Antiviral Research 155 (2018) 20–27

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Peptides P4 and P7 derived from E protein inhibit entry of dengue virus serotype 2 via interacting with β3 integrin

Xiaoyun Cui[a], Yanhua Wu[a], Dongying Fan[a], Na Gao[a], Ying Ming[a], Peigang Wang[a], Jing An[a,b,*]

[a] Department of Microbiology and Parasitology, School of Basic Medical Sciences, Capital Medical University, Beijing, China
[b] Center of Epilepsy, Beijing Institute for Brain Disorders, Beijing, China

ARTICLE INFO

Keywords:
Dengue virus
E protein
β3 integrin
Peptide
Domain III

ABSTRACT

Dengue virus (DENV) infection has become a severe public health problem worldwide. However, there is no specific antiviral drug available yet. In this study, we found that DENV serotype 2 (DENV2) infection enhanced the expression of β3 integrin on human umbilical vein endothelial cells (HUVECs) and that DENV2 antigens co-localized with β3 integrin. DENV2 envelope protein (E) directly interacted with β3 integrin, and their interacting sites were located at domain III of E protein (EDIII). Several synthetic peptides were designed based on the amino acid sequence of EDIII, and peptides P4 and P7 could inhibit DENV2 entry into HUVECs in a dose-dependent manner. The inhibitory concentration (IC_{50}) of the two peptides was 19.08 ± 2.52 μM for P4 and 12.86 ± 5.96 μM for P7. Moreover, P7 containing an FG-loop, but not P4, could also inhibit DENV1 entry into HUVECs. Our results suggest a novel mechanism in which interaction between β3 integrin and EDIII is involved in DENV entry. The findings on the inhibitory effect of the peptides on viral entry have significance for anti-DENV drug design.

1. Introduction

Dengue virus (DENV) is one of the most important emerging mosquito-borne viruses and belongs to the family *Flaviviridae*. It is composed of 4 serotypes (DENV1-4), and infection by one or more serotypes can cause classical dengue fever (DF) or severe dengue including dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). The incidence of dengue has increased 30-fold over the last 50 years. In 2013, DENV caused approximately 60 million symptomatic infections worldwide, with 18% hospitalization and approximately 13,600 deaths (Shepard et al., 2016). The worldwide cost of dengue case is estimated at US$9 billion. Thus, DENV infection has been a severe public health problem and economic burden.

In spite of dengue's prevalence, the pathogenesis of DENV infection is not completely clear and no specific antiviral drug is available yet. DHF/DSS is a fatal syndrome, mainly due to increased vascular permeability and plasma leakage. The endothelium is the first barrier vasculature in DENV infection. Interaction of vascular endothelial cells (VECs) with DENV and slight structural destruction of VECs are generally considered to contribute to this process (Dalrymple and Mackow, 2014; Nunes et al., 2016). It was reported that DENV attaches to a variety of cellular molecules for its entry into host cells including dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN) (Chen et al., 2008; Liu et al., 2017), heparan sulfate (HS) (Germi et al., 2002) and integrins (Wan et al., 2012; Zhang et al., 2007). Among them, integrins consist of two heterodimeric subunits, α and β, and play important roles in cell adhesion, migration and extracellular matrix protein recognition. β1 and β3 integrins are abundant surface receptors of VECs and platelets (Hussein et al., 2015) and crucial for maintaining capillary integrity and permeability. Additionally, αvβ3 integrin was shown to act as a receptor or co-receptor of many viruses (Chintakuntlawar et al., 2010; Schmidt et al., 2013).

Previously, we and other researchers found that DENV2 infection enhanced expression of β3 integrin in VECs, and pre-incubation of the virus with soluble β3 integrin could strongly inhibit DENV2 entry. Approximately 90% of virus entry was inhibited when β3 integrin expression was down-regulated by RNA interference, indicating that β3 integrin might act as a possible receptor for DENV2 entry into VECs, and interaction between β3 integrin and DENV might be involved in occurrence of DHF/DSS (Wan et al., 2012; Zhang et al., 2007). However, the molecular mechanisms underlying β3 integrin as receptor remain to be elucidated.

The genome of DENV encodes three structural proteins, capsid (C), pre-membrane (prM) and envelope (E) proteins, and seven non-structural (NS) proteins (NS1 to NS5). E is the envelope glycosylated protein and important for viral binding and entry into host cells (Chiu and

* Corresponding author. Department of Microbiology and Parasitology, School of Basic Medical Sciences, Capital Medical University, Beijing, China.
E-mail address: anjing@ccmu.edu.cn (J. An).

https://doi.org/10.1016/j.antiviral.2018.04.018
Received 30 August 2017; Received in revised form 28 December 2017; Accepted 24 April 2018
Available online 28 April 2018
0166-3542/

Yang, 2003). It is 500 amino acids (AAs) in length, and the N-terminal ectodomain of E protein contains 396 AAs. The ectodomain is further divided into three structural domains (EDI, EDII and EDIII). EDI and EDII are linearly discontinuous structures and share the N-terminal 296 AAs. EDII contains a conserved flavivirus fusion peptide (cd loop), which is responsible for low-pH-triggered fusion with the endosomal membrane and the cell membrane (Modis et al., 2004). EDIII contains the C-terminal 98 AAs and has an immunoglobulin-like structure, which is postulated to have receptor binding sites. EDIII also contains virus-specific neutralization sites (Roehrig, 2003). Experimental data have revealed that a considerable degree of post-attachment neutralization is associated with anti-EDIII antibodies (Crill and Roehrig, 2001; Hung et al., 1999). Thus, EDIII is a molecular target for designing anti-viral drugs and developing a DENV vaccine.

To address the molecular mechanism underlying interaction between DENV and β3 integrin, immunofluorescence staining (IFA), a Yeast two-hybrid (Y2H) membrane protein system and surface plasmon resonance (SPR) were used to investigate the interaction characteristics of the two proteins. Our data revealed that β3 integrin directly interacted with E protein and the binding domain located at EDIII. Moreover, two peptides derived from EDIII (P4 and P7) could block DENV2 entry into human umbilical vein endothelial cells (HUVECs) via interacting with β3 integrin. Our results would provide important evidence to well understand the pathogenesis of DENV infection and to design novel antiviral peptides for DENV therapy.

2. Materials and methods

2.1. Cell lines, viruses and plasmids

HUVECs (ATCC, USA) were cultured in RPMI medium 1640 basic (1640, Gibco, USA) containing 10% foetal bovine serum (FBS, Gibco, USA) at 37 ℃, 5% CO_2. Vero cells (ATCC, USA) were cultured in minimal essential medium (MEM, Gibco, USA) containing 5% FBS (PAN, Germany) at 37 ℃, 5% CO_2. *Aedes albopictus* cells (C6/36, ATCC, USA) were cultured in 1640 containing 10% FBS at 28 ℃, 5% CO_2.

DENV2 (strain Tr1751) and DENV1 (strain Hawaii) were propagated in C6/36 cells and stored at −80 ℃. The viral titres were quantified by plaque assay using Vero cells (Chen et al., 2016).

Plasmids carrying the gene for each DENV2 protein (GenBank accession numbers M84728), including C, M, E, NS1-2a, NS2b, NS3, NS4a, NS4b and NS5, were constructed previously in our lab. The coding sequences of each viral protein gene were amplified and cloned into the eukaryotic expression vector pReceiver-M01a (pRe). The primers used in PCR amplification and location of each gene are listed in Supplementary table 1. The sequences of PCR generated fragments were verified by DNA sequencing. These plasmids were used in the transfection experiment.

2.2. Antibodies, proteins and synthesis of peptides

Anti-DENV2 polyclonal antibody (PAb) was produced in our lab using Balb/c mouse challenged with DENV2. β3 integrin rabbit monoclonal antibody (MAb) and Cy3-conjugated goat anti-rabbit IgG were purchased from Abcam, UK and Sigma, USA, respectively. Fluorescein isothiocyanate- (FITC-) conjugated goat anti-mouse IgG was purchased from Earth, USA. Recombinant human β3 integrin and the DENV2 E protein were purchased from RD, USA and Biorbyt, UK, respectively.

All peptides were chemically synthesized by China Peptides Co, Ltd. (China). After being pooled, lyophilized and analysed by reversed-phase high-performance liquid chromatography (RP-HPLC), all peptide fractions with purity > 95% were obtained for SPR and a virus-binding blocking assay. All the peptides were solubilized in phosphate-buffered saline (PBS) to a final concentration of 10 mM and stored at −80 ℃ until use.

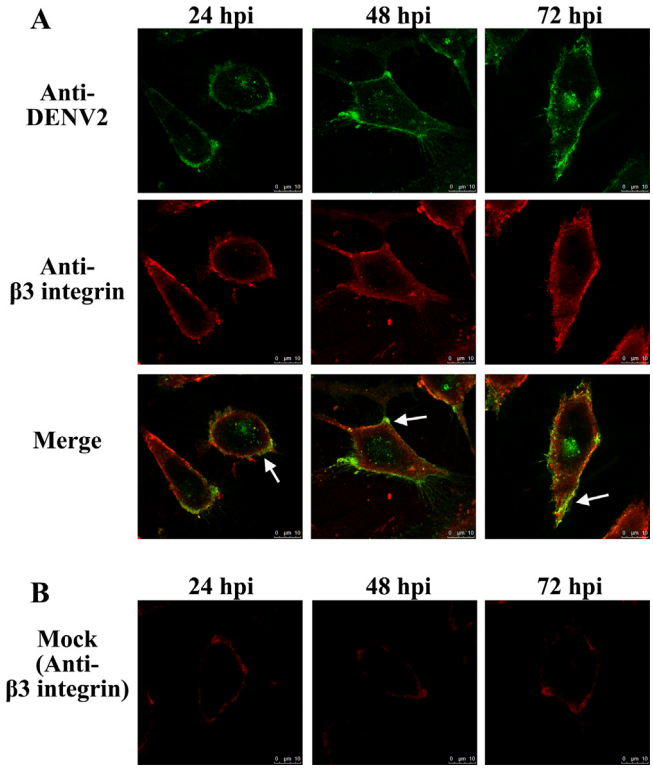

Fig. 1. The expression of β3 integrin in HUVECs at different time points after DENV2 infection. HUVECs were infected (A) with DENV2 (MOI = 1) or mock infected (B) with heat-inactivated DENV2 (at 60 ℃ for 30 min), and expression of β3 integrin was detected by IFA at 24, 48 and 72 h post infection (hpi). Anti-DENV2 PAb and anti-β3 integrin MAb were used as primary antibodies, and FITC-(green) and Cy3-(red) labelled anti-rabbit or anti-mouse IgG were used as secondary antibodies for DENV2 protein and β3 integrin, respectively. The merged image shows the co-localization of DENV2 and β3 integrin (Bar = 10 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. DENV infection

HUVECs grown on glass slides were infected with DENV2 or DENV1 (MOI = 1) at 37 ℃ for 1 hour (h). After being washed, the cells were continuously incubated at 37 ℃ for 24, 48 and 72 h.

2.4. Transfection

HUVECs seeded into a 24-well plate were transfected with 1 μg DNA (per well) of each plasmid using 5 μL Lipofectamine 2000 reagent (Thermo Scientific, USA). At 6 h post-transfection, the cells were washed and continuously cultured for 48 h in 1640 with 10% FBS.

2.5. Immunofluorescence staining assay

After DENV2 infection or transfection, HUVECs were continuously incubated at 37 ℃ for 24, 48 or 72 h. After being fixed, the cells were incubated with anti-DENV2 PAb and anti-β3 integrin MAb at 4 ℃ overnight, followed by incubation with Cy3- and FITC-conjugated antibodies. The specimens were observed under a confocal laser microscope (Leica TCS SP2, Germany).

2.6. Expression and purification of recombinant EDI/II and EDIII protein

The plasmids pET32a-EDIII and pGEX-6P-1-EDI/II, expressing EDIII or EDI/II of DENV2 E protein, were kindly provided by Dr. CF Qin (Beijing Institute of Microbiology and Epidemiology) and expressed in