Medical Microbiology and Immunology (2019) 208:703–714
https://doi.org/10.1007/s00430-019-00588-8

ORIGINAL INVESTIGATION

Innate immune response in patients with acute Zika virus infection

Marcelo Henrique Matias da Silva[1] · Raiza Nara Cunha Moises[2] · Brenda Elen Bizerra Alves[1,2,3] · Hannaly Wana Bezerra Pereira[1,2,3] · Anne Aline Pereira de Paiva[2,3] · Ingryd Câmara Morais[3] · Yasmim Mesquita Nascimento[2,3] · Joelma Dantas Monteiro[2,3] · Janeusa Trindade de Souto[1,2] · Manuela Sales Lima Nascimento[4] · Josélio Maria Galvão de Araújo[1,2,3] · Paulo Marcos Matta da Guedes[1,2] · José Veríssimo Fernandes[1,2]

Received: 30 March 2018 / Accepted: 25 February 2019 / Published online: 16 March 2019

Abstract

Innate immunity receptors (Toll-like receptors/TLRs and RIG-like receptors/RLRs) are important for the initial recognition of Zika virus (ZIKV), modulation of protective immune response, and IFN-α and IFN-β production. Immunological mechanisms involved in protection or pathology during ZIKV infection have not yet been determined. In this study, we evaluated the mRNA expression of innate immune receptors (TLR3, TLR7, TLR8, TLR9, melanoma differentiation-associated protein 5/MDA-5, and retinoic acid inducible gene/RIG-1), its adapter molecules (*Myeloid Differentiation Primary Response Gene 88*/Myd88, *Toll/IL-1 Receptor Domain-Containing Adaptor-Inducing* IFN-β/TRIF), and cytokines (IL-6, IL-12, TNF-α, IFN-α, IFN-β, and IFN-γ) in the acute phase of patients infected by ZIKV using real-time PCR in peripheral blood. Patients with acute ZIKV infection had high expression of TLR3, IFN-α, IFN-β, and IFN-γ when compared to healthy controls. In addition, there was a positive correlation between TLR3 expression compared to IFN-α and IFN-β. Moreover, viral load is positively correlated with TLR8, RIG-1, MDA-5, IFN-α, and IFN-β. On the other hand, patients infected by ZIKV showed reduced expression of RIG-1, TLR8, Myd88, and TNF-α molecules, which are also involved in antiviral immunity. Similar expressions of TLR7, TLR9, MDA-5, TRIF, IL-6, and IL-12 were observed between the group of patients infected with ZIKV and control subjects. Our results indicate that acute infection (up to 5 days after the onset of symptoms) by ZIKV in patients induces the high mRNA expression of TLR3 correlated to high expression of IFN-γ, IFN-α, and IFN-β, even though the high viral load is correlated to high expression of TLR8, RIG-1, MDA-5, IFN-α, and IFN-β in ZIKV patients.

Keywords Zika virus · Patient · Innate immune receptors · Toll-like receptors (TLRs) · RIG-like receptors (RLRs) · Cytokines

Edited by: Stipan Jonjic.

✉ Paulo Marcos Matta da Guedes
paulguedes@cb.ufrn.br

✉ José Veríssimo Fernandes
veris@cb.ufrn.br

1 Graduate Program in Parasitary Biology, Department of Microbiology and Parasitology, Federal University of Rio Grande do Norte, Natal, Rio Grande Do Norte, Brazil

2 Department of Microbiology and Parasitology, Federal University of Rio Grande do Norte, Natal, Rio Grande Do Norte, Brazil

3 Laboratory of Virology, Institute of Tropical Medicine, Federal University of Rio Grande do Norte, Natal, Brazil

4 Edmond and Lily Safra International Institute of Neuroscience (ELS-IIN), Santos Dumont Institute, Macaíba, RN, Brazil

Introduction

The Zika virus (ZIKV) is an arbovirus of the Flaviviridae family and *flavivirus* genus. This virus had been historically restricted to the African and Asian continents, but emerged on the Yap Islands in the Pacific Ocean in 2007 [1], and later on the French Polynesia archipelago in 2013 [2], Easter Island, Chile in 2014 [3], Northeastern Brazil in 2015 [4], thereafter spreading rapidly to the other regions of the country [5], and Americas, reaching 48 countries and territories in total [6]. ZIKV infects humans and is estimated that over 80% of individuals are asymptomatic, while 20% of cases typically exhibit mild fever, rash, and joint pain for a period of 7 days [7, 8]. Although most of the time ZIKV infection is asymptomatic, or only causes self-limiting mild symptoms, there are reports

of late neurological complications including Guillain–Barré syndrome [9]. In addition, the congenital transmission may result in fetuses and neonates with microcephaly or other neurodevelopment disorders [10].

The immune response with IFN-α, IFN-β, and IFN-γ production plays a critical role in the control of flaviviruses, demonstrated by the fact that knockout mice of these cytokines or components of the IFN pathway are more susceptible to flaviviral infection [11, 12]. ZIKV infection can also inhibit STAT1 and STAT2 phosphorylation, while NS5 protein has been shown to degrade STAT2 signaling molecules involved in the type I IFN pathway [13, 14]. ZIKV-infected patients have shown the increased levels of chemokines (CXCL-10, CXCL-12, CCL-2, and CCL-3) and cytokines (IL-1β, IL-2, IL-4, IL-6, IL-9, IL-10, IL-13, IL-17, TNF-α, and IFN-γ) during the acute phase, while serum levels of IL-1β, IL-6, IL-8, IL-10, IL-13, TNF-α, and IFN-γ were increased in the convalescent phase [15, 16]. Patients with neurological complications had higher expression of TNF-α, IFN-γ, IL-18, and CXCL-10, and lower expression of IL-10 when compared to ZIKV-infected patients without neurological manifestations [15]. Increased expression levels of CCL2, CCL-10, TNF-α, and IL-22 were found in ZIKV-infected mothers who gave birth to fetuses with congenital malformations of the central nervous system (CNS) when compared to pregnant women whose fetuses were normal [15].

At the present time, a few studies have evaluated the profile of immune response in patients infected with the ZIKV, since immunological mechanisms that confer protection or pathology in humans are still poorly understood. There are no studies describing the participation of innate immunity receptors during infection in patients. Virus detection can be largely attributed to the presence of intracellular innate immune receptors such as Toll-like receptors (TLRs) and RIG-like receptors (RLRs) in dendritic cells and macrophages. TLR3 (recognizes double-stranded RNA), TLR7, TLR8, RIG-1, and MDA-5 recognizes RNA viruses such as the ZIKV (recognize single-stranded RNA), and TLR9 can recognize double-stranded DNA fragments generated during the viral replication process [17, 18]. Virus recognition by innate immune receptors leads to type I IFNs production and other cytokines, as well as for modulating adaptative immune response. This study is a pioneer in evaluating the profile of innate immune receptors (TLRs and RLRs) in patients infected by ZIKV.

Materials and methods

Study population and ethics statement

Blood samples from patients diagnosed with Zika virus ($n = 30$) (Table 1) were kindly provided by the Laboratory of Molecular Biology for Infectious Diseases and Cancer at the Department of Microbiology and Parasitology, Federal University of Rio Grande do Norte, and by several Public Health Units in Rio Grande do Norte, Brazil. All patients involved in this study received medical care from the public healthcare system and their medical records showed the same symptoms' pattern, including fever, headache, nausea, rash, joint pain, itchiness, and muscular pain. Neurological patients and pregnant woman were not included. Viral infection was confirmed by nested reverse transcriptase PCR assay (RT-PCR). Patients included in this study had acute infection by ZIKV (up to 5 days after the onset of symptoms). Uninfected healthy individuals ($n = 20$) were used as controls. Informed consent was obtained from the participants and the study was approved by the Research Ethics Committee, with a Certificate from the National System of Ethics in Research (CAEE-SISNEP) with protocol number CAAE: 51057015.5.0000.5537. The study was performed according to human experimental guidelines of the Brazilian Ministry of Health and the Helsinki Declaration.

Viral RNA extraction and real-time RT-PCR

Viral RNA for the real-time RT-PCR assay was extracted using the QIAamp viral RNA Mini Kit (Qiagen, CA, USA), in accordance with the manufacturer's instructions. Real-time RT-PCR (TaqMan System) was applied for detecting ZIKV, following the protocol described by Faye et al. [19].

Total mRNA extraction, cDNA synthesis, and real-time RT-PCR to quantify innate immune receptors and cytokines expression in patients infected by ZIKV

Innate immune receptors (TLR3, TLR7, TLR8, TLR9, MDA-5, and RIG1), adapter molecules (Myd88 and TRIF) and cytokines (IL-6, IL-12, TNF-α, IFN-γ, IFN-α, and IFN-β) mRNA expression were detected by real-time PCR (qPCR) in peripheral blood obtained from ZIKV-infected patients. Total mRNA was extracted using TRIzol® reagent (Invitrogen™, CA, USA) and SV Total RNA Isolation System (Promega, CA, USA) using DNase treatment step following the manufacturer's instructions. cDNA synthesis was performed with the high-capacity cDNA Reverse Transcription kit (Applied Biosystems, CA, USA) using the Eppendorf Mastercycler gradient set (Eppendorf, CA, USA), and following the manufacturer's instructions. Quantitative analysis of gene expression was performed by real-time PCR reactions using the Fast SYBR Green® Master Mix system (Applied Biosystems) supported by 7500 Fast Real-time (Applied Biosystems) thermal cycler. Specific primers were obtained by the Primer Express software (Applied Biosystems, USA) or from the previous studies in the literature

Table 1 Viral load, and demographic and clinical characteristics of Zika virus-infected subjects, from Rio Grande do Norte State-Brazil, included in this investigation

Patient number	Gender	Age (years)	Birth city	Cycle threshold	Days of disease
1	F	26	Natal	36.65	–
2	F	59	Guamaré	35.91	–
3	F	02	Guamaré	37.26	–
4	M	04	Guamaré	33.32	–
5	F	03	Acari	34.85	–
6	F	16	Natal	34.75	–
7	F	43	Natal	36.64	–
8	F	–	Ceará Mirim	35.68	–
9	F	–	Natal	30.12	–
10	M	–	Natal	27.90	–
11	F	–	Natal	35.90	–
12	M	–	Caiçara do Rio dos Ventos	35.14	–
13	M	46	Natal	37.96	–
14	M	–	Ceará Mirim	37.73	–
15	F	–	Natal	35.73	–
16	M	–	–	36.44	–
17	M	–	–	37.09	–
18	M	67	Parnamirim	31.34	3
19	F	07	Parnamirim	37.52	3
20	F	12	São Paulo do Potengi	37.77	3
21	M	36	Natal	34.72	4
22	F	66	Natal	34.18	3
23	F	39	Parnamirim	36.60	2
24	F	28	Natal	35.83	3
25	M	53	Parnamirim	35.42	3
26	M	06	Parnamirim	36.84	1
27	M	73	Parnamirim	36.93	4
28	F	37	Natal	34.50	4
29	F	58	Parnamirim	37.72	3
30	F	70	Nova Cruz	34.28	4

M male, *F* female, – not found

(Table 1). Reactions were performed on 96-well plates (MicroAmp® Fast Optical, Applied Biosystems). Standard PCR conditions were as follows: 50 °C (2 min) and 95 °C (10 min) followed by 40 cycles of 94 °C (30 s), variable annealing primer temperature (Table 2) (30 s), and 72 °C (1 min). The mRNA expression levels were determined using the mean Ct values from triplicate measurements to calculate the relative expression levels of the target genes in the patients with acute infection by ZIKV compared to healthy individuals, and were normalized to the housekeeping β-actin gene using the $2^{-\Delta\Delta Ct}$ formula.

Statistical analysis

Data are reported as mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to verify parametric or non-parametric data distribution. The mRNA expression levels were compared using the Kruskal–Wallis test. Correlations among were performed using the Spearman test. Differences were considered significant when $p < 0.05$. The analyses were performed using the PRISM 5.0 software (GraphPad, CA, USA).

Results

Patients during acute infection by ZIKV have increase of TLR3, IFN-α, IFN-β, IFN-γ, and decrease of RIG-1, TLR8, Myd88, and TNF-α mRNA expression

In an attempt to elucidate the immunological profile involved in acute infection by ZIKV in patients, we analyzed the mRNA expression of innate immune receptors and cytokines. Interestingly, patients with acute infection

Table 2 Sequences of the primers used for RT-PCR reactions

Target	Sense and antisense sequences	Primer annealing temperature (°C)
β-actin	TGACTCAGGATTTAAAAACTGGAA GCCACATTGTGAACTTTGGG	56.5
TLR3	TGGGTCTGGGAACATTTCTCTTC TGAGATTTAAACATTCCTCTTCGC	58.5
TLR7	TTTACCTGGATGGAAACCAGCTA TCAAGGCTGAGAAGCTGTAAGCTA	59.3
TLR8	TTATGTGTTCCAGGAACTCAGAGAA TAATACCCAAGTTGATGATCGATAAGTTTG	59.0
TLR9	CCACCCTGGAAGAGCTAAACC GCCGTCCATGAATAGGAAGC	60.8
MDA-5	GCAGAGGTGAAGGAGCAGA AAACGATGGAGAGGGCAAG	54.5
RIG-1	CACACCAAGAGCCCAAAC TGACCCGATAGCAACAGC	56.0
Myd88	CAAGTACAAGGCAATGAAGAAAG AAGGCGAGTCCAGAACCA	56.9
TRIF	ACTGAACGCAGCCTACTCAGC ATGACATGTGGCTCCCAAAAG	59.9
TNF-α	TTCTGGCTCAAAAAGAGAATTG TGGTGGTCTTGTTGCTTAAAG	55.2
IL-6	CAAATTCGGTACATCCTCGA TGCTGCTTTCACACATGTTACT	56.0
IL-12	CACTCCCAAAACCTGCTGAG TCTCTTCAGAAGTGCAAGGGTA	59.0
IFN-γ	ATGCAGAGCCAAATTGTCTCC GGCAGGACAACCATTACTGG	58.9
IFN-α	GAAGAATCTCTCCTTTCTCCTGCC ATGGAGGACAGAGATGGCTTG	61.0
IFN-β	TGCCCTAAGGACAGGATGAAC GCGTCCTCCTTCTGGAACTG	60.9

by ZIKV presented higher expression of TLR3 transcripts (Fig. 1a) than healthy individuals. However, similar expression of mRNA for TLR7, TLR9, MDA5, and TRIF was observed in patients and healthy controls (Fig. 1b–e). Furthermore, infected patients showed lower expression of TLR8, Myd88, and RIG-1 (Fig. 1f–h). Next, we evaluated the cytokine profile in patients infected by ZIKV. Patients during acute infection (up to 5 days after the onset of symptoms) by ZIKV showed similar expression of mRNA for IL-6 and IL-12 when compared to healthy controls (Fig. 2a, b). However, the patients presented a reduced expression of TNF-α transcripts (Fig. 2c). In addition, we observed the higher mRNA expression of cytokines produced upon TLR activation, such as IFN-α, IFN-β, and IFN-γ in patients infected by ZIKV than in healthy individuals (Fig. 2d–f). Together, these data demonstrate that the ZIKV infection in humans upregulates the mRNA expression for TLR3, IFN-α, IFN-β, and IFN-γ suggesting that these components are involved in the immune response against ZIKV infection.

Upregulation of TLR3 mRNA expression in patients with ZIKV is correlated with higher expression of IFN-α and IFN-β mRNAs

We subsequently analyzed the correlation between immune innate receptor and cytokine mRNA expression. We initially observed a positive correlation between TLR3 expression and IL-12 (Fig. 3a), IFN-α (Fig. 3b) and IFN-β (Fig. 3c) mRNA expression. No correlation was observed between TLR3 with IL-6 (Fig. 3d) or with IFN-γ (Fig. 3e). We posteriorly verified a positive correlation between TLR-9 expression with IL-6 (Fig. 4a), IL-12 (Fig. 4b), IFN-α (Fig. 4c), and IFN-γ (Fig. 4d) mRNA expression. No significant correlation was observed between TLR9 and IFN-β mRNA expression (Fig. 4e). MDA-5 mRNA expression presented a positive correlation with IL-6 (Fig. 5a), IL-12 (Fig. 5b), IFN-α (Fig. 5c), and IFN-β (Fig. 5d). No significant correlation was observed between MDA-5 and IFN-γ mRNA expression (Fig. 5e). No significant correlations were observed between RIG-1 and cytokine expression.

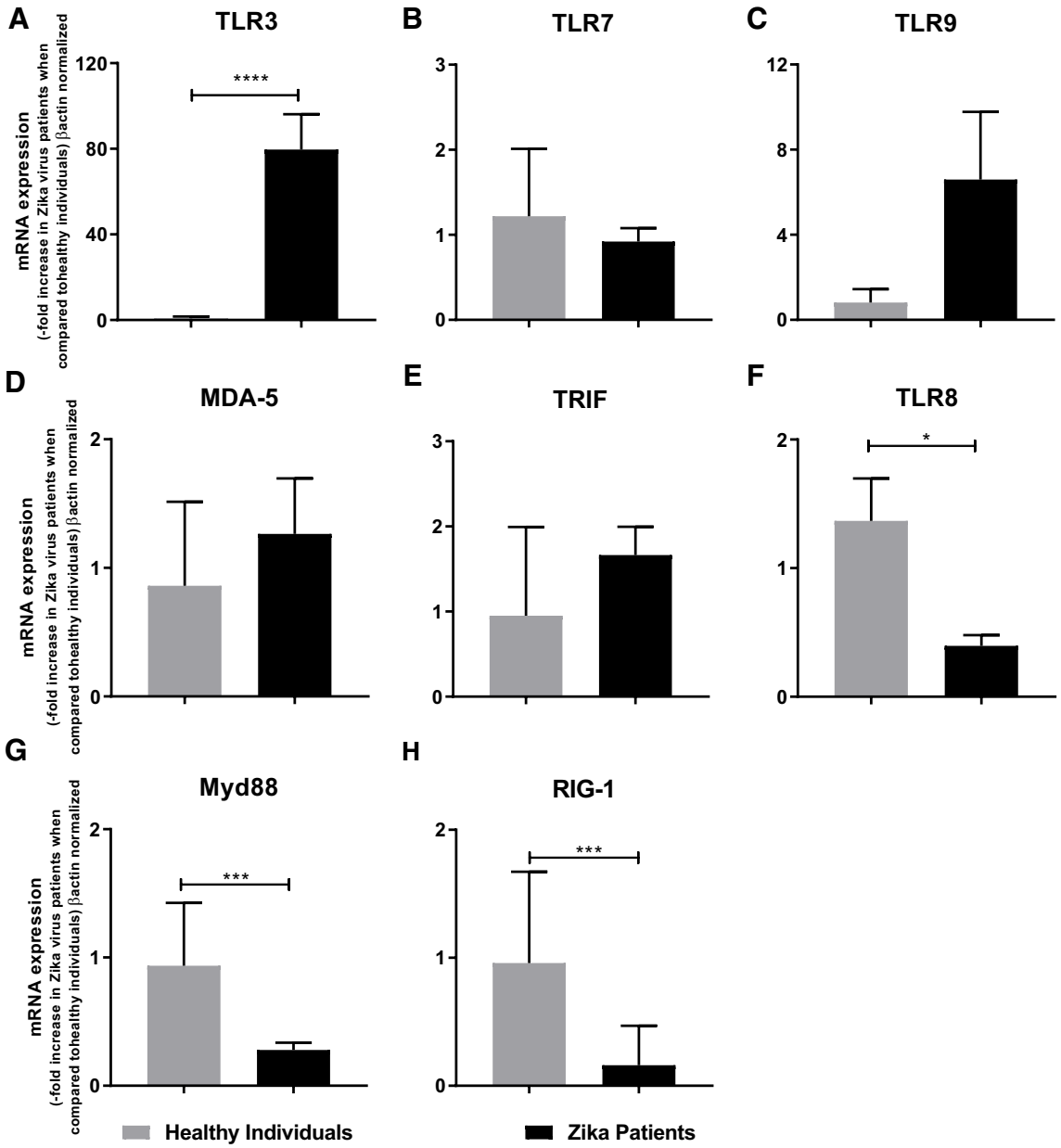

Fig. 1 Patients with acute Zika virus infection showed high TLR3 mRNA expression and low TLR8, Myd88, and RIG-1 mRNA expression. The mRNA expression levels of TLR3 (**a**) TLR7 (**b**) TLR9 (**c**) MDA-5 (**d**) TRIF (**e**) TLR8 (**f**) Myd88 (**g**), and RIG-1 (**h**) were determined by real-time PCR in peripheral blood of patients with acute Zika virus infection ($n=30$) and healthy individuals ($n=20$). The expression levels were normalized to the expression level of β-actin. The results are expressed as the means ± standard errors. *$p<0.05$

Altogether, these data suggest the possible involvement of TLR3 in production of IFN-α and IFN-β during ZIKV infection in patients.

Upregulation of TLR8, RIG1, MDA-5, IFN-α, and IFN-β mRNA expression in patients with ZIKV is correlated with higher viral load

Next, we evaluated the correlation between innate immune receptors/cytokines with viral load. No correlation was observed between TLR3 (Fig. 6a), TLR7 (Fig. 6b) and TLR9 (Fig. 6c) mRNA expression with viral load of patients during acute ZIKV infection. However, there was a negative correlation between C_t values for ZIKV RNA with TLR8 (Fig. 6d), RIG1 (Fig. 6e), MDA-5 (Fig. 6f), IFN-α (Fig. 6g), and IFN-β (Fig. 6h) mRNA expression. The negative correlation suggests that viral load is positively correlated with TLR8, RIG1, MDA-5, IFN-α, and IFN-β during acute infection of patients with ZIKV.

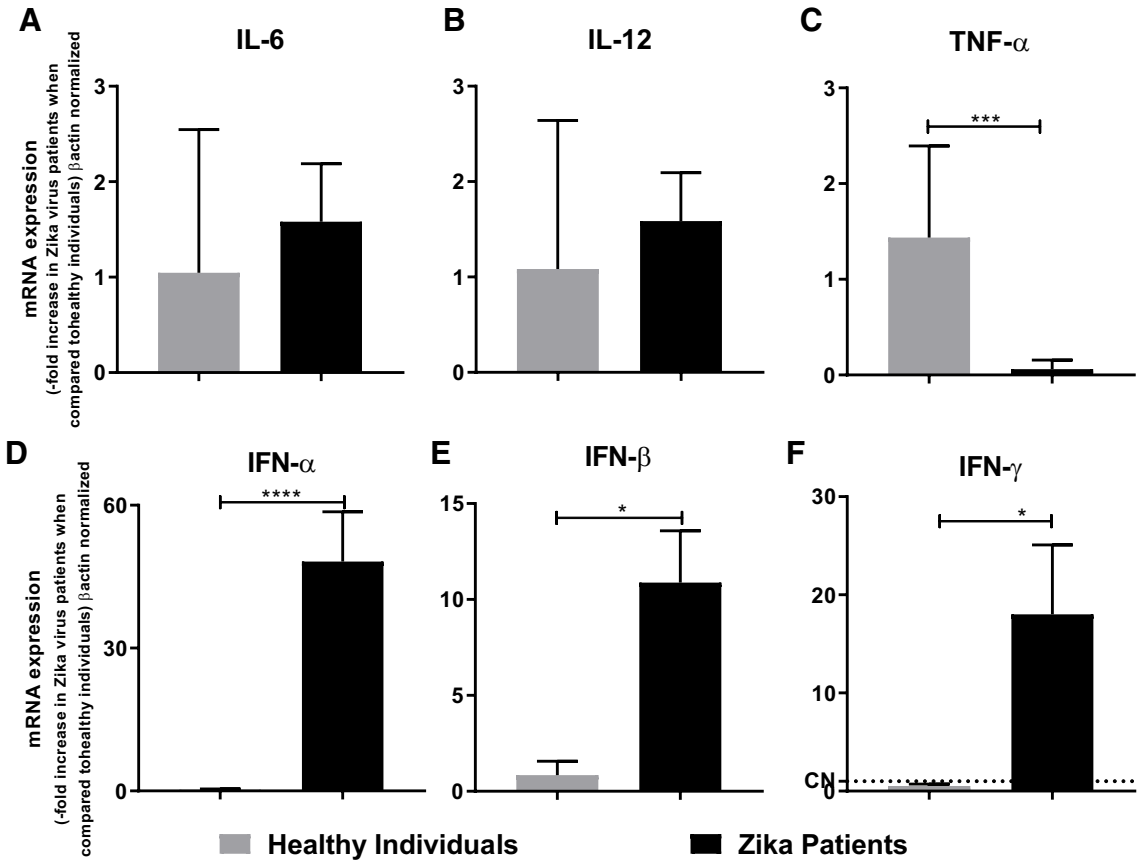

Fig. 2 Patients with acute Zika virus infection showed high IFN-γ, IFN-α, and IFN-β mRNA expression and low TNF-α mRNA expression. The mRNA expression levels of IL-6 (**a**) IL-12 (**b**) TNF-α (**c**) IFN-α (**d**), IFN-β (**e**), and IFN-γ (**f**) were determined by real-time PCR in peripheral blood of patients with acute Zika virus infection ($n=30$) and healthy individuals ($n=20$). The expression levels were normalized to the expression level of β-actin. The results are expressed as the means ± standard errors. *$p<0.05$

Discussion

In the present study, we evaluated the expression of innate immunity receptors (TLR3, TLR7, TLR8, TLR9, MDA-5, and RIG-1), adapter molecules (Myd88 and TRIF), and cytokines (IL-6, IL-12, TNF- IFN-α, IFN-β, and IFN-γ) in patients with acute ZIKV infection. Our results indicate that acute infection (up to 5 days after the onset of symptoms) by ZIKV induces the higher expression of TLR3 mRNA correlated with high IFN-γ, IFN-α, and IFN-β expression compared to healthy subjects. In addition, high viral load is correlated with TLR8, RIG1, MDA-5, IFN-α, and IFN-β. However, patients infected with ZIKV also have reduced expression of RIG-1, TLR8, Myd88, and TNF-α molecules, which are also involved in antiviral immunity.

We initially verified that patients with acute ZIKV infection have high TLR3 expression when compared to healthy controls. In fact, skin fibroblasts infected in vitro by ZIKV showed increased TLR3 expression, but not TLR7 [7]. The literature data demonstrate that the expression of IRF3 remained elevated during the course of infection by ZIKV in fibroblasts. On the other hand, TLR7 expression was reduced, indicating the participation of TLR3 in IRF7 activation and production of interferon type 1 [7, 20]. TLR3 expression contributes to antiviral immunity by inducing IFN-α, IFN-β, IL-6, and IL-12 production. However, high type I interferon production and other proinflammatory cytokines were also detected in the serum and amniotic fluid of children who had congenital transmission of the virus [20].

In the present study, we observed similar expression of TLR7 mRNA in the group of patients with ZIKV and healthy subjects. The literature data regarding experimental infection with West Nile virus demonstrated that TLR7 knockout mice produced less amounts of type 1 interferon [21]. Interestingly, this study showed reduced levels of RIG-1 and TLR8 mRNA expression in patients infected by ZIKV. These data suggest that patients infected by ZIKV have reduced TLR8 expression, an important receptor for interferon type 1 production. The reduced expression of RIG-1, TLR8, and the adapter molecule Myd88, both involved in the recognition of simple tape RNA genome virus during ZIKV infection,

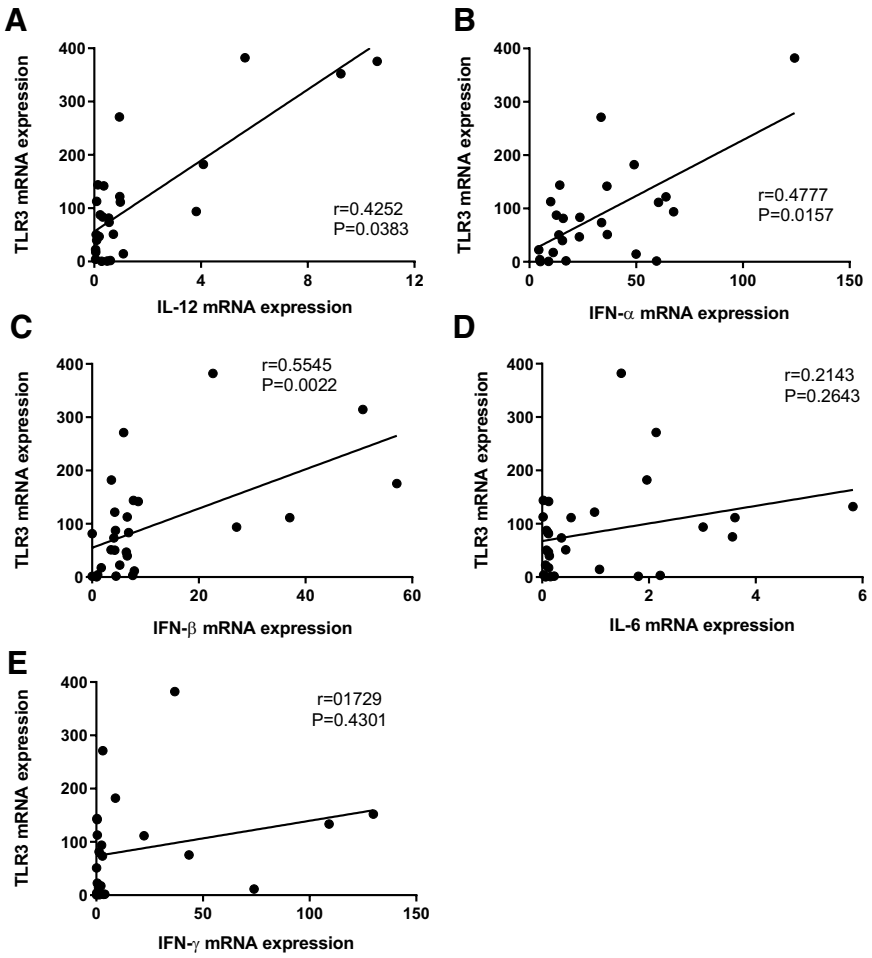

Fig. 3 High TLR3 mRNA expression is correlated with high IL-12, IFN-α, and IFN-β mRNA expression. mRNA expression levels were determined by real-time PCR in peripheral blood of patients with acute Zika virus infection ($n=30$) and healthy individuals ($n=20$). TLR3 mRNA expression was correlated with IL-12 (**a**), IFN-α (**b**), IFN-β (**c**), IL-6 (**d**), and IFN-γ (**e**). The mRNA expression levels were normalized to the expression level of β-actin. The results are expressed as the means ± standard errors and the Spearman test was used

suggests that this condition could be an escape mechanism used by the virus to evade the innate immune response. Inhibitory mechanisms of innate immunity are described for other flaviviruses as an immune evasion mechanism [22, 23]. NS5 protein of the Zika virus degrades STAT2 in human cells, a signaling molecule involved in the type I IFN pathway, which increases viral replication [13]. Study with Mexican patients infected with ZIKV demonstrated strong antiviral response with type I IFN production in peripheral blood mononuclear cells (PBMC) mediated by TLR3 and TLR8. However, in ZIKV-infected trophoblasts, a downregulation of both TLR3 and TLR8 and an upregulation of type I IFN and IFN-γ was observed [24]. Selective knockdown of either TLR3 or TLR8 decreased type I IFN production, but did not completely eliminate its production, suggesting the participation of other innate immune receptors in IFN-α and IFN-β production [24]. In the present study, we demonstrated the reduction of TLR8 mRNA levels during acute ZIKV infection, despite the positive correlation between TLR8 levels and the patient's viremia. The discrepancy between the low TLR8 expression and the positive correlation observed with the viremia could be due to the kinetics of TLR8 expression regulation, inhibitory viral mechanisms of cytokine production, and the participation of other innate immunity receptors (e.g., MDA-5 and RIG-1) in the production of antiviral cytokines, which could contribute to the protection during the infection [24, 25].

The outcome of a viral infection is determined by a balance between the rates of viral replication and spreading, as well as the immune response. The innate immune system acts as the first line of defense for sensing a viral infection, and responds by immediate protective defense mechanisms, including type I IFNs, inflammatory cytokine, complement response, NK cell, apoptosis, and autophagy. Moreover, the innate immune response modulates adaptive immune response [23].

The expression of TLR9 and MDA-5 was similar between the group of patients infected by ZIKV and healthy individuals. However, there is a positive correlation between MDA-5 expression with type I interferon mRNA expression (IFN-α and IFN-β) and proinflammatory cytokines (IL-6 and IL-12). These results suggest that MDA-5 plays a role in recognizing and protection against the ZIKV. Moreover, there was a positive correlation in the present study between

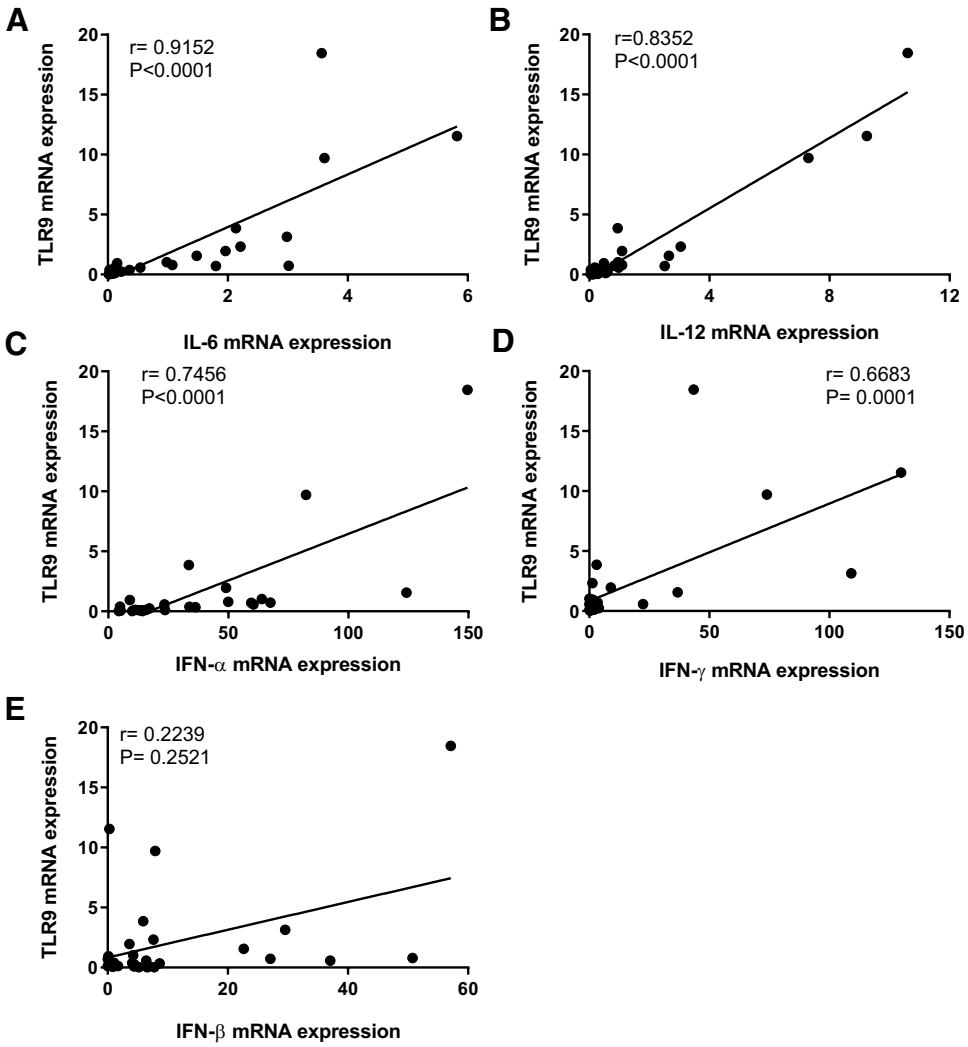

Fig. 4 High TLR9 mRNA expression is correlated with high IL-6, IL-12, IFN-α, and IFN-γ mRNA expression. mRNA expression levels were determined by real-time PCR in peripheral blood of patients with acute Zika virus infection ($n=30$) and healthy individuals ($n=20$). TLR9 mRNA expression was correlated with IL-6 (**a**), IL-12 (**b**), IFN-α (**c**), IFN-γ (**d**), and IFN-β (**e**). The mRNA expression levels were normalized to the expression level of β-actin. The results are expressed as the means ± standard errors and Spearman test was used

viral load with TLR8, RIG1, and MDA-5, thus suggesting that these receptors could play a role in virus recognition. The viral load was also correlated with IFN-α and IFN-β expression, which are induced by activation of TLR8, RIG1, and MDA-5. In fact, a culture of human fibroblasts infected in vitro by the ZIKV has shown increased MDA-5, IFN-α, and IFN-β mRNA expression [7].

In the present study, patients with acute ZIKV infection had high expression of IFN-γ, IFN-α, IFN-β, and reduced TNF-α mRNA expression. In addition, the high TLR3 expression shows positive correlation with IL-12, IFN-α, and IFN-β expression. Human monocytes obtained from healthy individuals and infected with an MR766 strain of African ZIKV strains showed higher expression of interferon type 1 (IFN-α and IFN-β) genes when compared to monocytes infected with H/PF/2013 strain of the Asian-origin virus [26]. The study also demonstrated that the infection of monocytes obtained from healthy pregnant women infected by the African ZIKV strain showed high IFN-β production when compared to the same cells infected with viruses of Asian origin. Type I interferon production in infected patients is correlated with protection against infection, but can also be correlated with pathogenesis, as in congenital ZIKV syndrome and the Guillain–Barré syndrome, and can result in microcephaly and/or other neurological damage [27–29]. The signaling pathway STAT1/STAT2/IRF9 leads to interferon type 1 production and can be inhibited by the NS5 protein of the ZIKV through the degradation of STAT2 [30]. Several flaviviruses such as Dengue, Chikungunya, and the West Nile virus inhibit the production of type I IFN using NS5 protein [31, 32]. Non-structural ZIKV proteins also interfere with the signaling stage of type I interferon and IFN-β production via RIG-I inhibition [33].

We also observed an increased level of IFN-γ mRNA expression in patients infected by ZIKV compared to healthy individuals. The NS5 ZIKV protein also induces the expression of type II interferons through forming STAT1–STAT1 protein complexes and activates genes leading to IFN-γ production. The literature data demonstrate that ZIKV infection induces Th1 response profile in CD4 T lymphocytes

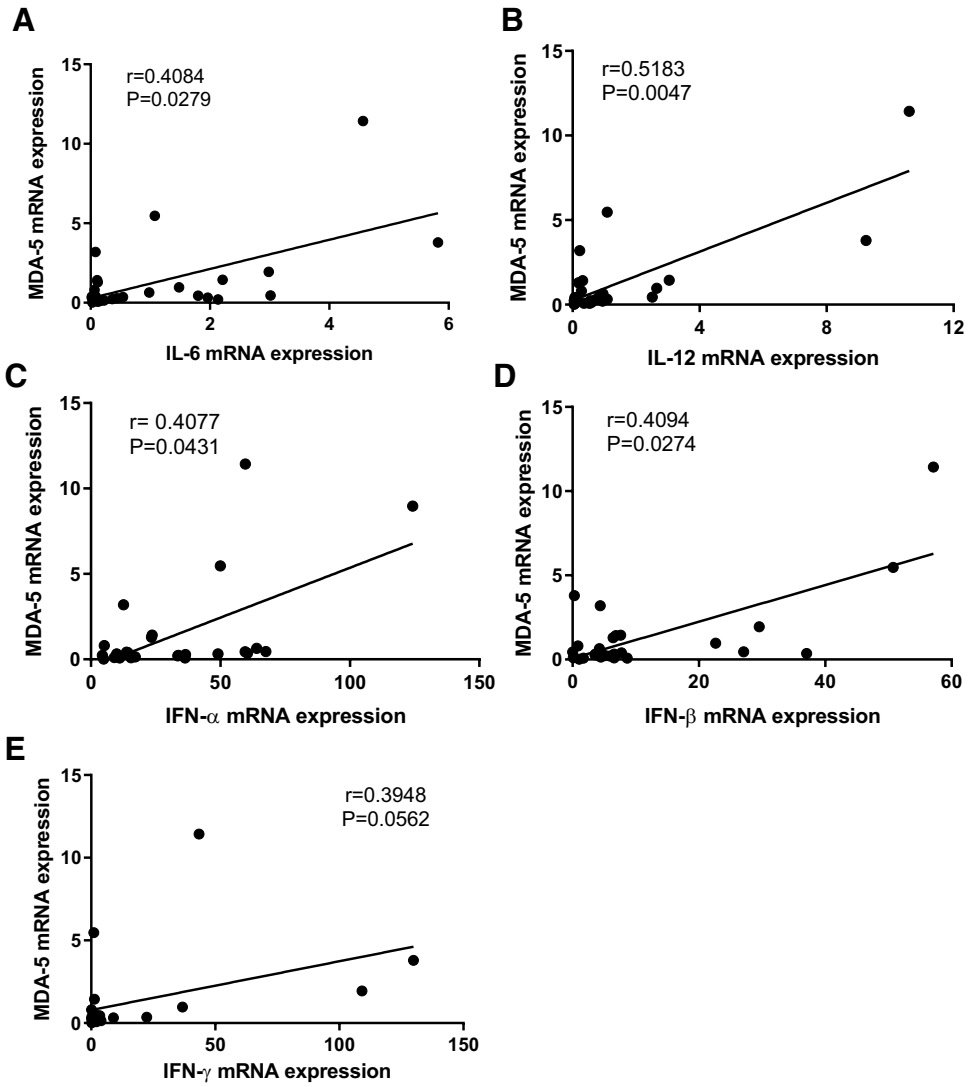

Fig. 5 High MDA-5 mRNA expression is correlated with high IL-6, IL-12, IFN-α, and IFN-β mRNA expression. mRNA expression levels were determined by real-time PCR in peripheral blood of patients with acute Zika virus infection ($n=30$) and healthy individuals ($n=20$). MDA-5 mRNA expression was correlated with IL-6 (**a**), IL-12 (**b**), IFN-α (**c**), IFN-β (**d**), and IFN-γ (**e**). The mRNA expression levels were normalized to the expression level of β-actin. The results are expressed as the means ± standard errors and Spearman test was used

with the production of IFN-γ, TNF-α and IL-2 [30, 34]. In this study, patients infected by ZIKV and healthy individuals showed similar expression of IL-6 and IL-12. Dentritic cells and monocytes infected with ZIKV isolated in Puerto Rico and phylogenetically related to the outbreak isolates in Brazil in 2015–2016 [35, 36] showed low production of IL6 and IL-12 [14]. Furthermore, pregnant women infected by ZIKV have similar serum levels of IL-12 when compared to infected and healthy women [26].

In the present study, we observed a reduction in TNF-α mRNA expression in patients infected by ZIKV when compared to healthy controls. Experimental infection of guinea pigs by ZIKV showed a reduction of TNF-α production starting from the 5th day of infection [37]. Patients infected with the ZIKV in the acute phase and convalescence phase showed reduced production of TNF-α. Low TNF-α production can be due to persistent elevated levels of IL-10 and IL-13 from the acute phase to convalescence phase [16]. On the other hand, in an atypical case of ZIKV infection, a patient with no cardiovascular disease history had typical symptoms of atrial fibrillation and elevated levels of chemokines, IL-6, TNF-α, and IFN-γ, among other cytokines. The authors also observed higher TNF-α, IFN-γ, and chemokine production in patients infected with ZIKV with typical clinical manifestations when compared to healthy controls [38]. Furthermore, differential cytokine expression seems to influence the natural history of the ZIKV infection and can lead to manifesting asymptomatic clinical conditions to those of more severe neurological symptomatology.

Immune response against ZIKV at the maternal–fetal interface may be responsible for the congenital malformation associated with ZIKV [26]. Data from pregnant patients infected by ZIKV who had neonates with microcephaly showed that there is a polyfunctional immune activation in the amniotic fluid, with the increased levels of IP-10, IL-6,

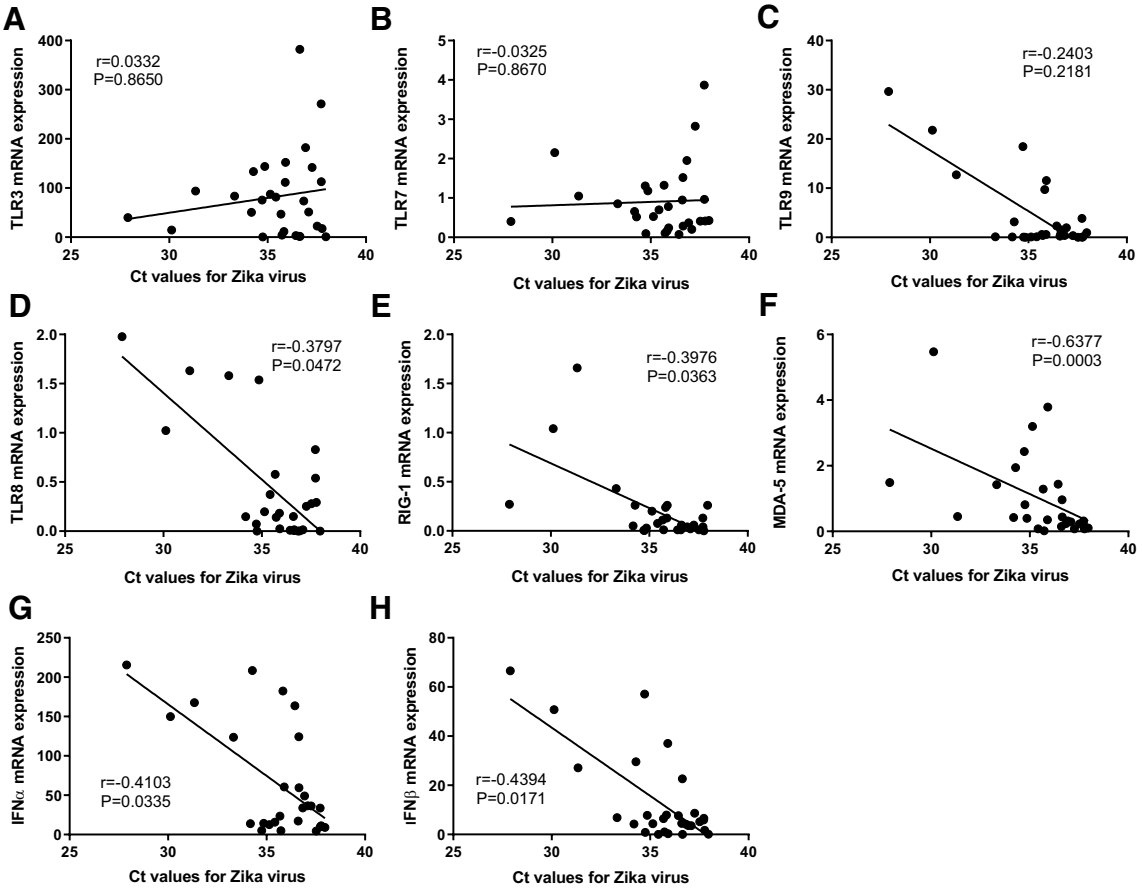

Fig. 6 Upregulation of TLR8, RIG1, MDA-5, IFN-α, and IFN-β mRNA expression in patients with ZIKV is correlated with higher viral load. mRNA expression levels were determined by real-time PCR in peripheral blood of patients with acute Zika virus infection ($n=30$) and healthy individuals ($n=20$). Viral load (cycle threshold for Zika virus) was correlated with TLR-3 (**a**), TLR-7 (**b**), TLR-9 (**c**), TLR8 (**d**), RIG-1 (**e**), MDA-5 (**f**), IFN-β (**g**), and IFN-γ (**h**). The mRNA expression levels were normalized to the expression level of β-actin. The results are expressed as the means ± standard errors and the Spearman test was used. C_t cycle threshold

IL-8, VEGF, MCP-1, and G-CSF-related cytokines to neuronal damage which impaired differentiation and proliferation of neural progenitor cells [39]. Increased IL-1β, IL-2, IL-4, IL-6, IL-9, IL-10, IL-13, and IL-17 were also seen in patients in the acute phase of ZIKV infection (Ornelas et al. [40]). Type I IFNs are upregulated in ZIKV-infected fibroblasts, and comprise key factors to mount the antiviral response [41]. However, they may be mediators of pregnancy complications, including miscarriages, abnormal placental development, intrauterine growth restriction, and fetal reabsorption in the context of congenital viral infections.

In the evaluated area of this study, there are other flavivirus infections circulating in humans such as Chikungunya virus (CKV), dengue virus (DENV), and yellow fever virus (YFV). The previous infections with these viruses can modulate or influence innate immune activation and adaptive immune response, thereby influencing immune response in posteriori viral infection in patients, such as in the case of ZIKV infection. Modulation of innate immune responses by flaviviruses affects the quality of the adaptive immune response. Particular focus has been given to cross reactivity between DENV and ZIKV. Dengue-specific antibodies are able to enhance cell infection with ZIKV in vitro [42–44]. Adoptive plasma transfer of patients in convalescent phase of DENV infection enhanced pathogenesis of ZIKV infection in a mouse model [45]. However, *Rhesus* macaques with the previous infection by DENV did not show more severe ZIKV infection/disease [46].

Innate immune receptors are responsible for the initial protective response against the virus and in modulating the adaptive immune response profile. Our results demonstrate that patients showed high TLR3 mRNA expression during acute infection by the ZIKV, correlating with high IFN-α and IFN-β expression, which are major innate immunity molecules with antiviral activity. Moreover, high viral load is correlated with high TLR8, RIG-1, MDA-5, IFN-α, and IFN-β mRNA expression. On the other hand, patients with acute ZIKV infection showed decreased levels of RIG-1,

TLR8, and Myd88 mRNA expression, which are also molecules involved in antiviral immunity. Studies which evaluate the role of innate immune activation in adaptive response as well as the role of previous viral infection in innate immune response modulation need to be performed. Better knowledge of immunological mechanisms that confer protection or pathology during ZIKV infection in patients can be a valuable tool for reducing morbidity and mortality associated with infection.

Acknowledgements This work was supported by the National Council for Scientific and Technological Development (*MEC*/*MCTI*/*CAPES*/*CNPq*/*FAPS-PVE* Grant no. 400328/2014-3 and *MCTI*/*CNPq*/Universal Grant no. 404904/2016-5). Post-graduation research fellows are supported by University Faculty Advanced Studies Coordination Unit (*CAPES*). The author PMMG acknowledges the *CNPq* for the research productivity fellowship.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

References

1. Hayes EB (2009) Zika virus outside Africa. Emerg Infect Dis 15:1347–1350. https://doi.org/10.3201/eid1509.090442
2. Kucharski AJ, Funk S, Eggo RM et al (2016) Transmission dynamics of Zika virus in island populations: a modelling analysis of the 2013–14 French Polynesia outbreak. PLoS Negl Trop Dis 10:1–15. https://doi.org/10.1371/journal.pntd.0004726
3. Tognarelli J, Ulloa S, Villagra E et al (2016) A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. Arch Virol 161:665–668. https://doi.org/10.1007/s00705-015-2695-5
4. Zanluca C, De Melo VCA, Mosimann ALP et al (2015) First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz 110:569–572. https://doi.org/10.1590/0074-02760150192
5. Calvet G, Aguiar RS, Melo ASO et al (2016) Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis 16:653–660. https://doi.org/10.1016/S1473-3099(16)00095-5
6. Ikejezie J, Shapiro CN, Kim J et al (2017) Zika virus transmission—region of the Americas, May 15, 2015–December 15, 2016. Am J Transplant 17:1681–1686. https://doi.org/10.1111/ajt.14333
7. Hamel R, Dejarnac O, Wichit S et al (2015) Biology of Zika virus infection in human skin cells. J Virol 89:8880–8896. https://doi.org/10.1128/JVI.00354-15
8. Petersen LR, Jamieson DJ, Powers AM, Honein MA (2016) Zika virus. N Engl J Med 374:1552–1563. https://doi.org/10.1056/NEJMra1602113
9. Nascimento OJM, Da Silva IRF (2017) Guillain–Barré syndrome and Zika virus outbreaks. Curr Opin Neurol 30:500–507. https://doi.org/10.1097/WCO.0000000000000471
10. De Oliveira Melo AS, Aguiar RS, Amorim MMR et al (2016) Congenital Zika virus infection: beyond neonatal microcephaly. JAMA Neurol 73:1407–1416. https://doi.org/10.1001/jamaneurol.2016.3720
11. Lazear HM, Govero J, Smith AM et al (2016) A mouse model of Zika virus pathogenesis. Cell Host Microbe 19:720–730. https://doi.org/10.1016/j.chom.2016.03.010
12. Shresta S, Kyle JL, Snider HM et al (2004) Interferon-dependent immunity is essential for resistance to primary dengue virus infection in mice, whereas T- and B-cell-dependent immunity are less critical. J Virol 78:2701–2710. https://doi.org/10.1128/JVI.78.6.2701-2710.2004
13. Grant A, Ponia SS, Tripathi S et al (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
14. Bowen JR, Quicke KM, Maddur MS et al (2017) Zika virus antagonizes type I interferon responses during infection of human dendritic cells. PLoS Pathog 13:1–30. https://doi.org/10.1371/journal.ppat.1006164
15. Kam Y-W, Leite JA, Lum F-M et al (2017) Specific biomarkers associated with neurological complications and congenital CNS abnormalities from Zika virus-infected patients in Brazil. J Infect Dis. https://doi.org/10.1093/neuonc/nox188/4265638
16. Tappe D, Pérez-Girón JV, Zammarchi L et al (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
17. Brubaker SW, Bonham KS, Zanoni I, Kagan JC (2016) Innate immune pattern recognition: a cell biological perspective. Annu Rev Immunol 33:257
18. Takeda K, Akira S (2003) Toll receptors and pathogen resistance. Cell Microbiol 5:143–153. https://doi.org/10.1046/j.1462-5822.2003.00264.x
19. Faye O, Faye O, Diallo D et al (2013) Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. Virol J 10:1–8. https://doi.org/10.1186/1743-422X-10-311
20. Morris G, Barichello T, Stubbs B et al (2017) Zika virus as an emerging neuropathogen: mechanisms of neurovirulence and neuro-immune interactions. Mol Neurobiol. https://doi.org/10.1007/s12035-017-0635-y
21. Welte T, Reagan K, Fang H et al (2009) Toll-like receptor 7-induced immune response to cutaneous West Nile virus infection. J Gen Virol 90:2660–2668. https://doi.org/10.1099/vir.0.011783-0
22. Pierson T, Fremont D, Kuhn R, Diamond M (2013) Structural insights into the mechanisms of antibody-mediated neutralization of flavivirus infection: implications for vaccine development. Cell Host Microbe 17:148–159. https://doi.org/10.1007/s10461-012-0143-z.Provider-patient
23. Ye J, Zhu B, Fu ZF et al (2013) Immune evasion strategies of flaviviruses. Vaccine 31:461–471. https://doi.org/10.1016/j.vaccine.2012.11.015
24. Luo H, Winkelmann ER, Fernandez-salas I et al (2018) Zika, dengue and yellow fever viruses induce differential anti-viral immune responses in human monocytic and first trimester trophoblast cells. Antivir Res 151:55
25. Kawai T, Akira S (2011) Review toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34:637–650. https://doi.org/10.1016/j.immuni.2011.05.006
26. Foo SS, Chen W, Chan Y et al (2017) Asian Zika virus strains target CD14+ blood monocytes and induce M2-skewed immunosuppression during pregnancy. Nat Microbiol 2:1558–1570. https://doi.org/10.1038/s41564-017-0016-3
27. Araujo LM, Ferreira MLB, Nascimento OJM (2016) Síndrome de Guillain–Barré associada ao surto de infecção por vírus Zika no Brasil. Arq Neuropsiquiatr 74:253–255. https://doi.org/10.1590/0004-282X20160035
28. Malkki H (2016) CNS infections: Zika virus infection could trigger Guillain–Barré syndrome. Nat Rev Neurol 12:187. https://doi.org/10.1038/nrneurol.2016.30

29. Garcez P, Loiola E, Costa RM et al (2005) Zika virus impairs growth in human neurospheres and brain organoids. Am J Hum Genet 76:717–728. https://doi.org/10.1086/429930
30. Chaudhary V, Yuen KS, Chan JF, Chan CP, Wang PH, Cai JP, Zhang S, Liang M, Kok KH, Chan CP, YuenKY Jin DY (2017) Selective activation of type II interferon signaling by Zika virus NS5 protein. J Virol. 91(14):e00163-17
31. Laurent-Rolle M, Boer EF, Lubick KJ et al (2010) The NS5 protein of the virulent West Nile virus NY99 strain is a potent antagonist of type I interferon-mediated JAK-STAT signaling. J Virol 84:3503–3515. https://doi.org/10.1128/JVI.01161-09
32. Lubick KJ, Robertson S, McNally K et al (2015) Flavivirus antagonism of type I interferon signaling reveals prolidase as a regulator of IFNAR1 surface expression. Cell Host Microbe 18:61–74. https://doi.org/10.1097/RCT.0000000000000239.Texture
33. Xia H, Luo H, Shan C et al (2018) An evolutionary NS1 mutation enhances Zika virus evasion of host interferon induction. Nat Commun. https://doi.org/10.1038/s41467-017-02816-2
34. Ngono AE, Vizcarra EA, Tang W et al (2017) Mapping and role of the CD8+ T cell response during primary Zika virus infection in mice. Cell Host Microbe 21:35–46. https://doi.org/10.1007/s10549-015-3663-1.Progestin
35. Faria (2016) Zika virus in the Americas: early epidemiological and genetic findings. Science 352:345–349. https://doi.org/10.1126/science.aaf5036.Zika
36. Lanciotti RS, Lambert AJ, Holodniy M et al (2016) Phylogeny of Zika virus in western hemisphere, 2015. Emerg Infect Dis 22:933–935. https://doi.org/10.3201/eid2205.160065
37. Kumar A, Hou S, Airo AM et al (2016) Zika virus inhibits type-I interferon production and downstream signaling. EMBO Rep 17:1766–1775. https://doi.org/10.15252/embr.201642627
38. Abdalla LF, Santos JHA, Barreto RTJ et al (2018) Atrial fibrillation in a patient with Zika virus infection. Virol J 15:4–9. https://doi.org/10.1186/s12985-018-0938-2
39. Silasi M, Cardenas I, Racicot K et al (2015) Viral infections during pregnancy. Am J Reprod Immunol 73:199–213. https://doi.org/10.1111/aji.12355.VIRAL
40. Ornelas AM, Pezzuto P, Silveira PP, Melo FO, Ferreira TA, Oliveira-Szejnfeld PS, Leal JI, Amorim MM, Hamilton S, Rawlinson WD, Cardoso CC, Nixon DF, Tanuri A, Melo AS, Aguiar RS (2017) Immuneactivation in amniotic fluid from Zika virus-associated microcephaly. Ann Neurol 81(1):152–156
41. Simoni M, Jurado KA, Abrahams VM et al (2017) Zika virus infection of Hofbauer cells. Am J ReprodImmunol 77:1–8
42. Barba-Spaeth G, Dejnirattisai W, Rouvinski A et al (2017) Structural basis of Zika and dengue virus potent antibody cross-neutralization. Nature 536:48–53
43. Dejnirattisai W, Supasa P, Wongwiwat W et al (2016) Dengue virus sero-cross-reactivity drives antibodydependent enhancement of infection with zika virus. Nat Immunol 17:1102–1108
44. Priyamvada L, Quicke KM, Hudson WH et al (2016) Human antibody responses after dengue virusinfection are highly cross-reactive to Zika virus. Proc Natl Acad Sci 113:7852–7857
45. Bardina SV, Bunduc P, Tripathi S et al (2017) Enhancement of Zika virus pathogenesis by preexistingantiflavivirus immunity. Science 356:175–180
46. Pantoja P, Pérez-Guzmán EX, Rodríguez IV et al (2017) Zika virus pathogenesis in rhesus macaques is unaffected by pre-existing immunity to dengue virus. Nat Commun. https://doi.org/10.1038/ncomms15674