Cellular Immunology 367 (2021) 104411

Contents lists available at ScienceDirect

Cellular Immunology

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

Research paper

Interleukin 27 as an inducer of antiviral response against chikungunya virus infection in human macrophages

Juan Felipe Valdés-López, Geysson J. Fernandez, Silvio Urcuqui-Inchima *

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

ARTICLE INFO

Keywords:
Transcriptomic
ISG
Interferon
IL27
Antiviral response
Innate immunity

ABSTRACT

Chikungunya virus (CHIKV) is known to have a wide range of tropism in human cell types throughout infection, including keratinocytes, fibroblasts, endothelial cells, monocytes, and macrophages. We reported that human monocytes-derived macrophages (MDMs) are permissive to CHIKV infection *in vitro*. We found that the peak of CHIKV replication was at 24 hpi; however, at 48 hpi, a significant reduction in viral titer was observed that correlated with high expression levels of genes encoding antiviral proteins (AVPs) in an IFN-independent manner. To explore the molecular mechanisms involved in the induction of antiviral response in CHIKV-infected MDMs, we performed transcriptomic analysis by RNA-sequencing.

Differential expression of genes at 24 hpi showed that CHIKV infection abrogated the expression of all types of IFNs in MDMs. However, we observed that CHIKV-infected MDMs activated the JAK-STAT signaling and induced a robust antiviral response associated with control of CHIKV replication. We identified that the IL27 pathway is activated in CHIKV-infected MDMs and that kinetics of IL27p28 mRNA expression and IL27 protein production correlated with the expression of AVPs in CHIKV-infected MDMs. Furthermore, we showed that stimulation of THP-1-derived macrophages with recombinant-human IL27 induced the activation of the JAK-STAT signaling and induced a robust pro-inflammatory and antiviral response, comparable to CHIKV-infected MDMs. Furthermore, pre-treatment of MDMs with recombinant-human IL27 inhibits CHIKV replication in a dose-dependently manner (IC50 = 1.83 ng/mL). Altogether, results show that IL27 is highly expressed in CHIKV-infected MDMs, leading to activation of JAK-STAT signaling and stimulation of pro-inflammatory and antiviral response to control CHIKV replication in an IFN-independent manner.

1. Introduction

Chikungunya virus (CHIKV) is a zoonotic arthropod-borne virus, a member of the *Togaviridae* family, *Alphavirus* genus. CHIKV is the etiological agent of chikungunya fever (CHIKF), a self-limiting disease characterized by fever and acute or chronic arthralgia (Reviewed in [1]). Currently, neither antiviral drugs nor candidate vaccine is available for the treatment of CHIKV infection. The limited resources to fight against CHIKV infection and the rapid re-emergence has led to the search for new compounds that could prevent CHIKV infection or control CHIKF progression.

Interferons (IFNs) are a group of cytokines that help regulate the activity of the immune system and induce the establishment of an antiviral state in infected cells [2]. Type I IFNs [IFN-I (IFNα, β, κ, ω, ζ)] bind to IFN alpha/beta receptor complex [IFNAR (IFNAR1/IFNAR2)] [3–4], whereas Type II IFN [IFN-II (IFNγ)] signals through IFNγ receptor complex [IFNGR (IFNGR1/IFNGR2)] [5]. Type III IFNs (IFN-III) have three members [IFNλ1 (IL29), IFNλ2 (IL28A), and IFNλ3 (IL28B)] that bind to the interferon-Lambda receptor complex [IFNLR (IFNLR1/IL10RB) [6]. The IFN-I/IFNAR and IFN-III/IFNLR interaction results in the activation of the Janus kinase (JAK) signaling pathway, which phosphorylates and activates signal transducer and activator of the transcription 1 (STAT1) and STAT2 that together with IFN regulatory factor 9 (IRF9) forms a transcription factor complex known as IFN-stimulated gene factor 3 (ISGF3) [7]. ISGF3 is translocated into the nucleus and binds to IFN-stimulated response elements (ISRE) to induce the coordinate up-expression of a large number of IFN-stimulated genes (ISGs), which encode a number of antiviral proteins (AVPs), cytokines, and chemokines that orchestrate antiviral state in cells [2,8–9]. AVPs, including Apolipoprotein B Editing Complex 3 (APOBEC3) family of

* Corresponding author.
E-mail addresses: felipe.valdes@udea.edu.co (J.F. Valdés-López), geysson.fernandez@udea.edu.co (G.J. Fernandez), silvio.urcuqui@udea.edu.co (S. Urcuqui-Inchima).

https://doi.org/10.1016/j.cellimm.2021.104411
Received 20 May 2021; Received in revised form 25 June 2021; Accepted 17 July 2021
Available online 21 July 2021

proteins, ISG15, double-stranded RNA-activated protein kinase R (PKR), oligoadenylate synthase (OAS) family of proteins, and Viperin, are a critical component of cellular antiviral state implicated in the control of CHIKV replication [1–2,10].

In addition to IFNs, other cytokines could mediate activation of JAK-STAT signaling, including members of interleukin 2 (IL2), IL6, IL10, and IL12 families [11]. IL12 family is soluble heterodimeric cytokines that consist of α (IL12p35, IL23p19, and IL27p28) and β [IL12p40 and Epstein-Barr virus-induced protein 3 (EBI3)] subunits. IL12 family includes four members, the IL12p70 (IL12p35/IL12p40), IL23 (IL23p19/IL12p40), IL27 (IL27p28/EBI3) and IL35 (IL12p35/EBI3) (Reviewed in [12]). These cytokines signal through their interaction with a specific heterodimeric receptor complex consisting of two protein chains on the cell surface. Thus, the IL12p70 bind to the IL12 receptor [IL12R (IL12RB1/IL12RB2)], IL23 signals through IL23 receptor [IL23R (IL23R/IL12RB1)], IL27 signaling employs IL27 receptor [IL27R (IL27Rα/Gp130)] and, IL35 signaled through a unique heterodimer of receptor chains IL12RB2 and Gp130 or homodimers of each chain [13–16]. Moreover, the IL12 family members signal through different components of the JAK-STAT signaling pathway; STAT4 by IL12p70; STAT3 and STAT4 by IL23; STAT1 and STAT3 by IL27, and STAT1, and STAT4 by IL35 (Reviewed in [17]).

IL27 is expressed by antigen-presenting cells, including activated macrophages and dendritic cells; it has been associated with pro-and anti-inflammatory properties [18]. This cytokine regulates T-helper 1 cell development [19], acts on naïve CD8 + T-cells to enhance the generation of cytotoxic T lymphocytes [20], induces isotype switching in B-cells [21], and maintain plasmacytoid DCs [22]. Furthermore, exogenous administration of IL27 has been shown to possess antiviral properties against HIV-1, Hepatitis C virus, Hepatitis B virus, and Zika virus in respective host cell types [23–26]. High serum levels of IL27 have been proposed as a biomarker of chronic CHIKV infection [27]. Although IL27 has been suggested to have antiviral activity, the role of IL27 in CHIKV control and immunopathogenesis has not been reported.

We previously reported that monocytes-derived macrophages (MDMs) are susceptible and permissive to CHIKV infection *in vitro* [10]. Furthermore, we found maximum CHIKV replication (in MDMs) at 24 h post-infection (hpi). However, at 48 hpi, we observed a significant reduction in viral titer, an event independent of cell death. Further, we observed a positive correlation between high expression of Toll-like receptor 3 (TLR3) and AVPs such as OAS1 and PKR, in IFN-I independent manner. Our aim here was to explore the molecular mechanism of induction of antiviral response in CHIKV-infected MDMs based on transcriptomic analysis by RNA-seq.

2. Material and methods

2.1. Viral stocks and CHIKV titration

A clinical isolate of CHIKV obtained following the protocol described in [28] from CHIKF patient (kindly gifted by Professor Francisco Javier Díaz, University of Antioquia), was amplified from a Colombian patient's serum and propagated in Vero cells (ATTC CCL-81), as we previously reported [10]. Briefly, 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 (Corning-Cellgro, New York, USA), and incubated at 37 °C and 5% CO_2 in cell culture flasks, to a cell density of $1x10^5$-$1x10^6$ cells/mL. 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, supernatants were collected, precleared by centrifugation (1650 × g for 10 min), and stored at −80 °C. CHIKV stocks were titrated by plaque assay on Vero cells, as previously described [10]. The virus titer was determined to be $2.1x10^8$ PFU/mL.

2.2. Culture of human monocytes and differentiation into monocytes-derived macrophages (MDMs).

The study was approved by the Ethics Committee of "Sede de Investigación Universitaria-Universidad de Antioquia". Written informed consent was obtained from all individuals who voluntarily participated in this study. Human peripheral blood mononuclear cells (PBMCs) from blood samples of healthy donors were isolated through density gradient with Lymphoprep (STEMCELL Technologies Inc, Vancouver, Canada) by centrifugation at 850 × g for 21 min, and mixed with 2% EDTA as previously described (Valdés et al., 2020). PBMCs from each healthy volunteer were prepared independently. Platelet depletion was performed by washing with PBS 1X (Sigma-Aldrich) three times at 250 × g for 10 min and the percentage of CD14 positive cells was determined by flow cytometry. To obtain monocytes, 24-well plastic plates were scratched with a 1000 μL pipette tip and seeded with, $5x10^5$ CD14 positive cells per well and allowed to adhere for 2 h in RPMI-1640 medium supplemented with 0.5 (v/v) autologous serum or plasma (to favor adherence of monocytes to the well), 4 mM L-glutamine and 0.3% (v/v) $NaCO_3$ and cultured at 37 °C and 5% CO_2. Non-adherent cells were removed by washing twice with PBS 1X and monocytes were 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 for 6 days to obtain MDMs, as previously described [10,29]

2.3. In vitro CHIKV infection of MDMs.

CHIKV infection of human primary MDMs was performed at MOI of 5 in serum-free RPMI-1640 medium as previously reported [10]. Samples were incubated at 37 °C for 1.5 h. An hour and a half after infection, the cells were washed with PBS 1X to remove the unbound virus and a fresh complete medium was added and incubated at 37 °C with 5% CO_2. Culture supernatants and cell lysates were collected at 6, 24, and 48 hpi and stored at −80 °C.

2.4. RNA extraction and cDNA libraries synthesis

Total RNA from uninfected or CHIKV-infected MDMs was obtained using Direct-zol ™ RNA Miniprep Plus (Zymo Research, Irvine, California) following the manufacturer's protocol. RNA samples were treated with DNase I column (Zymo Research, Irvine, California) to remove contaminating genomic DNA. RNA was quantified, and the quality of each RNA sample was checked by spectrophotometry (Thermo Scientific, Wilmington, DE, USA). Additionally, RNA quality was determined by analysis of ribosomal RNAs, using the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). cDNA libraries were constructed for each experimental group using the RevertAid Minus First Strand cDNA Synthesis Kit (Thermo Scientific), according to the manufacturer's instructions. The samples were stored at −80 °C. One μg of RNA was used for RNA-Seq.

2.5. RNA-seq data and bioinformatics analysis.

RNA sequencing was carried out on Illumina HiSeq 2000 platform. After sequencing, image data was transformed into raw reads and stored in FASTQ datasets for each sample, using FastQC (google/vFqiZ). Clean reads were obtained by removing the low-quality adapter, poly-N containing, and shorter-than-70 bp reads and mapped to the human transcriptome (RefSeq, HG19) using TopHat software (version 1.3.2.). Gene expression (mRNA) was normalized by calculating Reads per kilobase per million mapped reads (RPKM). The HTseq software for Python was used to generate counts for each gene from the mapped sequences. Differential expression of mRNAs in each experimental group was

identified using the DEseq2 software (version 1.8.261) implemented in R software (version 3.6.3). To determine the differentially expressed genes (DEG), we used the edgeR package of R software where the false discovery rate (FDR) < 0.05 and the $|\text{Log}_2$ Fold Change (FC) (CHIKV-infected MDMs/Uninfected MDMs) $|> 0.6$ ($|\log_2 FC| > 0.6$), were used as the threshold to determine the statistically significant difference in gene expression. Gene Ontology (GO) was performed with the BiNGO Cytoscape plugin, using a hypergeometric test with a Benjamini and Hochberg False Discovery Rate correction, to identify significant functions of the DEG. A $p < 0.05$ was used to identify enriched processes. Additionally, the ClueGO Cytoscape plugin was used to group and analyze the GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment. Gene interaction networks were visualized and analyzed with Cytoscape.

2.6. Gene expression analysis from THP-1 treated with recombinant human IL27

A publicly available dataset (MicroArray GSE143228, GEO) [26] was reanalyzed to confirm our results observed in CHIKV-infected MDMs; in this case, the THP-1 cells, a human promonocytic cell line, were pre-treated with Phorbol 12-myristate 13-acetate (PMA) to induce macrophage-like phenotype and treated with 100 ng/mL recombinant human IL27 for 18 h. Then, total RNA from unstimulated or IL27-treated THP-1 cells was obtained and Microarray was performed. Differential expression of mRNAs in each experimental group was identified using GEO2R. To define the top DEG, we selected genes with an FDR < 0.05 and |Log2 Fold Change (IL27-treated THP-1 cells/unstimulated THP-1 cells) $|> 0.6$.

2.7. Quantitative real-time PCR (RT-qPCR)

To verify the accuracy of the RNA-seq data, specific primer pairs were used to quantify IFNβ1, OAS1, PKR, B-Actin [10], and SOCS3 [30] as previously reported. Furthermore, cDNA products were amplified by RT-qPCR using a set of primers specific for diverse genes as follows: IL27p28 mRNA: forward: 5′-GAGCAGCTCCCTGATGTTTC-3′, and reverse: 5′-AGCTGCATCCTCTCCATGTT-3′. EBI3 mRNA: Forward: 5′-TGGCTCCCTACGTGCTCAAT-3′, and reverse 5′-GAGGGTCGGGCTTGATGATGT-3′. IFNα1 mRNA: Forward: 5′-CAGAGTCACCCATCTCAGCA-3′, and reverse 5′-CACCACCAGGACCATCAGTA-3′. IFNγ mRNA: Forward: 5′-TGCAGGTCATTCAGATGTAG-3′, and reverse 5′-AGCCATCACTTGGATGAGTT-3′. IFNλ1 mRNA: Forward: 5′-GGTGACTTTGGTGCTAGGCT-3′, and reverse 5′-TGAGTGACTCTTCCAAGGCG-3′. APOBEC3A mRNA: Forward: 5′-CATCCGGGCCCAGGCATAA −3′, and reverse 5′-TCTTGACCGAGGTGCCATTG-3′. ISG15 mRNA: Forward: 5′-GGTGGACAAATGCGACGAAC-3′, and reverse 5′-TCGAAGGTCAGCCAGAACAG-3′. Viperin (RSAD2) mRNA: Forward: 5′-AAATGCGGCTTCTGTTTCCAC-3′, and reverse 5′-TTGATCTTCTCCATACCAGCTTCC-3′. PCR amplifications were carried out using the SYBR system (Invitrogen, Oregon, USA). 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. Relative expression of each target gene was normalized to the uninfected control and housekeeping gene β-actin (ΔΔCt) and is reported as the Log_2 Fold Change.

2.8. Cytokine quantification

The LEGEND MAX™ Human IL27 ELISA Kit (BioLegend, San Diego, CA, USA) was used for the detection of mature IL27 in culture supernatants of MDMs infected with CHIKV, following the manufactureŕs instructions. The detection limit was 11 pg/mL.

2.9. In vitro antiviral assay

Human MDMs were pre-treated for 6 h with increasing concentrations of recombinant-human IL27 (BioLegend) or 25 ng/mL of recombinant-human IFNβ1 (STEMCELL Technologies Inc). Next, MDMs were infected with CHIKV at MOI 5 in serum-free RPMI-1640 medium. Samples were incubated at 37 °C for 1.5 h. An hour and a half after infection cells were washed with PBS to remove the unbound virus and a fresh complete medium was added and incubated at 37 °C with 5% CO_2. Culture supernatants were obtained at 24 hpi and stored at −80 °C. Samples were titrated by plaque assay on Vero cells.

2.10. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc. San Diego, CA). Statistical tests are indicated in the figure legends. Data are represented as mean ± SEM, Log2 Fold Change (Log2 FC), or RPKM. Significant results were defined as $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.002$ (***).

3. Results

3.1. Monocytes-derived macrophages are targets of CHIKV replication and express AVPs in an Interferon-independent manner

As described previously [10], human MDMs are susceptible and permissive to CHIKV infection. CHIKV replication in these cells is fast, with a peak of infectious viral particles production at 24 hpi and, a significant decrease in viral titer at 48 hpi (Fig. 1A), suggesting activation of antiviral response in MDMs. However, we did not observe a significant increase in the mRNA expression of IFN-I (IFNα1 and IFNβ1), IFN-II (IFNγ), and IFN-III (IFNλ1) in CHIKV-infected MDMs (Fig. 1B). Despite this result, we noted significant levels of genes encoding AVPs mRNAs, including APOBEC3A, ISG15, OAS1, PKR, and Viperin, at 6 hpi, and higher expression at 24 hpi (Fig. 1C), suggesting that AVPs induction in CHIKV-infected MDMs may be independent of classic IFNs expression. Since we observed elevated levels of AVPs mRNA and a decrease in viral titers, we suggest that MDMs mount an antiviral response that leads to the control of CHIKV replication in an Interferon-independent manner.

3.2. CHIKV infection induces a robust pro-inflammatory and antiviral program in human MDMs

To explore the molecular mechanism of induction of antiviral response in CHIKV-infected MDMs, we performed an RNA-Seq analysis of uninfected or infected human MDMs at 24 hpi. First, we analyzed sample variance in the RNA-Seq dataset using a principal component analysis (PCA), and a linear transformation of the data. According to the PCA, the first component (PC1), which separates uninfected MDMs from CHIKV-infected MDMs, explains 76.7% of the variance between samples and was due to the response of MDMs to CHIKV infection (Fig. 2A). To define the top DEG, we selected genes with an FDR < 0.05 and $|\text{Log}_2$ FC (CHIKV-infected MDMs/ Uninfected MDMs) $|> 0.6$. Of the 60,592 genes interrogated by RNA-Seq, 367 were differentially up-regulated and 61 were differentially down-regulated in CHIKV-infected MDMs (Fig. 2B). Among the up-regulated genes included AVPs [APOBEC3 family proteins, Guanylate-binding proteins (GBP) family proteins, interferon-inducible (IFI) family proteins, interferon-induced transmembrane (IFITM) family proteins, OAS family proteins, indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1), ISG15, ISG20, dynamin-like GTPase 1 (MX1), MX2, PKR, Viperin, and others] (Fig. 2B). Furthermore, CHIKV infection in MDMs induced a high expression of genes associated with pro-inflammatory pathways, including cytokines [Tumor necrosis factor-alpha (TNFα), Interleukin (IL)-1β (IL1β), IL6, IL7, IL12p40, IL15, IL18, IL27 (IL27p28 and EBI3), IL32, B-cell activating factor (BAFF) and TNF-

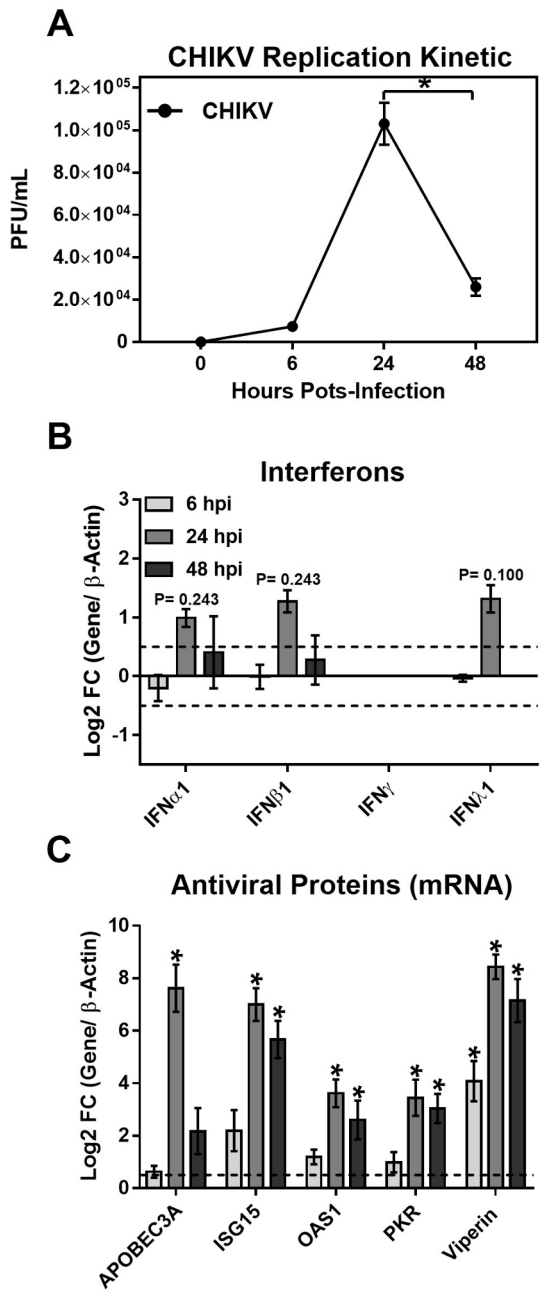

Fig. 1. Monocyte-derived macrophages are target cells of CHIKV replication and expressed AVPs in an Interferon-independent manner. Human MDMs cultures were left uninfected or infected with CHIKV at MOI 5. Culture supernatants were obtained at 6, 24, and 48 hpi, and quantification of PFU/mL was performed by plaque assay. Cell lysates were obtained at 6, 24, and 48 hpi, and RT-qPCR was performed. CHIKV replication kinetics in human MDMs (A). mRNA expression of IFN-I (IFNα1 and IFNβ1), IFN-II (IFNγ), IFN-III (IFNλ1) (B) and antiviral proteins (APOBEC3A, ISG15, OAS1, PKR, and Viperin) (C) in CHIKV-infected MDMs. Data are presented as the mean ± SEM. Mann-Whitney test was performed. Significant results are defined as $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.002$ (***). n = 4.

related apoptosis-inducing ligand (TRAIL)] (Fig. 2C); CC– chemokines (CCL2, CCL5, CCL7, and CCL8) and CXC- chemokines (CXCL9, CXCL10, CXCL11) (Fig. 2D). In addition, Pattern recognition receptor (PRRs) including absent in melanoma 2 (AIM2), nucleotide-binding oligomerization domain containing 2 (NOD2), retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5), laboratory of genetics and physiology 2 (LGP2), TLR2, TLR3, TLR7 and C-Type lectins, including C-Type lectin domain family 1 member A (CLEC1A), CLEC4E, CLEC5A, CLEC6A, CLEC7A, CLEC10A, CD205, and Galectin 2, were significantly up-regulated in CHIKV-infected MDMs (Supplementary Table 1). Altogether, the results suggest robust pro-inflammatory and antiviral responses in CHIKV-infected MDMs.

Next, the GO functional analysis of DEG was performed to gain an insight into the biological roles of the most significantly up-regulated genes. In brief, all DEG observed in CHIKV-infected MDMs were enriched for the biological process associated with negative regulation of cell death, inflammatory and antiviral response. As shown (Fig. 2E), we identified DEG involved in the expression of a robust antiviral program, including MDA5 signaling pathway, TLR3, and TLR7 signaling pathway, type-I and type-II interferon signaling (grouped as high diversity of AVPs and inflammatory molecules), and positive regulation of tyrosine phosphorylation of STAT protein (associated with JAK-STAT signaling pathway activation), suggesting that modulation of these genes may be essential for the control of CHIKV replication in MDMs. However, the expression of some of the genes that are involved in these signaling pathways was induced at 6 hpi (results not shown).

3.3. CHIKV infection activated the JAK-STAT signaling pathway and induced a robust and IFN-independent antiviral network in MDMs

RNA-Seq analysis showed that several transcripts (mRNAs) associated with ISGs were significantly increased in CHIKV-infected MDMs (Fig. 2B). Next, we evaluated whether the expression of IFN-I, IFN-II, and IFN-III was induced in CHIKV-infected MDMs. In agreement with the results shown in Fig. 1B, we found that CHIKV infection did not induce expression of all three types of IFNs and did not affect the expression of their receptors (IFNAR, IFNGR, and IFNLR) (Fig. 3A). Although we did not detect the expression of IFNs, we showed that CHIKV infection-induced JAK-STAT signaling pathway, as seen by up-regulation of STAT genes (STAT1, STAT2, and STAT3) and negative regulators of JAK-STAT signaling pathway [Suppressor of cytokine signaling 1 (SOCS1) and SOCS3] (Fig. 3B).

Additionally, CHIKV infection-induced expression of a diverse set of genes associated with a robust antiviral network; we observed consolidation of functional clusters involved in the control of CHIKV replication at different steps of the viral replication cycle (Fig. 3B), including negative regulation of viral replication/translation, regulators of ribonuclease activity, negative regulation of viral entry into the host cell, base conversion or substitution on nucleic acids, negative regulation of viral transcription and viral release from the host cell, ISG15-protein conjugation and the ubiquitin–proteasome system (Fig. 3B). Taken together all these results, we run into a key question, what is the inductor of the JAK-STAT signaling pathway that mediated the expression of AVPs in CHIKV-infected MDMs, without IFNs production?

3.4. The IL27 signaling is activated in CHIKV-infected MDMs

Cavalcanti et al., (2019) [27] reported high levels of IL27 in serum of patients with chronic CHIKV infection. Furthermore, Kwock et al. (2020) [26] shown that IL27 signaling activates skin cells to induce innate antiviral proteins and protect against ZIKV infection. We studied the expression of factors that could be associated with the JAK-STAT signaling pathway in CHIKV-infected MDMs. We focused on the IL12 family of cytokines because some of their members (IL27 and IL35) are known to mediate the activation of STAT1, a crucial inductor of AVPs in response to IFN stimulation [2,17]. In present studies, we investigated expression levels of genes that encode for AVPs in CHIKV-infected MDMs in an IFN-independent manner. We evaluated the expression of IL12p70, IL23, IL27, IL35, and their receptors. We observed significant up-regulation in IL12p40 expression (Fig. 4A). However, we did not observe the expression of either IL12p35 or IL12RB2, suggesting that mature IL12p70 and IL35 are not induced in CHIKV-infected MDMs. In the case of IL23, although both subunits of the cytokine were expressed

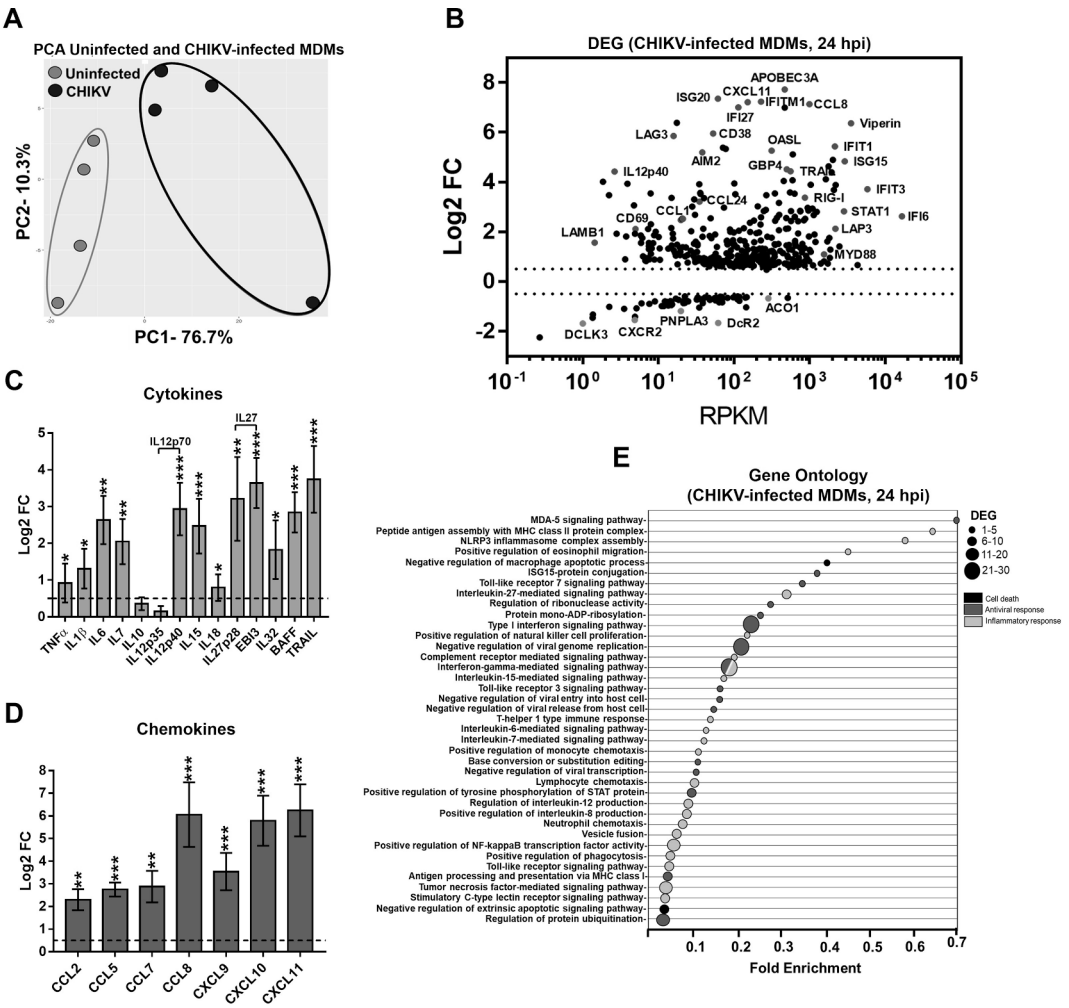

Fig. 2. CHIKV infection induces the expression of a robust pro-inflammatory and antiviral response in MDMs. Human MDMs cultures were left uninfected or infected with CHIKV at MOI 5. Cell lysates were obtained at 24 hpi and RNA-seq was performed. PCA plot (A) of differentially expressed genes (DEG) interrogated by RNA-Seq. Grey and black data points represent uninfected and CHIKV-infected MDMs, respectively. MA plot of DEG (B). Differentially expressed cytokines (C), and CC– and CXC-chemokines (D) in CHIKV-infected MDMs. GO enrichment analysis (E) from DEG regulated in CHIKV-infected MDMs. DEG in CHIKV-infected MDMs was selected from genes with an FDR < 0.05 and $|$Log2 Fold Change (CHIKV-infected MDMs/ Uninfected MDMs) $|> 0.6$. To quantify mRNAs abundance, we use the Reads per kilobase per million mapped reads (RPKM). n = 4.

in CHIKV-infected MDMs, IL23R was not expressed (Fig. 4A), suggesting that although mature IL23 could be present, its signaling pathway is not activated in MDMs. Of note, we observed that CHIKV infection induces a significantly high level of both IL27p28 and EBI3. Furthermore, both IL27 receptor subunits (IL27Rα and Gp130) were expressed in MDMs and Gp130 was up-regulated by CHIKV infection, suggesting that all IL27 subunits are expressed and therefore, IL27 could be biologically functional in response to CHIKV infection (Fig. 4A)

Next, we evaluated the expression of the IL27 signaling pathway in CHIKV-infected MDMs. We observed that CHIKV infection promotes up-regulation of Gp130 mRNA expression, as well the mRNA encoding receptor-associated kinases, such as JAK2. Additionally, we observed up-regulation in transcription factors expression, including STAT1, which was among the STATs, most highly expressed in CHIKV-infected MDMs (Fig. 4B); and STAT3, both previously reported implicated in the IL27 signaling transduction *in vivo* [18].

3.5. Validation of the RNA-Seq results by RT-qPCR and ELISA

Next, we proceeded to validate the RNA-Seq results related to IL27 signaling by RT-qPCR based on Log2 FC and p values. As shown in Fig. 4C, CHIKV infection of MDMs induced expression of both IL27p28, and EBI3, as well SOCS3, the most important negative regulator of the IL27 signaling pathway. The highest level of EBI3 mRNA level was observed at 6 hpi, whereas, for IL27p28 and SOCS3, the peak mRNA level was observed at 24 hpi. Furthermore, we quantified IL27 protein in CHIKV-infected MDMs culture supernatants, at 0, 6, 24, and 48 hpi, by ELISA. We found the highest level of IL27 protein at 24 hpi (Fig. 4D). Results are consistent with the kinetic of IL27p28 (Fig. 4C) and AVPs mRNAs (APOBEC3A, ISG15, PKR, OAS1, and Viprin) (Fig. 1C) observed by RT-qPCR. The RT-qPCR and ELISA data were consistent with the transcriptomic analysis, indicating the reliability of the RNA-Seq results and that the IL27 signaling pathway is activated in CHIKV-infected MDMs.

3.6. IL27-treatment and CHIKV-infection induced a common pro-inflammatory and antiviral program in human macrophages

To confirm the ability of IL27 to activate the JAK-STAT signaling pathway and increased the expression of ISGs observed in our transcriptomic analysis of CHIKV-infected MDMs, we reanalyzed a publicly dataset (MicroArray GSE143228) [26], where human promonocytic (THP-1) cells were pre-treated with PMA to induce macrophage-like phenotype followed by 100 ng/ml recombinant-human IL27 for 18 h.

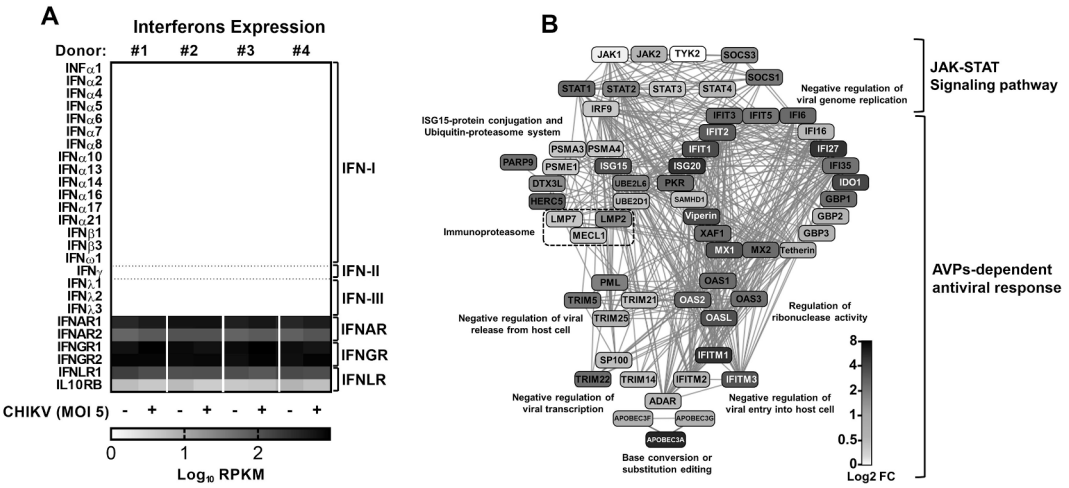

Fig. 3. CHIKV infection in MDMs activated the JAK-STAT signaling pathway and induced a robust antiviral network in an IFN-independent manner. Human MDMs cultures were left uninfected or infected with CHIKV at MOI 5. Cell lysates were obtained at 24 hpi and RNA-seq was performed. Heat map (A). IFNs/IFNRs mRNA abundance (RPKM) in uninfected (-) and CHIKV-infected MDMs (+). Each column corresponds to an independent donor. Mann-Whitney test was performed. Significant results are defined as $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.002$ (***). Differentially regulated mRNA network (B). JAK-STAT signaling pathway components and AVPs regulated in CHIKV-infected MDMs. Grayscale was normalized to Log2 Fold Change. n = 4.

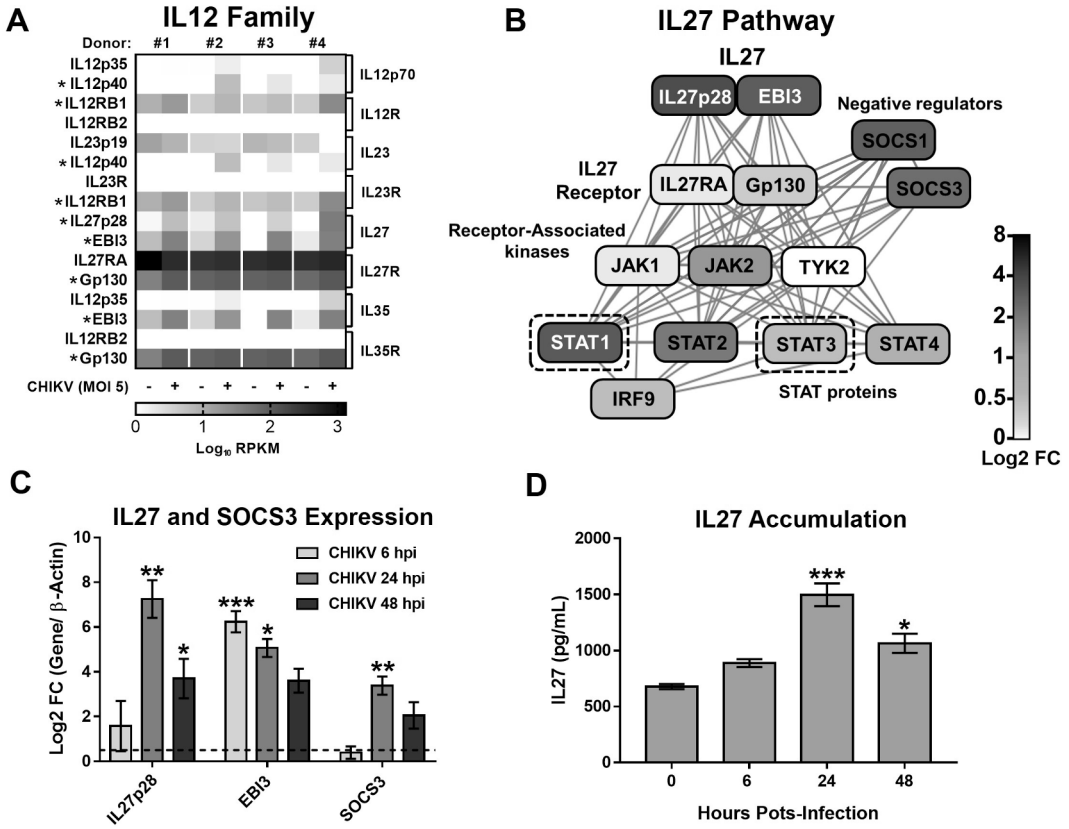

Fig. 4. The IL27 signaling pathway is activated in CHIKV-infected MDMs. Human MDMs cultures were left uninfected or infected with CHIKV at MOI 5. Cell lysates and culture supernatants were obtained at 6, 24, and 48 hpi, and RNA-seq, RT-qPCR, and ELISA were performed. Heat map (A). IL12-family of cytokines/Receptors mRNA abundance (RPKM) in uninfected (-) and CHIKV-infected MDMs (+) at 24 hpi. Each column corresponds to an independent donor. Mann-Whitney test was performed. Significant results are defined as $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.002$ (***). Differentially regulated mRNA network (B). IL27 signaling pathway components regulated in CHIKV-infected MDMs at 24 hpi. Grayscale was normalized to Log2 Fold Change. Dotted line: STAT proteins implicated in the biological activity of IL27 *in vivo*. Kinetics of IL27 and SOCS3 mRNA expression by RT-qPCR (C) and kinetics of mature IL27 accumulation by ELISA (D) in CHIKV-infected MDMs. Kruskal-Wallis test with Dunn's post-test was performed. Significant results are defined as $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.002$ (***). n = 4.

The analysis of data showed that IL27-treated THP-1 cells expressed a high diversity of mRNA encoding AVPs (APOBEC3-family proteins, GBP-family proteins, IFI-family proteins, IFITM-family proteins, OAS-family proteins, IDO1, ISG15, ISG20, MX1, MX2, PKR, Viperin, and others) (Fig. 5A); cytokines (TNFα, IL7, IL15, IL32, BAFF, and TRAIL) (Fig. 5B), and CC– and CXC- chemokines (CCL2, CCL7, CCL8, CXCL9, CXCL10, and

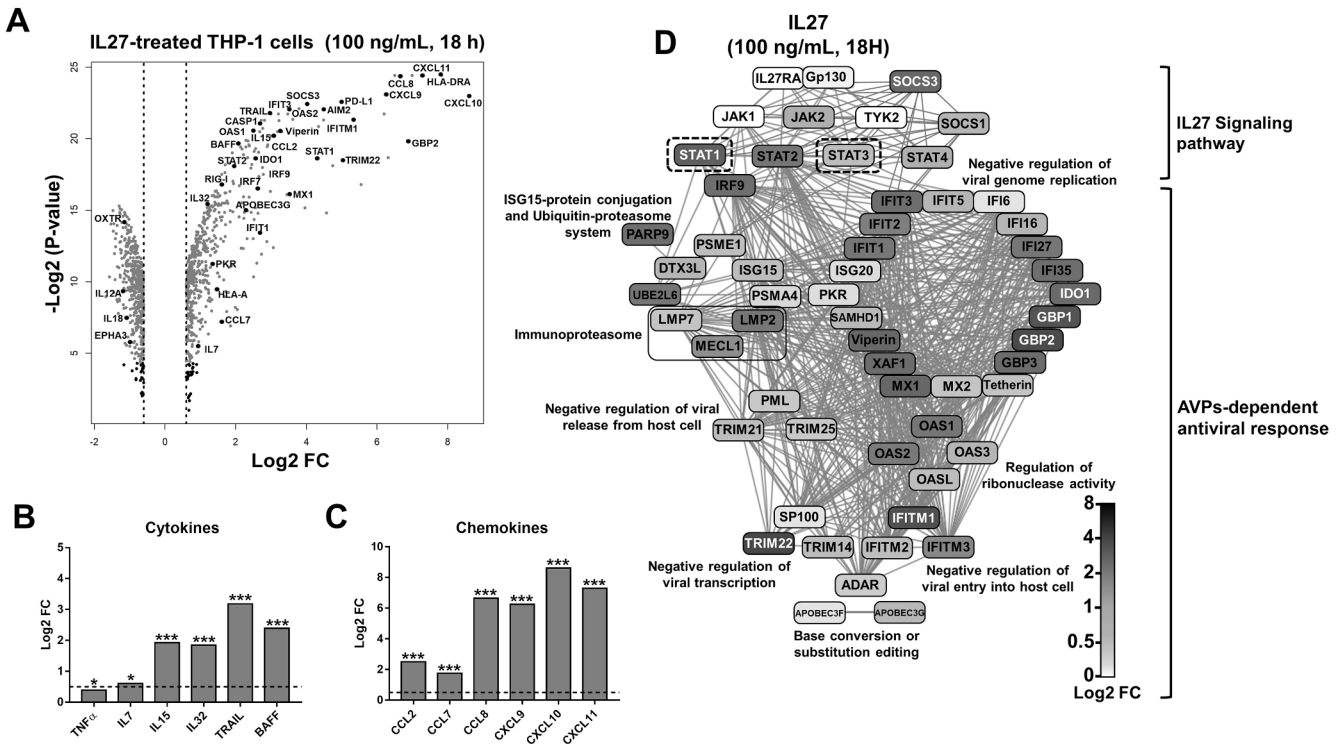

Fig. 5. IL27 treatment in THP-1-derived macrophages induced a robust pro-inflammatory and antiviral response. To determine whether IL27 plays a role in the induced pro-inflammatory and antiviral responses in human macrophages, we reanalyzed a publicly available dataset (MicroArray GSE143228, GEO) [26]. Volcano plot of DEG (A); Differentially expressed cytokines (B); CC– and CXC-chemokines (C); and Differentially regulated mRNA network (D). JAK-STAT signaling pathway components and AVPs regulated in IL27-treated THP-1 cells. Grayscale was normalized to Log2 Fold Change. Dotted line: STAT protein implicated in the biological activity of IL27 *in vivo*. DEG were selected from genes with an FDR < 0.05 and |Log2 Fold Change (IL27-treated THP-1 cells/unstimulated THP-1 cells) |> 0.6. n = 3.

CXCL11) (Fig. 5C). Additionally, we found that IL27-treated THP-1 cells, analogous to CHIKV-infected MDMs (Fig. 3B), up-regulated expression of the JAK-STAT signaling pathway, including the receptor-associated kinase JAK2 mRNA, transcription factors mRNAs such as STAT1, STAT2, and STAT3, and negative regulators SOCS1 and SOCS3 (Fig. 5D). Furthermore, IL27 treatment induced the expression of genes encoding proteins involved in the implementation of a robust antiviral network (Fig. 5D), with a pattern similar to what we observed in CHIKV-infected MDMs (Fig. 3B); as well as consolidation of the functional clusters involved in the control of viral replication cycle (Fig. 3B).

Considering the similarity of response between CHIKV-infected MDMs and IL27-treated THP-1 cells (Fig. 2A and 5A, respectively), we performed a comparative analysis of DEG for both treatment conditions. We found that CHIKV-infected MDMs and IL27-treated THP-1 cells

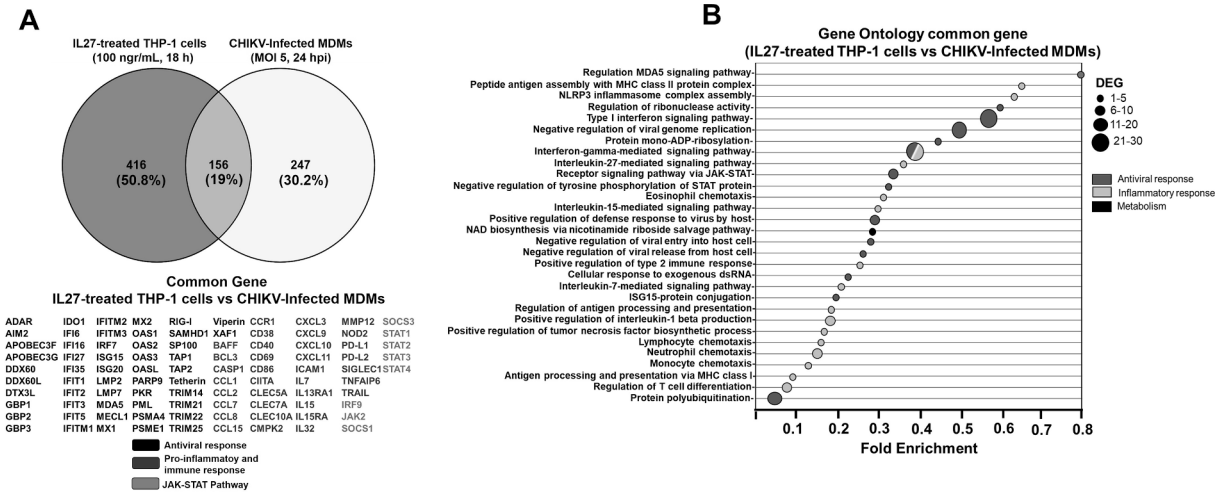

Fig. 6. IL27-treatment and CHIKV-infection induced a common pro-inflammatory and antiviral program in human macrophages. To determine whether the IL27 treatment of THP-1 cell line and CHIKV infection induced a common pro-inflammatory and antiviral program in human macrophages, we reanalyzed a publicly available dataset [26] and compared it with our transcriptomic analysis of CHIKV-infected MDMs. Human MDMs cultures were left uninfected or infected with CHIKV at MOI 5. Cell lysates were obtained at 24 hpi and RNA-seq was performed. Venn diagram (A) and GO enrichment analysis (B) of common DEG between IL27-treated THP-1 cells and CHIKV-infected MDMs.

expressed 156 common genes (19%) (Fig. 6A). Next, we performed GO analysis of common genes and found that many encoded AVPs, molecules associated with pro-inflammatory and immune response, and components of the JAK-STAT signaling pathway (Fig. 6B), confirming that commonly expressed genes are involved in both robust pro-inflammatory- and antiviral-response.

3.7. IL27 pre-treatment inhibits CHIKV replication in MDMs

To confirm the contribution of IL27 in inhibiting CHIKV replication, human MDMs were pre-treated for 6 h with increasing concentrations of recombinant-human IL27 (Biolegent) or 25 ng/mL of recombinant-human IFNβ1 (Biolegent) as a positive control for the inhibition of viral replication. Next, MDMs were infected with MOI 5 of CHIKV, and viral replication was evaluated at 24 hpi. We observed that like IFNβ1, IL27 treatment resulted in a significant decrease of CHIKV replication in a dose-dependent manner (Fig. 7A). We have shown earlier that IL27 displays antiviral properties against CHIKV infection, with an IC50 of 1.82 ng/mL (Fig. 7B). These results confirm that IL27, as well as IFNβ1, can induce an antiviral state in MDMs and reduce CHIKV replication *in vitro*.

4. Discussion

The first report of antiviral properties of IL27 was presented by Fakruddin et al. (2007) [23], who showed that IL27 was expressed in PBMCs and human MDMs in response to the non-infectious papilloma virus-like particles. Additionally, the authors reported that IL27 treatment inhibits HIV-1 replication in CD4 + T cells, PBMCs and MDMs, in a dose-dependent manner. However, it was not until 2020 that IL27 activated JAK-STAT signaling pathway in human normal epidermal keratinocytes (HNEKs) was reported; as well as induced expression of AVPs, including OAS1, OAS2, OASL, and MX1, in a STAT1-dependent but STAT2-independent manner [26]. Furthermore, the authors reported that IL27-treated HNEKs inhibited replication of ZIKV and Sendai virus *in vitro*. Additionally, using Ifnar1$^{-/-}$, Il27ra$^{-/-}$, and Ifnar1$^{-/-}$/Il27ra$^{-/-}$ mice infected with ZIKV, Kwock et al. (2020) [26] showed that subcutaneous administration of IL27 reduced mortality and onset of neurological symptoms in an interferon-independent manner, confirming the ability of IL27 to induce a protective antiviral response against ZIKV, both *in vitro* and *in vivo*.

Even though IL27 was described in 2002 [31], only in the last years its role in the control of viral infections has begun to be understood, which might be because its functionality was being masked by the effect of the three classical IFNs (IFN-I, IFN-II, and IFN-III) which are inducers of antiviral states [32]. The IFN-I/IFNAR and IFN-III/IFNLR interaction promote the JAK-STAT signaling pathway which activates STAT1 and STAT2, leading to the expression of ISGs encoding AVPs involved in the induction of an antiviral state [9]. On the other hand, the IFN-II/IFNGR interaction results in the JAK-STAT signaling pathway which mediates homodimerization, and activation of STAT1 leading to the expression of IFNγ-stimulated genes, including cytokines (IL7, IL15, and IL32), CXC chemokines (CXCL9, CXCL10, and CXCL11) and some AVPs, including IDO1, ADAR1, GBP family proteins, OAS family proteins, and IFITM-family proteins [33–37]. In both cases, IFNs result in the expression of AVPs that lead to the antiviral immune response [32,34]. Therefore, when IFNs are expressed in the context of viral infections, the induction of an antiviral state is attributed to them, masking the potential antiviral activity of other factors, such as IL27.

Along with the results described here, recently [10] we demonstrated that CHIKV infection induces expression of ISGs encoding AVPs, but not IFNs in human MDMs, suggesting an alternative pathway that regulates CHIKV infection in MDMs. In the present study, we show that CHIKV can abrogate the expression of classic IFNs in human MDMs; however, a robust ISGs-dependent antiviral activity is promoted in MDMs in response to CHIKV infection. We found that MDMs highly express IL27, its respective heterodimeric receptor complex, and components of signaling pathways in response to CHIKV infection, besides the expression of AVPs involved in the control of CHIKV replication. These findings are not limited to MDMs; Kwock et al (2020) [26] reported a comparable pattern of antiviral response in HNEKs stimulated with IL27 *in vitro* and demonstrated that IL27 treatment of mice protects against subcutaneous ZIKV infection in the absence of IFN-I. AVPs such as PKR, MX1, MX2, ISG20, OAS1, OAS2, OAS3, and Viperin among others, have previously been reported to be induced by IFN-I (Reviewed in [32]). However, Kwock et al (2020) [26] found that IL27 induces expression of these AVPs in a manner that is STAT1 and IL27Rα dependent. Based on these results, we propose that IL27 induces the expression of AVPs in an IFN-independent manner, both in MDMs and keratinocytes in response to the infectious agent. Thus, IL27 signaling may play a relevant novel mechanism of inhibition of CHIKV infection. Taken together, these findings suggest that induction of AVPs by IL27 in MDMs is independent of the three types IFNs described.

IFNs are crucial factors in the control of alphavirus replication (Reviewed in [1]). Hence, viruses including CHIKV have acquired the ability to block IFN signaling pathway. One of the proteins implicated in the control of antiviral effects IFNs-dependent is CHIKV nsP2, a polyfunctional protein with helicase activity, RNA triphosphatase, nucleoside triphosphatase, methyltransferase, and papain-like cysteine protease activity implicated in viral replication [38–39]. CHIKV nsP2 orchestrate down-regulation of IFN-dependent cellular antiviral state in multiple steps, including the blocking of IFN-I dependent JAK-STAT signaling pathway activation [40], and degradation of Rpb1 (a catalytic subunit of RNA Polymerase II, through the ubiquitin–proteasome system), blocks the activation of cellular gene transcription and down-regulation of cellular antiviral response late in CHIKV replication cycle [41]. However, the mechanism of CHIKV to block interferon expression in infected cells has not been previously reported. Fros *et al.*, (2010) [40] reported that CHIKV replication was resistant to IFN treatment and block IFN-induced JAK-STAT signaling downstream gene transcription

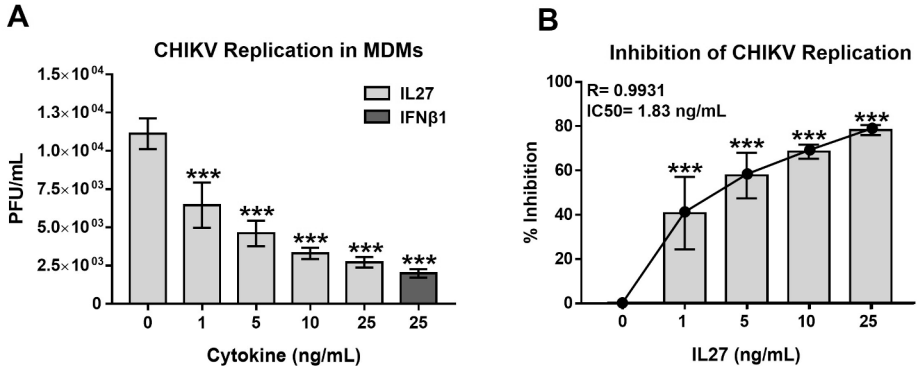

Fig. 7. IL27 pre-treatment inhibits CHIKV replication in MDMs. Human MDMs were pre-treated for 6 h with increasing concentrations of recombinant-human IL27 or 25 ng/mL of recombinant-human IFNβ1. Next, MDMs cultures were infected with CHIKV at MOI 5. Culture supernatants were obtained at 24 hpi and viral titration was performed by plaque assay on Vero cells. CHIKV replication in MDMs (A). Percentage of viral inhibition and IC50 of IL27 in MDMs (B). Kruskal-Wallis test with Dunn's post-test was performed. Significant results between unstimulated and pre-treated MDMs are defined as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.002$ (***). $n = 4$.

independently of host shutoff. Furthermore, when cells were infected with CHIKV 12 h before IFNs stimulation, STAT1 and STAT2 nuclear translocation were completely altered. Also, since CHIKV infection decreases the STAT2 expression, it has suggested that CHIKV might have evolved strategies to evade the antiviral response by modulating STAT2 activity [42–43]. If STAT2 is inhibited by CHIKV, thus, IL27 could play a novel host-defensive role to subvert viral decreases of all types IFNs regulatory molecules, including STAT2. In further support of the role of IL27 in specifically inducing STAT1 signaling, Kwock *et al* (2020) [26] reported that p-STAT1, but not p-STAT2, was rapidly activated in keratinocytes stimulated with IL27, and consequently, this cytokine directly signals via STAT1 to induces AVPs expression.

A characteristic of IL27 is that it consists of a heterogeneous dimer with an α subunit (IL27p28) and a β subunit (EBI3) [31]. Therefore, inhibition of either an IL27p28 or EBI3 subunit leads to the alteration of the biological function of IL27, since it is only active when both subunits are present [12,31]. Our IL27 expression kinetics shown a differential regulation of both IL27p28 and EBI3. We show that EBI3 is expressed early in CHIKV-infected MDMs with a peak of mRNA at 6 hpi, whereas, in the case of IL27p28, we observed a peak of mRNA expression at 24 hpi, a result consistent with kinetic of mature IL27 accumulation and AVPs expression, suggesting that both subunits of IL27 are required for induction of antiviral response in CHIKV-infected MDMs. Additionally, these results suggest that both IL27 subunits have different transcriptional and temporal regulation.

IL27 expression is induced in APCs in response to TLRs activation [18]. Recently, we reported a positive correlation between TLR3 and AVPs expression in CHIKV-infected MDMs [10], suggesting that TLR3 activation could be implicated in IL27 production by MDMs in response to CHIKV infection. However, the relevance of TLR3 activation in IL27 expression requires further investigation.

CHIKV pathogenesis has an important immunological component (Reviewed [1]). Higher levels of pro-inflammatory cytokines (IL1β, IL6, IL12p70, IL15), CC– chemokines (CCL2, CCL3, CCL5, and CCL8), and CXC- chemokines (CXCL9, CXCL10, CXCL11) are detected in CHIKV-infected patients. Some of these cytokines/chemokines, such as IL1β, IL6, CCL5, and CCL8 have been correlated with the severity of CHIKF, while others, including IL1Ra, IL6, IL12p70, IL16, IL17, IL18, CCL2, and CXCL10 were correlated with high CHIKV loads [1,44]. In addition, Cavalcanti *et al.* 2019 [27] shown that IL27 serum levels were higher in patients with CHIKV chronic symptoms than in ones in the acute or subacute stage of the disease. Furthermore, in patients with chronic symptoms of CHIKF, a significant correlation between IL27 levels and tender joint counts was observed. We show that IL27 promotes an antiviral state in CHIKV-infected MDMs; IL27 also mediates the expression of inflammatory factors. Among them, cytokines such as IL7, IL15, IL32, BAFF and TRAIL, CC– chemokines including CCL2, CCL7, CCL8, and CXC- chemokines, such as CXCL9, CXCL10, and CXCL11 (Fig. 5A). All arthritogenic factors up-regulated in Rheumatoid arthritis [45–46] as in CHIKV-infected patients and had been associated with the development of inflammation and joint pain [33,36,45–46], suggesting a possible role of IL27 in the pathogenesis of CHIKV-dependent arthralgia and arthritis.

In conclusion, these results have shown that IL27 is highly expressed in CHIKV-infected MDMs, leading to the activation of JAK-STAT signaling and induction of robust pro-inflammatory and antiviral response in an IFN-independent manner. Furthermore, based on our results and others already discussed above, we propose that IL27 signals through STAT1 and IL27Rα to generate ISGs transcripts encoding AVPs, cytokines, and chemokines in an IFN-independent manner, to generate an immune response against CHIKV infection in MDMs.

CRediT authorship contribution statement

Juan Felipe Valdés-López: Conceptualization, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Geysson J. Fernandez:** Conceptualization, Writing - review & editing. **Silvio Urcuqui-Inchima:** Conceptualization, Writing - original draft, Resources, Supervision, Project administration.

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.

Acknowledgments

The authors thank Ajit Kumar for reading and the English editing of the manuscript and her constructive and valuable comments. This research was supported by Minciencias/Colciencias [grant No. 111574455028 and contrato No. 455-2019], and Universidad de Antioquia-CODI, acta 2017-16389. The funders played no role in the study design, data collection, and analysis, decision to publish, or preparation of the manuscript. 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 and the personnel at the institutions where the study was performed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellimm.2021.104411.

References

[1] J.F. Valdés López, P.A. Velilla, S. Urcuqui-Inchima, Chikungunya Virus and Zika Virus, Two Different Viruses Examined with a Common Aim: Role of Pattern Recognition Receptors on the Inflammatory Response, J Interf CYTOKINE Res 39 (9) (2019) 507–521, https://doi.org/10.1089/jir.2019.0058.
[2] D.B. Stetson, R. Medzhitov, Type I Interferons in Host Defense, Immunity 25 (3) (2006) 373–381, https://doi.org/10.1016/j.immuni.2006.08.007.
[3] G. Uzé, G. Schreiber, J. Piehler, S. Pellegrin, The Receptor of the Type I Interferon Family, Interf 50th Anniv 1 (2007) 71–95.
[4] J. Piehler, C. Thomas, K.C. Garcia, G. Schreiber, Structural and dynamic determinants of type I interferon receptor assembly and their functional interpretation, Immunol Rev 250 (1) (2012) 317–334, https://doi.org/10.1111/imr.12001.
[5] S. Pestka, C.D. Krause, M.R. Walter, Interferons, interferon-like cytokines, and their receptors, Immunol Rev 202 (1) (2004) 8–32.
[6] S.V. Kotenko, G. Gallagher, V.V. Baurin, A. Lewis-Antes, M. Shen, N.K. Shah, J. A. Langer, F. Sheikh, H. Dickensheets, R.P. Donnelly, IFN- λ s mediate antiviral protection through a distinct class II cytokine receptor complex, Nat Immunol 4 (1) (2003) 69–77, https://doi.org/10.1038/ni875.
[7] T. Taniguchi, A. Takaoka, The interferon- α/β system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors, Curr Opin Immunol 14 (1) (2002) 111–116.
[8] C.E. Samuel, Antiviral Actions of Interferons, Clin Microbiol Rev 14 (2001) 778–800, https://doi.org/10.1128/CMR.14.4.778.
[9] N.A. Weerd, T. Nguyen, The interferons and their receptors-distribution and regulation, Immunol Cell Biol 90 (5) (2012) 483–491, https://doi.org/10.1038/icb.2012.9.
[10] V.L.J. Felipe, V. Paula A, U.-I. Silvio, Chikungunya virus infection induces differential inflammatory and antiviral responses in human monocytes and monocyte-derived macrophages, Acta Trop 211 (2020) 105619, https://doi.org/10.1016/j.actatropica.2020.105619.
[11] A. Salas, C. Hernandez-Rocha, M. Duijvestein, W. Faubion, D. McGovern, S. Vermeire, S. Vetrano, N. Vande Casteele, JAK–STAT pathway targeting for the treatment of inflammatory bowel disease, Nat Rev Gastroenterol Hepatol 17 (6) (2020) 323–337, https://doi.org/10.1038/s41575-020-0273-0.
[12] J. Ye, Y. Wang, Z. Wang, L. Liu, Z. Yang, M. Wang, Y. Xu, D. Ye, J. Zhang, Y. Lin, Q. Ji, J. Wan, Roles and Mechanisms of Interleukin-12 Family Members in Cardiovascular Diseases: Opportunities and Challenges, Front Pharmacol 11 (2020) 129, https://doi.org/10.3389/fphar.2020.00129c.
[13] D.H. Presky, H. Yang, L.J. Minetti, A.O. Chua, N. Nabavi, C.Y. Wu, M.K. Gately, U. Gubler, A functional interleukin 12 receptor complex is composed of two B-type cytokine receptor subunits, Proc Natl Acad Sci USA 93 (1996) 14002–14007.
[14] B. Oppmann, R. Lesley, B. Blom, J.C. Timans, Y. Xu, B. Hunte, F. Vega, N. Yu, J. Wang, K. Singh, F. Zonin, E. Vaisberg, T. Churakova, M.-r. Liu, D. Gorman, J. Wagner, S. Zurawski, Y.-J. Liu, J.S. Abrams, K.W. Moore, D. Rennick, R. de Waal-Malefyt, C. Hannum, J.F. Bazan, R.A. Kastelein, Novel p19 Protein Engages IL-

12p40 to Form a Cytokine, IL-23, with Biological Activities Similar as Well as Distinct from IL-12, Immunity 13 (5) (2000) 715–725.
[15] S. Pflanz, L. Hibbert, J. Mattson, R. Rosales, E. Vaisberg, J.F. Bazan, J.H. Phillips, T. K. McClanahan, R. de Waal Malefyt, R.A. Kastelein, WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27, J Immunol 172 (4) (2004) 2225–2231, https://doi.org/10.4049/jimmunol.172.4.2225.
[16] L.W. Collison, G.M. Delgoffe, C.S. Guy, K.M. Vignali, V. Chaturvedi, D. Fairweather, A.R. Satoskar, K.C. Garcia, C.A. Hunter, C.G. Drake, P.J. Murray, D. A.A. Vignali, The composition and signaling of the IL-35 receptor are unconventional, Nat Immunol 13 (3) (2012) 290–299, https://doi.org/10.1038/ni.2227.
[17] E.D. Tait Wojno, C.A. Hunter, J.S. Stumhofer, The Immunobiology of the Interleukin-12 Family: Room for Discovery, Immunity 50 (4) (2019) 851–870, https://doi.org/10.1016/j.immuni.2019.03.011.
[18] C. Hunter, R. Kastelein, Review Interleukin-27: Balancing Protective and Pathological Immunity, Immunity 37 (6) (2012) 960–969, https://doi.org/10.1016/j.immuni.2012.11.003.
[19] G. Murugaiyan, A. Mittal, R. Lopez-Diego, L.M. Maier, D.E. Anderson, H.L. Weiner, IL-27 Is a Key Regulator of IL-10 and IL-17 Production by Human CD4+ T Cells, J Immunol 183 (2009) 2435–2443, https://doi.org/10.4049/jimmunol.0900568.IL-27.
[20] N. Morishima, T. Owaki, M. Asakawa, S. Kamiya, J. Mizuguchi, T. Yoshimoto, Augmentation of Effector CD8+ T Cell Generation with Enhanced Granzyme B Expression by IL-27, J Immunol 175 (3) (2005) 1686–1693, https://doi.org/10.4049/jimmunol.175.3.1686.
[21] F. Larousserie, P. Charlot, E. Bardel, J. Froger, R.A. Kastelein, O. Devergne, Differential Effects of IL-27 on Human B Cell Subsets, J Immunol 176 (10) (2006) 5890–5897, https://doi.org/10.4049/jimmunol.176.10.5890.
[22] J.A. Harker, K.A. Wong, S. Dallari, P. Bao, A. Dolgoter, Y. Jo, E.J. Wehrens, M. Macal, E.I. Zuniga, A. García-Sastre, Interleukin-27R Signaling Mediates Early Viral Containment and Impacts Innate and Adaptive Immunity after Chronic Lymphocytic Choriomeningitis Virus Infection, J Virol 92 (12) (2018), https://doi.org/10.1128/JVI.02196-17.
[23] J.M. Fakruddin, R.A. Lempicki, R.J. Gorelick, J. Yang, J.W. Adelsberger, A. J. Garcia-Pineres, L.A. Pinto, H.C. Lane, T. Imamichi, Noninfectious papilloma virus–like particles inhibit HIV-1 replication: implications for immune control of HIV-1 infection by IL-27, Blood 109 (2007) 1841–1849, https://doi.org/10.1182/blood-2006-02-001578.
[24] A.C. Frank, X. Zhang, A. Katsounas, J.P. Bharucha, S. Kottilil, T. Imamichi, Interleukin-27, an Anti-HIV-1 Cytokine, Inhibits Replication of Hepatitis C Virus, J Interf CYTOKINE Res 30 (6) (2010) 427–431, https://doi.org/10.1089/jir.2009.0093.
[25] Y. Cao, R. Zhang, W. Zhang, C. Zhu, Y.i. Yu, Y.u. Song, Q. Wang, L. Bai, Y. Liu, K. Wu, J. Wu, IL-27, a Cytokine, and IFN- λ1, a Type III IFN, Are Coordinated To Regulate Virus Replication through Type I IFN, J Immunol 192 (2) (2014) 691–703, https://doi.org/10.4049/jimmunol.1300252.
[26] J.T. Kwock, C. Handfield, J. Suwanpradid, P. Hoang, M.J. McFadden, K. F. Labagnara, L. Floyd, J. Shannon, R. Uppala, M.K. Sarkar, J.E. Gudjonsson, D. L. Corcoran, H.M. Lazear, G. Sempowski, S.M. Horner, A.S. MacLeod, IL-27 signaling activates skin cells to induce innate antiviral proteins and protects against Zika virus infection, Sci Adv 6 (14) (2020) eaay3245, https://doi.org/10.1126/sciadv.aay3245.
[27] N.G. Cavalcanti, K. Melovilar, A. Luzia, B. Pinto, M. Jesus, B. Melo, M.C. Pereira, R. Pitta, C. Diniz, L. Marques, M. Galdino, IL-27 in patients with Chikungunya fever: A possible chronicity biomarker? Acta Trop 196 (2019) 48–51, https://doi.org/10.1016/j.actatropica.2019.05.005.
[28] B. Pastorino, M. Bessaud, M. Grandadam, S. Murri, H.J. Tolou, C.N. Peyrefitte, Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African Chikungunya viruses, J Virol Methods 124 (1-2) (2005) 65–71, https://doi.org/10.1016/j.jviromet.2004.11.002.
[29] J.F. Valdés López, S. Urcuqui-Inchima, Synergism between phorbol-12-myristate-13-acetate and vitamin D3 in the differentiation of U937 cells to monocytes and macrophages, Morphologie **102** 102 (338) (2018) 205–218, https://doi.org/10.1016/j.morpho.2018.06.001.
[30] K. Dhar, K. Rakesh, D. Pankajakshan, D.K. Agrawal, SOCS3 promotor hypermethylation and STAT3-NF-κB interaction downregulate SOCS3 expression in human coronary artery smooth muscle cells, Am J Physiol Heart Circ Physiol 304 (6) (2013) H776–H785, https://doi.org/10.1152/ajpheart.00570.2012.
[31] S. Pflanz, J.C. Timans, J. Cheung, R. Rosales, H. Kanzler, J. Gilbert, L. Hibbert, T. Churakova, M. Travis, E. Vaisberg, W.M. Blumenschein, J.D. Mattson, J. L. Wagner, W. To, S. Zurawski, T.K. McClanahan, D.M. Gorman, J.F. Bazan, R. de Waal Malefyt, D. Rennick, R.A. Kastelein, IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells, Immunity 16 (6) (2002) 779–790, https://doi.org/10.1016/S1074-7613(02)00324-2.
[32] W.M. Schneider, M.D. Chevillotte, C.M. Rice, Interferon-Stimulated Genes: A Complex Web of Host Defenses, Annu Rev Immunol 32 (1) (2014) 513–545, https://doi.org/10.1146/annurev-immunol-032713-120231.
[33] I. González-Álvaro, C.C. Domínguez-jiménez, A.M. Ortiz, V. Núñez-gonzález, P. Roda-navarro, E. Fernández-ruiz, D. Sancho, F. Sánchez-madrid, Interleukin-15 and interferon-γ participate in the cross-talk between natural killer and monocytic cells required for tumour necrosis factor production Research article, Arthritis Res Ther 8 (2006) R88, https://doi.org/10.1186/ar1955.
[34] X. Yang, M. Arslan, X. Liu, H. Song, M. Du, Y. Li, Z. Zhang, IFN-γ establishes interferon-stimulated gene-mediated antiviral state against Newcastle disease virus in chicken fibroblasts, Acta Biochim Biophys Sin 52 (2020) 268–280, https://doi.org/10.1093/abbs/gmz158.
[35] T.F. Decade, M.I. Musterman, Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases, Biomol Concepts 9 (2018) 64–79. https://doi.org/10.1515/bmc-2018-0007.
[36] S. Kim, H.J. Chang, M.V. Volin, S. Umar, K.V. Raemdonck, A. Chevalier, K. Palasiewicz, J.W. Christman, S. Volkov, S. Arami, M. Maz, A. Mehta, R.K. Zomorrodi, D.A. Fox, N. Sweiss, S. Shahrara, Macrophages are the primary effector cells in IL-7-induced arthritis, Cell Mol Immunol 17 (2019) 728–74. https://doi.org/10.1038/s41423-019-0235-z.
[37] V. Chaudhary, K. Yuen, J.F. Chan, C. Chan, Selective Activation of Type II Interferon Signaling by Zika Virus NS5 Protein, J Virol 91 (2017) 1–17.
[38] T. Ahola, P. Laakkonen, H. Vihinen, L. Kääriäinen, Critical residues of Semliki Forest virus RNA capping enzyme involved in methyltransferase and guanylyltransferase-like activities, J Virol 71 (1) (1997) 392–397.
[39] L. Vasiljeva, A. Merits, P. Auvinen, L. Kääriäinen, Identification of a novel function of the alphavirus capping apparatus. RNA 5'-triphosphatase activity of Nsp2, J Biol Chem 275 (23) (2000) 17281–17287, https://doi.org/10.1074/jbc.M910340199.
[40] J.J. Fros, W.J. Liu, N.A. Prow, C. Geertsema, M. Ligtenberg, D.L. Vanlandingham, E. Schnettler, J.M. Vlak, A. Suhrbier, A.A. Khromykh, G.P. Pijlman, Chikungunya Virus Nonstructural Protein 2 Inhibits Type I/II Interferon-Stimulated JAK-STAT Signaling, J Virol 84 (20) (2010) 10877–10887, https://doi.org/10.1128/JVI.00949-10.
[41] I. Akhrymuk, S.V. Kulemzin, E.I. Frolova, Evasion of the Innate Immune Response: The Old World Alphavirus nsP2 Protein Induces Rapid Degradation of Rpb1, a Catalytic Subunit of RNA Polymerase II, J Virol 86 (13) (2012) 7180–7191, https://doi.org/10.1128/JVI.00541-12.
[42] M. Paingankar, V. Arankalle, Identification of chikungunya virus interacting proteins in mammalian cells, jorurnal Biosci 39 (2014) 389–399, https://doi.org/10.1007/s12038-014-9436-x.
[43] S. Wichit, F. Diop, R. Hamel, L. Talignani, P. Ferraris, S. Cornelie, F. Liegeois, F. Thomas, H. Yssel, D. Missé, Aedes Aegypti saliva enhances chikungunya virus replication in human skin broblasts via inhibition of the type I interferon signaling pathway, Infect Genet Evol 55 (2017) 68–70, https://doi.org/10.1016/j.meegid.2017.08.032.
[44] A. Chow, Z. Her, E.K.S. Ong, J. Chen, F. Dimatatac, D.J.K. Kwek, T. Barkham, H. Yang, L. Rénia, Y.S. Leo, L.F.P. Ng, Persistent arthralgia induced by Chikungunya virus infection is associated with interleukin-6 and granulocyte macrophage colony-stimulating factor, J Infect Dis 203 (2011) 149–157, https://doi.org/10.1093/infdis/jiq042.
[45] A. Burska, M. Boissinot, F. Ponchel, Cytokines as Biomarkers in Rheumatoid Arthritis, Mediators Inflamm 2014 (2014) 1–25.
[46] H. Yap, S.Z. Tee, M.M. Wong, S. Chow, S. Peh, S. Teow, Pathogenic Role of Immune Cells in Rheumatoid Arthritis: Implications in Clinical Treatment and Biomarker Development, Cells 7 (2018) 161, https://doi.org/10.3390/cells7100161.