Acta Tropica 211 (2020) 105619

Contents lists available at ScienceDirect

Acta Tropica

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

Chikungunya virus infection induces differential inflammatory and antiviral responses in human monocytes and monocyte-derived macrophages

Valdés López Juan Felipe, Velilla Paula A, Urcuqui-Inchima Silvio*

Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia

ARTICLE INFO

Keywords:
Inflammation
Toll-like receptors
Antiviral activity
Double-stranded RNA
Chikungunya virus
Chikungunya fever
interferon
ISG

ABSTRACT

Chikungunya virus (CHIKV) is a zoonotic arthropod-borne virus that has caused several outbreaks in tropical and subtropical areas worldwide during the last 50 years. The virus is known to target different human cell types throughout the course of infection including epithelial and endothelial cells, fibroblasts, primary monocytes and monocyte-derived macrophages (MDMs). The two latter are phagocytic cell populations of the innate immune system which are involved in some aspects of CHIKV pathogenesis. However, monocytes and macrophages also potentially contribute to the control of viral replication through the expression of different pattern recognition receptors sensing viral pathogens and subsequently, inducing an type I interferone (IFN-I)-dependent antiviral immune response. The aim of this study was to determine the modulation of the expression of Toll-like receptors (TLRs), cytokine secretion capabilities and antiviral factor production in monocytes and MDMs following infection with CHIKV. Moreover, we sought to determine the replication kinetics of CHIKV in these two cell populations. We found that the maximum peak of CHIKV replication was observed between 18- and 24-hours post-infection (hpi), while after that the is strongly reduced. Furthermore, CHIKV infection induced the pro-inflammatory cytokine production starting from the first 6 hpi in both monocytes and MDMs, with similar kinetics but different protein levels. In contrast, the kinetics of transcriptional expression of some TLRs were different between both cell types. In addition, IFN-I, 2′,5′-oligoadenylate synthetase 1 (OAS1), and double-stranded RNA-activated protein kinase R (PKR) mRNA levels were detected in response to CHIKV infection of monocytes and MDMs, resulting the highest expression levels at 48 hpi. In conclusion, our data provides evidence that CHIKV infection activates the TLR pathways in primary monocytes and MDMs, which play a crucial role in CHIKV pathogenesis and/or host defense, differentially. However, additional studies are required to determine the functional role of TLRs in monocytes and MDMs.

1. Introduction

Chikungunya virus (CHIKV) is a zoonotic arthropod-borne virus, a member of the *Togaviridae* family, of the *Alphavirus* genus and is transmitted to humans by mosquitoes of the *Aedes* genus (Lo Presti et al., 2014). During the last 50 years, CHIKV has been responsible for explosive outbreaks in central and South Africa, Southeast Asia, the Indian Ocean and Central and South America (Schuffenecker et al., 2006; Morrison, 2014; Weaver and Lecuit, 2015). Although infection with CHIKV is usually nonfatal, the virus is the etiological agent responsible for chikungunya fever, a self-limiting illness that occurs in approximately 95% of the infected individuals [for a review, see (Staples et al., 2009)]. After the acute-phase of the infection, about 55% of affected individuals develop a subacute state of the disease in which pain in joints can last for several days/weeks or develop a chronic state of the disease in which pain in joints (chronic arthritis) can persist for several months or even years (Hoarau et al., 2010; Chow et al., 2011; Valdés et al., 2019; Gasque et al., 2015; Petitdemange et al., 2015; Goupil and Mores, 2016). A growing body of evidence suggests that age, underlying medical conditions, viral load, and host responses are factors predisposing to a more severe CHIKV presentation and long-term sequelae (Hoarau et al., 2010). Extensive studies on the host immune response during CHIKV infection has been reported an association between pro-inflammatory cytokines, such as interleukin (IL)−1β and IL-6, and severity during the acute-phase of the disease, while high levels of IL-6, monocyte chemotactic protein-1 (MCP-1) and IL-8 were linked to development of chronic phase or prolonged arthralgia (Ng et al., 2009; Chow et al., 2011; Dupuis-Maguiraga et al., 2012; Lohachanakul et al., 2012). In CHIKV-infected patients, a high viral load has been associated with elevated levels of IL-

* Corresponding autor
E-mail addresses: paula.velilla@udea.edu.co (V. Paula A), silvio.urcuqui@udea.edu.co (U.-I. Silvio).

https://doi.org/10.1016/j.actatropica.2020.105619
Received 13 May 2020; Received in revised form 3 July 2020; Accepted 3 July 2020
Available online 04 July 2020
0001-706X/

6 and tumor necrosis factor-alpha (TNF-α) during the acute-phase. However, upon progressing to a chronic manifestation of the disease, it is possible to detect MCP-1, interferon (IFN)-α, IL-6, IL-8, IL-17, and matrix metalloproteinase 2 (MMP2) in the synovial fluid (Hoarau et al., 2010; Chow et al., 2011; Dupuis-Maguiraga et al., 2012; Goupil and Mores, 2016; Valdés et al., 2019), indicating a persistent inflammatory response. Elevated levels of monocyte and macrophage infiltrate were also reported in arthritis associated with CHIKV infection (Gardner et al., 2010).

CHIKV is an enveloped virus, containing an 11.8 kb long single-stranded positive-sense RNA (ssRNA) genome with two open reading frames (ORFs). The first encodes for the non-structural proteins, nsP1–4, which are mainly involved in viral replication. The second ORF encodes for the 6 structural proteins, namely the capsid protein (CP), the envelope (E) E3-E2–6K-E1 and the transframe protein (TF) [for a review, see (Strauss and Strauss, 1994)]. Within the cell, CHIKV starts the viral RNA replication process and production of abundant viral ssRNAs, dsRNA intermediates and viral proteins that are recognized by pattern recognition receptors (PRRs) of the host cells, such as Toll-like receptors (TLRs). The TLR activation results in the expression of pro-inflammatory cytokines and immune responsive genes, such as type I IFN (IFN-I), which are involved in the regulation of viral replication, dissemination and immunopathogenesis (Janeway and Medzhitov, 2002; Lester and Li, 2014). Induction of IFN-I by intracellular TLRs (e.g. TLR3, TLR7 or TLR8) recognizing viral RNAs represents an early innate immune response against viruses (Iwasaki and Medzhitov, 2004; Lester and Li, 2014). The IFN-I response promotes the expression of various IFN-stimulated genes (ISGs), some of which are involved in the clearing of CHIKV infection, such as for isntance double-stranded RNA-activated protein kinase R (PKR), 2′−5′-oligoadenylate synthetases (OAS), viperin and ISG15 (Werneke et al., 2011; White et al., 2011; Teng et al., 2012; Li et al., 2016).

Recent research reports that certain polymorphisms of TLR7 and TLR8 are associated with CHIKV infection susceptibility (Dutta and Tripathi, 2017). Yet, the pretreatment of mice with poly (I:C), a TLR3 agonist, induces a reduction of CHIKV titers in the brain and protection of the mice by increased induction of TLR3, IFN-β and antiviral genes (Priya et al., 2014). Similar results were observed in human bronchial epithelial cells (Li et al., 2012a,b). Poo et al. (2014) using CCR2(-/-) mice infected with CHIKV observed that although monocytes and macrophages were recruited to infection sites and promoted inflammation, they appeared to be critical for preventing excessive pathology and resolving inflammation. Although monocytes and macrophages play an important role in the control and/or immunopathogenesis of viral infections (Dolganiuc et al., 2004; Pichlmair et al., 2006; Lang et al., 2010; Arboleda et al., 2017; Michlmayr et al., 2017), they are also important target cells of CHIKV infection both *in vitro* and *in vivo* (Her et al., 2010; Labadie et al., 2010; Sourisseau et al., 2007). However, despite the fact that these two cell populations express TLRs, their expression and their role in the induction of inflammatory and antiviral activity in response to CHIKV infection are poorly characterized. Therefore, here, we describe the response of both primary monocytes and monocyte-derived macrophages (MDMs) to CHIKV throughout the course of infection, establishing the kinetics of CHIKV replication, the TLR expression, the proinflammatory cytokine production and the expression of IFN-I and some ISGs.

2. Materials and methods

2.1. CHIKV stocks, titration and uv light inactivation of CHIKV

A clinical isolate of CHIKV (kind gift of Professor Francisco Javier Díaz, University of Antioquia) was amplified from a Colombian patient's serum sample and propagated in Vero cells (ATTC CCL-81). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, St. Louis, USA) supplemented with 5% heat-inactivated fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Massachusetts, USA), 4 mM L-glutamine (Sigma-Aldrich), 0.3% (v/v) sodium carbonate ($NaCO_3$; Sigma-Aldrich) and 1% (v/v) antibiotic-antimycotic solution 100X (Corning-Cellgro, New York, USA), and incubated at 37 °C and 5% CO_2 in cell culture flasks, at a density of 1×10^5–1×10^6 cells/mL. The Vero cells were inoculated with CHIKV at 0.1 multiplicity of infection (MOI), incubated at 37 °C and 5% CO_2 for 2 days or until an advanced cytopathic effect was observed. Next, the supernatants were collected, precleared by centrifugation (1650 x g for 10 min). CHIKV stocks were titrated by plaque assay on Vero cells, as previously described (Juarez et al., 2013). Virus titers were determined to be 1.9×10^8 PFU/ml. CHIKV inactivation was achieved by exposure to UV light (~365 nm, ~145 mW/cm^2) at a distance of 10 cm for 60 min, at room temperature (UV-CHIKV). Inactivation of the virus was confirmed by plaque assay.

2.2. Culturing of human monocytes and differentiation into monocyte-derived macrophages

Human peripheral blood mononuclear cells (PBMCs) were isolated through a density gradient with Lymphoprep (STEMCELL Technologies Inc, Vancouver, Canada) by centrifugation at 850 x g for 21 min, from whole blood, mixed with 2% EDTA, donated by healthy volunteers or leukocyte-enriched blood units from the healthy individual from the blood bank of the "Escuela de Microbiologia, UdeA, Medellín, Colombia", as described previously (Arboleda et al., 2017). The PBMC sample from each healthy volunteer was worked independently. Platelet depletion was performed by washing with PBS-1X (Sigma-Aldrich) three times at 250 x g for 10 min and the percentage of CD14 positive cells was determined by flow cytometry as we previously reported (Arboleda et al., 2017). To obtain monocytes, 24-well plastic plates were scratched with a 1000 μL pipette tip and then, 5×10^5 CD14 positive cells/well were plated and allowed to adhere for 2 h in RPMI-1640 medium supplemented with 0.5 (v/v) autologous serum or plasma, 4 mM L-glutamine and 0.3% (v/v) $NaCO_3$ and cultured at 37 °C and 5% CO_2. Nonadherent-cells were removed by washing twice with PBS-1X and cultured in RPMI-1640 medium supplemented with 10% (v/v) FBS, 4 mM L-glutamine, 0.3% (v/v) $NaCO_3$ and 1% (v/v) antibiotic-antimycotic solution 100X (complete medium) and incubated at 37 °C and 5% CO_2 overnight to obtain monocytes or 6 days to obtain MDMs, as we described previously (Arboleda et al., 2017; Valdés and Urcuqui-Inchima, 2018).

2.3. Microscopy

Cultures of primary human monocytes and MDMs were visualized and photographed in an Axio Vert. A1 microscope (ZEISS, Oberkochen, Germany) equipped with a DS-Fi1 camera (Nikon, Tokyo, Japan) and adapted to microscopy of transmitted light in phase-contrast. Pictures were processed and analyzed using the software IrfanView (version 4.54), ImageJ (version 1.8.0) and GIMP (version 2.10.12). The cells were not fixed before visualization by microscopy. For the generation of scale bars, the IrfanView software was calibrated using photomicrographs taken with the 40X objective to a stage micrometer (ZEISS).

2.4. Flow cytometry analysis

The flow cytometer was set up to determine the size and granularity of monocytes and MDMs. In addition to the cell surface markers that we previously reported to characterized monocytes and MDMs (Arboleda et al., 2017; Valdés and Urcuqui-Inchima, 2018), we quantified CD86 and CD206 (mannose receptor). For this, the cells were routinely resuspended in PBS 1X and stained with anti-human CD86-PE antibody (clone: FUN-1, BD Biosciences, New Jersey, USA), anti-human CD206-FITC antibody (clone: 19.2, BD Biosciences) or the relevant

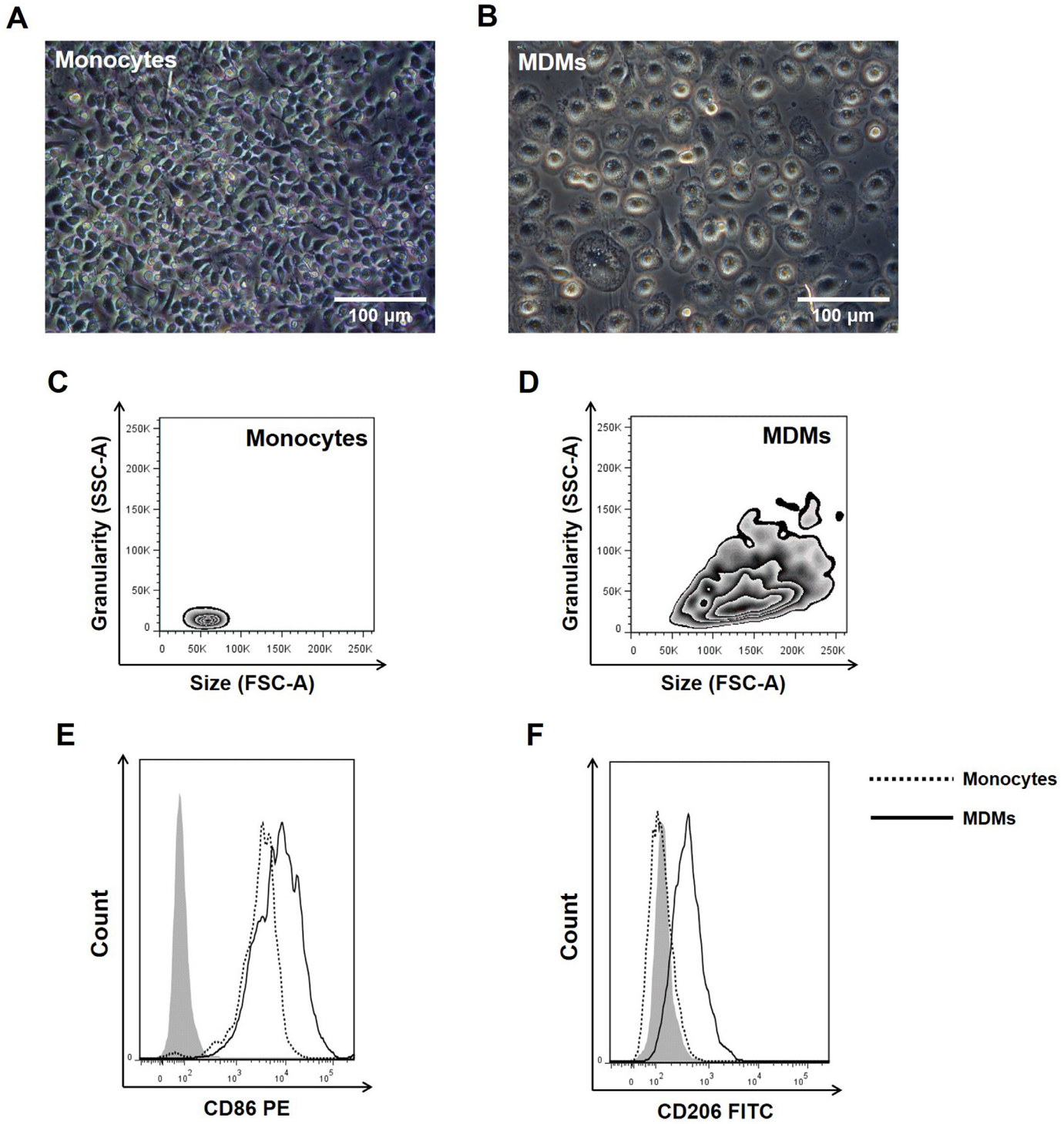

Fig. 1. Characterization of monocytes and monocyte-derived macrophages (MDMs). Representative images of the morphology of monocytes (A) and MDMs (B) by phase-contrast microscopy. Size and complexity (granularity) of monocytes (C) and MDMs (D) by flow cytometry. Expression of CD86 (E) and CD206 (F) on cell surface of primary human monocytes and MDMs was analyzed by flow cytometry. The gray areas correspond to an appropriate isotype-control reaction. Data are representative of one of at least three independent experiments. Scale bar equals 100 µm.

isotypes for 40 min at 4 °C. The cells were further washed via centrifugation and resuspended in PBS 1X. Surface staining samples were read on a FACScanto flow cytometry (BD Biosciences). Positive labeling cells were defined based on isotype-controls and a compensation matrix was performed to compensate the spectral overlap. At least 10.000 events were acquired per sample and data were analyzed using FlowJo software (version 7.6.2). Debris and dead cells were gated out using forward and side light scatter.

2.5. *In vitro virus infections*

CHIKV infection of primary human monocytes and MDMs (from healthy donors) were performed at MOI of 10 or 5, respectively, in serum-free RPMI-1640 medium. Samples were incubated at 37 °C for

1.5 h. Infection using UV-CHIKV was performed in a similar manner. An hour and a half after infection the cells were washed with PBS-1X to remove unbound virus and fresh complete medium was added and left at 37 °C with 5% CO_2. Cell culture supernatants and cell lysates were obtained at 6, 12, 18, 24 and 48 h post-infection (hpi) and stored at −80 °C. Cell viability of CHIKV-infected cells was determined at 6, 24 and 48 hpi by flow cytometry using the Fixable Viability Dye eFluor™ 506 (Thermo Fisher Scientific, Waltham, Mass, USA) according to the manufacturer's instructions.

2.6. Cytokine quantification

The Human Inflammatory Cytokines Cytometric Bead Array (CBA) Kit (BD Biosciences) was used for the detection of IL-1β, IL-6, IL-8, IL-10 and TNF-α in culture supernatants of monocytes and MDMs infected with the CHIKV, following manufacturer´s instructions. The detection limit was 20 pg/mL.

2.7. Quantitative real-time PCR for TLRs, IFN-I, and ISGs

Total RNA was extracted with TRIzol reagent (Thermo Fisher Scientific) following the manufacturer's instructions. For cDNA synthesis, the RevertAid Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) was used according to the manufacturer's instructions. The primers used to quantify the TLR2, TLR4, TLR7, and TLR8 mRNAs were previously reported (Giraldo et al., 2016). Furthermore, the cDNA products were amplified by RT-qPCR using a set of primers specific for diverse genes as follows: TLR3 mRNA: forward: 5´-GTCAGATTTAAAC ATTCCTCTTCGC-3´, and reverse: 5´-ATTGGGTCTGGGAACATTTCTC TTC-3´. IFN-α1 mRNA: forward: 5´-CAGAGTCACCCATCTCAGCA-3´, and reverse: 5´-CACCACCAGGACCATCAGTA-3´. IFN-β1 mRNA: 5´-CGCCGCATTGACCATCTA-3´, and reverse: 5´-GACATTAGCCAGGAGG TTTCTCA-3´. Protein kinase R (PKR) mRNA: forward: 5´-GGTACAGG TTCTACTAAACA-3´, and reverse: 5′-GAAAACTTGGCCAAATCCACC-3′. 2′−5′ Oligoadenylate synthase 1 (OAS1) mRNA: forward: 5´-GTGTGT CCAAGGTGGTAAAGG-3´, and reverse: 5´-CTGCTCAAACTTCACGGAA-3´. β-Actin mRNA: forward: 5´-ATCTGGCACCACACCTTCTACAATGA-3´, and reverse: 5´-CGTCATACTCCTGCTTGCTGATCCAC-3´. The Bio-Rad CFX manager was used to obtain the cycle thresholds (Ct) that were determined for each sample using a regression fit in the linear phase of the PCR amplification curve. The qRT-PCR was carried out using SYBR system (Invitrogen, Oregon, USA). The relative expression of each target gene was normalized to the uninfected control and to the housekeeping gene β-actin (ΔΔCt) and is reported as the fold change. A fold-change of 0.5 and 1.5 were considered as down- and up-regulation of gene expression, respectively.

2.8. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc. San Diego, CA, USA). The statistical tests are indicated in the figure legends. Data are represented as mean ± SEM. Two-Way ANOVA with Bonferroni multiple comparisons tests were performed. The production levels of pro-inflammatory cytokines were compared between CHIKV vs uninfected cells (*); UV-CHIKV vs uninfected cells (#) and CHIKV vs UV-CHIKV (+). Significant results are defined as $p<0.05$ (*, #, +), $p<0.01$ (**, ##, ++), $p<0.002$ (***, ###, +++) and $p<0.001$ (****, ####, ++++)

3. Results

3.1. Morphological and phenotypic characterization of primary human monocytes and MDMs

As has been previously reported (Daigneault et al., 2010; Holder et al., 2014; Valdés and Urcuqui-Inchima, 2018), primary human monocytes are adherent cells (monolayers) with spherical morphology and cytoplasmic projections (Fig. 1A). The average purity of the enriched monocytes in all our experiments was at least 95%. After 6 days in culture, the monocytes were differentiated into MDMs that acquired an oval cell morphology (Fig. 1B). The phenotype of monocytes and MDMs was determined by several parameters as we described previously (Arboleda et al., 2017; Valdés and Urcuqui-Inchima, 2018). Another feacture of MDMs differentiation is enhanced size and granularity versus monocytes, as demonstrated by flow cytometry (Fig. 1C and D, respectively). An increase in the cell-surface expression of CD86 and CD206 was observed on MDMs compared to monocytes (Fig. 1E and F, respectively).

3.2. CHIKV infects human monocytes and MDMs with comparable efficiencies

To evaluate the susceptibility of primary monocytes and MDMs to CHIKV infection, we performed growth kinetics of virus, where monocytes were infected with CHIKV or UV-CHIKV [We first confirmed that UV-inactivation resulted in inhibition of virus titers production by analyzing samples of inactivated virus stocks via plaque assay (data not shown)] at MOI of 10 and MDMs at MOI of 5. To examine the growth and release of the new infectious virus particles, a plaque assay was performed using the supernatants collected at 6, 12, 18, 24 and 48 hpi. We found that both, monocytes and MDMs, began to release newly produced progeny viruses at 6 hpi. The amount of virus released increased gradually over time reaching a maximum peak of 8.0–9.0 × 10^{4} PFU/ml between 18 and 24 hpi in both monocytes and MDMs (Fig. 2A and B). Thereafter, the amount of virus detected in the supernatant of both cell populations decreased strongly and independent of cell death in the following 24 h (Fig. 2A and B). Since the supernatant was collected at 6 h intervals during the first 24 hpi, the virus titer we estimated reflects the newly generated virus particles that were released during the 48 hpi evaluated. The UV-CHIKV was unable to replicate in either cell populations (Fig. 2A and B). The obtained results therefore suggest that the growth kinetics of CHIKV are equivalent in both cell populations evaluated. Moreover, the comparable yields of released infectious virus particles therefore indicate an effective replication (permissiveness) of CHIKV in both primary monocytes and MDMs.

3.3. CHIKV infection promotes a pro-inflammatory response in both monocytes and MDMs

Since we observed that CHIKV could successfully infect and replicate in human monocytes and MDMs *in vitro*, we next evaluated the production kinetics of pro-inflammatory cytokines in the infected cells. To accomplish this, the supernatants from monocyte and MDMs cultures infected with CHIKV or UV-CHIKV were collected at different time points and the production of TNF-α, IL-1β, IL-6, IL-8 and IL-10 were monitored by CBA. In monocytes, different amounts of cytokines were released and the accumulation kinetics changed according to post-infection time evaluated. The maximum peak for TNF-α was observed at 6 hpi (9558 pg/mL) for infection with both viruses, followed by a time-dependent decrease (Fig. 3A). A statistically significant difference in TNF-α secretion was found between monocyte populations infected with either CHIKV or UV-CHIKV at 6 and 24 hpi, but was no longer detected at 48 hpi. The maximum levels of production of IL-1β and IL-8 were found at 24 hpi (697 pg/ml and 62326 pg/ml, respectivelly) with the detected levels decreasing slightly after 48 hpi (Fig. 3B and D). IL-6 production levels increased from 6 hpi, reaching the highest level at 48 hpi (Fig. 3C). Remarkably, IL-10 production was significantly higher in CHIKV infection than UV-CHIKV at 48 h hpi (126 pg/mL) (Fig. 3E).

In order to clarify a potential role for MDM-derived cytokines, we examined the cytokine concentrations in culture supernatant of CHIKV or UV-CHIKV infected MDMs. As previously observed on monocytes,

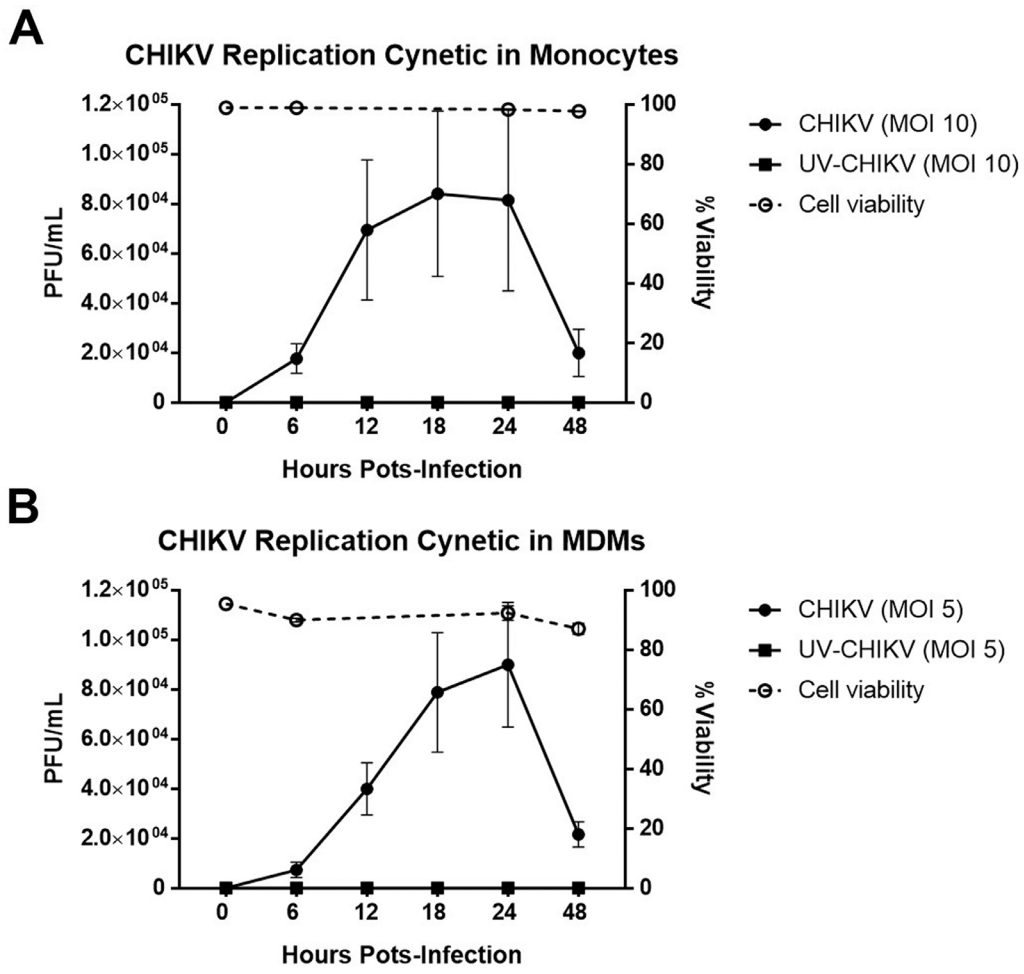

Fig. 2. Human monocytes and MDMs are target cells of CHIKV replication. Human monocytes and MDMs cultures were left uninfected or infected with CHIKV or UV-CHIKV at MOI of 10 (monocytes) or 5 (MDMs). Culture supernatants were obtained 6, 12, 18, 24 and 48 hpi and quantification of PFU/mL was performed by plaque assay. Cell viability in CHIKV-infected cells was measured at 6, 24 and 48 hpi using the Fixable Viability Dye eFluor™ 506 by flow cytometry and was expressed as a percentage of viable cells. CHIKV replication kinetics in human monocytes (A) and MDMs (B) infected with CHIKV or UV-CHIKV. Data are represented as mean ± SEM. $n = 3$ to monocytes and $n = 4$ to MDMs.

the maximum peak of TNF-α production was found at 6 hpi (2107 pg/mL). Thereafter, a drastic decrease was observed, reaching levels below 500 pg/ml at 48 hpi (Fig. 3F), for both CHIKV and UV-CHIKV. For IL-1β as well as IL-6 release, a continuous increase was observed over time (Figs. 3G and H, respectively), reaching the maximum concentration at 48 hpi (78 pg/mL and 2028 pg/mL, respectively). However, CHIKV infection led to release of higher levels of IL-1β compared to UV-CHIKV at 48 hpi. The maximum concentration of IL-8 was observed at 6 hpi and from that time, the detected concentration was stable (Fig. 3I). Neither CHIKV, nor UV-CHIKV infection led to the release of significant amounts of IL-10 and detected concentrations in the supernatant were comparable with uninfected cells (Fig. 3J). Although UV-CHIKV was unable to release infectious viral particles, we observed a pronounced response in both monocytes and MDMs. Except for the determined levels of IL-10 in monocytes and IL-1β in MDMs at 48 hpi, the UV-CHIKV-infected cells showed similar levels of cytokine production compared to CHIKV-infected cells. This suggests that the recognition of viral particles by monocytes and MDMs induces a pro-inflammatory response, independent of productive infection. However, the production of pro-inflammatory cytokines on monocytes was observed to be higher than MDMs.

3.4. CHIKV infection promotes Toll-like receptor expression in primary human monocytes and MDMs

The modulation of TLR expression could potentially explain the observed response in the examined cell types to CHIKV infection. To date and to our knowledge, there are no other reports that describe the TLR expression on primary monocytes and macrophages in response to CHIKV infection, and much less in the context of a growth kinetic. Upon analyzing the fold change patterns of TLRs in human monocytes upon infection with CHIKV, we observed an increase of the transcriptional expression of TLR2 (from 3.48-fold at 6 hpi to 4.49-fold at 24 hpi, $p = 0.07$) and TLR8 (from 1.27-fold at 6 hpi to 3.17-fold at 24 hpi, $p = 0.177$) (Fig. 4A and E). A higher level of TLR8 mRNA was detected upon infection with CHIKV compared to infection with UV-CHIKV, at 24 hpi. The mRNA expression of both TLRs decreased at 48 hpi compared to 24 hpi. High expression of TLR7 mRNA was observed at 6 hpi, followed by a significant decrease of mRNA levels at 48 hpi (from 4.48-fold at 6 hpi to 1.86-fold at 48 hpi, $p = 0.267$) (Fig. 4D). No significant changes were observed in TLR4 expression among the time points evaluated (Fig. 4C). TLR3 mRNA expression could not be detected in monocytes (Fig. 4B). Of note, similar changes were observed in the expression of TLRs evaluated after UV-CHIKV treatment, with the exception of TLR8, where no changes in the induction or modulation were observed over time (Fig. 4E).

In contrast to monocytes, no significant changes in the transcriptional expression of TLR2 and TLR8 were detected at the different time points evaluated in MDMs (Fig. 4F and J). We found statistically significant up-regulation of TLR3 mRNA expression from 6 until 48 hpi (fold change of 1.98 and 38.37, respectively, $p = 0.0149$) in response to CHIKV infection; whereas the infection with UV-CHIKV did not induce any expression of TLR3 (Fig. 4G). This indicates that TLR3 expression in MDMs is dependent of viral replication. A significant up-regulation of TLR7 mRNA was observed at 48 hpi upon both, CHIKV or CHIKV-UV infection, compared to 6 hpi (from 1.72-fold and 1.49-fold, vs 3.56-fold and 3.64-fold, respectively) (Fig. 4I). No significant changes were observed in TLR4 expression among the time points evaluated (Fig. 4H).

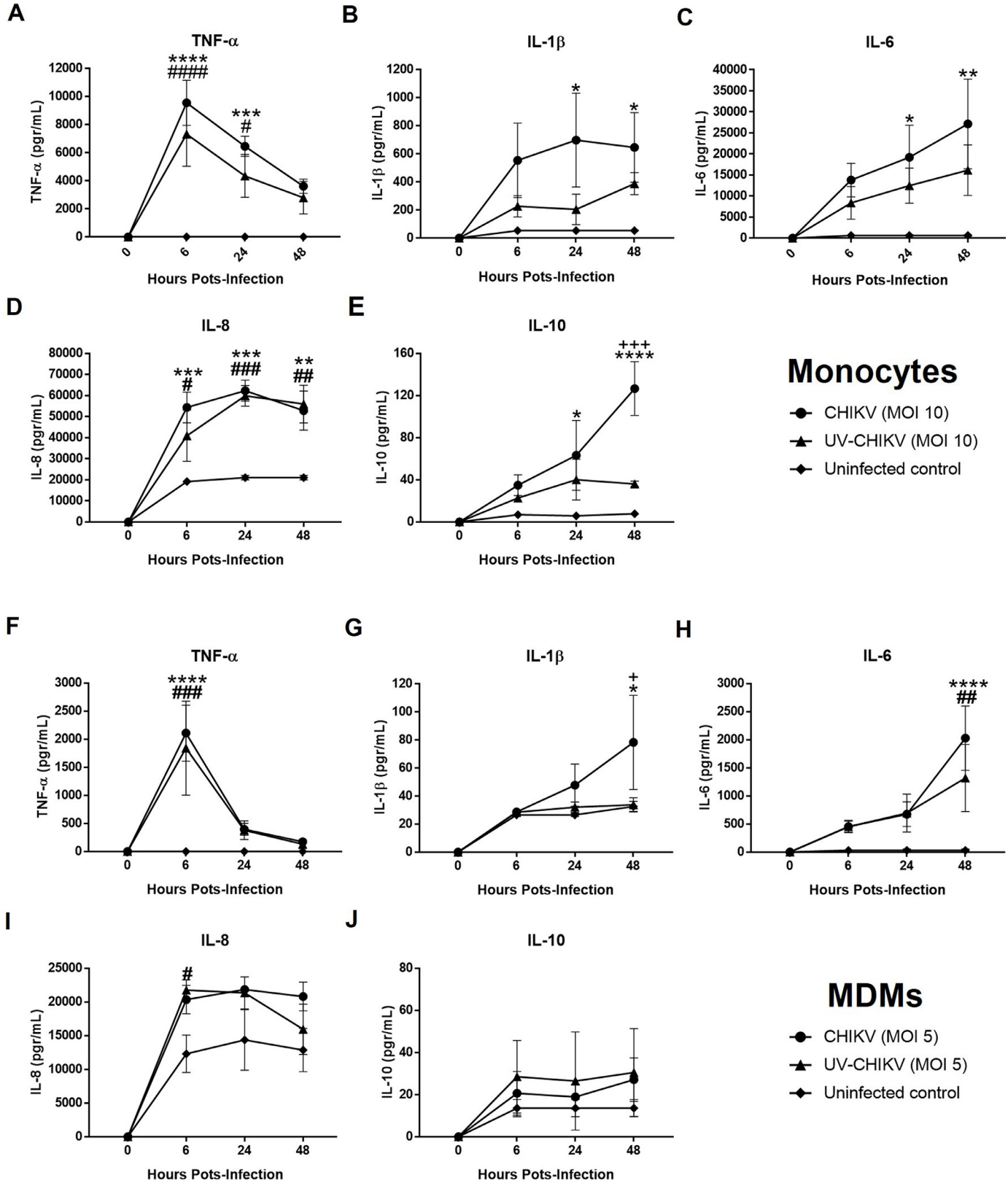

Fig. 3. CHIKV infection induces the production of pro-inflammatory cytokines in human monocytes and MDMs. Human monocytes and MDMs cultures were left uninfected or infected with CHIKV or UV-CHIKV at MOI of 10 (monocytes) or 5 (MDMs) of. Culture supernatants were obtained 6, 24 and 48 hpi and the concentrations of TNF-α, IL-1β, IL-6, IL-8 and IL-10 were quantified by CBA. Kinetics of TNF-α (A), IL-1β (B), IL-6 (C), IL-8 (D) and IL-10 (E) production in cultures of human monocytes left uninfected or infected with CHIKV or UV-CHIKV are depicted next to kinetics of TNF-α (F), IL-1β (G), IL-6 (H), IL-8 (I) and IL-10 (J) production in cultures of MDMs uninfected or infected with CHIKV or UV-CHIKV. Data are represented as the mean ± SEM. Two-Wey ANOVA with Bonferroni multiple comparisons tests were performed. Significative differences between CHIKV and uninfected control are indicated as $*p<0.05$, ** $p<0.01$, *** $p<0.002$, **** $p<0.001$; significant differences between UV-CHIKV and uninfected controls are indicated as $\#p<0.05$, ## $p<0.01$, ### $p<0.002$, #### $p<0.001$; significant differences between CHIKV and UV-CHIKV are indicated as + $p<0.05$, ++ $p<0.01$, +++ $p<0.002$, ++++ $p<0.001$. $n = 3$ to monocytes and MDMs.

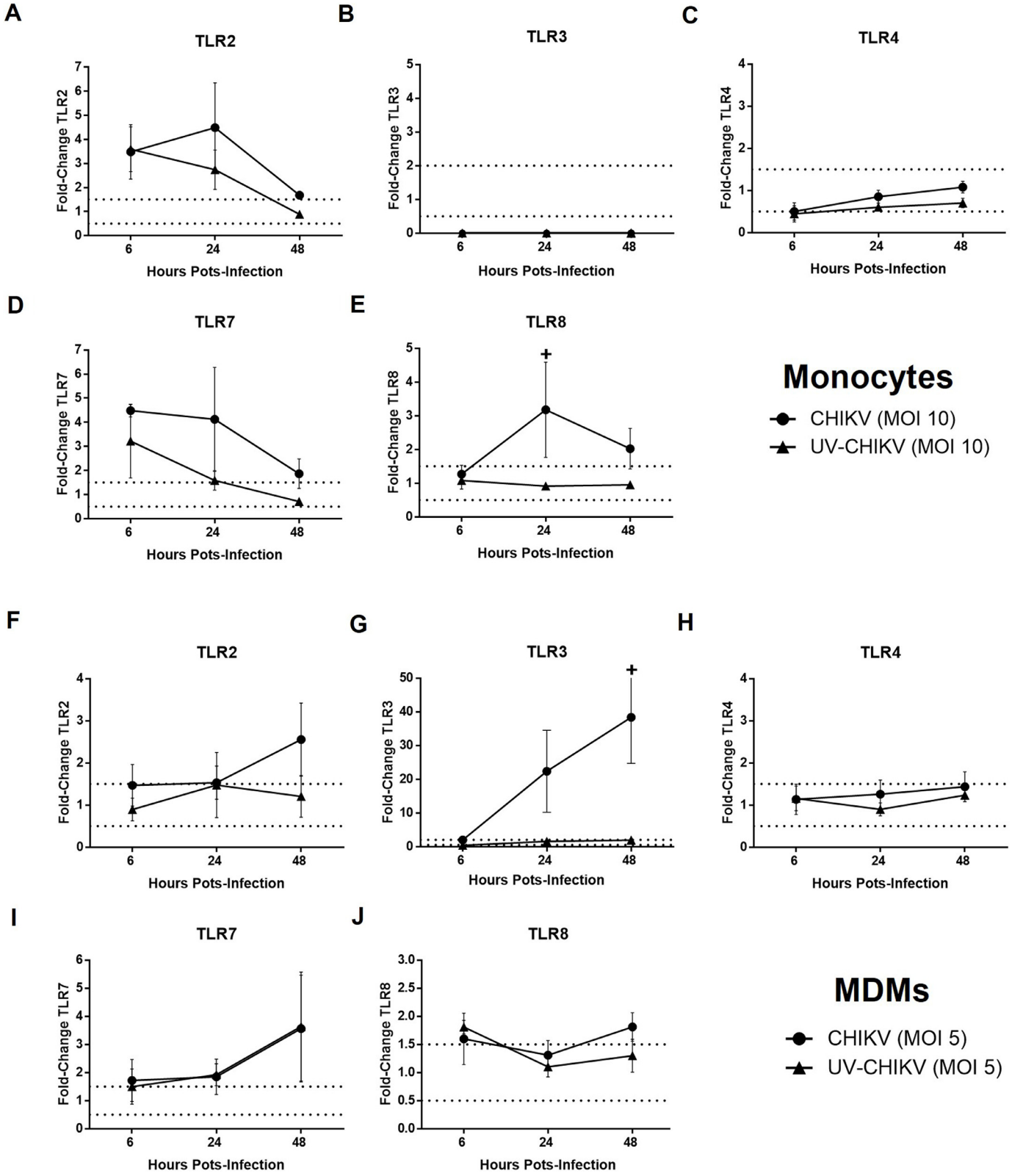

Fig. 4. CHIKV infection up-regulates the mRNA expression of TLRs in human monocytes and MDMs. Human monocytes and MDMs cultures were left uninfected or infected with CHIKV or UV-CHIKV at MOI of 10 (monocytes) or 5 (MDMs). Cell lysates were obtained 6, 24 and 48 hpi and RT-qPCR was performed. Fold-change in mRNA expression of TLR2 (A), TLR3 (B), TLR4 (C), TLR7 (D) and TLR8 (E) in human monocytes infected with CHIKV or with UV-CHIKV is depicted next to fold-change in mRNA expression of TLR2 (F), TLR3 (G), TLR4 (H), TLR7 (I) and TLR8 (J) in MDMs infected with CHIKV or with UV-CHIKV. A fold-change of 0.5 and 1.5 was considered as down-regulation and up-regulation of gene expression, respectively. Data are represented as the mean ± SEM. Two-Wey ANOVA with Bonferroni multiple comparisons tests were performed; significant differences between CHIKV and UV-CHIKV are indicated as + $p<0.05$, + + $p<0.01$, + + + $p<0.002$, + + + + $p<0.001$. $n = 3$ to monocytes and MDMs.

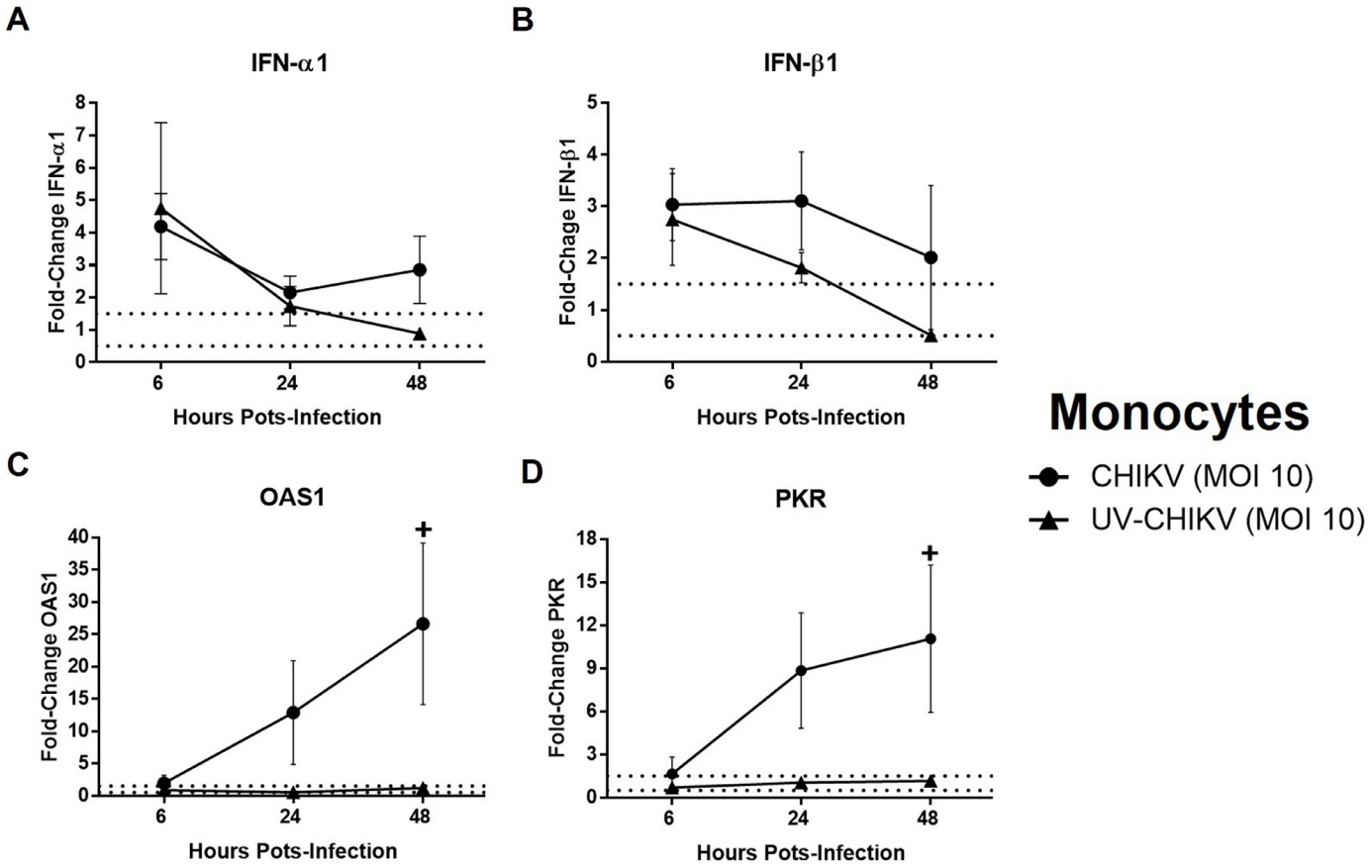

Fig. 5. CHIKV infection modulates the mRNA expression of IFN-I and ISGs in human monocytes. Human monocytes cultures were left uninfected or infected with CHIKV or UV-CHIKV at MOI of 10. Cell lysates were obtained 6, 24 and 48 hpi and RT-qPCR was performed. Fold-changes in mRNA expression of IFN-α1 (A), IFN-β1 (B), PKR (C) and OAS1 (D) in human monocytes infected with CHIKV or UV-CHIKV were determind. A fold-change of 0.5 and 1.5 was considered as down-regulation or up-regulation of gene expression, respectively. Data are represented as the mean ± SEM. Two-Wey ANOVA with Bonferroni multiple comparisons tests was performed; significant differences between CHIKV and UV-CHIKV are indicated as + $p<0.05$, + + $p<0.01$, + + + $p<0.002$, + + + + $p<0.001$. $n = 3$ to monocytes and MDMs.

Interestingly, these differences in the profile of TLRs expressed by monocytes and MDMs in response to CHIKV indicate a distinct response of both cell populations to CHIKV infection.

3.5. CHIKV infection of monocytes and MDMs induces an antiviral response

Activation of TLRs leads to the production of pro-inflammatory cytokines including IFN-I. Since we observed early modulation of TLR expression in response to CHIKV infection, we attempted to assess the mRNA expression levels of IFN-α1, IFN-β1, and various ISGs, such as PKR and OAS1 in monocytes and MDMs in response to CHIKV or UV-CHIKV infection. As expected, infection with both CHIKV and UV-CHIKV increased the mRNA expression levels of IFN-α1 (fold change of 4.19 and 4.75, respectively) (Fig. 5A) and IFN-β1 (fold change of 3.03 and 2.75, respectively) (Fig. 5B) in monocytes rapidly. In the following, the expression of both IFNs decreases gradually over time, resulting in low transcriptional levels at 48 hpi compared to 6 hpi ($p = 0.844$ for IFN-α1 and $p = 0.249$ for IFN-β1). However, different kinetic was observed in the expression of ISGs. A significant increase in the mRNA levels of OAS1 (1.95 and 26.63, respectively, $p = 0.0428$) from 6 until 48 hpi (Fig. 5C) and PKR (1.65 and 11.1, respectively, $p = 0.09$) from 6 until 48 hpi (Fig. 5D) was observed. Since this increases were only found in response to CHIKV infection, the obtained results suggest that OAS1 and PKR could play a critical role in the antiviral response against CHIKV infection in human monocytes.

In contrast to monocytes, the level expression of both IFN-I in MDMs was substantially lower in response to CHIKV infection at the time points evaluated (Fig. 6A and B). However, the infected CHIKV-MDMs also showed an up-regulation of OAS1 mRNA levels from 6 to 48 hpi (fold change of 5.79 vs 18.31, $p = 0.153$, respectively) (Fig. 6C) and PKR mRNA levels from 6 to 48 hpi (fold change of 3.94 vs 11.95, $p = 0.06$, respectively) (Fig. 6D). UV-CHIKV infection had no effect on the OAS1 and PKR mRNA expression levels in human MDMs. The observed induction of the expression of these ISGs as well as TLR3 could be related to the marked decrease of viral titer over time observed in supernatants of monocytes and MDMs cultures infected with CHIKV at 48 hpi (compare Fig. 2 with Figs. 4G, 5 and 6), suggesting an important role of ISGs in the induction of the antiviral state and control of CHIKV replication in monocytes and macrophages.

4. Discussion

Infections caused by emerging RNA viruses, such as CHIKV, have had a major economic impact and are a threat to public health worldwide. There are neither effective treatments nor vaccines available against CHIKV and therefore studies on the pathophysiological characteristics are of crucial importance for combating these viral pathogens. Monocytes and macrophages are the main components of the innate immune response and have been shown to be involved in the immunopathogenesis of different diseases of viral origin (Grieder and Nguyen, 1996; Coleman and Wu, 2009). Both cell populations have been linked to the pathogenesis of CHIKV and demonstrated to be recruited to CHIKV-infected tissues, promoting an inflammatory response

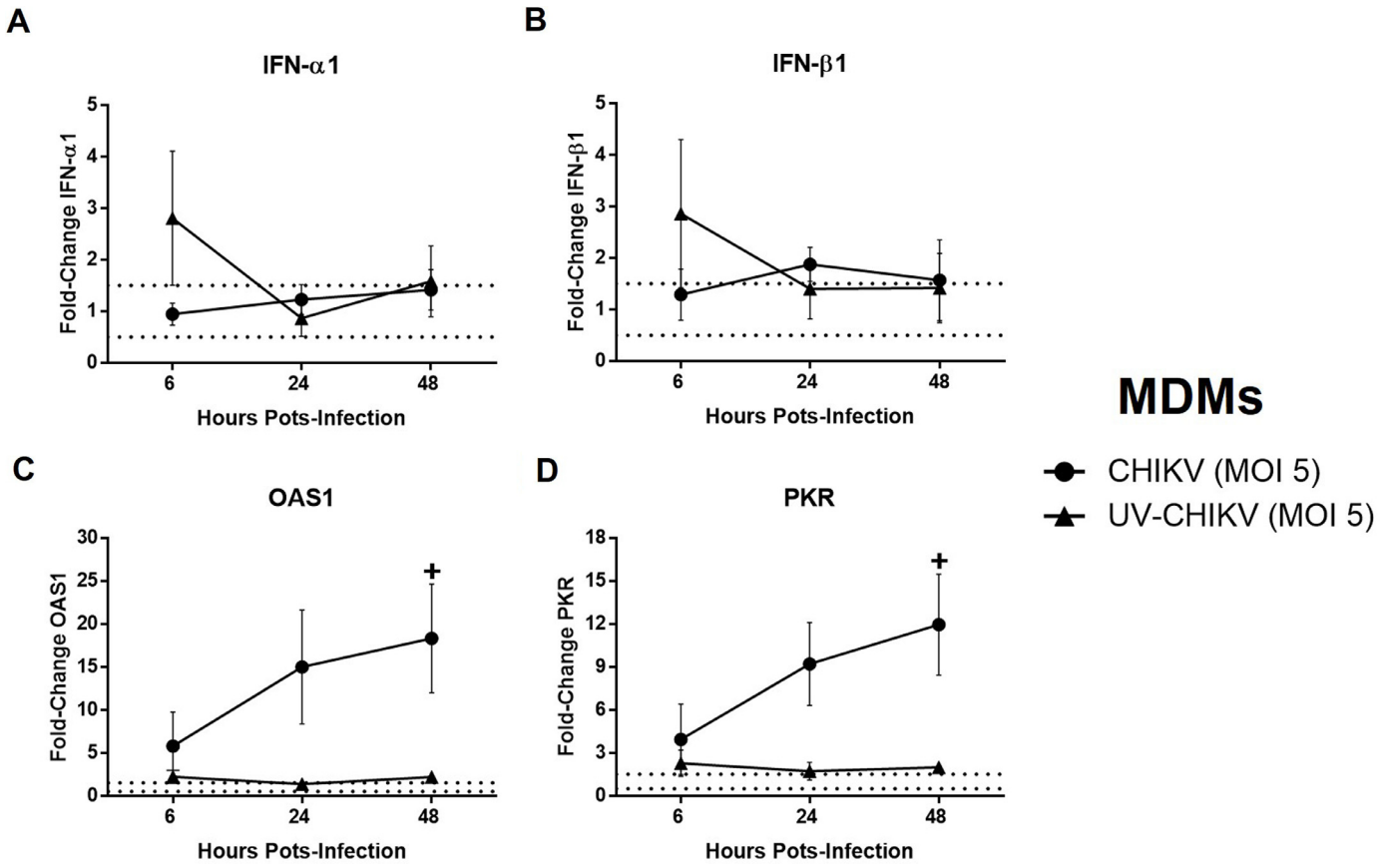

Fig. 6. CHIKV infection modulates the mRNA expression of IFN-I and ISGs in human MDMs. Human MDMs cultures were left uninfected or infected with CHIKV or UV-CHIKV at MOI of 5. Cell lysates were obtained 6, 24 and 48 hpi and RT-qPCR was performed. Fold-changes in mRNA expression of IFN-α1 (A), IFN-β1 (B), PKR (C) and OAS1 (D) in MDMs infected with CHIKV or UV-CHIKV are presented. A fold-change of 0.5 and 1.5 was considered as down-regulation or up-regulation of gene expression, respectively. Data are represented as the mean ± SEM. Two-Wey ANOVA with Bonferroni multiple comparisons tests was performed; significant differences between CHIKV and UV-CHIKV are indicated as + $p<0.05$, ++ $p<0.01$, +++ $p<0.002$, ++++ $p<0.001$. $n = 3$ to monocytes and MDMs.

(Poo et al., 2014). In addition, it has been suggested that macrophages could act as reservoirs of the virus in persistent infections in macaques (a non-human primate model) (Labadie et al., 2010). However, the role of monocytes and macrophages in the control of CHIKV replication and the establishment of an innate antiviral response is poorly understood. As previously reported (Sourisseau et al., 2007; Her et al., 2010), we confirmed that human monocytes and MDMs are target cells of CHIKV replication *in vitro* and both induce a pro-inflammatory response to CHIKV and UV-CHIKV infection. Nevertheless, Monocytes and macrophages have important differences in morphological and phenotypic features that lead to both cell populations presenting a differential response to the same stimulus (McCullough et al., 1999; Italiani and Boraschi, 2014; Gautier and Yvan-Charvet, 2014; Valdés and Urcuqui-Inchima, 2018).

Here we show that primary monocytes and MDMs are susceptible to CHIKV infection and that an immediate constant increase in virus titer occurs as soon as 6 hpi, with a maximum peak between 18 and 24 hpi, indicating that the replication cycle of this virus is comparably short. Since viral production declined quickly over time, our results suggest that there was no *de novo* production at later time points as evidenced by a decreased titer determined at 48 hpi, potentially associated with the develpment of an antiviral state in the infected cell. In our virus growth kinetics analysis, we found no differences between the behavior of CHIKV infection in monocytes and MDMs. At MOI of 5 and 10, we observed comparable infection rates between monocytes and MDMs. Previously, it was reported that monocytes from acute chikungunya fever patients were the main targets for CHIKV in peripheral blood (Her et al., 2010). Similar results were observed when the authors used purified CD14+ monocytes, i.e. these cells were infected with CHIKV. In contrast, Sourisseau et al. (2007) reported that CHIKV did not replicate in primary human monocytes, but macrophages derived from human monocyte cultures supplemented with macrophage colony-stimulating factor were able to support CHIKV infection and replication. A possible explanation for this discrepancy could be found in the differences in monocyte culture conditions used in the two studies. Contrary to Sourisseau et al. (2007), we obtained the CD14+ cells by adhesion to the culture dish for 2 h and supplemented with 0.5% autologous serum. However, CHIKV infection did occur in monocytes using a medium with heat-inactivated FBS. The reason why viral production declined quickly over time in both, monocytes and macrophages, could be due to the inhibition of CHIKV replication by ISGs such as PKR and OAS1, whose expression is involved in mediating the inhibition of CHIKV replication. Furthermore, It has been reported that CHIKV is highly sensitive to the antiviral activity mediated by IFN in infected human epithelial, endothelial and nonhematopoietic cells (Schilte et al., 2010).

We observed three important moments in the CHIKV replication cycle in monocytes: at an early time (6 hpi), characterized by an initial release of infectious viral particles, a high production of pro-inflammatory cytokines (IL-6 and TNF-α) and up-regulation of mRNA expression of type I IFNs, TLR2 and TLR7; at an intermediate time (18–24 hpi) in which viral replication was maximal and is linked to the second peak of pro-inflammatory cytokine production (IL-1β and IL-8) which coincided with the up-regulation of mRNA expression of TLR2 and TLR8, and also with up-regulation of mRNA expression of antiviral genes such as PKR and OAS1. Finally, we reported a late time point (48 hpi) characterized by a high production/accumulation of IL-6 and IL-10

and down-regulation of mRNA expression of TLRs and IFN-β, which is potentially associated with the initial control of the inflammatory response. However, in the latest time point a higher transcriptional expression of PKR and OAS1 was observed, which coincided with the arrest in the production/accumulation of new infective viral particle, suggesting the establishment of an antiviral state in the infected cells.

In the case of MDMs, also three important moments in the CHIKV replication cycle were observed: an early time point (6 hpi), characterized by an initial release of infectious viral particles, a high production of pro-inflammatory cytokines, but not at the same level as monocytes, and a stronger up-regulation of mRNA expression of PKR, OAS1 and TLR3 compared to monocytes. However, and unlike what occurred in monocytes, there was no increase in mRNA expression of TLR2 and TLR7, suggesting a lower activation of these TLRs in MDMs, which might be associated with a lower pro-inflammatory response of MDMs to CHIKV infection. We defined a middle time (18–24 hpi) in which viral replication was maximal with a decreasing production/accumulation of TNF-α and an up-regulation of mRNA expression of TLR3, TLR7, PKR and OAS1. Finally, a late time point (48 hpi), characterized by the accumulation/production of low levels of IL-6 and IL-1β, accompanied by a higher transcriptional expression of TLR3 and TLR7, and also PKR and OAS1 mRNAs which coincided with a marked decrease in viral titers in supernatants. These characteristics of the late time point suggest, similar to earlier observations in infected human monocytes, the establishment of an antiviral state in MDMs and the crucial role of ISGs in their control of CHIKV replication.

Like CHIKV infection, we reported that UV-CHIKV infection in monocytes and MDMs induced the production of pro-inflammatory cytokines and expression of TLRs in a comparable level to CHIKV, suggesting that the recognition of viral genome and/or structural components present in viral particles lead to the activation of monocytes and MDMs and subsequent induction of an inflammatory, but not antiviral response.

Differences in the response to TLR activation have been observed between monocytes and some populations of macrophages. For example, strong induction of IL-1β following TLR2 or TLR4 activation was reported in monocytes, while this response is down-regulated in alveolar macrophages (Wewers et al., 1984; Daigneault et al., 2010). Our results show that monocytes and MDMs exhibit differential responses to CHIKV infection. While monocytes induced a stronger pro-inflammatory response (high levels of IL-1β, IL-6, IL-8 and TNF-α) to CHIKV infection at early time points of infection (6 hpi), which appear stable until later time points (48 hpi); the production of these cytokines in the MDMs was low. Previous studies on the cytokine expression patterns in patients infected with CHIKV yielded broadly heterogeneous results (Hoarau et al., 2010; Chow et al., 2011; Dupuis-Maguiraga et al., 2012; Lohachanakul et al., 2012). However, a correlation between increased levels of serum IL-6 in patients with CHIKV fever (CHIKF), was determined to be associated with further progress of the disease into persistent arthralgia and severe CHIKF (Chow et al., 2011; Lohachanakul et al., 2012). Furthermore, high levels of TNF-α have been hypothesized to be linked to the development of a chronic inflammatory condition resulting in arthritis and rheumatoid arthritis in affected patients (Vasanthi et al., 2007). This effect has been reported for CHIKV patients which were found to exhibit increased levels of TNF-α after infection (Dupuis-Maguiraga et al., 2012). Her et al. (2010), using whole blood samples from normal healthy donors, observed that the stimulation with CHIKV furthermore induced a significantly increased production of immune mediators including IL-6, with the virus being detected predominantly in the monocytes after CHIKV inoculation.

TLRs, perhaps the best-characterized class of PRRs in mammalian species, play an important role in the recognition of viral components and activation of innate immunity, which then leads to the development of adaptive immune responses (Aderem and Ulevitch, 2000). Up-regulation of TLR-expression was reported to be involved in priming pro-inflammatory responses against various viruses (Tsai et al., 2009; Okamoto et al., 2017). Until now there are no other reports describing TLR expression patterns for CHIKV-infected primary human monocytes and MDMs. One of the advantages of the presented study is that the mRNA expression of TLRs was studied at three distinct time points. In this way, we can infer the specific time at which the TLRs are induced in response to CHIKV infection, establishing their possible role. Our results show that the expression of TLR2 and TLR7 mRNAs was up-regulated in primary human monocytes infected with CHIKV at early time points of infection, whereas the expression of TLR8 mRNA was up-regulated at 24 hpi. However, after 24 hpi, the expression of these TLRs was downregulated, reaching minimum levels at 48 hpi, possibly by the reduction of the viral titer in culture. In MDMs, we observed a poor upregulation of TLR2 and TLR7 mRNA-expression early (6 hpi) in CHIKV infection, which could explain the low production of pro-inflammatory cytokines by MDMs in response to CHIKV infection. However, the mRNA expression of TLR3 and TLR7 were up-regulated at 24 hpi, reaching higher levels at 48 hpi, suggesting a possible role of both TLRs in the recognition of viral RNAs and control of viral replication. It has previously been proposed that TLR3 activation in response to CHIKV infection plays a crucial role in the control of CHIKV replication and the establishment of an antiviral response. Her et al. (2015) reported that susceptibility to CHIKV infection was markedly increased in human and mouse fibroblasts with defective TLR3 signaling. Moreover, Li et al. (2012a,b) reported that treatment of human bronchial epithelial-derived cells, BEAS-2B, with poly(I:C), a synthetic ligand of TLR3, suppressed the cytopathic effect induced by CHIKV infection and inhibited virus replication. This inhibition was hypothesized to be mediated through the induction of IFN production and the subsequent activation of ISGs involved in the antiviral response, such as the OAS/RNase L pathway and human myxovirus resistance protein A (MxA). We reported that MDMs, but not monocytes, strongly up-regulate TLR3 expression in response to CHIKV infection, indicating a possible role of TLR3 in the recognition of CHIKV replicative intermediates and induction of antiviral response in MDMs. On the other hand, Dutta and Tripathi (2017) reported a probable association of different single nucleotide polymorphisms among TLR7/8 with CHIKV infection susceptibility and level of pro-inflammatory cytokine production. We report that human monocytes up-regulate TLR7/8 expression whereas MDMs only up-regulate TLR7 expression, both in response to CHIKV and UV-CHIKV infection, indicating a potential role of these TLRs in recognition of CHIKV RNAs and the downstream induction of pro-inflammatory and the antiviral response. TLR2 is a cell surface TLR that has been found to recognize viral proteins, such as the non-structural protein 1 (NS1) of DENV or the core and NS3 proteins of Hepatitis C virus (Dolganiuc et al., 2004; Chen et al., 2015). TLR2 activation in response to CHIKV infection has not been previously reported; therefore, the role of TLR2 in the induction of inflammatory and/or antiviral response to CHIKV infection is unknown. We report that human monocytes up-regulated TLR2 mRNA expression in response to CHIKV and UV-CHIKV infection, suggesting a potential role of TLR2 in recognition of PAPMs present in the viral particle and the subsequent induction of inflammatory and/or antiviral responses to CHIKV infection. However, the role of TLR2, 3 and TLR7/8 in the induction of inflammatory and antiviral response to CHIKV infection in monocytes and macrophages needs further investigation.

IFN-I is the first barrier to control CHIKV replication and dissemination in various tissues (Schilte et al., 2010; Prow et al., 2017; Lane et al., 2018). IFN-I induces an antiviral response in infected and uninfected cells through induction of the expression of manifold ISGs, such as PKR, OAS/RNase L, MxA, viperin and ISG15, all of which have been reported to play a role in the control of CHKV replication (Werneke et al., 2011; White et al., 2011; Teng et al., 2012; Li et al., 2016). We report that human monocytes express higher levels of mRNA of IFN-α1 and β1 in response to CHIKV and UV-CHIKV infection than MDMs infected with CHIKV. However, both cell populations control

CHIKV replication and establish an antiviral response possibly through the expression of ISGs such as PKR and OAS1 in response to CHIKV infection. Notably, monocytes and MDMs induced IFN-I mRNA expression in response to UV-CHIKV infection at early time points. However, only infection with the replicative virus up-regulated ISGs expression, indicating a possible post-transcriptional control of IFN-I production that is dependent on active viral replication. Different reports have shown that miRNAs regulate the 3′-UTRs of IFN-I. Witwer et al. (2010) reported that poly(I:C)-stimulated miRNAs let7b, miR-145, miR-26a, and miR-34a act as regulators of IFN-β in human and macaque MDMs. Interestingly, these miRNAs repress IFN-β expression and protein output, thereby modulating IFN production in response to TLR3 stimulation. Additionally, it has been reported that miR-4661 targets the 3′ UTRs of 9 subtypes of IFN-α and its overexpression in human macrophages led to robust down-regulation of IFN-α gene expression in a subtype-specific manner (Li et al., 2012a,b). However, further investigation is necessary to shed light on a possible role of miRNAs in control of IFN-I production in monocytes and MDMs in response to CHIKV.

As previously discussed, monocytes and macrophages have been implicated in some aspects of CHIKV pathogenesis. However, even if these cells are targets exploited for CHIKV replication, we report that CHIKV infection in human monocytes and MDMs induces a pro-inflammatory response and the establishment of an antiviral response. These might lead to the control of CHIKV replication in the infected cells, possibly through the expression of IFN-I and the induction of the antiviral state through the expression of numerous ISGs. This shows an important role of monocytes and macrophages in the induction of pro-inflammatory and antiviral responses to control CHIKV replication in humans.

5. Conclusions

We reported that human monocytes and MDMs are susceptible and permissive cells to CHIKV infection *in vitro*. In addition, CHIKV challenge induces monocyte and macrophage activation, leading to high pro-inflammatory cytokine production and IFN-I expression. This response, although it could be involved in the pathogenesis of CHIKV, could also involve in induction of an antiviral state through the expression of ISGs, thus contributing to the control of viral replication. Furthermore, CHIKV challenge induced expression of TLR2, 7 and 8 on monocytes, and TLR3 and 7 on MDMs, suggesting a differential response of both cell populations to CHIKV infection.

According to our results with UV-CHIKV, we suggested that recognition of structural components on CHIKV leads to monocyte and macrophage activation, involving a high production of pro-inflammatory cytokines, but not the expression of ISGs.

Funding

This study was supported by Colciencias [grant Number 111574455028], Colombia and Universidad de Antioquia, UdeA(CODI-2017-16389). The funders had no role in study design, data collection and analyses, decision to publish, or preparation of the manuscript.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to thank the blood bank of the “Escuela de Microbiologia, UdeA, Medellín Colombia” for providing us with leukocyte-enriched blood units from healthy individuals.

References

Aderem, A., Ulevitch, R.J., 2000. Toll-like receptors in the induction of the innate immune response. Nature 406 (6797), 782–787. https://doi.org/10.1038/35021228.
Arboleda, A., F., J., Rodenhuis-Zybert, I.A., Hernández, J.C., Smit, J.M., Urcuqui-Inchima, S., 2017. Human macrophages differentiated in the presence of vitamin D3 restrict dengue virus infection and innate responses by downregulating mannose receptor expression. PLoS Neglect. Trop. D 11 (10), e0005904. https://doi.org/10.1371/journal.pntd.0005904.
Chen, J., Ng, M.M.L., Chu, J.J.H., 2015. Activation of TLR2 and TLR6 by dengue NS1 protein and its implications in the immunopathogenesis of dengue virus infection. PLoS Pathog. 11 (7), e1005053. https://doi.org/10.1371/journal.ppat.1005053.
Chow, A., Her, Z., Ong, E.K.S., Chen, J., Dimatatac, F., Kwek, D.J.C., Barkham, T., Yang, H., Rénia, L., Leo, Y.S., Ng, L.F.P., 2011. Persistent arthralgia induced by Chikungunya virus infection is associated with interleukin-6 and granulocyte macrophage colony-stimulating factor. J. Infect. Dis. 203 (2), 149–157. https://doi.org/10.1093/infdis/jiq042.
Coleman, C.M., Wu, L., 2009. HIV interactions with monocytes and dendritic cells: viral latency and reservoirs. Retrovirology 6 (51), 1–12. https://doi.org/10.1186/1742-4690-6-51.
Daigneault, M., Preston, J.a., Marriott, H.M., Whyte, M.K.B., Dockrell, D.H., 2010. The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages. PLoS ONE 5 (1), e8668. https://doi.org/10.1371/journal.pone.0008668.
Dolganiuc, A., Oak, S., Kodys, K., Golenbock, D.T., Finberg, R.W., Kurt-Jones, E., Szabo, G., 2004. Hepatitis C core and nonstructural 3 proteins trigger toll-like receptor 2-mediated pathways and inflammatory activation. Gastroenterology 127 (5), 1513–1524. https://doi.org/10.1053/j.gastro.2004.08.067.
Dupuis-Maguiraga, L., Noret, M., Brun, S., Le Grand, R., Gras, G., Roques, P., 2012. Chikungunya disease: infection-associated markers from the acute to the chronic phase of arbovirus-induced arthralgia. PLoS Neglect. Trop. D 6 (3), e1446. https://doi.org/10.1371/journal.pntd.0001446.
Dutta, S.K., Tripathi, A., 2017. Association of toll-like receptor polymorphisms with susceptibility to chikungunya virus infection. Virology 511 (March), 207–213. https://doi.org/10.1016/j.virol.2017.08.009.
Gardner, J., Anraku, I., Le, T.T., Larcher, T., Major, L., Roques, P., Schroder, W.A., Higgs, S., Suhrbier, A., 2010. Chikungunya virus arthritis in adult wild-type mice †. J. Virol. 84 (16), 8021–8032. https://doi.org/10.1128/JVI.02603-09.
Gasque, P., Couderc, T., Lecuit, M., Roques, P., Ng, L.F.P., 2015. Chikungunya virus pathogenesis and immunity. Vector Borne Zoonotic Dis. 15 (4), 241–249. https://doi.org/10.1089/vbz.2014.1710.
Gautier, E.L., Yvan-Charvet, L., 2014. Understanding macrophage diversity at the ontogenic and transcriptomic levels. Immunol. Rev. 262 (1), 85–95. https://doi.org/10.1111/imr.12231.
Giraldo, D.M., Juan, G., Hernandez, C., Velilla, P., Hernandez, J.C., Urcuqui-inchima, S., 2016. HIV-1 – neutrophil interactions trigger neutrophil activation and Toll-like receptor expression. Immunol. Res. 64 (1), 93–103. https://doi.org/10.1007/s12026-015-8691-8.
Goupil, B.A., Mores, C.N., 2016. A review of chikungunya virus-induced arthralgia: clinical manifestations, therapeutics, and pathogenesis. Open Rheumatol. J. 10, 129–140. https://doi.org/10.2174/1874312901610010129.
Grieder, F.B., Nguyen, H.T., 1996. Virulent and attenuated mutant Venezuelan equine encephalitis virus show marked differences in replication in infection in murine macrophages. Microb. Pathog. 21 (2), 85–95. https://doi.org/10.1006/mpat.1996.0045.
Her, Z., Malleret, B., Chan, M., Ong, E.K.S., Wong, S.C., Kwek, D.J.C., Tolou, H., Lin, R.T.P., Tambyah, P.A., Rénia, L., Ng, L.F.P., 2010. Active infection of human blood monocytes by chikungunya virus triggers an innate immune response. J. Immunol. 184 (10), 5903–5913. https://doi.org/10.4049/jimmunol.0904181.
Her, Z., Teng, T.-.S., Tan, J.J., Teo, T.-.H., Kam, Y.-.W., Lum, F.M., Lee, W.W., Gabriel, C., Melchiotti, R., Andiappan, A.K., Lulla, V., Lulla, A., Win, M.K., Chow, A., Biswas, S.K., Leo, Y.-.S., Lecuit, M., Merits, A., Renia, L., Ng, L.F., 2015. Loss of TLR3 aggravates CHIKV replication and pathology due to an altered virus-specific neutralizing antibody response. EMBO Mol. Med. 7 (1), 24–41. https://doi.org/10.15252/emmm.201404459.
Hoarau, J.-.J., Jaffar Bandjee, M.-.C., Krejbich Trotot, P., Das, T., Li-Pat-Yuen, G., Dassa, B., Denizot, M., Guichard, E., Ribera, A., Henni, T., Tallet, F., Moiton, M.P., Gauzere, B.A., Bruniquet, S., Jaffar Bandjee, Z., Morbidelli, P., Martigny, G., Jolivet, M., Gay, F., Grandadam, M., Tolou, H., Vieillard, V., Debre, P., Autran, B., Gasque, P., 2010. Persistent chronic inflammation and infection by Chikungunya arthritogenic alphavirus in spite of a robust host immune response. J. Immunol. 184 (10), 5914–5927. https://doi.org/10.4049/jimmunol.0900255.
Holder, G.E., McGary, C.M., Johnson, E.M., Zheng, R., John, V.T., Sugimoto, C., Kuroda, M.J., Kim, W.K., 2014. Expression of the mannose receptor CD206 in HIV and SIV encephalitis: a phenotypic switch of brain perivascular macrophages with virus infection. J. Neuroimmune Pharmacol. 9 (5), 716–726. https://doi.org/10.1007/s11481-014-9564-y.
Italiani, P., Boraschi, D., 2014. From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. Front. Immunol. 5, 514. https://doi.org/10.3389/fimmu.2014.00514.
Iwasaki, A., Medzhitov, R., 2004. Toll-like receptor control of the adaptive immune responses. Nat. Immunol. 5 (10), 987–995. https://doi.org/10.1038/ni1112.
Janeway, C.A., Medzhitov, R., 2002. Innate immune recognition. Annu. Rev. Immunol. 20

(1), 197–216. https://doi.org/10.1146/annurev.immunol.20.083001.084359.
Juarez, D., Long, K.C., Aguilar, P., Kochel, T.J., Halsey, E.S., 2013. Assessment of plaque assay methods for alphaviruses. J. Virol Methods. 187, 185–189. https://doi.org/10.1016/j.jviromet.2012.09.026.
Labadie, K., Larcher, T., Joubert, C., Mannioui, A., Delache, B., Brochard, P., Guigand, L., Dubreil, L., Lebon, P., Verrier, B., de Lamballerie, X., Suhrbier, A., Cherel, Y., Le Grand, R.R.P., 2010. Chikungunya disease in nonhuman primates leads to long-term viral persistence in macrophages. J. Clin. Invest. 120 (3), 894–906. https://doi.org/10.1172/JCI40104.
Lane, W.C., Dunn, M.D., Gardner, C.L., Lam, L.K.M., Watson, A.M., Hartman, A.L., Ryman, Kate, D., Klimstra, W.B., 2018. The efficacy of the interferon alpha/beta response versus arboviruses is temperature dependent. MBio 9 (2), 1–22. https://doi.org/10.1128/mBio.00535-18.
Lang, P.A., Recher, M., Honke, N., Scheu, S., Borkens, S., Gailus, N., Krings, C., Meryk, A., Kulawik, A., Cervantes-Barragan, L., Van Rooijen, N., Kalinke, U., Ludewig, B., Hengartner, H., Harris, N., Häussinger, D., Ohashi, P.S., Zinkernagel, R.M., Lang, K.S., 2010. Tissue macrophages suppress viral replication and prevent severe immunopathology in an interferon-I-dependent manner in mice. Hepatology 52 (1), 25–32. https://doi.org/10.1002/hep.23640.
Lester, S.N., Li, K., 2014. Toll-like receptors in antiviral innate immunity. J. Mol. Biol. 426 (6), 1246–1264. https://doi.org/10.1016/j.jmb.2013.11.024.
Li, Y.G., Siripanyaphinyo, U., Tumkosit, U., Noranate, N., A-Nuegoonpipat, A., Pan, Y., Kameoka, M., Kurosu, T., Ikuta, K., Takeda, N., Anantapreecha, S., 2012a. Poly (I:C), an agonist of toll-like receptor-3, inhibits replication of the Chikungunya virus in BEAS-2B cells. Virol. J. 9, 1–8. https://doi.org/10.1186/1743-422X-9-114.
Li, Y., Fan, X., He, X., Sun, H., Zou, Z., Yuan, H., Xu, H., Wang, C., Shi, X., 2012b. MicroRNA-466l inhibits antiviral innate immune response by targeting interferon-alpha. Cell Mol. Immunol. 9 (6), 497–502. https://doi.org/10.1038/cmi.2012.35.
Li, Y., Banerjee, S., Wang, Y., Goldstein, S.A., Dong, B., Gaughan, C., Silverman, R.H., Weiss, S.R., 2016. Activation of RNase L is dependent on OAS3 expression during infection with diverse human viruses. PNAS 113 (8), 2241–2246. https://doi.org/10.1073/pnas.1519657113.
Lo Presti, A., Lai, A., Cella, E., Zehender, G., Ciccozzi, M., 2014. Chikungunya virus, epidemiology, clinics and phylogenesis: a review. Asian Pac. J. Trop. Med. 7 (12), 925–932. https://doi.org/10.1016/S1995-7645(14)60164-4.
Lohachanakul, J., Phuklia, W., Thannagith, M., Thonsakulprasert, T., 2012. High concentrations of circulating interleukin-6 and monocyte chemotactic protein-1 with low concentrations of interleukin-8 were associated with severe chikungunya fever during the 2009 – 2010 outbreak in Thailand. Microbiol. Immunol. 56, 134–138. https://doi.org/10.1111/j.1348-0421.2011.0417.x.
McCullough, K.C., Basta, S., Knötig, S., Gerber, H., Schaffner, R., Kim, Y.B., Saalmüller, A., Summerfield, A., 1999. Intermediate stages in monocyte-macrophage differentiation modulate phenotype and susceptibility to virus infection. Immunology 98 (2), 203–212. https://doi.org/10.1046/j.1365-2567.1999.00867.x.
Michlmayr, D., Andrade, P., Gonzalez, K., Balmaseda, A., Harris, E., 2017. CD14 + CD16 + monocytes are the main target of Zika virus infection in peripheral blood mononuclear cells in a paediatric study in Nicaragua. Nat. Microbiol. 2 (11), 1462–1470. https://doi.org/10.1038/s41564-017-0035-0.
Morrison, T.E., 2014. Reemergence of Chikungunya virus. J. Virol. 88 (20), 11644–11647. https://doi.org/10.1128/JVI.01432-14.
Ng, L.F.P., Chow, A., Sun, Y., Kwek, D.J.C., Lim, P., Ng, L., Ooi, E., Choo, K.-H., Her, Z., Kourilsky, P., Leo, S., 2009. IL-1B, IL-6, and RANTES as biomarkers of Chikungunya severity. PLoS ONE 4 (1), e4261. https://doi.org/10.1371/journal.pone.0004261.
Okamoto, M., Tsukamoto, H., Kouwaki, T., Seya, T., Oshiumi, H., 2017. Recognition of Viral RNA by pattern recognition receptors in the induction of innate immunity and excessive inflammation during respiratory viral infections. Viral Immunol. 30 (6), 408–420. https://doi.org/10.1089/vim.2016.0178.
Petitdemange, C., Wauquier, N., Vieillard, V., 2015. Control of immunopathology during chikungunya virus infection. J. Allergy Clin. Immunol. 135 (4), 846–855. https://doi.org/10.1016/j.jaci.2015.01.039.
Pichlmair, A., Schulz, O., Tan, C.P., Na¨slund, T.I., Liljestro¨m, P., Weber, F., Sousa, C.R., Reis, E., Sousa, C., 2006. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. Science 314 (5801), 997–1001. https://doi.org/10.1126/science.1132998.
Poo, Y.S., Nakaya, H., Gardner, J., Larcher, T., Schroder, W.A., Le, T.T., Major, L.D., Suhrbier, A., 2014. CCR2 deficiency promotes exacerbated chronic erosive neutrophil-dominated chikungunya virus arthritis. J. Virol. 88 (12), 6862–6872. https://doi.org/10.1128/JVI.03364-13.
Priya, R., Patro, I.K., Parida, M.M., 2014. TLR3 mediated innate immune response in mice brain following infection with Chikungunya virus. Virus Res. 189, 194–205. https://doi.org/10.1016/j.virusres.2014.05.010.
Prow, N.A., Tang, B., Gardner, J., Le, T.T., Taylor, A., Poo, Y.S., Nakayama, E., Hirata, T.D.C., Nakaya, H.I., Slonchak, A., Mukhopadhyay, P., Mahalingam, S., Schroder, W.A., Klimstra, W., Suhrbier, A., 2017. Lower temperatures reduce type I interferon activity and promote alphaviral arthritis. PLoS Pathog. 13 (12), e1006788. https://doi.org/10.1371/journal.ppat.1006788.
Schilte, C., Couderc, T., Chretien, F., Sourisseau, M., Gangneux, N., Guivel-Benhassine, F., Kraxner, A., Tschopp, J., Higgs, S., Michault, A., Arenzana-Seisdedos, F., Colonna, M., Peduto, L., Schwartz, O., Lecuit, M., Albert, M.L., 2010. Type I IFN controls chikungunya virus via its action on nonhematopoietic cells. J. Exp. Med. 207 (2), 429–442. https://doi.org/10.1084/jem.20090851.
Schuffenecker, I., Iteman, I., Michault, A., Murri, S., Frangeul, L., Vaney, M.C., Lavenir, R., Pardigon, N., Reynes, J.M., Pettinelli, F., Biscornet, L., Diancourt, L., Michel, S., Duquerroy, S., Guigon, G., Frenkiel, M.P., Bréhin, A.C., Cubito, N., Desprès, P., Kunst, F., Rey, F.A., Zeller, H., Brisse, S., 2006. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med. 3 (7), e263. https://doi.org/10.1371/journal.pmed.0030263.
Sourisseau, M., Schilte, C., Casartelli, N., Trouillet, C., Guivel-Benhassine, F., Rudnicka, D., Sol-Foulon, N., Le Roux, K., Prevost, M.C., Fsihi, H., Frenkiel, M.P., Blanchet, F., Afonso, P.V., Ceccaldi, P.E., Ozden, S., Gessain, A., Schuffenecker, I., Verhasselt, B., Zamborlini, A., Sai¨b, A., Rey, F.A., Arenzana-Seisdedos, F., Després, P., Michault, A., Albert, M.L., Schwartz, O., 2007. Characterization of reemerging chikungunya virus. PLoS Pathog. 3 (6), e89. https://doi.org/10.1371/journal.ppat.0030089.
Staples, J.E., Breiman, R.F., Powers, A.M., 2009. Chikungunya fever : an epidemiological review of a re-emerging infectious disease. Clin. Infect. Dis. 49 (6), 942–948. https://doi.org/10.1086/605496.
Strauss, J.H., Strauss, E.G., 1994. The alphaviruses: gene expression, replication, and evolution. Microbiol. Rev. 58 (3), 491–562. https://doi.org/10.1074/jbc.M109.090936.
Teng, T.S., Foo, S.S., Simamarta, D., Lum, F.M., Teo, T.H., Lulla, A., Yeo, N.K.W., Koh, E.G.L., Chow, A., Leo, Y.S., Merits, A., Chin, K.C., Ng, L.F.P., 2012. Viperin restricts chikungunya virus replication and pathology. J. Clin. Invest. 122 (12), 4447–4460. https://doi.org/10.1172/JCI63120.
Tsai, Y.T., Chang, S.Y., Lee, C.N., Kao, C.L., 2009. Human TLR3 recognizes dengue virus and modulates viral replication in vitro. Cell. Microbiol. 11 (4), 604–615. https://doi.org/10.1111/j.1462-5822.2008.01277.x.
Valdés López, J.F., Urcuqui-Inchima, S., 2018. Synergism between phorbol-12-myristate-13-acetate and vitamin D3 in the differentiation of U937 cells to monocytes and macrophages. Morphologie 102 (338), 205–218. https://doi.org/10.1016/j.morpho.2018.06.001.
Valdés López, J.F., Velilla, P.A., Urcuqui-inchima, S., 2019. Chikungunya virus and zika virus, two different viruses examined with a common aim: role of pattern recognition receptors on the inflammatory response. J. Interferon Cytokine Res. 39 (9), 507–521. https://doi.org/10.1089/jir.2019.0058.
Vasanthi, P., Nalini, G., Rajasekhar, G., 2007. Role of tumor necrosis factor-alpha in rheumatoid arthritis : a review. APLAR J. Rheumatol. 10 (4), 270–274. https://doi.org/10.1111/j.1479-8077.2007.00305.x.
Weaver, S.C., Lecuit, M., 2015. Chikungunya virus and the global spread of a mosquito-borne disease. N. Engl. J. Med. 372 (13), 1231–1239. https://doi.org/10.1056/NEJMra1406035.
Werneke, S.W., Schilte, C., Rohatgi, A., Monte, K.J., Michault, A., Arenzana-Seisdedos, F., Vanlandingham, D.L., Higgs, S., Fontanet, A., Albert, M.L., Lenschow, D.J., 2011. ISG15 is critical in the control of chikungunya virus infection independent of UbE1l mediated conjugation. PLoS Pathog. 7 (10), e1002322. https://doi.org/10.1371/journal.ppat.1002322.
Wewers, M.D., Rennard, S.I., Hance, A.J., Bitterman, P.B., Crystal, R.G., 1984. Normal human alveolar macrophages obtained by bronchoalveolar lavage have a limited capacity to release interleukin-1. J. Clin. Invest. 74 (6), 2208–2218. https://doi.org/10.1172/JCI111647.
White, L.K., Sali, T., Alvarado, D., Gatti, E., Pierre, P., Streblow, D., DeFilippis, V.R., 2011. Chikungunya Virus Induces IPS-1-Dependent Innate Immune Activation and Protein Kinase R-Independent Translational Shutoff. J. Virol. 85 (1), 606–620. https://doi.org/10.1128/JVI.00767-10.
Witwer, K.W., Sisk, J.M., Gama, L., Clements, J.E., 2010. MicroRNA regulation of IFN-β protein expression: rapid and sensitive modulation of the innate immune response. J. Immunol. 184 (5), 2369–2376. https://doi.org/10.4049/jimmunol.0902712.