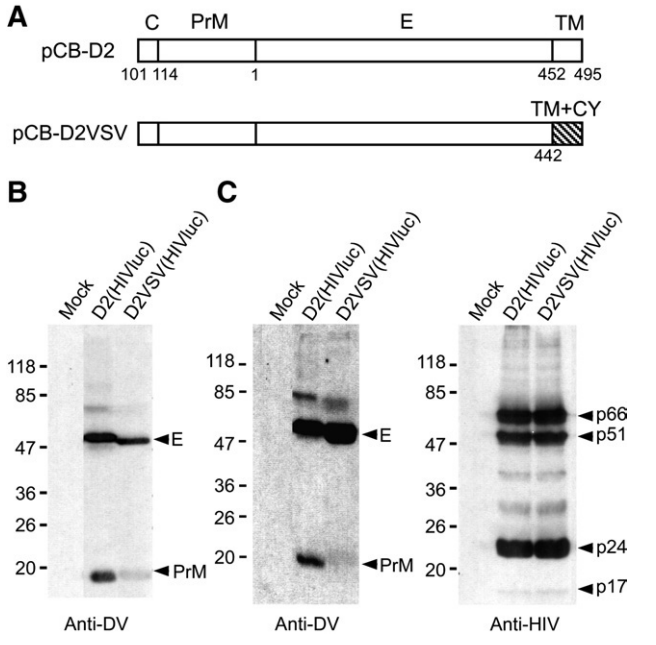

Fig. 1. Generation of pseudotype reporter viruses, D2(HIVluc) and D2VSV (HIVluc). (A) Schematic drawing of constructs expressing the PrM/E proteins of DENV2. PCR product containing the entire *PrM/E* gene and the 3′ 42-nucleotides of *C* gene (amino acid residues 101 to 114) of DENV2 was cloned into a pCDNA3-based vector (Chang et al., 2003) to generate pCB-D2. For pCB-D2VSV, the TM domain of the E protein of DENV2 was replaced with the TM and CY domains of VSV-G (hatched bar). The numbers indicate amino acid positions of the C and E proteins. (B, C) Plasmid pCB-D2 or pCB-D2VSV was co-transfected with pNL4-3.Luc.R-E− to 293T cells (Connor et al., 1995). 60 h later, cell lysates (B) and pellet lysates (C) derived from sucrose cushion ultracentrifugation were subjected to Western blot analysis by using serum from dengue patient (anti-DV) or HIV-1 patient (anti-HIV). Arrowheads indicate the structure proteins of dengue virus and HIV-1. The size of molecular weight markers is shown in kilodaltons.