Virus Research 325 (2023) 199040

Contents lists available at ScienceDirect

Virus Research

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

American-Asian- and African lineages of Zika virus induce differential pro-inflammatory and Interleukin 27-dependent antiviral responses in human monocytes

Lady Johana Hernández-Sarmiento, Juan Felipe Valdés-López, Silvio Urcuqui-Inchima *

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

ARTICLE INFO

Keywords:
Zika virus
Monocytes
Innate immune response
Antiviral response
Interleukin 27
RNA-seq

ABSTRACT

Zika virus (ZIKV) is an arbovirus that belongs to the *Flaviviridae* family and inflammatory responses play a critical role in ZIKV pathogenesis. As a first-line defense, monocytes are key components of innate immunity and host response to viruses. Monocytes are considered the earliest blood cell type to be infected by ZIKV and have been shown to be associated with ZIKV pathogenesis. The first ZIKV epidemic was reported in Africa and Asia although, it is less well known whether African- and Asian- lineages of ZIKV have different impacts on host immune response. We studied the pro-inflammatory and antiviral response of ZIKV-infected monocytes using publicly available RNA-seq analysis (GSE103114). We compared the transcriptomic profiles of human monocytes infected with ZIKV Puerto Rico strain (PRVABC59), American-Asian lineage, and ZIKV Nigeria strain (IBH30656), African lineage. We validated RNA-seq results by ELISA or RT-qPCR, in human monocytes infected with a clinical isolate of ZIKV from Colombia (American-Asian lineage), or with ZIKV from Dakar (African lineage). The transcriptomic analysis showed that ZIKV Puerto Rico strain promotes a higher pro-inflammatory response through TLR2 signaling and NF-kB activation and induces a strong IL27-dependent antiviral activity than ZIKV Nigeria strain. Furthermore, human monocytes are more susceptible to infection with ZIKV from Colombia than ZIKV from Dakar. Likewise, Colombian ZIKV isolate activated IL27 signaling and induced a robust antiviral response in an IFN-independent manner. Moreover, we show that treatment of monocytes with IL27 results in decreased release of ZIKV particles in a dose-dependent manner with an EC50 =2.870 ng/mL for ZIKV from Colombia and EC50 =10.23 ng/mL to ZIKV from Dakar. These findings highlight the differential inflammatory response and antiviral activity of monocytes infected with different lineages of ZIKV and may help better management of ZIKV-infected patients.

1. Introduction

Zika virus (ZIKV) is an arbovirus member of *Flaviviridae* family, *Flavivirus* genus. ZIKV has a single-strand positive-sense RNA (ssRNA+) genome that encodes a single polyprotein, which is processed by viral and cellular proteases into three structural proteins, capsid (C), pre-membrane (prM), and envelope (E), involved in the assembly of new viral particles; and seven non-structural (NS) proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5, involved in viral genome replication, polyprotein processing, particle assembly and evasion of innate antiviral response (Chambers et al., 1990; Wang et al., 2017). ZIKV is transmitted to humans by biting of infected female mosquitoes of the *Aedes* genus. However, human-to-human transmission of ZIKV has also been reported, including sexual contact, blood transfusion, and vertical transmission from mothers to the fetus (Magnus et al., 2018; Miner and Diamond, 2017; Pierson and Diamond, 2018).

ZIKV was identified in Africa in 1947 (Dick et al., 1952). Two phylogenetic lineages, Asian and African were reported (Basarab et al., 2016). African prototype strain (MR-766) was isolated in Uganda, while the Asian lineage was responsible for the epidemic outbreaks in Micronesia (2007), French Polynesia (2013), and South and Central America in 2014 (Weaver et al., 2016), causing increasing public health concern. Phylogenetic analyses using ZIKV genomes show that Asian lineage strains are grouped into the American, Pacific, and Southeast Asian subtypes, with several differences in their amino acid sequences (Hashimoto et al., 2017). African ZIKV isolate (MR-766) is more virulent

* Corresponding author at: Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellin, Colombia.
E-mail address: silvio.urcuqui@udea.edu.co (S. Urcuqui-Inchima).

https://doi.org/10.1016/j.virusres.2023.199040
Received 28 September 2022; Received in revised form 23 December 2022; Accepted 4 January 2023
Available online 5 January 2023

and causes more severe brain damage than asian ZIKV (MEX1–44) after intracranial infection in mice (Shao et al., 2017); african ZIKV strains also induces a higher cytopathic effect in human placental trophoblast as compared to asian ZIKV strains *in vitro* (Sheridan et al., 2018). Further studies in mice models of ZIKV infection showed that American ZIKV strains induce more severe neurological disorders and a higher inflammatory responses as compared with Southeast Asian strain (Zhang et al., 2017).

Although most ZIKV–infected patients are asymptomatic, ZIKV is the etiological agent of Zika fever (ZIKF), a self-limited disease characterized by maculopapular rash, headache, joint pain, conjunctivitis, and myalgia (Kelser, 2016). However, during the ZIKV outbreak in South-center America, there was increase in severe pathologies associated with ZIKV infection, including neurological disorders such as Guillain-Barré and alice in wonderland syndromes in adults (Paniz-Mondolfi et al., 2018), teratogenesis and microcephaly in newborns (Azevedo et al., 2018; de Oliveira et al., 2017). Thus, African- and Asian-lineage of ZIKV appear to differ in virulence from the newly evolved American-subtype ZIKV emerging as important global health problem (Esser-Nobis et al., 2019). Indeed, neurological disorders associated with American-subtype ZIKV has been associated with viral genetic changes present only in contemporary American strains of Asian lineage, including an additional N-glycosylation motif in E protein which is absent in ancestral African and Asian ZIKV lineages, and which has been associated with an increase in ZIKV virulence (Carbaugh et al., 2019; Pierson and Diamond, 2018; Valdés López et al., 2019).

In mammals, the first line of defense against viral infections is the innate immune response through the pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) (Brubaker et al., 2015). TLRs sense foreign and conserved microbial components called pathogen-associated molecular patterns (PAMPs), that activate signaling pathways and induce both pro-inflammatory and antiviral responses (Kawai and Akira, 2010). Among TLRs, it has been reported that TLR3 (Dang et al., 2016; Faizan et al., 2017; Hamel et al., 2015), and TLR7/8 (Luo et al., 2018; Vanwalscappel et al., 2018) play a crucial role in detecting ZIKV RNAs. Induction of pro-inflammatory response by TLRs is dependent of nuclear factor-κB (NF-κB) activation, a family of structurally related transcription factors [NF-κB1, NF-κB2, RELA, RELB, c-REL, and IκBα (negative regulator)] activated by diverse stimuli, including ligands of some cytokine receptors and PRRs. NF-kB activation induces coordinated transcription of NF-kB-target genes, including pro-inflammatory cytokines [Tumoral necrosis factor-α (TNFα)], Interleukins [(IL)−1β (IL1β), IL6, and IL12p40], chemokines (CXCL1 and CXCL8/IL8), and enzymes such as cyclooxygenase 2 (COX2) that promote inflammatory response in different cell types (Valdés-López et al., 2022).

The acute phase of ZIKF is characterized by high serum levels of pro-inflammatory cytokines in patients, including TNFα, IL1β, IL2, IL4, IL6, IL9, IL10, IL13, and IL17, which is correlated with disease severity (da Silva et al., 2019; Kam et al., 2017; Tappe et al., 2016). Furthermore, ZIKV-infected cells secrete cytokines with antiviral activity, including Interferons (IFNs). Interferons are a group of cytokines that regulate the immune system and induce the antiviral state in cells (Stetson and Medzhitov, 2006). Three types of IFNs have been reported (Sen, 2001), Whereas type I IFN [IFN-I (IFNα, β, ε, κ, ω, ζ)] bind to IFN alpha/beta receptor complex [IFNAR (IFNAR1/IFNAR2)] (Piehler et al., 2012), type II IFN [IFN-II (IFNγ)] signals through IFN gamma receptor complex [IFNGR (IFNGR1/IFNGR2)] (Pestka et al., 2004). Type III IFNs (IFN-III) have three members [IFNλ1 (IL29), IFNλ2 (IL28A), and IFNλ3 (IL28B)] that bind to the IFN-lambda receptor complex [IFNLR (IFNLR1/IL10RB) (Kotenko et al., 2003). Interaction of IFNs with their receptors on cell surface activate Janus kinase (JAK) signaling pathway which phosphorylates and activates heterodimers of signal transducer and activator of the transcription 1 (STAT1) and STAT2 (in case of IFN-I and IFN-III), or homodimers of STAT1 (in case of IFN-II). Subsequently, these protein complex are translocated to the nucleus where they induce coordinated expression of IFN-stimulated genes (ISGs) encoding antiviral proteins (AVPs) such as ISG15, 2′−5′-oligoadenylate synthetases (OAS) family proteins, double-stranded RNA-activated protein kinase R (PKR), and viperin (Faizan et al., 2017; Sen, 2001; Stetson and Medzhitov, 2006; Tappe et al., 2016) among others, implicated in control of ZIKV replication (Piehler et al., 2012). Several studies have reported that ZIKV replication can be inhibited by type I IFNs *in vitro* and *in vivo*. In primary skin fibroblast pre-treated with IFNα or IFNβ, ZIKV replication was shown to be decreased as compared to untreated cells (Hamel et al., 2015). Mice lacking IFNAR1 or IRF3/5 and 7 showed more severe neurological disease compared to wild type mice (Lazear et al., 2016). This was linked to the expression of ISGs, including IFIT1–3, Viperin and OAS1 in primary human dendritic cells (DCs; Hertzog et al., 2018). Furthermore, the silencing or overexpression of the ISG protein IFITM3 enhanced or reduced, respectively, ZIKV replication in HeLa cells (Savidis et al., 2016). Together, the results suggest a key role for IFNs in control of ZIKV replication.

In recent years, a non-canonical ISGs induction pathway dependent on interleukin 27 (IL27) has been reported. IL27, a member of the IL12 family of cytokines, is a heterodimeric protein composed of IL27p28 and Epstein-Barr virus-induced 3 (EBI3) subunits (Pflanz et al., 2002; Rousseau et al., 2010). Both IL27 subunits have diferential transcriptional regulation and are induced by activation of transcription factors IRF1 and IRF7 for IL27p28, and NF-kB1 for EBI3 (Valdés-López et al., 2022). IL27 signal through IL27 receptor (IL27R), a heterodimeric complex that consists of signal-transducing glycoprotein 130 (gp130) and the orphan cytokine receptor IL27Rα (Yoshida and Hunter, 2015), that trigger JAK-STAT signaling and activate STAT1 and STAT3 transcription factors (Huber et al., 2008; Kwock et al., 2020; Pflanz et al., 2004). IL27 is secreted by activated endothelial cells and antigen presenting cells (APC), including DCs, macrophages and monocytes (Hall et al., 2012), and induces both pro- and anti-inflammatory responses. Further, it has been reported that IL27 induces robust antiviral response through STAT1 and IL27RA, independent of STAT2, TYK2, and IFNAR1. Thus, IL27 activates STAT1, which translocates into the nucleus to induces transcription of ISGs containing gamma-interferon activated site (GAS) elements in the promoter (Kwock et al., 2020; Valdés-López et al., 2022). Interestingly, Kwock reported that human epidermal keratinocytes (hNEK) treated with recombinant IL27, induce innate antiviral proteins expression that protect against ZIKV infection (Kwock et al., 2020).

Although monocytes are phagocytic cells of innate immune sytem that act as first-line of defense against viral infection, they have been shown to associate with ZIKV infection/transmission and pathogenesis. Monocytes are the main target cells of ZIKV infection in humans. Both *in vitro* and *ex vivo* experiments have shown that monocytes constitute 84% of peripheral blood mononuclear cells (PBMCs) infected by ZIKV (Michlmayr et al., 2017). Further, ZIKV-infected monocytes can infiltrate immunoprotective organs, leading to the spread of ZIKV in different tissues, including the central nervous system through a mechanism known as "Trojan horses". Eventhough monocytes are the main targets of ZIKV infection in humans, their role in the induction of pro-inflammatory and antiviral response is less well understood. Here, we performed comparative analysis of the funtional response of human monocytes to infection by ZIKV Puerto Rico strain (PRVABC59; American-Asian lineage), and ZIKV Nigeria strain (IBH30656; African lineage), using publicly available RNA-seq (Khaiboullina et al., 2017). Furthermore, the expression of a set of genes identified by transcriptome analysis was confirmed in human monocytes infected with ZIKV isolates from Colombia (American-Asian lineage), or with ZIKV from Dakar (African lineage) by ELISA and/or RT-qPCR.

2. Materials and methods

2.1. Ethics statement

The protocols for individual enrollment and sample collection were approved by the Committee of Bioethics Research of Sede de Investigacion Universitaria, Universidad de Antioquia (Medellín, Colombia), and inclusion was preceded by a signed informed consent form, according to the principles expressed in the Declaration of Helsinki. Between 4–5 healthy donors from Medellín, Colombia were involved in this study.

2.2. Cells lines, ZIKV stocks and viral titration

Aedes albopictus derived C6/36-HT cells (ATCC) were grown in Leibovitz's L-15 Medium (L-15; Sigma-Aldrich) supplemented with 5% heat-inactivated fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Massachusetts, USA) and 1% antibiotic-antimycotic solution (Corning, New York, USA), and incubated at 34 °C in cell culture flasks at a density of 1×10^5–1×10^6 cells/mL.

ZIKV Colombia strain (GenBank: MH179341.1) was isolated from mosquitoes (kindly gifted by Professor Blanco P. Universidad de Sucre, Colombia) and is a member of America-Asian lineage ZIKV. ZIKV Dakar 41,525 (GenBank: MG758785.1) is a member of African lineage ZIKV. All Zika virus strains were obtained by grown in C6/36-HT cells, using a multiplicity of infection (MOI) of 0.01. Virus culture supernatants were stored at −80 °C and titrated by plaque assay on BHK-21 cells (clone 15, ATCC).

2.3. Culture of primary human monocytes

Human peripheral blood mononuclear cells (PBMCs) from blood samples of healthy donors and mixed with 2% (v/v) EDTA, were isolated through density gradient with Lymphoprep (STEMCELL Technologies Inc, Vancouver, Canada) by centrifugation at 850 x g for 21 min, as previously described (Valdés López et al., 2020). 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. To obtain monocytes, 24-well plastic plates were scratched with a 1000 μL pipette tip and seeded with 5×10^5 CD14 positive cells per well and allowed to adhere for 2 h in RPMI-1640 medium (Sigma-Aldrich) supplemented with 0.5% autologous serum or plasma, 4 mM L-glutamine, and 0.3% $NaCO_3$ 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% FBS, 4 mM L-glutamine, 0.3% $NaCO_3$, and 1% antibiotic-antimycotic solution 100X (complete medium) and incubated at 37 °C and 5% CO_2 overnight, as previously described (Valdés López et al., 2020; Valdés López and Urcuqui-Inchima, 2018).

2.4. In vitro ZIKV infection of human monocytes

ZIKV from Colombia (1.1×10^7 PFU/mL) and ZIKV from Dakar (5.0×10^7 PFU/mL) were used to infected human primary monocytes at MOI 5 in serum-free RPMI-1640 and the 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 fresh complete medium was added. Cells were incubated at 37 °C and 5% CO_2, and culture supernatants and cell lysates were collected at 6, 12, 24, 48, and 72 hours post-infection (hpi) and stored at −80 °C.

2.5. Bioinformatic analysis of previously published RNA-seq datasets and prediction of cell signaling pathways activation

We reanalyzed the publicly available RNA-seq GSE103114 (GEO) (Khaiboullina et al., 2017) obtained from human monocytes infected with an American-Asian ZIKV strain [ZIKV Puerto Rico strain (PRVABC59)] or an African ZIKV strain [ZIKV Nigeria strain (IBH30656)] at MOI 0.1. The data analysis was performed using the DESeq library in RStudio (Version 3.6.3). mRNA expression dataset was filtered to remove low-expressed genes (less than 32 counts). 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) (ZIKV-Infected monocytes/Uninfected monocytes) $|> 0.6$ ($|\log_2 FC| > 0.6$), were used as the threshold to determine the statistically significant difference in gene expression. Each Gene Ontology (GO) was performed with the BiNGO Cytoscape plugin, using a hypergeometric test with a Benjamini-Hochberg False Discovery Rate correction, to identify significant functions of the DEGs. A $p < 0.05$ was used to identify enriched processes.

To predict the activation of cell signaling pathways, we quantify mRNA expression ($\log_2 FC$) of some immune biomarkers associated with TLR signaling pathway, NF-κB-complex, NF-κB-target genes, IRFs, inductors of antiviral response, IL27 signaling pathway, ISGs, STAT1-dependent cytokines, and STAT1-dependent CC- and CXC-chemokines as was previously reported (Valdés-López et al., 2022).

2.6. Real-time RT-qPCR

Total RNA was extracted from ZIKV-infected monocytes using the Quick-RNA™ Miniprep Kit (Zymo Research, USA), following the manufacturer's instructions and the concentration was determined using NanoDrop-1000 spectrophotometer (Thermo Scientific, Wilmington, DE). The RNA was used to synthesize copy DNA (cDNA) using the commercial RevertAid Minus First Strand cDNA Synthesis Kit (Thermo Scientific, NH, USA), following the manufacturer's instructions. The following gene-specific primer pairs were used TLR2 forward: 5′-GGCCAGCAAATTACCTGTGTG-3′, and reverse: 5′- CCAGGTAGGT CTTGGTGTTCA-3′. TLR7 forward:TCTACCTGGGCCAAAACTGTT, and reverse: GGCACATGCTGAAGAGAGTTA. IL27p28 mRNA: forward: 5′-GAGCAGCTCCCTGATGTTTC-3′, and reverse: 5′-AGCTGCATCCTCTC-CATGTT-3′. EBI3 forward: 5′- TGGCTCCCTACGTGCTCAAT-3′, and reverse: 5′-GAGGGTCGGGCTTGATGATGT-3′. STAT1 forward: 5′-GGCAAAGAGTGATCAGAAACAA-3′, and reverse: 5′-GTTCAGTGA-CATTCAGCAACTC-3′. PKR forward: 5′-GGTACAGGTTCTACTAAACA-3′, and reverse: 5′- GAAAACTTGGCCAAATCCACC-3′. Viperin forward: 5′-AAATGCGGCTTCTGTTTCCAC-3′, and reverse: 5′-TTGATCTTCTC-CATACCAGCTTCC-3′. OAS1 forward: 5′-GTGTGTCCAAGGTGG-TAAAGG-3′, and reverse: 5′-CTGCTCAAACTTCACGGAA-3′. β- Actin forward: 5′-ATCTGGCACCACACCTTCTACAATGA-3′, and reverse: 5′-CGTCATACTCCTGCTTGCTGATCCAC-3′. Real-Time RT-qPCR amplifications were carried out using the Maxima SyBR-Green system (Thermo Scientific, Wilmington, DE, 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 (mRNA) of each target gene was normalized to the uninfected control and housekeeping gene β-actin, using ΔΔCt method and $|\text{Log}_2$ Fold Change$| > 0.6$ were used as the threshold to determine the significant difference in gene expression. $n = 4$ or 5.

2.7. Cytokines and chemokines quantification

Protein levels of TNFα, IL1β, IL6, CXCL8/IL8, and IL10 were quantified in culture supernatants of ZIKV-infected monocytes using ELISA (MAX™ Deluxe Set Human) following the manufacturer's instructions (BD Biosciences, San Jose, CA, USA).

The detection limit was 2 pg/mL for TNFα, 0.5 pg/mL for IL1β, 4 pg/mL for IL6, 8 pg/mL for CXCL/8IL8, and 2 pg/mL for IL10.

2.8. In vitro antiviral assay

Human monocytes 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) as control. Then, human monocytes were infected with ZIKV Colombia or ZIKV Dakar at MOI 5, as describe above, and culture supernatants were obtained at 24 hpi and stored at −80 °C.

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0.1 (GraphPad Software Inc. San Diego, CA, USA). Shapiro-Wilks test for normality of data was performed. The statistical tests are indicated in the figure legends. Data are represented as mean ± SEM. Significant results were defined as $p<0.1$ (.), $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

3. Results

3.1. Human monocytes are more susceptible to infection with ZIKV Colombia (American-Asian lineage) than with ZIKV Dakar (African lineage)

Previous reports showed that primary monocytes are susceptible to ZIKV infection *in vitro* (Michlmayr et al., 2017). However, a detailed kinetics of ZIKV replication in human monocytes has not been reported. We evaluated the permissiveness of primary human monocytes to ZIKV Colombia infection and evaluated viral replication at 6, 12, 24, 48, and 72 hpi (Fig. 1A). We observed low levels of infectious viral particles released at 6 and 12 hpi. However, at 24 to 72 hpi higher production of infectious viral particles were observed, with a peak at 48 hpi (~2×10^5 PFU/mL; Fig. 1A).

Next, we performed a comparative analysis of monocytes susceptibility to ZIKV Colombia and ZIKV Dakar infections. We observed that ZIKV Dakar-infected monocytes released higher amounts of infectious viral particles than ZIKV Colombia-infected monocytes at 12 hpi (Fig. 1B). However, ZIKV Colombia-infected monocytes showed significantly higher infectious viral particles than ZIKV Dakar-infected monocytes at 24 and 48 hpi (Fig. 1B). Overall, results suggest that human monocytes are susceptible to both ZIKV Colombia and ZIKV Dakar infection, but ZIKV Dakar has faster viral replication as compared to ZIKV Colombia.

3.2. Human monocytes infected with ZIKV Puerto Rico strain (PRVABC59) and ZIKV Nigeria strain (IBH30656) induce different transcriptional profiles

To evaluate transcriptional response of human monocytes to American-Asian ZIKV and African ZIKV infection, we reanalyzed publicly available RNA-seq data GSE103114 (GEO) (Khaiboullina et al., 2017) from human monocytes infected with ZIKV Puerto Rico strain (PRVABC59, an American-Asian ZIKV strain), or with ZIKV Nigeria strain (IBH30656, an African ZIKV strain) for 12 h. Sample variance in the RNA-seq datasets were done using principal component analysis (PCA) (Fig. 2A). PCA plot showed three clusters: ZIKV Puerto Rico-infected monocytes, ZIKV Nigeria-infected monocytes and uninfected monocytes (Mock), indicating differential transcriptional profiles between groups. The principal components (PC1 and PC2) explain 92.3% of the variance in the RNA-seq, suggesting that changes in transcriptional profile of monocytes is dependent of ZIKV infection.

To define differentially expressed genes (DEGs), we selected genes with a FDR< 0.05 and |Log2 FC (ZIKV-infected monocytes/Uninfected monocytes) |> 0.6. Of the 57.774 genes interrogated by RNA-Seq; 930 were up-regulated and 917 down-regulated in ZIKV Puerto Rico strain infected monocytes (Fig. 2B). Further, 748 genes were up-regulated and 724 down-regulated in ZIKV Nigeria strain infected monocytes (Fig. 2C). We noted that ZIKV Puerto Rico strain -infected monocytes up-regulated genes related to inflammatory and antiviral response. Among these were pro-inflammatory cytokines TNFα, IL1β, and IL6; chemokines CCL2, CCL3, CCL5, CCL7, CCL8, CXCL1, CXCL2, CXCL3, and CXCL5; and AVPs including GPB2, ISG15, DDX58/RIG-I, MX1, MX2, OAS1–3, and RSAD2/Viperin. Although the number of genes involved in the induction of inflammatory response was similar in ZIKV Nigeria strain -infected monocytes (Fig. 2C), the number of antiviral response genes was lower. Together, results show that although the monocytes infected with ZIKV Puerto Rico strain or ZIKV Nigeria strain induce expression of genes involved in the induction of pro-inflammatory response, only ZIKV Puerto Rico-infected monocytes strongly up-regulated the expression of genes involved in the induction of antiviral response. These results suggest that infection with American-Asian and African lineage of ZIKV induces a diferential antiviral response in human monocytes.

We identified 601 commonly up-regulated DEGs in human monocytes infected with ZIKV Puerto Rico or ZIKV Nigeria, that are associated with the induction of immune and antiviral response (Fig. 3A), including pro- and anti-inflammatory cytokines (TNFα, IL1α, IL1β, IL6, IL10, and IL19), CC- and CXC-chemokines (CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL20, CCL24, CXCL1, CXCL2, CXCL3, CXCL5, CXCL8/IL8, and CXCL16), NF-kB complex (NF-kB1, NF-kB2, and IκBα), inflammasome components (NLRP3, and CASP1), antiviral proteins (IDO1, IFI6, IFITM3, ISG15, OAS3, and Viperin), and JAK-STAT signaling pathway (SOCS1, and SOCS3). Furthermore, ZIKV Puerto Rico strain-infected monocytes induced 415 unique DEGs (Fig. 3A), including AVPs such as GBP2, IFI35, IFIT3, MX1, MX2, OAS1, OAS2, OASL, RIG-I, and TRIM-family proteins, suggesting that ZIKV Puerto Rico infection induces higher level of antiviral response than ZIKV Nigeria. On the other hand, ZIKV Nigeria-infected monocytes up-regulated 211 unique DEGs, including the proteasome subunit (PSM) family of genes (Fig. 3A), that negatively regulate NF-kB expression during viral infections (Krishnan

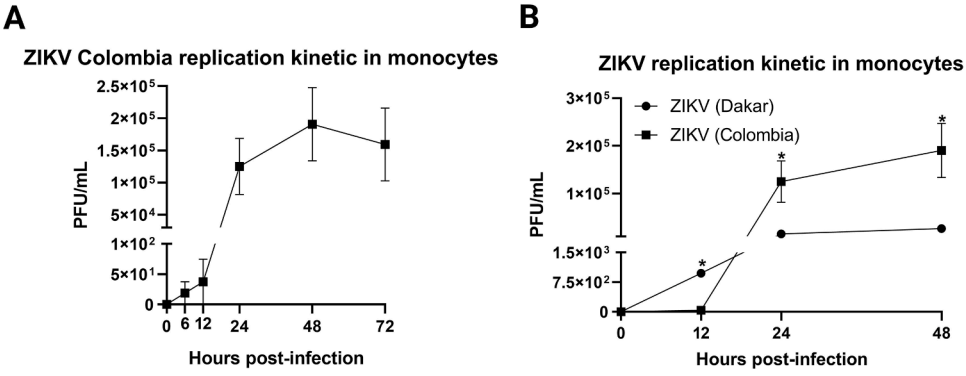

Fig. 1. ***Primary human monocytes are susceptible and permissive to Zika virus infection.*** Primary human monocytes were infected using a ZIKV clinical isolate from Colombia, (MH179341.1) or ZIKV strain from Dakar, (MG758785.1) at MOI 5. (***A***) ZIKV Colombia replication kinetic in monocytes. Viral replication was quantified by plaque assay at 6,12,24,48 and 72 hpi. Data are represented as mean ± SEM. $n = 5$. Repeated measures ANOVA test was performed ($p = 0.022$). ***B.*** ZIKV Colombia and ZIKV Dakar replication kinetic in monocytes. Viral replication was quantified by plaque assay at 12,24,48 hpi. Data are represented as mean ± SEM. $n = 4$. Mann-Whitney test was performed for each time. Signif. codes: '***' 0.001; '**' 0.01; '*' 0.05.

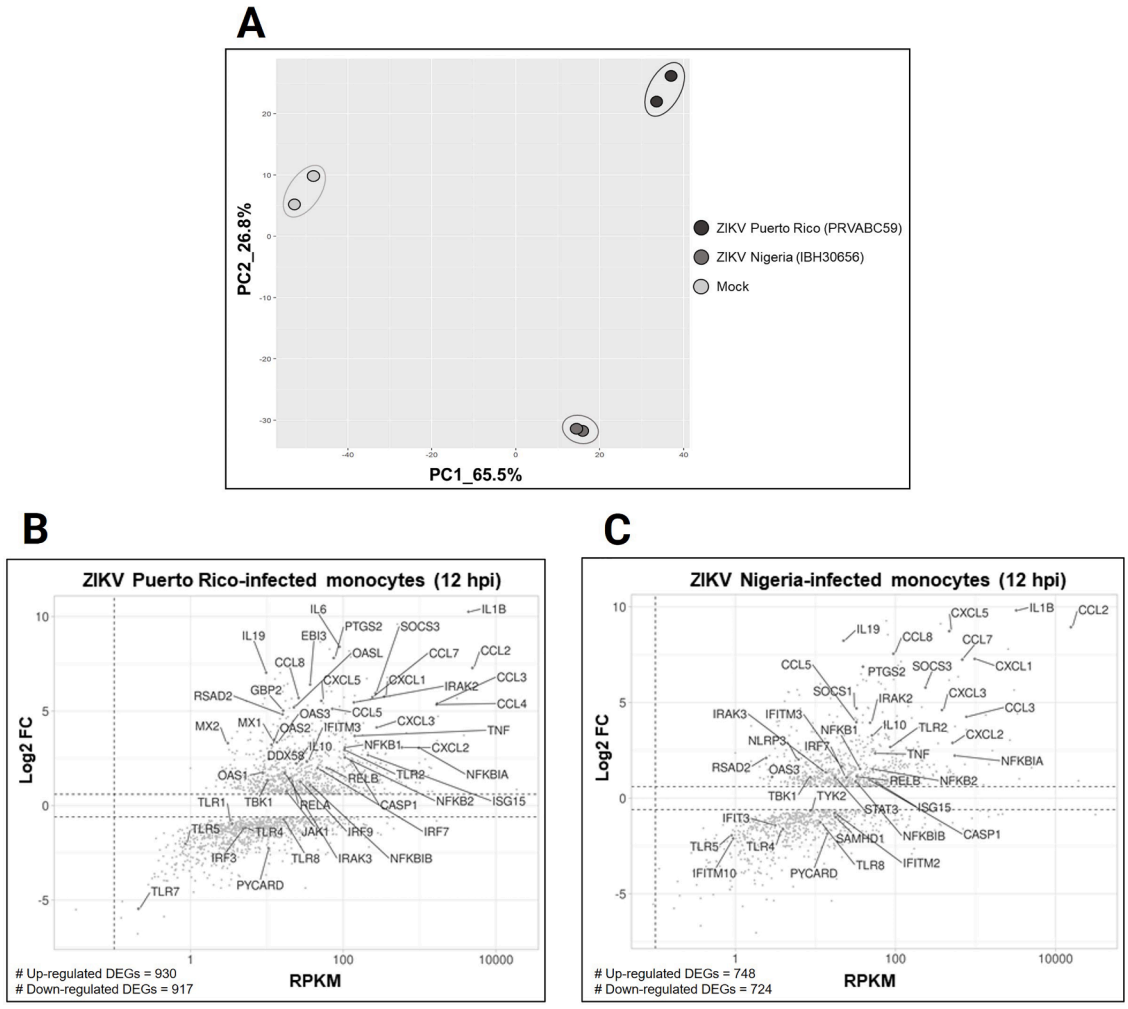

Fig. 2. ***Transcriptional response of human monocytes to ZIKV Puerto Rico and ZIKV Nigeria strains infection.*** The publicly available RNA-seq GSE103114 (GEO) (Khaiboullina et al., 2017) was reanalized. Differentially expressed genes (DEGs) in ZIKV Puerto Rico (PRVABC59) and ZIKV Nigeria (IBH30656)- infected monocytes. Monocytes were infected with two ZIKV strains, at MOI 0.1 for 2 h, independently. Monocytes were collected at 12 hpi and RNA was subjected for differential gene expression by RNA-seq. **A.** Principal component analysis (PCA). Principal Component Analysis (PCA) plot shows three clusters: ZIKV Puerto Rico-infected monocytes (Black), ZIKV Nigeria-infected monocytes (Dark gray), Mock (Light gray), each one with $n = 2$ biological replicates. MA plot of Differentially expressed genes (DEGs). MA plot shows DEGs in ZIKV Puerto Rico-infected monocytes **(B)** and DEGs in ZIKV Nigeria-infected monocytes **(C)**. The Log_2 FC indicates the mean expression level for each gene and RPKM indicates Reads Per Kilobase Million for each gene. A Log_2 FC of 0.6 and −0.6 was considered as up-regulation or down-regulation of gene expression, respectively.

et al., 2021). Next we performed Gene Ontology analysis of common (Fig. 3B) and unique DEGs induced by ZIKV Puerto Rico (Fig. 3C) or ZIKV Nigeria (Fig. 3D) infection of human monocytes. Common DEGs were classified into 23 biological processes; 20 associated with inflammatory responses and 3 with antiviral response and one linked with inflammatory and antiviral response (cellular response to virus process). TLR2 signaling pathway was the most enriched (Fold Enrichment= 28.26) with 4 up-regulated genes (TLR2, RIPK2, PIK3AP1, and TNIP2) associated with induction of inflammatory response (Fig. 3B). Unique DEGs of ZIKV Puerto Rico were classified into 14 biological processes; 8 associated with inflammatory responses and 6 with antiviral responses (Fig. 3C), whereas unique DEGs of ZIKV Nigeria were classified into 6 biological processes linked mainly with proteasome complex (Fig. 3D). Together, results suggest that ZIKV Puerto Rico and ZIKV Nigeria infection in human monocytes lead to differential transcriptomic profiles associated with the induction of pro-inflammatory and antiviral response.

3.3. ZIKV infection modulates mRNA levels of TLR2 signaling components and induces robust NF-kB-dependent pro-inflammatory response in human monocytes

Since our reanalyzed RNA-seq dataset GSE103114 (GEO) (Khaiboullina et al., 2017) showed that TLR2 signaling was the most enriched process, we focused in the signaling pathway of this PRR. We observed that both ZIKV Puerto Rico- and ZIKV Nigeria-infected monocytes up-regulated expression of TLR2 signaling compounds, including TLR2, but TLR1, TLR4, TLR5, TLR6, TLR7, and TLR8 mRNA levels were down-regulated (Fig. 4A); Among the components of TLRs pathway IRAK2 and IRAK3 were up-regulated (Fig. 4B). Further, we observed significant expression of NF-κB components, including NF-κB1, NF-κB2, RELA, RELB, and IκBα (Fig. 4C), and NF-κB-target genes such as COX2, CXCL1, CXCL8/IL8, IL1β, IL6, and TNFα (Fig. 4D).

The RNA-seq results were validated by monitoring expression levels of TLR2 and TLR7 in monocytes infected with ZIKV Colombia (American-Asian lineage) or ZIKV Dakar (African lineage) at 12, 24, and 48 hpi by RT-qPCR. We found significant upregulation of TLR2 (Fig. 4E) and TLR7 (Fig. 4F) mRNA in ZIKV Colombia-infected monocytes (at 12 hpi),

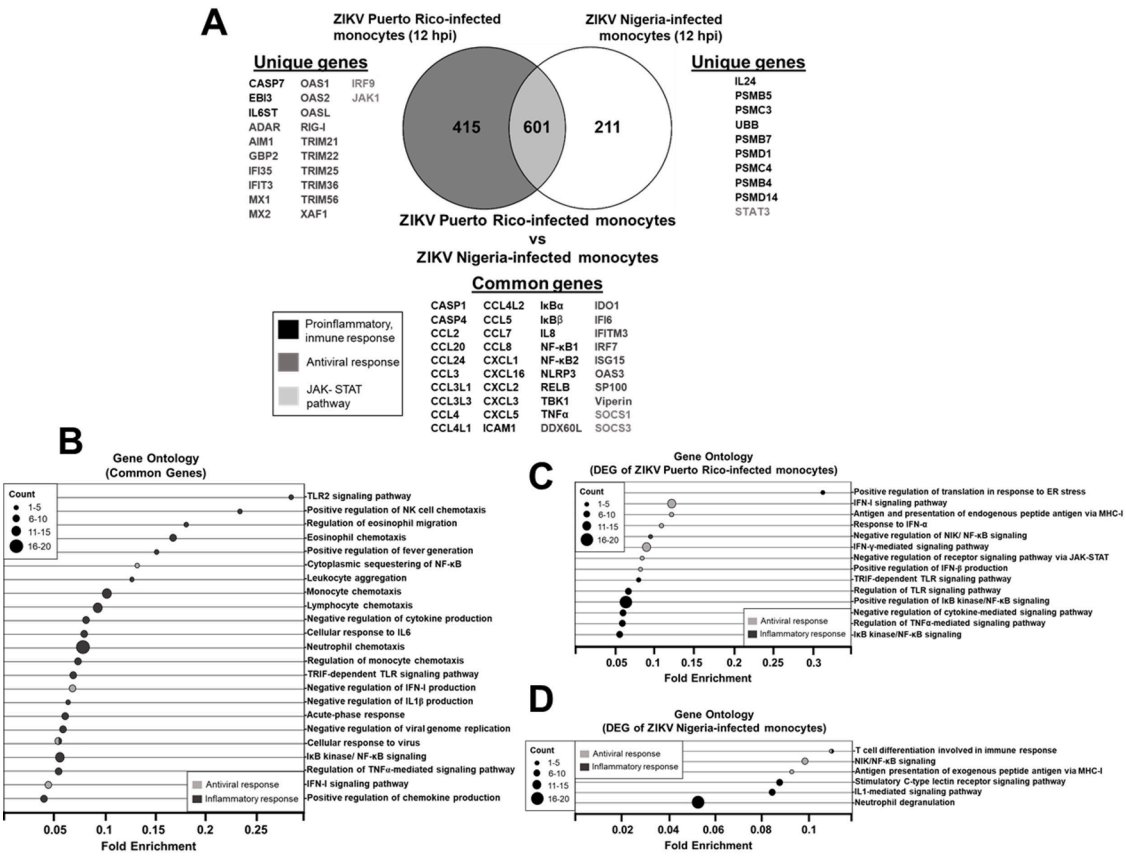

Fig. 3. ***ZIKV Puerto Rico and ZIKV Nigeria infection-induced differential transcriptional profiles in human monocytes.*** **The publicly available RNA-seq GSE103114 (GEO)** (Khaiboullina et al., 2017) **was reanalized.** Differentially expressed genes (DEGs) in ZIKV Puerto Rico (PRVABC59) and ZIKV Nigeria (IBH30656)- infected monocytes. Monocytes were infected with two ZIKV strains, at MOI 0.1 for 2 h, independently. Monocytes were collected at 12 hpi and RNA was subjected for differential gene expression by RNA-seq. ***A.*** A Venn diagram shows the overlap of DEGs and unique genes for each infection at 12 h. DEGs were chosen considering a Log_2 (Fold Change) > |0.6|. The names of the unique genes and common genes are written in different colors classified according to three processes: Pro-inflammatory and immune response (Black), antiviral response (Dark gray), JAK-STAT signaling pathway (Light gray). Gene Ontologies (GO). GO of common genes between ZIKV infected monocytes from Puerto Rico or Nigeria **(B).** GO of unique genes in ZIKV Puerto Rico-infected monocytes **(C)** or GO of unique genes in ZIKV Nigeria-infected monocytes **(D).** Each GO diagram shows the common biological processes between infection by two strains. Differentially expressed genes (DEGs) common were classified into biological processes associated with a function such as antiviral response (Light gray) and Inflammatory response (Dark gray). The biological processes were chosen considering a Fold Enrichment > 4 and $p < 0.05$. For each biological process, the number of the genes regulated in infection (Count) by the two ZIKV strains in monocytes is shown. The count is represented by 4 circles of ascending size that represent, respectively, 4 groups of different gene numbers: 1–5, 6–10, 10–15, 16–20 for **(B)**, 1–6, 7–12, 13–18, 19–24 for **(C)**, 1–7, 8–14, 15–21, 22–28 for **(D)**.

and lower expression was observed in ZIKV Dakar-infected monocytes. Additionally, we quantifited the production of pro- and anti-inflammatory cytokines in culture supernatants by ELISA. As observed in the transcriptomic analysis, we found that ZIKV Colombia-infected monocytes induce higher levels of IL1β (Fig. 5A), IL6 (Fig. 5B), TNFα (Fig. 5C), and IL10 (Fig. 5D) as compared to ZIKV Dakar-infected monocytes at 12, 24, and 48 hpi. Our results suggest that ZIKV Colombia induces a higher pro-inflammatory response than ZIKV Dakar in human monocytes. However, ZIKV Dakar-infected monocytes produce significantly higher protein levels of CXCL8/IL8 at 12, 24, and 48 hpi than ZIKV Colombia-infected monocytes or uninfected cells (Fig. 5E), consistent with CXCL8/IL8 mRNA expression observed in the RNA-seq (Fig. 4D).

Together, the results indicate that infection of human monocytes by American-asian ZIKV strains induces TLR2 signaling and activates NF-kB complex to promote a robust NF-kB-dependent pro-inflammatory response which could be associated with immunopathogenesis of ZIKF in humans.

3.4. American-Asian ZIKV strains induce robust interleukin 27-dependent antiviral response in human monocytes

Analysis of RNA-seq dataset GSE103114 (GEO) (Khaiboullina et al., 2017) of monocytes infected with ZIKV Puerto Rico and ZIKV Nigeria showed neither IFN-I (IFNα1 and IFNβ1), nor IFN-II (IFNγ) IFN-III (IFNλ1) expression at 12 hpi (Fig. 6A). However, unlike ZIKV Nigeria that induces IL27p28 expression, ZIKV Puerto Rico-infected monocytes induced expression level of IL27 subunits (IL27p28 and EBI3) (Fig. 6A), and transcription factors involved in IL27 gene expression, including IRF1 and IRF7 for IL27p28 subunit (Fig. 6B), and NF-kB1 for EBI3 (Fig. 4C). Similarly, we found that ZIKV Puerto Rico infection significantly up-regulated mRNA levels of IL27 signaling components, including gp130, JAK1, STAT3, and SOCS3 (Fig. 6C). Further, IL27 expression and IL27 signaling components were linked to significant increase in AVPs mRNA levels, including APOBEC3A, GBP2, ISG15, MX1, OAS1, RIG-I, and Viperin at 12 hpi (Fig. 6D). Results suggest that whereas infection of monocytes with ZIKV Puerto Rico activated IL27 signaling leading to induction of robust antiviral response in an IFN-independent manner, infection with ZIKV Nigeria only induces the expression of one of the two IL27 subunits (EBI3), not related to IL27-dependent antiviral response.

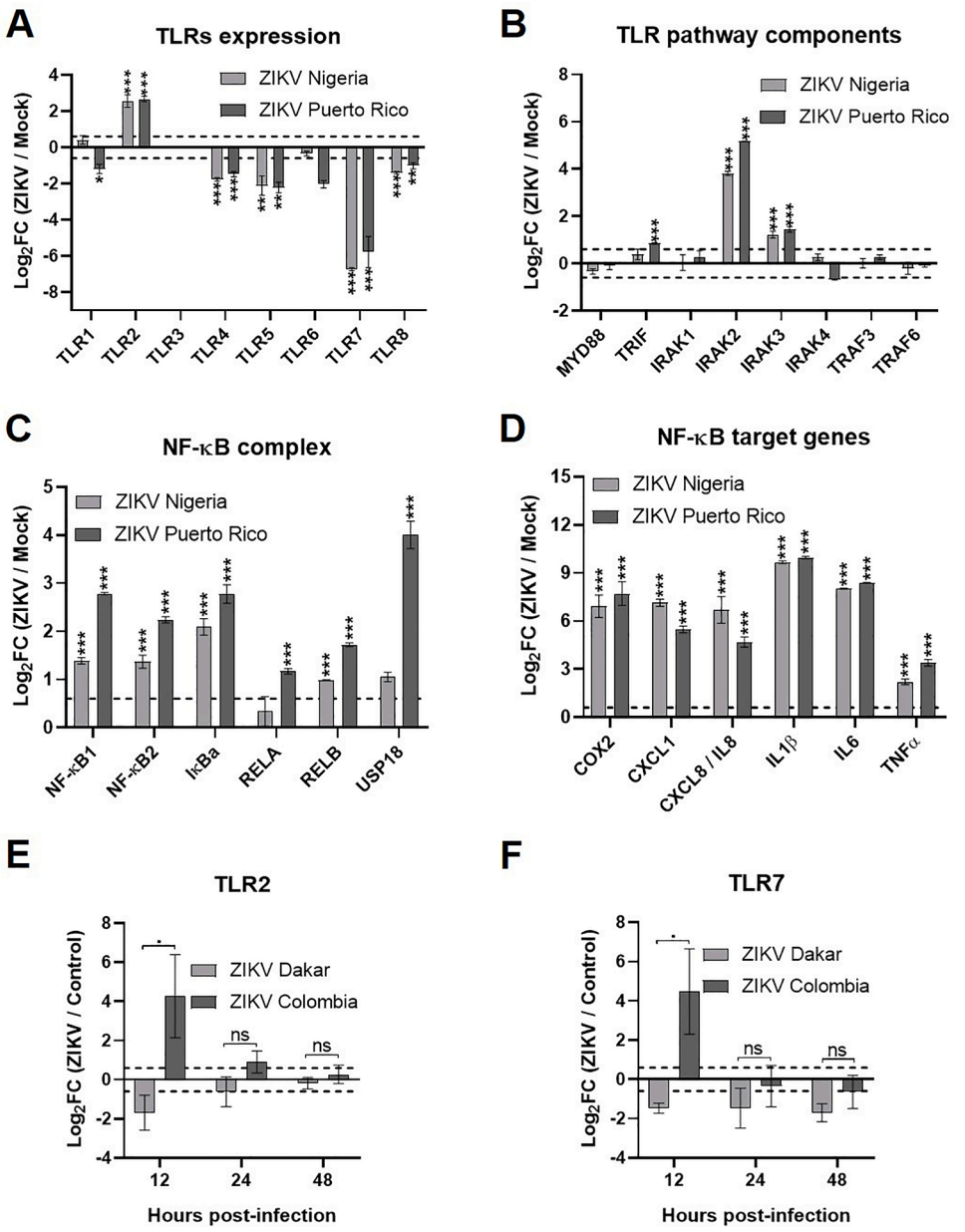

Fig. 4. ZIKV infection in human monocytes activated TLR2 signaling and induced NF-kB activation. RNA-seq data (Accession number GSE103114 in GEO, (Khaiboullina et al., 2017)) were obtained from independent infections of CD14+ monocytes with ZIKV Puerto Rico strain, (Dark gray) and ZIKV Nigeria strain (Light gray) at 12 hpi; each infection with $n = 2$ biological replicates. Statistical significance according to the p-value obtained in the DEGs final matrix. Logarithm of fold change (Log_2FC) ratios for eight TLR expression **(A),** TLR pathway components expression **(B),** NF-κB complex expression **(C),** NF-κB target genes expression **(D)** in ZIKV infected monocytes / Mock. Primary human monocytes cultures were left uninfected or infected with ZIKV clinical isolate from Colombia or ZIKV strain from Dakar. The MOI was 5. Cell lysates were obtained at 12, 24, and 48 hpi and RT-qPCR was performed. Logarithm of fold change (Log_2FC) ratios for TLR2 **(E),** TLR7 **(F)** in ZIKV infected monocytes / Control. Student's *t*-test was performed for each time in normally distributed data and contrary to this, Mann-Whitney test was performed for each time. $n = 4$. Significant. codes: '***' 0.001; '**' 0.01; '*' 0.05; '.' 0.1, 'ns' no significance. In general, a Log_2FC of 0.6 and −0.6 were considered as up-regulation or down-regulation of gene expression, respectively. Data are represented as mean ± SEM.

In addition to the antiviral response, we evaluated the expression of STAT-dependent cytokines (Fig. 6E) and STAT-dependent chemokines (Fig. 6F). We found that ZIKV Puerto Rico-infected monocytes moderately increased the expression of STAT-dependent pro-inflammatory cytokines, including IL7 and IL15, as compared to ZIKV Nigeria at 12 hpi (Fig. 6E). While the mRNA expression level of TNF-related apoptosis-inducing ligand (TRAIL) and B-cell activating factor (BAFF) were down-regulated in response to infection with both ZIKV Puerto Rico and ZIKV Nigeria (Fig. 6E), the mRNA expression of STAT-dependent CC chemokines, CCL2, CCL5, and CCL7 were upregulated, but not STAT-dependent CXC chemokines at 12 hpi (Fig. 6F).

Next, we validated the expression levels of representative DEGs, including IL27, STAT1 by RT-qPCR analysis of human monocytes infected with ZIKV Colombia or ZIKV Dakar. We found that ZIKV Colombia-infected monocytes induced significant expression of mRNA levels of IL27p28 (Fig. 7A), EBI3 (Fig. 7B), and STAT1 (Fig. 7C) than ZIKV Dakar at 12, 24, and 48 hpi. Furthermore, ZIKV Colombia-infected monocytes induced higher expression of AVPs mRNAs, including Viperin, PKR, and OAS1 as compared to ZIKV Dakar (Fig. 7D; 7E and 7F, respectively), with a peak at 12 hpi. ZIKV Colombia-infected monocytes showed significant positive correlation between STAT1 and AVPs mRNA expression levels (STAT1 *vs* Viperin: $R = 0.6864$, $p = 0.0009$; STAT1 *vs* PKR: $R = 0.8634$, $p = <0.0001$ and STAT1 *vs* OAS1: $R = 0.9328$, $p = <0.0001$; Supplementary Fig. 1). As well, we observed significant positive correlation between STAT1 and IL27 subunits mRNA expression levels (STAT1 *vs* IL27p28: $R = 0.5520$, $p = 0.0056$, STAT1 *vs* EBI3: $R = 0.6316$, $p = 0.0020$) (Supplementary Fig. 1). The results are consistent with the RNA-seq analysis (Fig. 6), suggesting that IL27 is involved in the induction of antiviral response against American-Asian ZIKV infection of human monocytes.

3.5. Interleukin 27 inhibits ZIKV replication in human monocytes

We validated RNA-seq results by monitoring the antiviral effects of IL27 and its capability to control ZIKV replication in human monocytes. For this, monocytes were pre-treated for 6 h with increasing concentrations (1, 5, 10, 25 ng/mL) of recombinant human IL27, or with 25 ng/mL recombinant human IFNβ1, a critical mediator of protection against

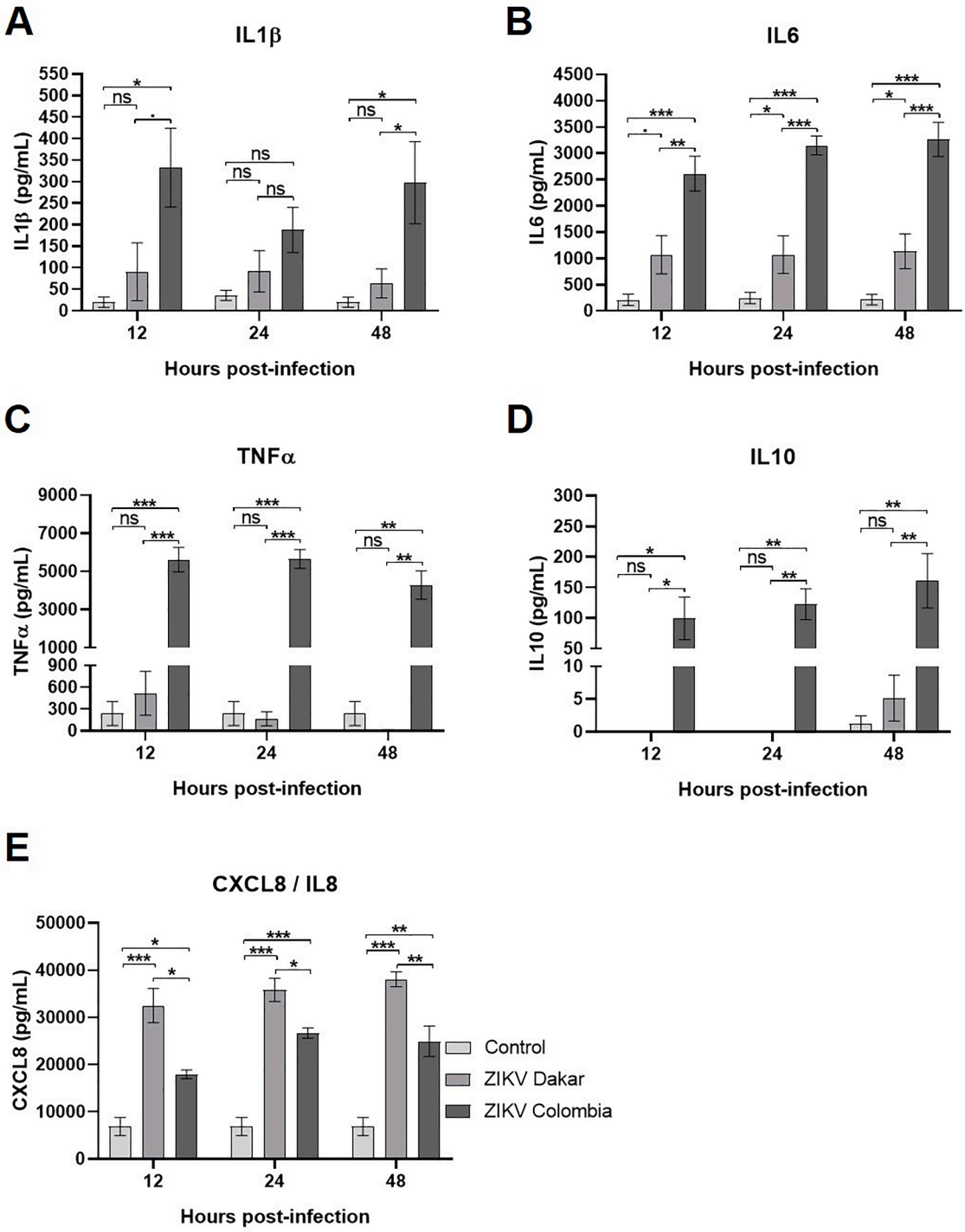

Fig. 5. Infection with ZIKV Colombia promotes a higher inflammatory response than ZIKV Dakar in human monocytes. Primary human monocytes cultures were left uninfected or infected with ZIKV clinical isolate from Colombia or ZIKV strain from Dakar. The MOI was 5. The quantification of cytokines were by ELISA from the culture supernatants collected at 12, 24 and 48 hpi. Data are represented as mean ± SEM. Kinetic of IL1β **(A),** IL6 **(B),** TNFα **(C),** IL10 **(D),** CXCL8/IL8 **(E)** production in primary human monocytes infected with ZIKV clinical isolate from Colombia and ZIKV strain from Dakar. Repeated measures ANOVA test was performed. $n = 4$. Significant. codes: '***' 0.001; '**' 0.01; '*' 0.05; '.' 0.1; 'ns' no significance.

most viruses. Subsequently, monocytes were infected with ZIKV Colombia or ZIKV Dakar and viral replication was evaluated at 24 hpi by plaque assay on BHK-21 cells. As the case with IFNβ1, the results show dose-dependent reduction of replication of both strains of ZIKV in monocytes pretreated with IL27, with an EC50= 2.870 ng/mL (Fig. 8A and C) and EC50= 10.23 ng/mL (Fig. 8B and C), for ZIKV Colombia and ZIKV Dakar, respectively. Results confirm that IL27 induces robust antiviral response to control ZIKV replication in human monocytes.

4. Discussion

ZIKV is an emergent arbovirus that causes important outbreaks in tropical and subtropical areas worldwide. Unfortunately, there are no specific or effective treatments or vaccines available to control ZIKV infections (Kazmi et al., 2020). Despite the advances in ZIKV molecular biology studied in different cell lines (Lazear et al., 2016), there are few studies in primary human (innate immune) cells. Thus far, there is little

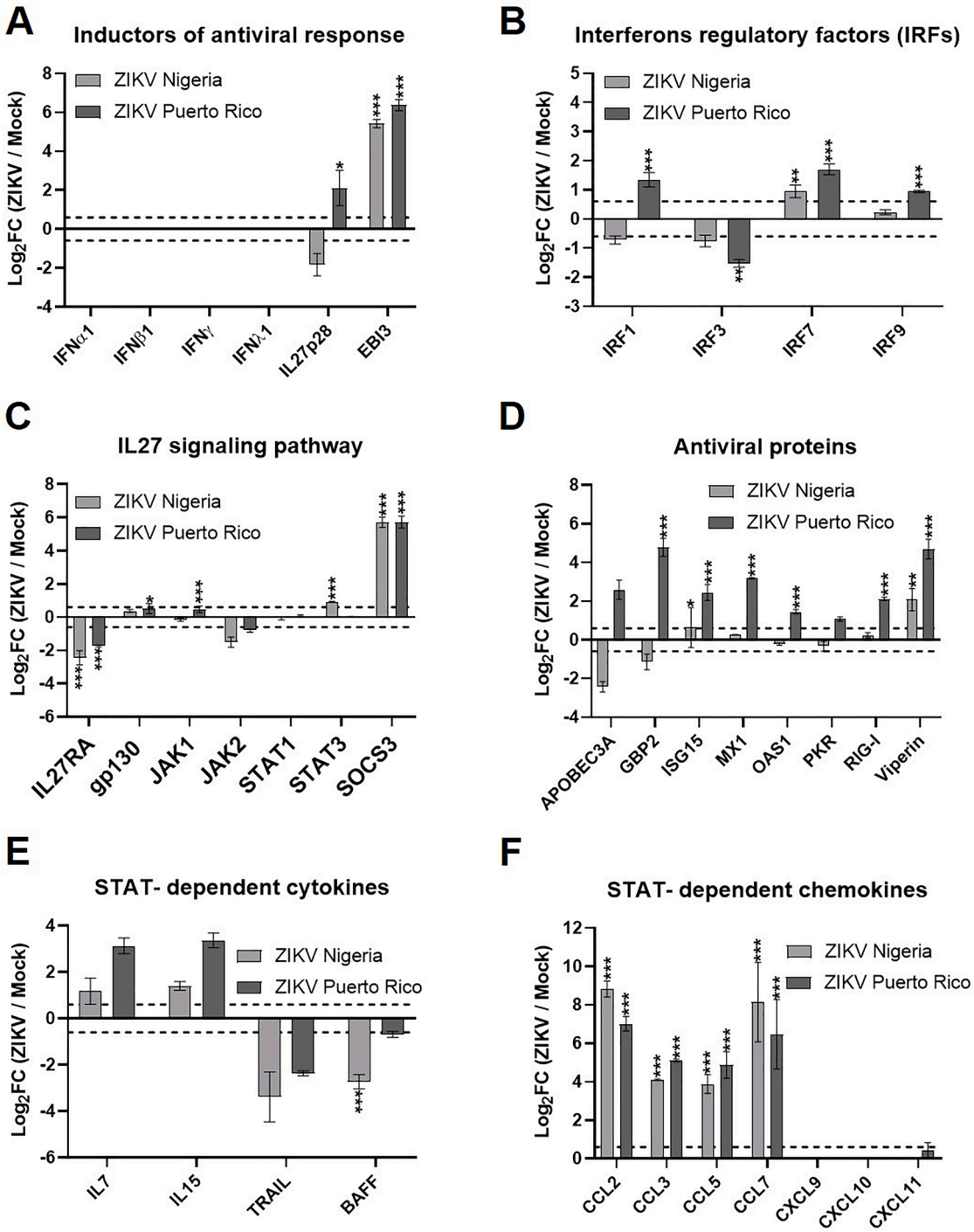

Fig. 6. ZIKV infection regulated STAT-dependent cytokines expression and only ZIKV Puerto Rico upregulated IL27 expression and induced the antiviral response, in human monocytes. RNA-seq data (Accession number GSE103114 in GEO, (Khaiboullina et al., 2017)) were obtained from independent infections of CD14+ monocytes with a ZIKV Puerto Rico strain (Dark gray) and a ZIKV Nigeria strain (Light gray) at 12 hpi; each infection with $n = 2$ biological replicates. Logarithm of fold change (Log_2FC) ratios for inductors antiviral response expression **(A),** Interferons regulatory factors (IRFs) expression **(B),** IL27 signaling pathway expression **(C),** antiviral proteins expression **(D),** STAT-dependent cytokines **(E),** STAT-dependent chemokines **(F)** in ZIKV infected monocytes / Mock. A Log_2FC of 0.6 and −0.6 were considered as up-regulation or down-regulation of gene expression, respectively. Data are represented as mean ± SEM. Statistical significance according to the p-value obtained in the DEGs final matrix. Significant codes: '***' 0.001; '**' 0.01; '*' 0.05.

information on whether ZIKV from different lineages show differences in pathogenesis, nor is much known about the relationship between ZIKV and monocytes and its impact on inflammatory and antiviral responses. We reasoned that a better understanding of the relationship between the pathogenesis of different ZIKV could be vital to search for optimal ZIKF treatment. We focused our analysis on ZIKV infection of monocytes since monocytes are the main targets of ZIKV infection in humans (Michlmayr et al., 2017), and play a central role in initiation and resolution of inflammatory and antiviral response (Auffray et al., 2009; Parihar et al., 2010).

As was previously reported (Khaiboullina et al., 2017; Michlmayr et al., 2017), we observed that primary human monocytes are targets of ZIKV infection *in vitro*. Our analysis of the infection kinetics has shown that African lineages (ZIKV Dakar) replicated well and the release of

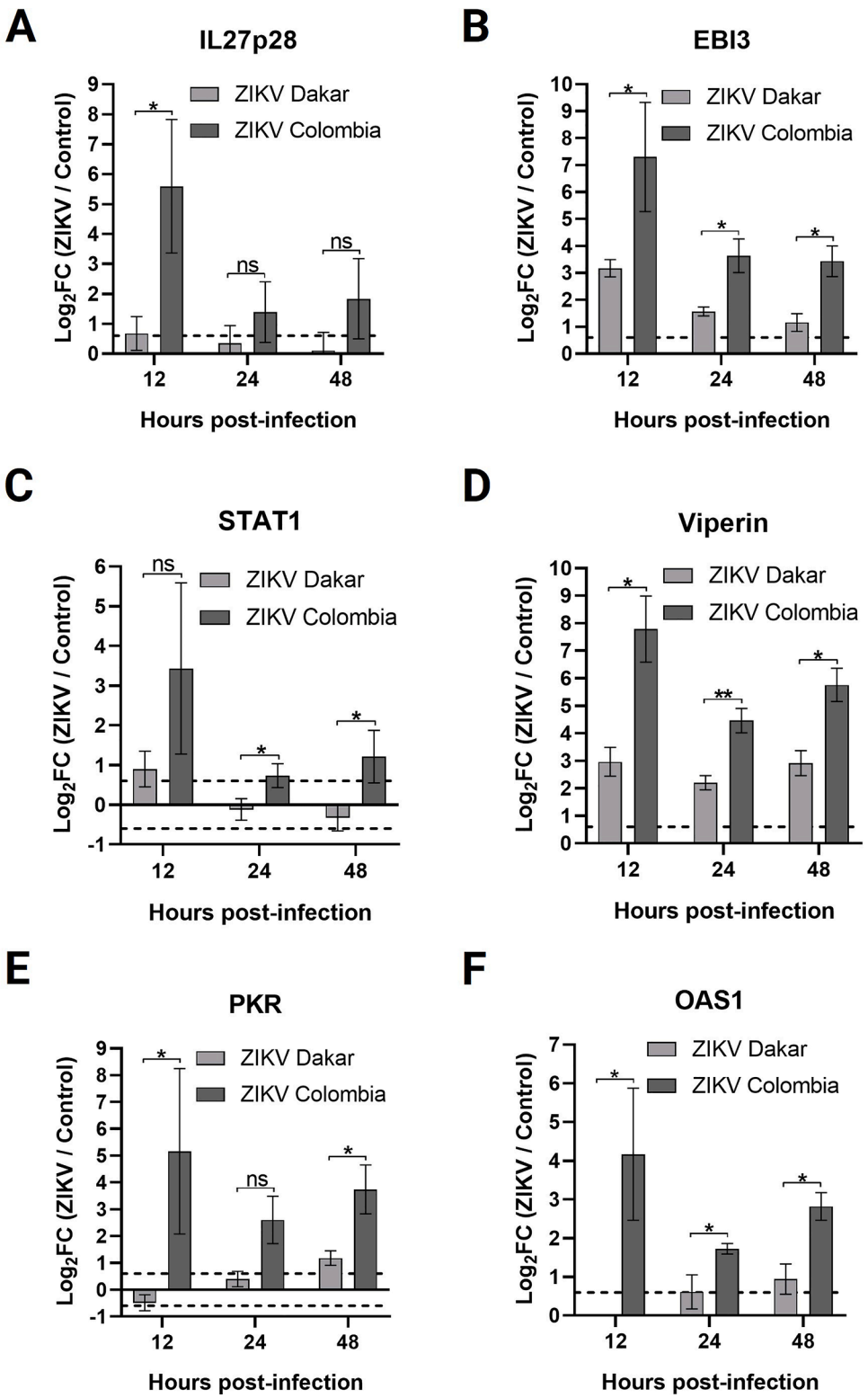

Fig. 7. Infection with ZIKV Colombia induces the IL27 production and the antiviral proteins expression in human monocytes. Primary human monocytes cultures were left uninfected or infected with ZIKV clinical isolate from Colombia or ZIKV strain from Dakar. The MOI was 5. Cell lysates were obtained at 12, 24, and 48 hpi. and RT-qPCR was performed. Data are represented as mean ± SEM. Logarithm of fold change (Log_2FC) ratios for IL27p28 **(A),** EBI3 **(B),** STAT1 **(C),** Viperin **(D),** PKR **(E),** OAS1 **(F)** in ZIKV infected monocytes / Control. A Log_2FC of 0.6 and −0.6 were considered as up-regulation or down-regulation of gene expression, respectively. Student's *t*-test was performed for each time in normally distributed data and contrary to this, Mann-Whitney test was performed for each time. $n = 4$. Significant. codes: '***' 0.001; '**' 0.01; '*' 0.05; 'ns' no significance.

infectious viral particles was relatively rapid (at 12 hpi), while the American/Asian lineages (ZIKV Colombia) showed slower replication and the release of viral particles (after 12 hpi). However, the release of viral particles was higher for ZIKV Colombia as compared to ZIKV Dakar at 24 and 48 hpi. It was observed previously that Asian/American lineage Zika viruses replicated well in DCs but poorly in macrophages, while the African lineage virus showed limited infectivity in both cell types (Österlund et al., 2019). Bowen and co-workers observed a faster infection kinetics in DCs with the African strain ZIKV as compared to Asian lineage ZIKV (Bowen et al., 2017); Vielle et al., however, did not find lineage-specifc diferences in infections of DCs (Vielle et al., 2018).

Since these results demonstrated that virus strains from different ZIKV lineages show differential replication capacity, we hypothesized that during the first 12 hpi, the monocytes have the ability to induce effective antiviral response against ZIKV Colombia, but not to ZIKV Dakar. To address the issue a comparative transcriptome analysis of

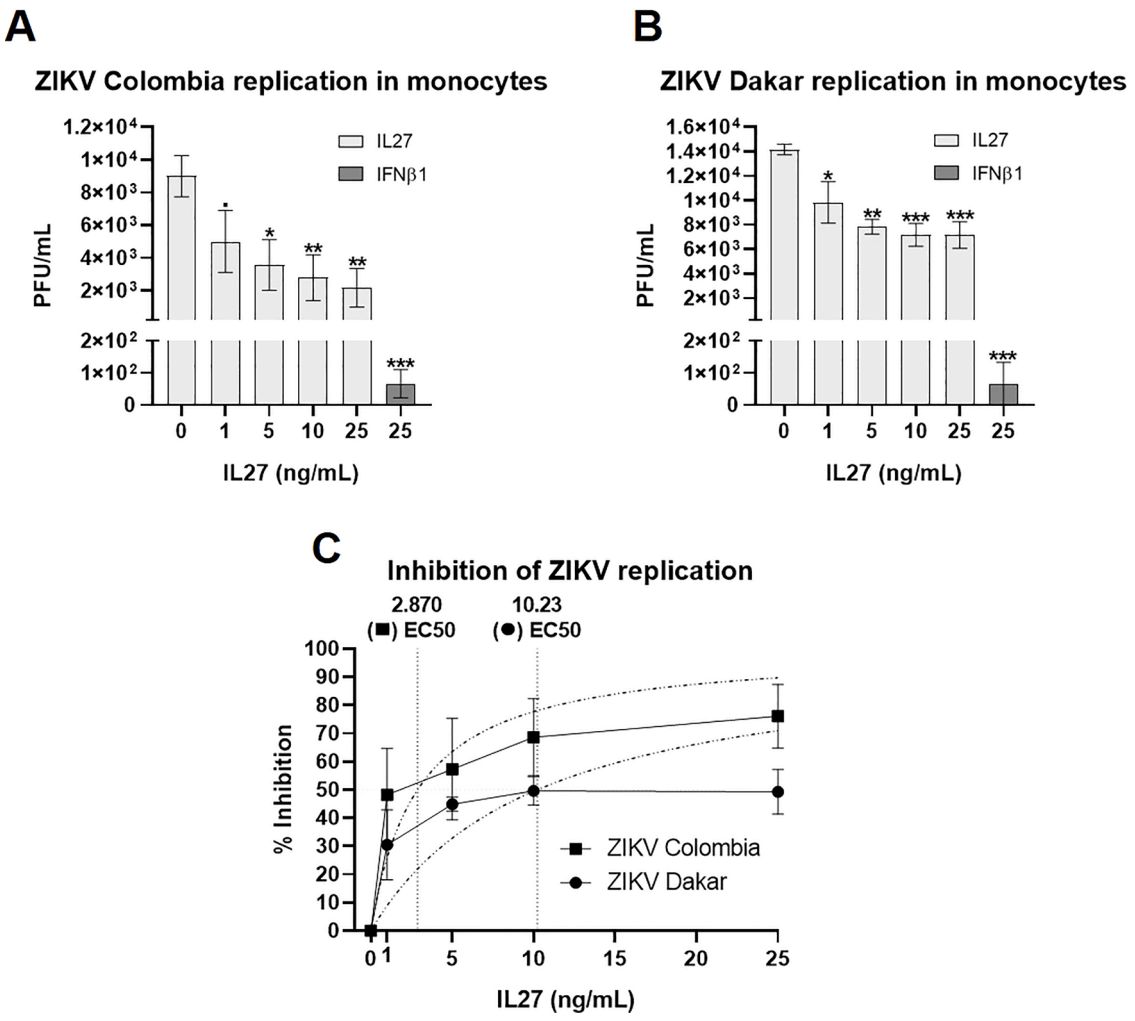

Fig. 8. ZIKV replication in human monocytes is inhibited by IL27 pre-tratment. Human monocytes were pre-treated for 6 h with increasing concentrations of recombinant- human IL27 (1,5,10, 25 ng/mL) or 25 ng/mL of recombinant-human IFNβ1, later, monocytes were infected with ZIKV at MOI of 5. Culture supernatans were obtained at 24 hpi where viral titration was calculated by plaque assay on BHK-21 cells. ZIKV Colombia replication in monocytes (**A**). ZIKV Dakar replication in monocytes (**B**). Percentage inhibition of ZIKV (Colombia (■) and Dakar (●)) replication and EC50 of IL27 in human monocytes (**C**). Repeated measures ANOVA test to ZIKV Dakar ($p = 0.00118$) and ZIKV Colombia ($p = 0.00964$) with Dunnett's post-test were performed. $n = 3$. Significant. codes: '***' 0.001; '**' 0.01; '*' 0.05; '. ' 0.1; 'ns' no significance.

ZIKV Puerto Rico (PRVABC59) and ZIKV Nigeria (IBH30656) was performed. We found 415 unique DEGs in ZIKV Puerto Rico; 211 unique DEGs in ZIKV Nigeria, and 601 common DEGs in infected monocytes, and enriched in GO links with innate immune-related pathways. Moreover, we found that both ZIKV strains could activate inflammatory response, but ZIKV Puerto Rico activated host anti-viral innate immunity more intensely as compared to ZIKV Nigeria. Initial recognition of ZIKV by innate immune system is mediated by at (least) two distinct class of PRRs, TLRs and RIG-I (Brubaker et al., 2015; Plociennikowska et al., 2021; Schilling et al., 2020; Vanwalscappel et al., 2018). The role of TLR pathway that controls ZIKV infection and pathogenesis has not been well studied, despite the fact that TLR signaling activation results in the induction of NF-kB complex (Kawai and Akira, 2007). We observed that TLR2 and TLR2 signaling components were up-regulated in human monocytes infected with African- and American-Asian-lineages, leadding to NF-kB activation and a robust NF-kB-dependent pro-inflammatory response, suggesting that recognition of ZIKV-PAMPs by TLR2 induces a robust NF-kB-dependent pro-inflammatory response that could be involved in ZIKV control and/or immunopathogenesis. Aguilar-Briseño et al. (2020) reported that infection by Dengue virus (DENV), a ZIKV close-related *Flavivirus*, activated TLR2 signaling in human monocytes, and was correlated with dengue severity in patients (Aguilar-Briseño et al., 2020). In contrast to Vanwalscappel et al. (2018) who reported TLR7/8 activation in ZIKV-infected monocytes (Vanwalscappel et al., 2018), the transcriptomic analysis showed downregulation of TLR7 and 8 expression, as well as TLR1, TLR4, TLR5 and TLR6, in monocytes infected with ZIKV Puerto Rico and ZIKV Nigeria at 12 hpi. Expression of TLR2 was confirmed by RT-qPCR, which detected an increased accumulation of TLR2 mRNA in monocytes infected with ZIKV Colombia, but not with ZIKV Dakar. We found an increase in TLR7 mRNA levels in monocytes infected with ZIKV Colombia which could be a consequence of the MOI used (we used a MOI of 5 and the RNA-seq was performed with a MOI of 0.1). In addition, NF-kB activation and expression of NF-kB-target genes were confrmed by ELISA, which detected increased production of TNFα, IL1β, IL6, and IL10 in monocytes infected with ZIKV Colombia, but not with ZIKV Dakar. Furthermore, as observed in the transcriptomic analysis, ZIKV Dakar infection induces higher production of CXCL8/IL8 than ZIKV Colombia.

Expression of NF-kB-target genes is essentials to induce robust pro-inflammatory response associated with the control and immunopathogenesis of several viral infections (Liu et al., 2017). For example, COX2 facilitates DENV-2 replication in Huh-7 cells and plays a crucial role in DENV immunopathogenesis (Lin et al., 2017). Moreover, IL1β, IL6,

TNFα are pyrogenic cytokines associated with fever induction (Mackowiak, 1998). IL1β has been implicated in development of pain and inflammation (Ren and Torres, 2009), promotes monocytes differentiation to mDC and M1-like macrophages and supports B-lymphocytes proliferation and their differentiation to plasma cells (Kaneko et al., 2019). Other studies have shown that ZIKV infection activates the NLRP3 inflammasome which leads the secretion of mature IL1β from ZIKV-infected monocytic cell lines, PBMCs, or monocyte-derived macrophages (Khaiboullina et al., 2017; Wang et al., 2018; Zheng et al., 2018). Serum samples from acute DENV, ZIKV, CHIKV, DENV/ZIKV, and CHIKV/ZIKV-infected adult patients presented increased secretion of TNFα, CC and CXC chemokines as compared to healthy donors (Sánchez-Arcila et al., 2020). We found that ZIKV Colombia, unlike to ZIKV Dakar, increased the production of IL10 at 12, 24, and 48 hpi that is associated with the induction of anti-inflammatory response by down-regulation of TNFα, IL1β, IL6, and CXCL8/IL8 secretion. Similar results have been shown in monocytes stimulated with LPS, that control inflammatory response downregulating those cytokines (de Waal Malefyt et al., 1991; Sánchez-Arcila et al., 2020).

The induced expression of CC- and CXC- chemokines such as CCL2, CCL3, CCL5, CCL7, and CXCL8/IL8, contribute to inflammation through chemotaxis of monocytes, DCs, neutrophils, eosinophils, natural killer cells, basophils, and lymphocytes (Proost et al., 1996). Both cytokines and chemokines are closely related to the ZIKV severity, and ZIKF patients with moderate symptoms and viremia phase have higher levels of CXCL10 and CCL2 as compared with patients with mild symptoms or no viremia (Lum et al., 2018). We found that both American-Asian and African lineage ZIKV induced the expression of STAT-dependent cytokines and CC chemokines, including IL7, IL15, CCL2, CCL3, CCl5 and CCL7 in infected monocytes.

PRRs also trigger sequential activation of intracellular signaling pathways, leading to the IFNs induction, which induce a range of ISGs and not only exert an antiviral effect but also promote antiviral proteins to make the host enter an antiviral state. Monocytes play an important role in regulating antiviral response by producing IFNα (Valdés López et al., 2020). However, Grant et al. (2016) reported that ZIKV infection inhibits IFN-dependent antiviral response through ZIKV-NS5 which degrades STAT2 (Grant et al., 2016), as well as NS1, NS4B, and NS2B, that inhibit IFN-I signaling in infected cells (Wu et al., 2017). In addition, ZIKV-NS5 inhibits the RIG-I signaling and the activation of IFNλ1 promoter in HEK293 cells (Lundberg et al., 2019). As was reported by Khaiboullina et al. (2017), we found that monocytes infected with Puerto Rico and Nigeria ZIKV strains did not show activation of the three types of IFNs, however, we did find ISGs expression that encode AVPs, including APOBEC3A, GBP2, MX1, OAS1, PKR, among others in ZIKV Puerto Rico-infected monocytes, but not in ZIKV Nigeria. Similar findings were reported by Bowen et al. (2017), they also showed reduced IFN secretion by ZIKV-infected DCs, suggesting that ZIKV could block the translation of IFN mRNAs yet induce an antiviral state (Bowen et al., 2017). Here, we report that the antiviral state in ZIKV-infected monocytes is independent of IFNs expression. We report that American-Asian ZIKV strains induce the expression IL27p28 and EBI3, the two IL27 subunits, to induce IL27 an IL27-dependent antiviral response in human monocytes. Further, IL27 expression was linked with increased expression of divers ISGs. Activation of IL27 pathway was confirmed by RT-qPCR, which detected an increased accumulation of IL27p28 and EBI3, STAT1, and ISGs, including Viperin, PKR, and OAS1 mRNAs, whose expression was very fast (12 hpi) in ZIKV Colombia-infected monocytes than ZIKV Dakar, suggesting that African lineages prevented activation of innate antiviral defense IL27-dependent. IL27 activates JAK-STAT signaling in hNEKs and induces the AVPs expression through IL27RA and STAT1, that is independent of IFNAR1, TYK2, and STAT2 (Kwock et al., 2020). Further, we show that pretreatment of monocytes cultures with recombinant-human IL27 inhibited ZIKV Colombia and ZIKV Dakar replication in a dose-dependent manner, confirming that IL27 induces a robust antiviral response in an interferon-independent manner to control ZIKV replication. Interestingly, the required concentration of recombinant human IL27 to inhibit at least 50% of the release of viral particles in monocytes is lower in ZIKV Colombia (EC50=2.870 ng/mL) than in ZIKV Dakar (EC50=10.23 ng/mL) (Fig. 8C), indicating that IL27 has higher potency to inhibit the replication of an America-asian-ZIKV strain *in vitro*. Together, our results suggest that infection of human monocytes by American-Asian-ZIKV strains induce IL27 expression which plays an important role in induction of antiviral response and control ZIKV replication in an IFN-independent manner.

Previously, we reported that EBI3 is a NF-kB-target gene (Valdés-López et al., 2022). Here, we propose that American-Asian and African ZIKV induce EBI3 mRNA expression in response to TLR2, which activate NF-kB complex, whereas activation of IRF1 and IRF7 leads to the IL27p28 mRNA expression (Fig. 9), as was previously reported (Valdés-López et al., 2022, 2021). IL27p28 mRNA expression was induced only in American-Asian-ZIKV infected monocytes (Fig. 6A) and was associated with up-regulation of IRF1 mRNA expression in these cells (Figs. 6B and 9).

Our comparative analysis of ZIKV Colombia replication and IL27 expression kinetics in human monocytes (Fig. 1A and 7A, respectively), suggests that early in viral cycle (12 hpi), American-Asian-ZIKV strains evade and block interferon-mediated antiviral response, but induce IL27 pathway and increase the antiviral response at 12 hpi. We suggest that IL27 production could activate IL27 signaling pathway involved in the induction both inflammatory response and antiviral activity through ISGs expression (Fig. 9). Between 12–24 hpi and up 48 hpi, there is a higher and significant increase in ZIKV Colombia replication, which coincides with a decrease in mRNAs expression of IL27, STAT1, and AVPs (Fig. 7), suggesting a block of IL27 pathway in later stages of virus life cycle to increased viral replication. Altogether, our results show that human monocytes are an important target cells of ZIKV infection and play a critical role in the induction of pro-inflammatory and antiviral responses to control ZIKV replication in humans. Collectively, the published resulst and our present data indicate that ZIKV is able to induce IL27 and IL27-regulated antiviral gene expression in human monocytes.

5. Conclusion

Overall, our results show that primary human monocytes are more susceptible to infection by American-asian ZIKV strains (ZIKV Colombia) than African ZIKV strains (ZIKV Dakar). Additionally, ZIKV-PAMPs modulated TLR2 signaling and a robust NF-kB-dependent pro-inflammatory response (Fig. 9A). Early in viral cycle (12 hpi), infection by American-asian ZIKV strains, but not African ZIKV strains, induce the expression of both IL27 subunits (IL27p28 and EBI3) and activate IL27 signaling in human monocytes (Fig. 9B), leading to the induction of ISGs that encode AVPs, cytokines, and chemokines involved in the antiviral state and the control of ZIKV replication in an IFN-independent manner (Fig. 9C).

Funding

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.

CRediT authorship contribution statement

Lady Johana Hernández-Sarmiento: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Juan Felipe Valdés-López:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Silvio Urcuqui-Inchima:** Conceptualization, Writing – original draft, Resources,

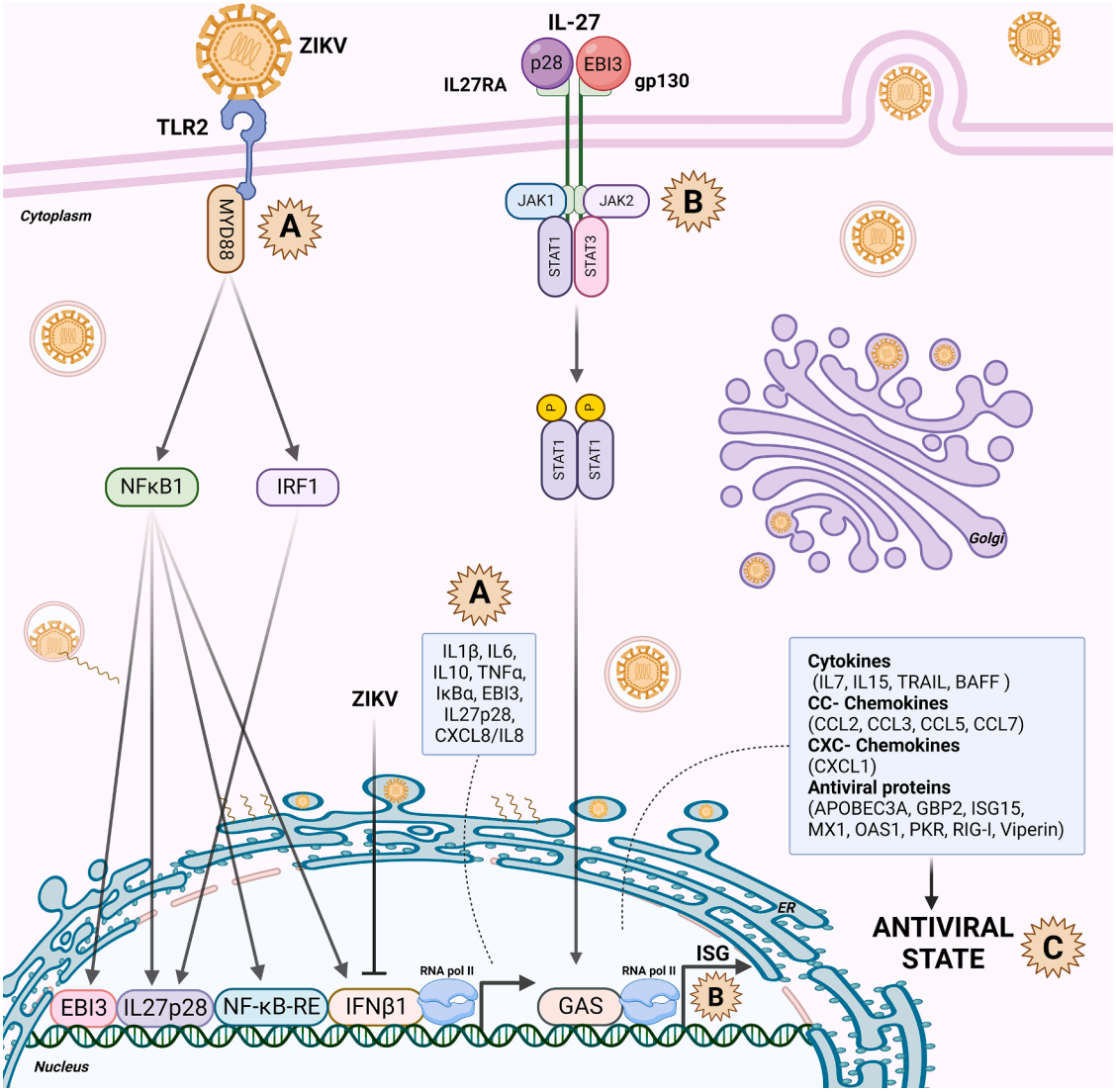

Fig. 9. Induction model of IL27-dependent pro-inflammatory and antiviral response in American-asian ZIKV-infected monocytes. We proposed that IL27 production in American-asian ZIKV -infected monocytes is dependent on recognition of ZIKV-PAMPs by TLR2-MyD88 to activate NF-κB-complex (**A**). Furthermore, IL27 signaling pathway (**B**) activates robust STAT1-dependent pro-inflammatory and antiviral response including cytokines, CC- and CXC- chemokines, antiviral proteins in an IFN-independent manner; to control ZIKV replication in human monocytes (**C**). Created with BioRender.com.

Funding acquisition, Writing – review & editing, Visualization, 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.

Data availability

We reanalyzed publicly available RNA-seq data

Acknowledgments

The authors thank Ajit Kumar for reading the manuscript and his valuable comments and 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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.virusres.2023.199040.

References

Aguilar-Briseño, J.A., Upasani, V., Ellen, B.M.teter, Moser, J., Pauzuolis, M., Ruiz-Silva, M., Heng, S., Laurent, D., Choeung, R., Dussart, P., Cantaert, T., Smit, J.M., Rodenhuis-Zybert, I.A., 2020. TLR2 on blood monocytes senses dengue virus infection and its expression correlates with disease pathogenesis. Nat. Commun. 11, 3177. https://doi.org/10.1038/s41467-020-16849-7.

Auffray, C., Sieweke, M.H., Geissmann, F., 2009. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. Annu. Rev. Immunol. 27, 669–692. https://doi.org/10.1146/annurev.immunol.021908.132557.

Azevedo, R.S.S., de Sousa, J.R., Araujo, M.T.F., Martins Filho, A.J., de Alcantara, B.N., Araujo, F.M.C., Queiroz, M.G.L., Cruz, A.C.R., Vasconcelos, B.H.B., Chiang, J.O., Martins, L.C., Casseb, L.M.N., da Silva, E.V., Carvalho, V.L., Vasconcelos, B.C.B., Rodrigues, S.G., Oliveira, C.S., Quaresma, J.A.S., Vasconcelos, P.F.C., 2018. In situ immune response and mechanisms of cell damage in central nervous system of fatal cases microcephaly by Zika virus. Sci. Rep. 8, 1. https://doi.org/10.1038/s41598-017-17765-5.

Basarab, M., Bowman, C., Aarons, E.J., Cropley, I., 2016. Zika virus. BMJ i1049. https://doi.org/10.1136/bmj.i1049.
Bowen, J.R., Quicke, K.M., Maddur, M.S., O'Neal, J.T., McDonald, C.E., Fedorova, N.B., Puri, V., Shabman, R.S., Pulendran, B., Suthar, M.S., 2017. Zika virus antagonizes type I interferon responses during infection of human dendritic cells. PLOS Pathog 13, e1006164. https://doi.org/10.1371/journal.ppat.1006164.
Brubaker, S.W., Bonham, K.S., Zanoni, I., Kagan, J.C., 2015. Innate immune pattern recognition: a cell biological perspective. Annu. Rev. Immunol. 33, 257–290. https://doi.org/10.1146/annurev-immunol-032414-112240.
Carbaugh, D.L., Baric, R.S., Lazear, H.M., 2019. Envelope protein glycosylation mediates Zika virus pathogenesis. J. Virol. 93, 1–16. https://doi.org/10.1128/JVI.00113-19.
Chambers, T.J., Hahn, C.S., Galler, R., Rice, C.M., 1990. Flavivirus genome organization, expression, and replication. Annu. Rev. Microbiol. 44, 649–688. https://doi.org/10.1146/annurev.mi.44.100190.003245.
da Silva, M.H.M., Moises, R.N.C., Alves, B.E.B., Pereira, H.W.B., de Paiva, A.A.P., Morais, I.C., Nascimento, Y.M., Monteiro, J.D., de Souto, J.T., Nascimento, M.S.L., de Araújo, J.M.G., da Guedes, P.M.M., Fernandes, J.V., 2019. Innate immune response in patients with acute Zika virus infection. Med. Microbiol. Immunol. 208, 703–714. https://doi.org/10.1007/s00430-019-00588-8.
Dang, J., Tiwari, S.K., Lichinchi, G., Qin, Y., Patil, V.S., Eroshkin, A.M., Rana, T.M., 2016. Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3. Cell Stem Cell 19, 258–265. https://doi.org/10.1016/j.stem.2016.04.014.
de Oliveira, W.K., de França, G.V.A., Carmo, E.H., Duncan, B.B., de Souza Kuchenbecker, R., Schmidt, M.I., 2017. Infection-related microcephaly after the 2015 and 2016 Zika virus outbreaks in Brazil: a surveillance-based analysis. Lancet 390, 861–870. https://doi.org/10.1016/S0140-6736(17)31368-5.
de Waal Malefyt, R., Abrams, J., Bennett, B., Figdor, C.G., de Vries, J.E., 1991. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J. Exp. Med. 174, 1209–1220. https://doi.org/10.1084/jem.174.5.1209.
Dick, G.W., Kitchen, S., Haddow, A., 1952. Zika Virus (I). Isolations and serological specificity. Trans. R. Soc. Trop. Med. Hyg. 46, 509–520. https://doi.org/10.1016/0035-9203(52)90042-4.
Esser-Nobis, K., Aarreberg, L.D., Roby, J.A., Fairgrieve, M.R., Green, R., Gale, M., 2019. Comparative analysis of african and asian lineage-derived Zika virus strains reveals differences in activation of and sensitivity to antiviral innate immunity. J. Virol. 93, 1–18. https://doi.org/10.1128/JVI.00640-19.
Faizan, M.I., Abdullah, M., Ali, S., Naqvi, I.H., Ahmed, A., Parveen, S., 2017. Zika virus-induced microcephaly and its possible molecular mechanism. Intervirology. https://doi.org/10.1159/000452950.
Grant, A., Ponia, S.S., Tripathi, S., Balasubramaniam, V., Miorin, L., Sourisseau, M., Schwarz, M.C., Sánchez-Seco, M.P., Evans, M.J., Best, S.M., García-Sastre, A., 2016. Zika virus targets human STAT2 to inhibit Type I interferon signaling. Cell Host Microbe 19, 882–890. https://doi.org/10.1016/j.chom.2016.05.009.
Hall, A.O.H., Silver, J.S., Hunter, C.A., 2012. The immunobiology of IL-27. Adv. Immunol. 1–44. https://doi.org/10.1016/B978-0-12-394299-9.00001-1.
Hamel, R., Dejarnac, O., Wichit, S., Ekchariyawat, P., Neyret, A., Luplertlop, N., Perera-Lecoin, M., Surasombatpattana, P., Talignani, L., Thomas, F., Cao-Lormeau, V.-.M., Choumet, V., Briant, L., Desprès, P., Amara, A., Yssel, H., Missé, D., 2015. Biology of Zika virus infection in human skin cells. J. Virol. 89, 8880–8896. https://doi.org/10.1128/JVI.00354-15.
Hashimoto, T., Kutsuna, S., Tajima, S., Nakayama, E., Maeki, T., Taniguchi, S., Lim, C.-K., Katanami, Y., Takeshita, N., Hayakawa, K., Kato, Y., Ohmagari, N., 2017. Importation of Zika virus from Vietnam to Japan, November 2016. Emerg. Infect. Dis. 23, 1223–1225. https://doi.org/10.3201/eid2307.170519.
Hertzog, J., Dias Junior, A.G., Rigby, R.E., Donald, C.L., Mayer, A., Sezgin, E., Song, C., Jin, B., Hublitz, P., Eggeling, C., Kohl, A., Rehwinkel, J., 2018. Infection with a Brazilian isolate of Zika virus generates RIG-I stimulatory RNA and the viral NS5 protein blocks type I IFN induction and signaling. Eur. J. Immunol. 48, 1120–1136. https://doi.org/10.1002/eji.201847483.
Huber, M., Steinwald, V., Guralnik, A., Brustle, A., Kleemann, P., Rosenplanter, C., Decker, T., Lohoff, M., 2008. IL-27 inhibits the development of regulatory T cells via STAT3. Int. Immunol. 20, 223–234. https://doi.org/10.1093/intimm/dxm139.
Kam, Y.-.W., Leite, J.A., Lum, F.-.M., Tan, J.J.L., Lee, B., Judice, C.C., Teixeira, D.A., de, T., Andreata-Santos, R., Vinolo, M.A., Angerami, R., Resende, M.R., Freitas, A.R. R., Amaral, E., Junior, R.P., Costa, M.L., Guida, J.P., Arns, C.W., Ferreira, L.C.S., Rénia, L., Proença-Modena, J.L., Ng, L.F.P., Costa, F.T.M., 2017. Specific biomarkers associated with neurological complications and congenital central nervous system abnormalities from Zika virus–infected patients in Brazil. J. Infect. Dis. 216, 172–181. https://doi.org/10.1093/infdis/jix261.
Kaneko, N., Kurata, M., Yamamoto, T., Morikawa, S., Masumoto, J., 2019. The role of interleukin-1 in general pathology. Inflamm. Regen. 39, 12. https://doi.org/10.1186/s41232-019-0101-5.
Kawai, T., Akira, S., 2010. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat. Immunol. https://doi.org/10.1038/ni.1863.
Kawai, T., Akira, S., 2007. Signaling to NF-κB by Toll-like receptors. Trends Mol. Med. 13, 460–469. https://doi.org/10.1016/j.molmed.2007.09.002.
Kazmi, S.S., Ali, W., Bibi, N., Nouroz, F., 2020. A review on Zika virus outbreak, epidemiology, transmission and infection dynamics. J. Biol. Res. 27, 5. https://doi.org/10.1186/s40709-020-00115-4.
Kelser, E.A., 2016. Meet dengue's cousin. Zika. Microbes Infect. 18, 163–166. https://doi.org/10.1016/j.micinf.2015.12.003.
Khaiboullina, S.F., Uppal, T., Sarkar, R., Gorzalski, A.Z., St Jeor, S., Verma, S.C., 2017. ZIKV infection regulates inflammasomes pathway for replication in monocytes. Sci. Rep. 7, 16050. https://doi.org/10.1038/s41598-017-16072-3.
Kotenko, S.V., Gallagher, G., Baurin, V.V., Lewis-Antes, A., Shen, M., Shah, N.K., Langer, J.A., Sheikh, F., Dickensheets, H., Donnelly, R.P., 2003. IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex. Nat. Immunol. 4, 69–77. https://doi.org/10.1038/ni875.
Krishnan, R., Kim, J.-.O., Jang, Y.-.S., Oh, M.-.J., 2021. Proteasome subunit beta type-8 from sevenband grouper negatively regulates cytokine responses by interfering NF-κB signaling upon nervous necrosis viral infection. Fish Shellfish Immunol. 113, 118–124. https://doi.org/10.1016/j.fsi.2021.04.004.
Kwock, J.T., Handfield, C., Suwanpradid, J., Hoang, P., McFadden, M.J., Labagnara, K.F., Floyd, L., Shannon, J., Uppala, R., Sarkar, M.K., Gudjonsson, J.E., Corcoran, D.L., Lazear, H.M., Sempowski, G., Horner, S.M., MacLeod, A.S., 2020. IL-27 signaling activates skin cells to induce innate antiviral proteins and protects against Zika virus infection. Sci. Adv. 6 https://doi.org/10.1126/sciadv.aay3245.
Lazear, H.M., Govero, J., Smith, A.M., Platt, D.J., Fernandez, E., Miner, J.J., Diamond, M. S., 2016. A Mouse Model of Zika virus pathogenesis. Cell Host Microbe 19, 720–730. https://doi.org/10.1016/j.chom.2016.03.010.
Lin, C.-.K., Tseng, C.-.K., Wu, Y.-.H., Liaw, C.-.C., Lin, C.-.Y., Huang, C.-.H., Chen, Y.-.H., Lee, J.-.C., 2017. Cyclooxygenase-2 facilitates dengue virus replication and serves as a potential target for developing antiviral agents. Sci. Rep. 7, 44701. https://doi.org/10.1038/srep44701.
Liu, T., Zhang, L., Joo, D., Sun, S.-.C., 2017. NF-κB signaling in inflammation. Signal Transduct. Target. Ther. 2, 17023. https://doi.org/10.1038/sigtrans.2017.23.
Lum, F.-.M., Lye, D.C.B., Tan, J.J.L., Lee, B., Chia, P.-.Y., Chua, T.-.K., Amrun, S.N., Kam, Y.-.W., Yee, W.-.X., Ling, W.-.P., Lim, V.W.X., Pang, V.J.X., Lee, L.K., Mok, E.W. H., Chong, C.-.Y., Leo, Y.-.S., Ng, L.F.P., 2018. Longitudinal study of cellular and systemic cytokine signatures to define the dynamics of a balanced immune environment during disease manifestation in Zika virus–infected patients. J. Infect. Dis. 218, 814–824. https://doi.org/10.1093/infdis/jiy225.
Lundberg, Melén, Westenius, Jiang, Österlund, Khan, Vapalahti, Julkunen, Kakkola, 2019. Zika virus non-structural protein NS5 inhibits the RIG-I pathway and interferon Lambda 1 promoter activation by targeting IKK epsilon. Viruses 11, 1024. https://doi.org/10.3390/v11111024.
Luo, H., Winkelmann, E.R., Fernandez-Salas, I., Li, L., Mayer, S.V., Danis-Lozano, R., Sanchez-Casas, R.M., Vasilakis, N., Tesh, R., Barrett, A.D., Weaver, S.C., Wang, T., 2018. Zika, dengue and yellow fever viruses induce differential anti-viral immune responses in human monocytic and first trimester trophoblast cells. Antiviral Res. 151, 55–62. https://doi.org/10.1016/j.antiviral.2018.01.003.
Mackowiak, P.A., 1998. Concepts of fever. Arch. Intern. Med. 158, 1870. https://doi.org/10.1001/archinte.158.17.1870.
Magnus, M.M., Espósito, D.L.A., Costa, V.A.da, Melo, P.S.de, Costa-Lima, C., Fonseca, B. A.L.da, Addas-Carvalho, M., 2018. Risk of Zika virus transmission by blood donations in Brazil. Hematol. Transfus. Cell Ther. 40, 250–254. https://doi.org/10.1016/j.htct.2018.01.011.
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, 1462–1470. https://doi.org/10.1038/s41564-017-0035-0.
Miner, J.J., Diamond, M.S., 2017. Zika virus pathogenesis and tissue tropism. Cell Host Microbe 21, 134–142. https://doi.org/10.1016/j.chom.2017.01.004.
Österlund, P., Jiang, M., Westenius, V., Kuivanen, S., Järvi, R., Kakkola, L., Lundberg, R., Melén, K., Korva, M., Avšič – Županc, T., Vapalahti, O., Julkunen, I., 2019. Asian and African lineage Zika viruses show differential replication and innate immune responses in human dendritic cells and macrophages. Sci. Rep. 9, 15710. https://doi.org/10.1038/s41598-019-52307-1.
Paniz-Mondolfi, A.E., Giraldo, J., Rodríguez-Morales, A.J., Pacheco, O., Lombó-Lucero, G.Y., Plaza, J.D., Adami-Teppa, F.J., Carrillo, A., Hernandez-Pereira, C.E., Blohm, G.M., 2018. Alice in Wonderland syndrome: a novel neurological presentation of Zika virus infection. J. Neurovirol. 24, 660–663. https://doi.org/10.1007/s13365-018-0645-1.
Parihar, A., Eubank, T.D., Doseff, A.I., 2010. Monocytes and macrophages regulate immunity through dynamic networks of survival and cell death. J. Innate Immun. 2, 204–215. https://doi.org/10.1159/000296507.
Pestka, S., Krause, C.D., Walter, M.R., 2004. Interferons, interferon-like cytokines, and their receptors. Immunol. Rev. 202, 8–32. https://doi.org/10.1111/j.0105-2896.2004.00204.x.
Pflanz, S., Hibbert, L., Mattson, J., Rosales, R., Vaisberg, E., Bazan, J.F., Phillips, J.H., McClanahan, T.K., de Waal Malefyt, R., Kastelein, R.A., 2004. WSX-1 and Glycoprotein 130 constitute a signal-transducing receptor for IL-27. J. Immunol. 172, 2225–2231. https://doi.org/10.4049/jimmunol.172.4.2225.
Pflanz, S., Timans, J.C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E., Blumenschein, W.M., Mattson, J.D., Wagner, J.L., To, W., Zurawski, S., McClanahan, T.K., Gorman, D.M., Bazan, J.F., de Waal Malefyt, R., Rennick, D., Kastelein, R.A., 2002. IL-27, A heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4+ T cells. Immunity 16, 779–790. https://doi.org/10.1016/S1074-7613(02)00324-2.
Piehler, J., Thomas, C., Garcia, K.C., Schreiber, G., 2012. Structural and dynamic determinants of type I interferon receptor assembly and their functional interpretation. Immunol. Rev. 250, 317–334. https://doi.org/10.1111/imr.12001.
Pierson, T.C., Diamond, M.S., 2018. The emergence of Zika virus and its new clinical syndromes. Nature 560, 573–581. https://doi.org/10.1038/s41586-018-0446-y.
Plociennikowska, A., Frankish, J., Moraes, T., Del Prete, D., Kahnt, F., Acuna, C., Slezak, M., Binder, M., Bartenschlager, R., 2021. TLR3 activation by Zika virus stimulates inflammatory cytokine production which dampens the antiviral response induced by RIG-I-like receptors. J. Virol. 95 https://doi.org/10.1128/JVI.01050-20.
Proost, P., Wuyts, A., Van Damme, J., 1996. The role of chemokines in inflammation. Int. J. Clin. Lab. Res. 26, 211–223. https://doi.org/10.1007/BF02602952.

Ren, K., Torres, R., 2009. Role of interleukin-1β during pain and inflammation. Brain Res. Rev. 60, 57–64. https://doi.org/10.1016/j.brainresrev.2008.12.020.

Rousseau, F., Basset, L., Froger, J., Dinguirard, N., Chevalier, S., Gascan, H., 2010. IL-27 structural analysis demonstrates similarities with ciliary neurotrophic factor (CNTF) and leads to the identification of antagonistic variants. Proc. Natl. Acad. Sci. 107, 19420–19425. https://doi.org/10.1073/pnas.1005793107.

Sánchez-Arcila, J.C., Badolato-Correa, J., de Souza, T.M.A., Paiva, I.A., Barbosa, L.S., Nunes, P.C.G., Lima, M.da R.Q., Santos, dos, Damasco, F.B., da Cunha, P.V., Azeredo, R.V., de, E.L., de Oliveira-Pinto, L.M., 2020. Clinical, virological, and immunological profiles of DENV, ZIKV, and/or CHIKV-infected Brazilian patients. Intervirology 63, 33–45. https://doi.org/10.1159/000510223.

Savidis, G., Perreira, J.M., Portmann, J.M., Meraner, P., Guo, Z., Green, S., Brass, A.L., 2016. The IFITMs inhibit Zika virus replication. Cell Rep. 15, 2323–2330. https://doi.org/10.1016/j.celrep.2016.05.074.

Schilling, M., Bridgeman, A., Gray, N., Hertzog, J., Hublitz, P., Kohl, A., Rehwinkel, J., 2020. RIG-I plays a dominant role in the induction of transcriptional changes in Zika virus-infected cells, which protect from virus-induced cell death. Cells 9, 1476. https://doi.org/10.3390/cells9061476.

Sen, G.C., 2001. Viruses and Interferons. Annu. Rev. Microbiol. 55, 255–281. https://doi.org/10.1146/annurev.micro.55.1.255.

Shao, Q., Herrlinger, S., Zhu, Y.-N., Yang, M., Goodfellow, F., Stice, S.L., Qi, X.-P., Brindley, M.A., Chen, J.-F., 2017. The African Zika virus MR-766 is more virulent and causes more severe brain damage than current Asian lineage and Dengue virus. Development. https://doi.org/10.1242/dev.156752.

Sheridan, M.A., Balaraman, V., Schust, D.J., Ezashi, T., Roberts, R.M., Franz, A.W.E., 2018. African and Asian strains of Zika virus differ in their ability to infect and lyse primitive human placental trophoblast. PLoS ONE 13, e0200086. https://doi.org/10.1371/journal.pone.0200086.

Stetson, D.B., Medzhitov, R., 2006. Type I interferons in host defense. Immunity 25, 373–381. https://doi.org/10.1016/j.immuni.2006.08.007.

Tappe, D., Pérez-Girón, J.V., Zammarchi, L., Rissland, J., Ferreira, D.F., Jaenisch, T., Gómez-Medina, S., Günther, S., Bartoloni, A., Muñoz-Fontela, C., Schmidt-Chanasit, J., 2016. Cytokine kinetics of Zika virus-infected patients from acute to reconvalescent phase. Med. Microbiol. Immunol. 205, 269–273. https://doi.org/10.1007/s00430-015-0445-7.

Valdés-López, J.F., Fernandez, G.J., Urcuqui-Inchima, S., 2022. Synergistic effects of toll-like receptor 1/2 and toll-like receptor 3 signaling triggering interleukin 27 gene expression in Chikungunya virus-infected macrophages. Front. Cell Dev. Biol. 10 https://doi.org/10.3389/fcell.2022.812110.

Valdés-López, J.F., Fernandez, G.J., Urcuqui-Inchima, S., 2021. Interleukin 27 as an inducer of antiviral response against chikungunya virus infection in human macrophages. Cell. Immunol. 367 https://doi.org/10.1016/j.cellimm.2021.104411.

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, 205–218. https://doi.org/10.1016/j.morpho.2018.06.001.

Valdés López, J.F., Velilla, P.A., Urcuqui-Inchima, S., 2020. Chikungunya virus infection induces differential inflammatory and antiviral responses in human monocytes and monocyte-derived macrophages. Acta Trop 211, 105619. https://doi.org/10.1016/j.actatropica.2020.105619.

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. Interf. Cytokine Res. 39, 507–521. https://doi.org/10.1089/jir.2019.0058.

Vanwalscappel, B., Tada, T., Landau, N.R., 2018. Toll-like receptor agonist R848 blocks Zika virus replication by inducing the antiviral protein viperin. Virology 522, 199–208. https://doi.org/10.1016/j.virol.2018.07.014.

Vielle, N.J., Zumkehr, B., García-Nicolás, O., Blank, F., Stojanov, M., Musso, D., Baud, D., Summerfield, A., Alves, M.P., 2018. Silent infection of human dendritic cells by African and Asian strains of Zika virus. Sci. Rep. 8, 5440. https://doi.org/10.1038/s41598-018-23734-3.

Wang, A., Thurmond, S., Islas, L., Hui, K., Hai, R., 2017. Zika virus genome biology and molecular pathogenesis. Emerg. Microbes Infect. 6, 1–6. https://doi.org/10.1038/emi.2016.141.

Wang, W., Li, G., De Wu, Luo, Z., Pan, P., Tian, M., Wang, Y., Xiao, F., Li, A., Wu, K., Liu, X., Rao, L., Liu, F., Liu, Y., Wu, J., 2018. Zika virus infection induces host inflammatory responses by facilitating NLRP3 inflammasome assembly and interleukin-1β secretion. Nat. Commun. 9, 106. https://doi.org/10.1038/s41467-017-02645-3.

Weaver, S.C., Costa, F., Garcia-Blanco, M.A., Ko, A.I., Ribeiro, G.S., Saade, G., Shi, P.-Y. Y., Vasilakis, N., 2016. Zika virus: history, emergence, biology, and prospects for control. Antiviral Res 130, 69–80. https://doi.org/10.1016/j.antiviral.2016.03.010.

Wu, Y., Liu, Q., Zhou, J., Xie, W., Chen, C., Wang, Z., Yang, H., Cui, J., 2017. Zika virus evades interferon-mediated antiviral response through the co-operation of multiple nonstructural proteins *in vitro*. Cell Discov. 3, 17006. https://doi.org/10.1038/celldisc.2017.6.

Yoshida, H., Hunter, C.A., 2015. The Immunobiology of Interleukin-27. Annu. Rev. Immunol. 33, 417–443. https://doi.org/10.1146/annurev-immunol-032414-112134.

Zhang, F., Wang, H.-J., Wang, Q., Liu, Z.-Y., Yuan, L., Huang, X.-Y., Li, G., Ye, Q., Yang, H., Shi, L., Deng, Y.-Q., Qin, C.-F., Xu, Z., 2017. American strain of Zika virus causes more severe microcephaly than an old asian strain in neonatal mice. EBioMedicine 25, 95–105. https://doi.org/10.1016/j.ebiom.2017.10.019.

Zheng, Y., Liu, Q., Wu, Y., Ma, L., Zhang, Z., Liu, T., Jin, S., She, Y., Li, Y., Cui, J., 2018. Zika virus elicits inflammation to evade antiviral response by cleaving cGAS via NS1-caspase-1 axis. EMBO J. 37 https://doi.org/10.15252/embj.201899347.