Virology 384 (2009) 216–222

Contents lists available at ScienceDirect

Virology

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

The large form of human 2′,5′-Oligoadenylate Synthetase (OAS3) exerts antiviral effect against Chikungunya virus

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ARTICLE INFO

Article history:
Received 14 September 2008
Accepted 9 October 2008
Available online 3 December 2008

Keywords:
Chikungunya virus
Alphaviruses
Antiviral innate immunity
Interferon-stimulated genes
2′,5′-Oligoadenylate Synthetases
OAS3
Genetic polymorphism

ABSTRACT

Chikungunya virus (CHIKV) becomes one of the most important mosquito-borne alphavirus in the medical field. CHIKV is highly sensitive to antiviral activity of Type-I interferons (IFN-α/β). Here, we investigated the role of IFN-induced 2′,5′-Oligoadenylate Synthetase (OAS) family in innate immunity to CHIKV. We established inducible human epithelial HeLa cell lines expressing either the large form of human OAS, OAS3, or the genetic variant OAS3-R844X which is predicted to lack about 20% of the OAS3 protein from the carboxy terminus. HeLa cells respond to ectopic OAS3 expression by efficiently inhibiting CHIKV growth. The characteristic of the antiviral effect was a blockade in early stages of virus replication. Thus, OAS3 pathway may represent a novel antialphaviral mechanism by which IFN-α/β controls CHIKV growth. HeLa cells expressing the truncated form of OAS3 were less resistant to CHIKV infection, raising the question on the involvement of OAS3 genetic polymorphism in human susceptibility to alphavirus infection.

Introduction

Chikungunya fever is an arbovirosis of major impact in public health in Asia and Africa. Chikungunya virus (CHIKV) has been responsible for an unprecedented magnitude of outbreaks in the Indian Ocean (Schuffenecker et al., 2006) and in India where hundreds of thousands of people have been infected since 2005 (Epstein, 2007). Humans infected with CHIKV typically experience acute illness with incapacitating polyarthralgia, severe muscle pain, and stiffness in the joints. CHIKV infection in muscle tissue could explain some features of clinical manifestations (Ozden et al., 2007; Couderc et al., 2008).

CHIKV, Sindbis virus (SINV), and Semliki Forest virus (SFV) are members of the *Alphavirus* genus of the Togaviridae family. Alphaviruses are lipid enveloped, positive-sense, single-stranded (ss) RNA viruses that replicate in the cytoplasm of the infected cells (for review Strauss and Strauss, 1994; Griffin, 2007). The alphavirion contains an 11.5 kb, single-stranded RNA genome of positive polarity. The genomic RNA is directly translated into the non-structural proteins 1 to 4 (nsP-1 to -4), which are encoded by the 5′ two-thirds of the genome. The structural proteins are encoded within the 3′ one-third of the genomic RNA and are translated from the subgenomic 26S RNA. The three major structural proteins C (capsid), E2 and E1 (the both envelope glycoproteins) together with genomic RNA form the alphavirion. Alphavirus infection results in rapid inhibition of cellular machinery, which favours the production of viral RNA and proteins inside the host cells (for review Strauss and Strauss, 1994).

Alphaviruses such as CHIKV are highly sensitive to the antiviral activity of Type-I interferons (IFN-α/β) (Desprès et al., 1995; Ryman et al., 2005; Lenschow et al., 2007; Shabman et al., 2007; Sourisseau et al., 2007; Zhang et al., 2007; Couderc et al., 2008; Tesfay et al., 2008).

Innate antiviral mechanisms mediated by IFN-α/β are potentially the most important pathways of host cell defence limiting viral replication (for review, Samuel, 2001). Recent reports have shown that IFN-α/β is able to trigger the activation of a specific signal transduction pathway leading to induction of IFN-stimulated genes (ISGs) that are responsible for the establishment of an antialphaviral state (Lenschow et al., 2007; Zhang et al., 2007). To date, the ISGs thought to affect alphavirus replication are ISG15, ISG20, P56, ZAP, and

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doi:10.1016/j.virol.2008.10.021

Table 1
Amino acid differences between available OAS3 sequence (GenBank access NM-006178) and OAS3 cDNAs used in this study

AA position	NM-006178	OAS3	OAS3-R844X
18	Arg	Lys	Lys
844	Arg	Arg	*Opal*
865	Trp	Ser	–

Viperin (Lenschow et al., 2007; MacDonald et al., 2007; Zhang et al., 2007).

It is currently unknown whether any members of IFN-induced 2′,5′-Oligoadenylate Synthetase (OAS) family may play a role in the establishment of an antialphaviral state. The OAS/RNase L is a RNA decay pathway known to play an important role in the established endogenous antiviral pathway (Justesen et al., 2000; Sarkar et al., 1999; for review, Samuel, 2001; Ryman et al., 2005; Silverman, 2007; Randall and Goodbourn, 2008). Human OAS is a family of enzymes encoded by three closely linked genes on chromosome 12q24.2, with the following order: small (OAS1, p40/p46), medium (OAS2, p69/71), and large (OAS3, p100) OAS isoforms. The OAS proteins contain one (p40/p46), two (p69/71), or three (p100) repeats of the basic OAS module. Very little information is available on the biological properties of OAS3 (for review, Rebouillat and Hovanessian, 1999). The IFN-dependent OAS proteins are a group of double-strand (ds) RNA-dependent enzymes (for review Rebouillat and Hovanessian, 1999; Rebouillat et al., 2000). Binding of enzymatically active OAS to activator viral RNA results in the production of 2′- to 5′-linked oligoadenylates (2-5A). Latent, monomeric RNase L is enzymatically activated through homo-dimerization induced by binding to 2-5A oligomers. Once activated, RNase L degrades single-stranded RNA molecules including mRNA and viral RNA (for reviews, Samuel, 2001; Silverman, 2007).

We and others demonstrated that 1b isoform of mouse OAS gene (Oas1b) is a critical component of innate immunity to West Nile virus *in vivo* and *in vitro* (Mashimo et al., 2002; Perelygin et al., 2002; Kajaste-Rudnitski et al., 2006). We also reported that Oas1b is capable of suppressing flavivirus infection in RNase L-deficient mouse cells (Kajaste-Rudnitski et al., 2006). In the case of alphaviruses, a body of evidence exists to suggest that IFN-mediated inhibition of virus growth does not require RNase L (Ryman et al., 2005; for review, Silverman, 2007). Because the large form of human OAS is presumably not involved in RNase L activation (for review, Rebouillat and Hovanessian, 1999), we investigated whether OAS3 exerts an antiviral effect on CHIKV infection in human epithelial cells.

Results and discussion

Susceptibility of HeLa.Tet-Off cells to CHIKV

Our preliminary data showed that a high level of OAS3 expression after transfection with non-inducible DNA expression vectors resulted in great instability of OAS3 in transfected human cell lines (data not shown). To assess the antiviral activity of the large form of human OAS, we decided to establish a tetracycline-inducible human epithelial cell line with a stably integrated human OAS3 cDNA (Table 1) that allows induction of OAS3 expression by the removal of tetracycline (Tet) repressor.

To our knowledge, no stable cell lines expressing the large form of human OAS have been reported to date. Our previous attempts to generate a stable human cell line which allows inducible expression of OAS3 showed that HeLa.Tet-Off cell line was available for production of OAS3. To validate the susceptibility of HeLa.Tet-Off cells to CHIKV infection, these cells were exposed to an increasing input of La Réunion CHIKV strain 06-49 (Schuffenecker et al., 2006; Bréhin et al., 2008) (Fig. 1A). An analysis of CHIKV replication in HeLa.Tet-Off cells infected at 1 multiplicity of infection (MOI) showed that production of progeny virus reached ~7 log FFU.mL^{-1} at 18 h p.i. At this time point of infection, CHIKV infection resulted in detection of ~50% cells that were positive in flow cytometry analysis with anti-CHIK.E2 MAb 3E4 (Bréhin et al., 2008) (data not shown). Thus, HeLa.Tet-Off cells show susceptibility to CHIKV growth.

Recent attention has focused on the role of IFN-α/β in the antiviral innate immune responses against CHIKV in human cells (Sourisseau et al., 2007; Couderc et al., 2008). Thus, we examine the ability of human IFN-α to establish an antiviral state in HeLa.Tet-Off cells. Treatment of HeLa.Tet-Off cells with 1000 IU.mL^{-1} human IFN-α 5 h prior CHIKV exposure (1 MOI) resulted in ~1.5 log reduction in virus progeny production at 18 h p.i. (Fig. 1B). Thus, IFN-dependent antiviral pathways are functional in HeLa.Tet-Off cells and provide protection against CHIKV at cellular level. However, CHIKV-infected HeLa.Tet-Off cells showed complete resistance to IFN-α at 5 h p.i. once virus replication is well established (Fig. 1C).

Establishment of OAS3-expressing HeLa cell lines

To investigate the antiviral effect of OAS3 against CHIKV, we established a HeLa.Tet-Off/OAS3 cell clone that up-regulates OAS3 protein expression under the control of the Tet-Off expression system. The recombinant OAS3 protein is composed of three adjacent OAS units (domains I, II, and III) including three potential active catalytic

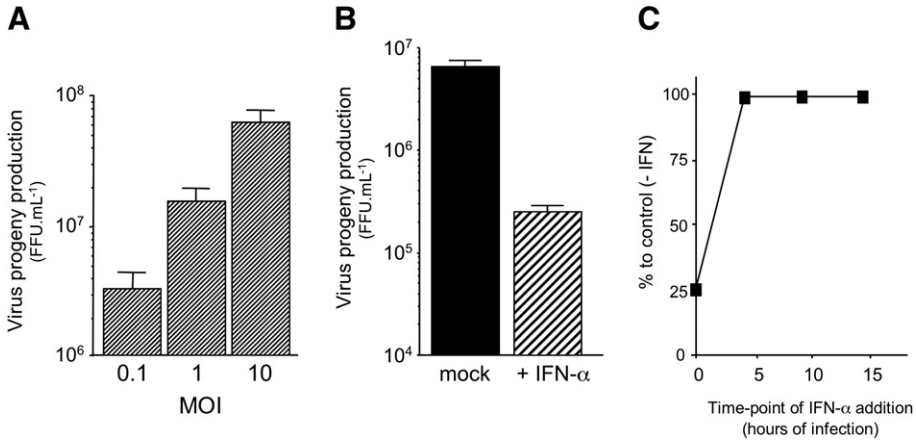

Fig. 1. Susceptibility of HeLa cells to CHIKV. In (A), HeLa.Tet-Off cells were infected with CHIKV at different MOI. At 20 h p.i., virus particles produced in the supernatants were titered on AP61 cells. In (B), HeLa.Tet-Off cells were treated with 1000 IU.mL^{-1} human IFN-α or mock-treated (control) 5 h prior to CHIKV 06-49 input at 1 MOI. Virus progeny productions were determined at 18 h p.i as described above. In (C), antiviral action of IFN-α on CHIKV growth. HeLa.Tet-Off cells were infected with CHIKV at 1 MOI. Cells were treated with 1000 IU.mL^{-1} human IFN-α at various time points p.i. Percentage of CHIKV progeny production in IFN-treated cells relative to that in mock-treated cells (% to control) at 20 h p.i.