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Inflammasome signaling pathways exert antiviral effect against Chikungunya virus in human dermal fibroblasts

Peeraya Ekchariyawat [a], Rodolphe Hamel [a], Eric Bernard [b], Sineewanlaya Wichit [a], Pornapat Surasombatpattana [c], Loïc Talignani [a], Frédéric Thomas [a], Valérie Choumet [d], Hans Yssel [e], Philippe Desprès [f], Laurence Briant [b], Dorothée Missé [a,*]

[a] Laboratoire MIVEGEC, UMR 224 IRD/CNRS/UM1, Montpellier, France
[b] Centre d'études d'agents Pathogènes et Biotechnologies pour la Santé, CNRS-UMR 5236/UM1/UM2, Montpellier, France
[c] Prince of Songkla University, Pathology Department, Faculty of Medicine, Songkla, Thailand
[d] Unit Environment and Infectious Risks, Institut Pasteur, Paris, France
[e] Centre d'Immunologie et des Maladies Infectieuses, Inserm, U1135, Sorbonne Universités, UPMC, APHP Hôpital Pitié-Salpêtrière, Paris, France
[f] Département infections et Epidémiologie, Institut Pasteur, 75724 Paris et UMR PIMIT (I2T team), Université de La Réunion, INSERM U1187, CNRS 9192, IRD 249, GIP-CYROI, 97491 Saint Clotilde, La Réunion, France

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ABSTRACT

Arboviruses represent an emerging threat to human. They are transmitted to vertebrates by the bite of infected arthropods. Early transmission to vertebrates is initiated by skin puncture and deposition of virus in this organ. However, events at the bite site remain largely unknown. Here, we report that Chikungunya virus (CHIKV) and West Nile virus (WNV), despite belonging to distinct viral families, elicit a common antiviral signature in primary human dermal fibroblasts, attesting for the up regulation of interferon signaling pathways and leading to an increased expression of IFN-β, interleukins and chemokines. Remarkably, CHIKV and WNV enhance IL-1β expression and induce maturation of caspase-1, indicating the capacity of these pathogens to elicit activation of the inflammasome program in resident skin cells. CHIKV and WNV also induce the expression of the inflammasome sensor AIM2 in dermal fibroblasts, whereas inhibition of caspase-1 and AIM2 with siRNA interferes with both CHIKV- and WNV-induced IL-1β production by these cells. Finally, inhibition of the inflammasome *via* caspase-1 silencing was found to enhance CHIKV replication in dermal fibroblasts. Together, these results indicate that the skin contributes to the pro-inflammatory and anti-viral microenvironment *via* the activation of the inflammasome in the early stages following infection with arboviruses.

1. Introduction

Chikungunya virus (CHIKV) and West Nile virus (WNV) are transmitted to humans by the bite of infected *Aedes* or *Culex* mosquitoes. In the recent years these viruses have propagated worldwide, causing millions of infections not only in countries with tropical climate, but also in temperate areas colonized by competent vectors. The global threat represented by these viruses is particularly well illustrated by CHIKV spreading from Africa to countries around the Indian Ocean (La Réunion, India and several South-Asian countries) where the virus infected millions of people and by the recent propagation of this pathogen across the Caribbean Islands (WHO, 2013). Together with the detection of autochthonous transmission in Italy (Rezza et al., 2007) and France (Gould et al., 2010), this situation is symptomatic of the spreading capacity of arboviruses.

Once transmitted to humans, the alphavirus CHIKV and the flavivirus WNV generate an acute syndrome, sharing high fever, headache, myalgia and cutaneous manifestations that generally resolve in a few days or weeks. However, they differentiate regarding the induction of some specific clinical manifestations. CHIKV is arthrogenic and may cause long lasting arthralgia related to bone erosion (Schwartz and Albert, 2010; Burt et al., 2012), while WNV is the

Abbreviations: AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; CHIKV, Chikungunya virus; ds, double-stranded; hpi, hours post infection; ISG, interferon-stimulated gene; MDA5, Melanoma differentiation-associated gene 5; NLR, NOD-like receptor; OAS2, oligoadenylate synthetase; RIG-I, retinoic acid-inducible gene-1; TLR, Toll-like receptor; WNV, West Nile virus.

* Corresponding author.
E-mail address: dorothee.misse@ird.fr (D. Missé).

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causative agent of severe meningoencephalitis, cognitive dysfunction, seizures and flaccid paralysis in infected humans. For both viruses, the skin is the first human organ supporting viral replication following a mosquito bite (for review see (Briant et al., 2014). In this body compartment, fibroblasts present in the dermis layer have been identified as a common target for CHIKV and WNV (Arnold et al., 2004; Kurane et al., 1992; Schilte et al., 2010). The dermis is an essential component of the skin, not only producing and organizing the extracellular matrix of this organ, but also communicating with other cell types through the paracrine production of immune modulators, such as peptide growth factors, cytokines, chemokines and other inflammatory mediators (Smith et al., 1997). This function is exacerbated during viral infection as dermal fibroblasts are equipped with a set of sensors specialized in pathogen detection and antiviral proteins required for the set-up of antiviral responses (Arnold et al., 2004). Because of their dual function as early skin sentinels and targets for arboviruses, host-pathogen interactions engaged in dermal fibroblasts may have a determining influence on further host colonization. The characterization of the antiviral responses and the viral countermeasures in these cells is therefore critical for the understanding of mechanisms supporting early transmission events at the skin entry site, a prerequisite for elaboration on therapeutic strategies capable to prevent further dissemination of the virus into the host. To provide insight in this field, the present study examines the antiviral signatures elicited by CHIKV and WNV in the human skin. We have characterized the dynamics of expression of antiviral genes modulated by these viruses following cutaneous infection and demonstrate the implication of inflammasome activation in the antiviral response.

2. Results

2.1. Antiviral genes differentially expressed in human dermal fibroblasts following CHIKV or WNV infection

Dermal fibroblasts are known targets for CHIKV (Schilte et al., 2012) and WNV (Hoover and Fredericksen, 2014). Indeed, following infection of human dermal fibroblasts with CHIKV or WNV (MOI of 1), viral replication was detected as soon as 6 h post infection (hpi), thus confirming the permissiveness of skin cells to infection with these arboviruses. The expression of both CHIKV and WNV RNA peaked at 24 hpi (Fig. 1a and c), whereas the production of virions was still detectable at 48 hpi (Fig. 1b and d), without any apparent alteration in cell viability (data not shown). The peak of replication was chosen to assess the antiviral program elicited by each virus in human fibroblasts. Total RNA was extracted from cells at 24 hpi and antiviral gene expression was investigated using a human quantitative PCR array covering 84 human antiviral genes. Comparative analysis with uninfected cells revealed that CHIKV and WNV infection affected the expression of 51 and 40 antiviral genes, respectively (Table 1), with 31 genes in common. CHIKV and WNV each modulated the expression of additional and specific sets of 20 and 9 genes, respectively, as shown in the Venn diagram in Fig. 2. These results show that a common gene pattern is elicited in dermal fibroblasts by arboviruses belonging to different viral families.

A detailed analysis of these upregulated genes pointed to the activation of several gene clusters. As expected, intracytoplasmic sensors that are able to detect the presence of foreign RNA, such as Toll-like receptor (TLR)-3, -7, -8, -9, retinoic acid-inducible gene-1 (RIG-I) and Melanoma differentiation-associated gene 5 (MDA 5), were activated upon infection. Increased pattern recognition receptor expression levels were confirmed by individual real time PCR showing that the expression of both RIG-I and MDA5 was upregulated as soon as 6 hpi in CHIKV- and WNV-infected fibroblasts with maximal levels detected at 24 hpi (Fig. 3).

Transcription factors known to mobilize the antiviral machinery were also upregulated in infected cells. NF-kB expression was stimulated by both viruses and IRF5, IRF7, RelA and Fos mRNA expression levels were increased in CHIKV- or WNV-infected cells (Table 1). The expression of IFN-β and interferon-stimulated genes (ISGs) (OAS2, MX1 and ISG15) was upregulated by CHIKV and WNV (Table 1 and Fig. 3), whereas infection with each of the viruses also resulted in enhanced levels of TNF-α, IL-8, CCL3 (MIP-1α) and CCL5 transcripts in the infected cells. Interestingly, the expression of the two CXCR3 ligands, CXCL10 and CXCL11, was induced as well by CHIKV and WNV. Altogether, these data attest for the early responsiveness of primary dermal fibroblasts to arbovirus infection, as demonstrated by the increased expression of several canonical Th1 cytokines and chemokines, susceptible to attract permissive and/or immunocompetent cells.

2.2. Arbovirus infection of primary skin fibroblasts results in inflammasome activation

Beside activation of IFN signaling pathways and upregulation of ISGs, enhanced levels of caspase-1 mRNA were observed in CHIKV- and WNV-infected fibroblasts (Table 1). Real time PCR analysis confirmed that transcription levels of caspase-1 increased over time in CHIKV- and WNV-infected cells, in a statistically significant manner, as compared to those in uninfected cells (Fig. 4a and b). Caspase-1 activation is regulated by a two-step mechanism that includes transcriptional regulation and conversion from an inactive procaspase to an active protease by autoproteolytic processing. To confirm the induction of caspase-1 activation by both CHIKV and WNV, the expression of active caspase-1 protein was therefore determined by Western blotting analysis. As shown in Fig. 4c and d either arbovirus was able to induce the expression of the catalytically active caspase-1 p10 subunit in dermal fibroblasts. Results from the PCR array analysis showed that CHIKV and WNV also induced the expression of IL-1β mRNA (Table 1). The induction of IL-β transcripts was found to be time-dependent (Fig. 4e and f) and to result in an enhanced production of this cytokine, as measured by ELISA, in the culture supernants of cells infected by each of the viruses, in comparison to those of uninfected cells (Fig. 4g).

Caspase-1 activation is coordinated by several inflammasome platforms controlled by members of the NLR (e.g., NLRP1, NLRP3, and NLRC4) or the PYHIN protein families (e.g., AIM2) (Aachoui et al., 2013). These sensors are linked to caspase-1 through pyrin-domain containing proteins such as ASC. As shown in Table 1, the results of the PCR array analysis indicated that several members of this signaling pathway were activated, following the infection of dermal fibroblasts with arboviruses. NLRP3 expression was upregulated 15-fold upon CHIKV infection, whereas CHIKV- and WNV-infection resulted in a 24- and 2-fold increase in ASC1 and ASC2 expression, respectively. The results furthermore show that 24 h after CHIKV or WNV infection, AIM2 mRNA expression was increased 33- and 51-fold, respectively (Table 1). The latter results were confirmed by real time PCR analysis, showing a time-dependent induction of AIM2 transcripts in CHIKV or WNV-infected dermal fibroblasts (Fig. 4h and i). Finally, the presence of enhanced AIM2 protein levels in these cells at 48 hpi were detected using Western blotting analysis (Fig. 4j and k), underscoring the capacity of both arboviruses to induce the expression of this inflammasome sensor.

2.3. Production of IL-1β by arbovirus-infected dermal fibroblasts is dependent on caspase-1 and AIM2 activation

To directly demonstrate the involvement of caspase-1 and AIM2 in the induction of IL-β production, the effect of suppressing the