Virology 434 (2012) 27–37

Contents lists available at SciVerse ScienceDirect

Virology

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

The E2-E166K substitution restores Chikungunya virus growth in OAS3 expressing cells by acting on viral entry

Hans Henrik Gad [a], Sylvie Paulous [a], Essia Belarbi [a], Laure Diancourt [b], Christian Drosten [c], Beate M. Kümmerer [c], Aileen E. Plate [a], Valérie Caro [b], Philippe Desprès [a,*]

[a] Unité Interactions Moléculaires Flavivirus-Hôtes, Institut Pasteur, 75724 Paris, France
[b] Plate-forme de Génotypage des Pathogènes et Santé Publique, Institut Pasteur, 75724 Paris, France
[c] Institut für Virologie, Universitätsklinikum Bonn, 53127 Bonn, Germany

ARTICLE INFO

Article history:
Received 9 July 2012
Accepted 13 July 2012
Available online 11 August 2012

Keywords:
Chikungunya virus
2′,5′-oligoadenylate synthetase
Innate immunity
Viral evasion
Myoblasts
Envelope glycoprotein
Molecular clone
Viral diversity

ABSTRACT

Human 2′,5′-oligoadenylate synthetase 3 (OAS3) exerts antiviral effect against alphaviruses including Chikungunya virus (CHIKV) by inhibiting viral RNA accumulation. Here, we identified a CHIKV variant exhibiting a remarkable resistance to the antiviral action of OAS3 in human epithelial HeLa cells. Using a molecular clone of CHIKV with *Renilla* luciferase inserted as a reporter gene in the non-structural region, we demonstrated that a single glutamine-to-lysine amino acid change at position 166 of the envelope E2 glycoprotein restores CHIKV replication in OAS3 expressing HeLa cells. Viral entry assays showed that CHIKV with a lysine at position E2-166 was more efficient at entering the replicative pathway. The E2-E166K substitution promotes a greater efficiency of CHIKV replication in human myoblasts leading to severe apoptosis through a more robust activation of the PKR pathway. These observations provide a new insight into the role of E2 into the pathogenicity of CHIKV in human cells.

Introduction

Chikungunya virus (CHIKV) is an arthropod-borne virus of the *Alphavirus* genus within the *Togaviridae* family. In 2005, CHIKV reemerged in Eastern Africa from where it spreads to several islands in the Indian Ocean and later on to India and South East Asia causing a major epidemic with millions of infected people. In Europe, the increased number of imported cases and the local outbreaks of CHIKV observed in Italy in 2007 and in France in 2010 have raised concern whether CHIKV may become a serious problem in temperate regions including Europe (Angelini et al., 2007; Grandadam et al., 2011). In humans, acute CHIKV infection is characterized by high fever, rash, headache, nausea, and severe pain in muscle and joints with the latter symptoms persisting in some patients for months or even years. (Borgherini et al., 2007, 2008; Robinson, 1955).

CHIKV, like other alphaviruses, is a small lipid enveloped virus with a positive-sense single-stranded 11.5 kb RNA genome. Together with the capsid protein, the genomic RNA forms the icosahedral nucleocapsid at the core of the virion. The nucleocapsid is surrounded by a host-derived lipid membrane with heterodimers of the envelope proteins, E1 and E2, embedded within. The E1–E2 heterodimer is responsible for viral entry through receptor-mediated endocytosis with E2 binding cell surface receptors and E1 facilitating membrane fusion. The acidic environment within the endosome leads to dissociation of the E1–E2 heterodimer and a conformational rearrangement of E1 that triggers fusion of viral and endosomal membranes thus releasing the viral genome into the cytoplasm. The 5′ two-thirds of the genome are directly translated into the non-structural polyprotein precursor which is cleaved by itself to produce the non-structural proteins 1 to 4 (nsP1 to 4). Together with cellular co-factors, the nsPs form the viral replication complex that synthesizes first negative-sense and secondly positive-sense genomic RNA. Besides the full-length positive-sense genomic RNA, a smaller positive-sense subgenomic 26S RNA corresponding to approximately 3′ one-third of the genome is also being synthesized. The structural polyprotein precursor is translated from the subgenomic 26S RNA and is later cleaved into the individual structural proteins: capsid, the two major envelope proteins E1 and E2 and two smaller peptides E3 and 6 K (Griffin, 2007; Schwartz and Albert, 2010; Solignat et al., 2009; Strauss and Strauss, 1994).

At the early stages of alphavirus life cycle, virus replication leads to a marked shutoff of mRNA translation which is mediated by activation of the RNA dependent protein kinase (PKR) pathway

* Correspondence to: Unité Interactions Moléculaires Flavivirus-Hôtes, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris, France.
Tel.: +33 1 40 61 35 63; fax: +33 1 40 61 37 74.
E-mail address: philippe.despres@pasteur.fr (P. Desprès).

0042-6822/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2012.07.019

(Gorchakov et al., 2004). Upon binding to double-stranded RNA (dsRNA) molecules, a byproduct of alphaviral replication, PKR becomes activated through auto-phosphorylation. Once active, PKR phosphorylates eukaryotic initiation factor 2α (eIF2α) which in turn renders eIF2α inactive and blocks initiation of translation. However, alphaviruses have developed strategies to evade blockage of PKR-mediated inhibition of translation. Indeed, the subgenomic RNA contains stem loop structure that allows initiation of translation without requirement of eIF2α (McInerney et al., 2005; Ventoso et al., 2006). At the late stages of alphavirus life cycle, shutoff of mRNA translation is followed by induction of apoptosis and cell death (Krejbich-Trotot et al., 2011; Sourisseau et al., 2007).

Alphaviruses are highly sensitive to the antiviral activity of type I interferon (IFN) and several IFN-stimulated genes (ISG) including ISG15, ISG20, P56, ZAP, Viperin, and 2′,5′-oligoadenylate synthetases (OAS) exert antiviral activity against alphaviruses (Brehin et al., 2009; Lenschow et al., 2007; MacDonald et al., 2007; Schilte et al., 2010; Zhang et al., 2007). The OAS proteins are a family of ISGs which are produced as latent enzymes and are activated by binding to dsRNA. The interaction of OAS with dsRNA leads to formation of the catalytically active OAS-RNA complex and triggers the antiviral activity of OAS. The active OAS-RNA complex polymerizes ATP into 2′,5′-linked oligoadenylates ranging from dimers up to 30-mers. The 2′,5′-linked oligoadenylates then binds a latent endoribonuclease, RNase L, triggering the formation of catalytically active dimeric RNase L. This leads to degradation of single stranded RNA, and subsequently to a decrease in protein synthesis thereby inhibiting viral replication (Hovanessian and Justesen, 2007; Kristiansen et al., 2011).

We have previously reported that the large form of human OAS (OAS3) exerts antiviral effect against CHIKV in human epithelial cells (Brehin et al., 2009). In the present study, we identified a CHIKV variant exhibiting a remarkable resistance towards the antiviral activity of OAS3 through an enhancement of viral RNA replication. We showed that a single amino acid change in the envelope E2 glycoprotein allows the rescue of viral growth in OAS3 expressing HeLa cells in acting on the early stages of viral life cycle.

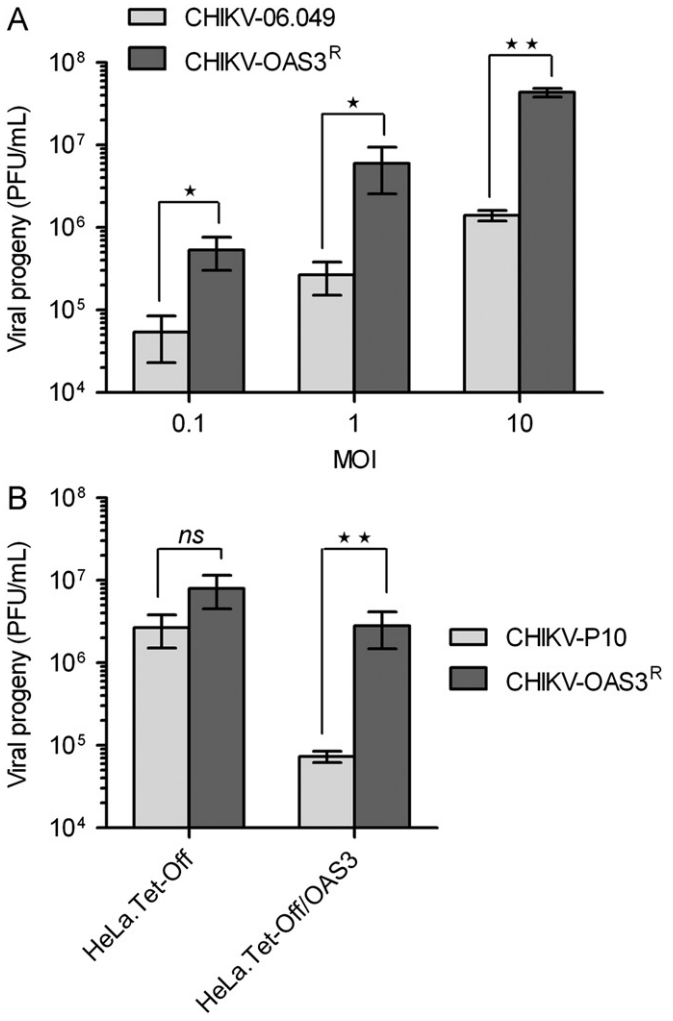

Fig. 1. CHIKV-OAS3^R shows resistance towards OAS3. In (A), HeLa.Tet-Off/OAS3 cells were infected with CHIKV-OAS3^R or CHIKV-06.49 at different MOIs and virus progeny production was determined at 24 h p.i. In (B), HeLa.Tet-Off or HeLa.Tet-Off/OAS3 cells were infected with CHIKV-OAS3^R or CHIKV-P10 at 1 MOI and virus progeny production was determined 24 h p.i. Values represent the mean and standard deviation of triplicates and are representative of two independent experiments. The values were compared according to Student's *t* test ($^{**}P \leq 0.01$).

Results

Identification of CHIKV-OAS3^R showing resistance towards OAS3

We were interested to investigate whether CHIKV is able to subvert the antiviral effect of OAS3. For this, CHIKV-06.049 strain was repeatedly passaged in induced HeLa.Tet-Off/OAS3 cells using a previously reported selection procedure (Mertens et al., 2010). After 10 serial passages and two steps of cloning by limiting dilution, a single virus clone, hereafter named CHIKV-OAS3^R, was selected for further studies. CHIKV-06.049 was also serially passaged on wild-type HeLa.Tet-Off cells resulting in a CHIK-P10 which served as a virus control. The CHIKV-OAS3^R variant exhibited a smaller plaque phenotype than the parental CHIKV-06.049 or CHIKV-P10 (Suppl. data 1). In HeLa.Tet-Off/OAS3 cells, progeny production was at least 1.5 $\log_{10}$ higher for the CHIKV-OAS3^R variant than for CHIKV-06.049 (Fig. 1A) and CHIKV-P10 (Fig. 1B) at multiplicities of infection (MOI) from 0.1 to 10 PFU/cell at 24 h post-infection (p.i.) (Fig. 1A). At 1 MOI, there was no significant difference in viral growth between CHIKV-OAS3^R and CHIKV-P10 in wild-type HeLa.Tet-Off cells (Fig. 1B). These results suggest that CHIKV-OAS3^R displays a greater efficiency at replicating in OAS3 expressing cells than CHIKV-06.49 or CHIKV-P10. Interestingly, infection of mosquito cell cultures with CHIKV-OAS3^R showed that it was not attenuated for viral growth in invertebrate cells (Suppl. data 2). Also, infection of adult BALB/c mice with CHIKV-OAS3^R was similar to what had been seen with parental virus after inoculation by intraperitoneal route (data not shown).

Genetic analysis of CHIKV-OAS3^R shows mutations in nsP2, C and E2 genes

Comparative analysis of the complete sequences of CHIKV-OAS3^R and CHIKV-06.049 identified one synonymous and three non-synonymous nucleotide changes between the two viruses (Table 1). The synonymous nucleotide change T8277C was located in the C gene whereas the three non-synonymous mutations A1016G, C3090T, and G9037A resulted in nsP1-M314V, nsP2-T470I and E2-E166K substitutions, respectively. Sequencing of genomic RNA from CHIKV-P10 suggested that the A1016G mutation resulted from cell culture adaptation (Table 1). The G9037A mutation was not observed in CHIKV-P10 indicating that a glutamine-to-lysine change at position E2-166 was not due to any positive selection towards epithelial cells.

Sequencing of genomic RNA from CHIKV-OAS3^R from all ten passages as well as the two cloning steps showed that the G9037A mutation arose at P3 where only nucleotide A was observed at