Zika, dengue and yellow fever viruses induce differential anti-viral immune responses in human monocytic and first trimester trophoblast cells

Huanle Luo[1], **Evandro R Winkelmann**[1], **Ildefonso Fernandez-Salas**[2], **Li Li**[3], **Sandra V Mayer**[3], **Rogelio Danis-Lozano**[2], **Rosa Ma Sanchez-Casas**[4], **Nikos Vasilakis**[3,5,6], **Robert Tesh**[3,5,6], **Alan D Barrett**[3,5,6], **Scott C Weaver**[1,3,5,6], and **Tian Wang**[1,3,5,6,*]

[1]Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston, TX, 77555, USA

[2]Centro Regional de Salud Pública, Instituto Nacional de Salud Pública, Tapachula, Mexico

[3]Department of Pathology, University of Texas Medical Branch, Galveston, TX, 77555, USA

[4]FMVZ/CIDICS. Universidad Autonoma de Nuevo Leon, Monterrey NL Mexico

[5]Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX, 77555, USA

[6]Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, Texas, USA

Abstract

Zika virus (ZIKV) is a mosquito-borne flavivirus associated with severe neonatal birth defects, but the causative mechanism is incompletely understood. ZIKV shares sequence homology and early clinical manifestations with yellow fever virus (YFV) and dengue virus (DENV) and are all transmitted in urban cycles by the same species of mosquitoes. However, YFV and DENV have been rarely reported to cause congenital diseases. Here, we compared infection with a contemporary ZIKV strain (FSS13025) to YFV17D and DENV-4 in human monocytic cells (THP-1) and first-trimester trophoblasts (HTR-8). Our results suggest that all three viruses have similar tropisms for both cells. Nevertheless, ZIKV induced strong type 1 IFN and inflammatory cytokine and chemokine production in monocytes and peripheral blood mononuclear cells. Furthermore, ZIKV infection in trophoblasts induced lower IFN and higher inflammatory immune responses. Placental inflammation is known to contribute to the risk of brain damage in preterm newborns. Inhibition of toll-like receptor (TLR)3 and TLR8 each abrogated the inflammatory cytokine responses in ZIKV-infected trophoblasts. Our findings identify a potential link between

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*Correspondence: Dr. Tian Wang, Department of Microbiology & Immunology, The University of Texas Medical Branch, Keiller 3.118B, Galveston, TX, 77555-0609, USA. Telephone: +1-409-772-3146; Fax: +1-409-772-3338; ti1wang@utmb.edu.

maternal immune activation and ZIKV-induced congenital diseases, and a potential therapeutic strategy that targets TLR-mediated inflammatory responses in the placenta.

Keywords

Zika virus; immunity; flavivirus; trophoblast

1. INTRODUCTION

Zika virus (ZIKV), an emerging mosquito-borne virus has caused outbreaks in Africa, Asia, the south Pacific and more recently in the Americas (Dyer, 2015; Fauci and Morens, 2016; Grard et al., 2014). The virus belongs to the genus *Flavivirus*, a group of single -stranded, positive sense RNA viruses, also including dengue (DENV), West Nile (WNV), yellow fever (YFV) and Japanese encephalitis viruses (JEV) (Dick et al., 1952). ZIKV is closely related to YFV and DENV, as they are all transmitted to humans predominantly by the same *Aedes* mosquitoes. They are also fast-growing global health threats and share structure and genetic homologies. The majority of human infections induced by these three viruses are either asymptomatic or result in self-limiting flu - like febrile illness at the acute stage (Weaver et al., 2017). Nevertheless, ZIKV has been linked to more severe diseases such as the neurological autoimmune disorder Gullian-Barré Syndrome in adults and birth malformations and microcephaly in fetuses and infants (congenital Zika syndrome or CZS) (McCarthy, 2016; Mlakar et al., 2016). In addition to mosquito bites, ZIKV can be transmitted vertically and, less frequently, by sexual contact (Cao-Lormeau et al., 2016; Musso, 2015; Oliveira Melo et al., 2016). DENV and YFV are known to cause hemorrhagic disease (dengue hemorrhagic fever, or dengue shock syndrome), and acute hepatic and renal failure, respectively (Fernandez-Garcia et al., 2009). Vertical transmission has rarely been reported for DENV and YFV infection. Currently, there are no treatments or approved vaccines for use in humans to protect against ZIKV infection, though there is an effective live attenuated vaccine (YFV-17D) for YFV (Collins and Barrett, 2017) and a recently licensed live attenuated DENV vaccine (Gack and Diamond, 2016).

The mechanisms of CZS are incompletely understood. It is believed that ZIKV pathogenesis is associated with its broad cell and tissue tropisms during the first trimester of pregnancy (El Costa et al., 2016; Miner and Diamond, 2017). For example, ZIKV replicates within different types of maternal and fetal cells, including Hofbauer cells, trophoblasts, fibroblasts, and fetal endothelial cells (Quicke et al., 2016; Tabata et al., 2016). Maternal immune activation (MIA) as a result of viral infection is known to have the potential to increase the risk of fetal developmental problems. The role of MIA in CZS is largely unknown. The objective of this study was to compare ZIKV, YFV, and DENV infection and immune responses in two human cell types: monocytic cells and the first trimester placental trophoblasts. We found that while all three viruses share similar tropisms in both cell types; however, ZIKV induces differential innate cytokine responses compared to the other two viruses, which may contribute to ZIKV-mediated clinical disease.

2. MATERIALS AND METHODS

2.1. Cell lines, and PBMCs

Human monocytic leukemia cells (THP-1), were a kind gift from Dr. J. McBride (University of Texas medical Branch, Galveston, TX) and were propagated in RPMI medium 1640 with L-glutamine and 25 mM HEPES buffer (Invitrogen, Carlsbad, CA) supplemented with 1 mM sodium pyruvate (Sigma, St. Louis, MO), and 10% fetal bovine serum (FBS, Sigma). Trophoblast (HTR-8/SVneo) cell line was purchased from the American Type Culture Collection (Manassas, VA). Cells were maintained in RPMI 1640 supplemented with 10% heat-inactivated FBS. PBMCs were isolated using Histopaque®-1083(Sigma) from ZIKV PCR-positive acute patients collected in a recent outbreak in southern Mexico and from healthy volunteers with no history of Flavivirus exposure as described in a previous report (Guerbois et al., 2016). Blood samples were collected by venipuncture from acute patients or healthy donors who volunteered and provided written informed consent.

2.2. Viruses

The Asian lineage FSS13025 strain of ZIKV was obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA, Galveston, TX) and was amplified once in Vero cells. YFV vaccine strain 17D and DENV-4 strain 703-4, a low passage human isolate collected in Thailand in 1994 (Milligan et al., 2015; Wang et al., 2000). Cells were cultured in 24-well plates and were infected with ZIKV, YFV, or DENV at a multiplicity of infection (MOI) of 1 or 5 units/cell. At various times post-infection, cells and supernatant were collected for measurement of viral load and cytokine production.

2.3. Focus-forming assay (FFA) for viral titer

At 48 h, the overlay was removed, cell monolayers were washed with PBS, air dried, and fixed with 1:1 solution of acetone and methanol for at least 30 min at −20 °C. Cells were next subjected to immunohistochemical (IHC) staining with either a ZIKV hyperimmune mouse ascitic fluid (obtained through the WRCEVA) or 4G2 mouse monoclonal antibody followed by goat anti-rabbit HRP-conjugated IgG (KPL, Gaithersburg, MD) at room temperature for 1 h respectively. After addition of the secondary antibody, cells were incubated with a peroxidase substrate (Vector Laboratories, Burlingame, CA) until color developed. The number of foci was determined and used to calculate viral titers expressed as focus-forming unit (FFU)/ml.

2.4. Quantitative PCR (Q-PCR) and PCR array

Viral-infected samples were re-suspended in Trizol ((Invitrogen, Carlsbad, CA) for RNA extraction. Complementary (c)DNA was synthesized by using a qScript cDNA synthesis kit (Bio-Rad, Hercules, CA). The sequences of the primer sets for ZIKV and cytokines cDNA, and PCR reaction conditions, were described previously (Fernandez-Garcia et al., 2016; Lanciotti et al., 2008; Waggoner et al., 2013; Xie et al., 2015). The assay was performed using iQ SYBR Green Supermix kit (product # 1708884, Biorad) in the CFX96 real-time PCR system (Bio-Rad) according to the manufacturer's instructions. Gene expression was calculated either based on C_t values by using the formula $2^{\wedge -[C_t \text{ (target gene)} - C_t \text{ (GAPDH)}]}$ as

described before (Welte et al., 2011). A 45-gene PrimePCR assay (Biorad) was performed following the manufacturer's protocol. Briefly, RNA was purified from non-infected and viral-infected cells by using an RNeasy extraction kit (Qiagen, Valencia, CA) and quantitated by spectrometry. cDNA was synthesized by using iScript family reverse transcription kits and then loaded onto 96-well PCR array plates for amplification on the CFX96 real-time PCR system (Biorad). A total of 45 genes were assayed (listed in Supplementary Table 1). Data analysis was performed by using CFX manager software. Six genes including IL12A, IL1B, GAPDH, IL23A, NFKB1, and TGFB1 were selected as reference genes for PrimePCR array of HTR8 cells; seven genes including CASP3, IFIT2, IFITM2, IRF7, ISG15, GAPDH, and IFNAR2 were selected for THP-1 cells; and six genes including OAS1, OAS3, IFIT2, RSAD2, GAPDH, and IFNAR2 were selected for human PBMCs.

2.5. SiRNA knockdown for Toll-like receptor (TLR)-3 and TLR8

HTR-8 cells (5×10^4) or THP-1 cells (1×10^5) were transfected with 37.5 pmol TLR3 siRNA, or human TLR8 siRNA, or control siRNA (Santa Cruz Biotechnology, Dallas, TX) according to the manufacturer's instructions. Transfected cells were grown in RPMI medium containing 10% FBS and the effects of siRNA knockdown were confirmed by Q-PCR assay. After 48 h transfection, cells were infected with ZIKV at an MOI of 2 or 1. Cells and culture supernatant were harvested at days 1 and 4 post-infection.

2.6. Cytokine analysis

Culture supernatant was collected for analysis of cytokine production by using 5-plex Bio-Plex Pro Human Cytokine Assays (Bio-Rad).

2.7. Statistical analysis

Data analysis was performed using Prism software (Graph-Pad) statistical analysis. Values for viral burden, and cytokine production experiments were presented as means ± SEM. *P* values of these experiments were calculated with a non-paired Student's t test. Statistical significance was accepted at $P < 0.05$.

3. RESULTS

3.1. ZIKV replicates in human monocytic cells at a low rate, similar to YFV and DENV, but it induces more robust anti-viral innate cytokine responses

In this study, we first compared the immune response of human monocytes to ZIKV infection versus two other closely related flaviviruses (YFV and DENV). The Asian lineage ZIKV strain FSS13025, YFV vaccine strain 17D, and a non-mouse adapted human DENV-4 strain 703-4 were used to infect THP-1 cells. Replication kinetics were analyzed on days 1 and 4 post-infection by Q-PCR and FFA. Viral RNA levels were decreased on day 4 versus day 1 for all three viruses (Fig. 1A–C). Although Q-PCR assay showed several-fold lower ZIKV RNA compared to the other two viruses, FFA results showed similarly low viral titers of all three viruses on days 1 and 4 post-infection (pi, Fig. 1D). Next, a PCR array of 45-genes of innate signaling pathways (Supplementary (S) Table 1) was used to identify anti-viral gene profiles in THP-1 cells on day 1 post-infection (pi). It revealed that ZIKV (18

genes) induced more genes than YFV (13 genes) and DENV (10 genes), which included interferon (IFN)s, interferon stimulated gene (ISG)s, inflammatory cytokines, chemokines, and factors involved in pathogen recognition receptor (PRR) signaling and apoptosis pathways (STable 2 & SFig. 1). ZIKV also downregulated fewer genes than YFV and DENV, particularly the inflammatory cytokine and chemokine genes. Compared to YFV and DENV infection, ZIKV- infected cells had 12 genes (IL1B, IL28A, IL29, SOCS3, IFIT1, CCR5, CCL3, CCL4, CXCL10, TLR8, IL10, and CSF2) and 8 genes (IL-12B, IL28A, IL-29, SOCS3, CXCL10, TLR8, IL-10, CSF2) respectively with more than 2- fold higher expression (Fig. 2A, **in red**). These results were next verified by Q-PCR and multi-cytokine Bioplex assays. As shown in Fig. 2B, ZIKV-induced higher IFNα expression than DENV on day 1 and YFV on day 4 pi. IFNβ expression was also augmented in ZIKV-infected THP-1 cells compared to DENV infection. The production of IL-6, IL-8, CCL2, CCL3, and CCL5 was between 0.5 – 86 -fold higher in ZIKV-infected THP-1 cells compared to YFV and DENV (Fig. 2C). Overall, all three flaviviruses, in particular ZIKV, replicated poorly in human monocytic cells. However, ZIKV triggered stronger IFN, inflammatory and chemotactic cytokine responses than YFV and DENV.

3.2. Peripheral blood mononuclear cell (PBMC)s of ZIKV acute patients displayed strong innate cytokine genes expression

During an outbreak of Zika fever in southern Mexico, near the Guatemalan border in late 2015 (Guerbois et al., 2016), PBMCs of PCR-confirmed patients were collected 1 – 10 days after the onset of signs and/or symptoms (STable 3). PCR array of PBMCs from two ZIKV-positive patients (0031, 0163) each showed 30 genes with more than 2- fold higher expression than healthy controls (Fig. 3A–B & STable 4), including genes for chemokines, inflammatory cytokines, IFNs, ISGs, PRRs, apoptosis and regulatory cytokines. Although ZIKV downregulated TLR3, and TLR8 mRNA expression in THP-1 cells (STable 2), TLR3 and TLR8 mRNA were upregulated in PBMCs of patient 0031 and patient 0163 respectively. Furthermore, all patients showed enhanced levels of expression of type 1 IFNs, and IL-6 versus controls (Fig. 3C). These results indicate that ZIKV boosted strong anti-viral innate cytokine responses in human PBMCs at the acute stage of infection, which was likely to be mediated by TLR3 or TLR8.

3.3. ZIKV replicates efficiently in first trimester human placental trophoblasts and triggers stronger inflammatory cytokine and chemokine production but weaker IFN responses compared to YFV and DENV

ZIKV-induced birth defects have been reported to be more likely to be associated with infection during the first-trimester of pregnancy (Brasil et al., 2016; Kleber de Oliveira et al., 2016). Trophoblasts are one major cell type of placental villi and are permissive to ZIKV infection (Quicke et al., 2016; Tabata et al., 2016). Here, we compared replication of ZIKV, YFV, and DENV in first trimester human extravillous trophoblast cells (HTR8). Viral RNA levels in ZIKV- or DENV-infected HTR8 cells were significantly enhanced on day 1 and continued to increase on day 4 and day 6 pi (Fig. 4A–C), while YFV viral RNA reached peak levels on day 4 (Fig. 4B). There were lower viral RNA levels in ZIKV-infected HTR8 cells compared to the cells infected with the other two viruses, although FAA results showed that all three viruses reached to a titer of 6 ($\log_{10}$) FFU/ml on day 4 (Fig. 4D). Next, the

PCR array revealed that ZIKV infection (30 genes) elevated a similar number of anti-viral genes as YFV (31 genes) and slightly more than DENV (26 genes) on day 1 pi (STable 5 & SFig. 2). ZIKV-infected HTR8 cells displayed 7 genes with more than 2-fold higher expression compared to YFV infection, and 11 genes with more than 2-fold higher expression compared to DENV. These altered genes included mostly chemokines and inflammatory cytokines. ZIKV-infected HTR8 cells also exhibited lower expression of ISGs compared to YFV and DENV (Fig. 5A). Q-PCR assay showed no differences in IFNα expression between ZIKV, YFV, and DENV (Fig. 5B), whereas IFN-β was diminished in ZIKV-infected HTR8 cells compared to the other two viruses at all time points. On day 4 pi, IL-6, TNF-α, IL-8, and CCL2 production was augmented in ZIKV-infected HTR8 cells compared to YFV and DENV; however, CCL3 and CCL5 production was decreased in this cell type (Fig. 5C–D). In summary, these results suggest that, while ZIKV-replicates as efficiently as YFV and DENV in HTR8 cells; it induces lower IFN responses but higher levels of inflammatory cytokines and chemokines compared to YFV and DENV.

3.4. TLR3 and TLR8 are involved in induction of differential innate immune responses in monocytic cells and trophoblasts following ZIKV infection

THP-1 cells express several different toll-like receptor (TLR)s (Indoh et al., 2007; Uehara et al., 2007; Verma et al., 2014). ZIKV- infected THP-1 cells had higher TLR8 expression than YFV or DENV-infected cells (Fig. 5), although the virus downregulated TLR gene expression on these cells. Selective knockdown of either TLR3 or TLR8 diminished type 1 IFNs, but not IL-6 production (Fig. 6). Thus, both TLR3 and TLR8 are required for triggering type 1 IFNs in THP-1 cells upon ZIKV infection. TLRs have also been involved in induction of inflammatory and IFN responses in trophoblasts (Gierman et al., 2015; Nakada et al., 2009; Potter et al., 2015; Yang et al., 2016). Upon stimulation with the TLR ligands, the expression of TLR3 mRNA was down-regulated while the expression of TLR-4, 7, 8, and 9 mRNA was upregulated (Xu et al., 2013). Here, we noted that TLR3 and TLR8 was either downregulated or upregulated after ZIKV infection of trophoblasts (STable 5). Selective inhibition of either TLR3 or TLR8 via siRNA diminished inflammatory cytokine production (IL6 and TNF-α), while neither IFN-β (Fig. 7) nor levels of chemokines (IL-8 and CCL2, data not shown) were affected. Together, these data suggest that TLR3 and TLR8 are important for induction of inflammatory cytokines in HTR8 cells.

4. DISCUSSION

ZIKV, YFV and DENV are flaviviruses predominantly transmitted to humans by the *Aedes* (*Stegomyia*) mosquitoes. Monocytes are an important innate immune cell type in the periphery that is targeted by many flaviviruses, and exhibit robust innate cytokine responses upon infection. Here, we found that ZIKV strain FSS13025, YFV vaccine strain 17D, and DENV-4 strain 703-4 all have low replication rates in human monocytic cells. Compared to YFV and DENV, ZIKV induces more robust IFN, inflammatory and chemotactic cytokine responses. Consistent with a previous report (Tappe et al., 2016), PBMCs of acute ZIKV patients displayed strong anti-viral immunity profiles in a PCR array. It was also noted that PBMCs of ZIKV patients displayed a more pronounced elevation of innate cytokine production than in ZIKV-infected THP-1 cells. This could be due to the differences between

immune responses in primary versus cell lines. In addition, the T cell population of PBMCs was reported to have poly-functional activities during the acute phase of ZIKV infection and could contribute to the enhanced inflammatory cytokine and chemokine responses in ZIKV patients (Tappe et al., 2016).

Vertical transmission has not been reported for DENV. Vaccination of pregnant women with YFV-17D reportedly caused no major clinical complications, even in one case an infant was found to be infected with the vaccine virus (Suzano et al., 2006; Tsai et al., 1993). However, ZIKV has been associated with severe birth defects. ZIKV infection of the fetus may be acquired during primary maternal infection by the passage of virus through the trophoblasts (Benirchke et al., 2006). It is believed that ZIKV pathogenesis correlates with its cell and tissue tropisms during the first trimester of pregnancy (El Costa et al., 2016; Miner and Diamond, 2017). Here, our results indicate that ZIKV replicates efficiently in placental trophoblasts and could reach a viral titer similar to YFV 17D and DENV-4. Thus, the tropism for trophoblasts is less likely to be associated with CZS. Furthermore, we observed that ZIKV induces stronger inflammatory responses and weaker IFN responses than YFV and DENV. ZIKV inhibition of type I IFNs has been documented in several human and mouse cell types (Kumar et al., 2016). It is possible that ZIKV suppresses type 1 IFN responses to facilitate its replication and dissemination into other placental cell types, in particular the more permissive Hofbauer cells. MIA, such as inflammation, is known to contribute to the risk of brain damage in preterm newborns (Yanni et al., 2017). In particular, pro-inflammatory cytokine responses can indirectly induce apoptosis in trophoblasts (Aldo et al., 2010) or elicit morphological changes in the fetal brain and tissues even in the absence of viral infection (Cardenas et al., 2010; Mor, 2016). Thus, inflammatory responses in placental trophoblasts are associated with the CZS phenotype. Further understanding and identifying the host factors involved in placenta inflammation during ZIKV infection will provide important insights into viral pathogenesis. We also demonstrated that knockdown of TLR3 or TLR8 decreases inflammatory cytokine production in placental trophoblasts, which suggests a role of these innate immune receptors in MIA during ZIKV infection of the placenta. Activation of TLR3 was previously reported to contribute to the depletion of neural progenitors in human cerebral organoids upon ZIKV infection (Dang et al., 2016). Our results suggest a novel role of these TLRs in ZIKV pathogenesis. Neither TLR3 nor TLR8 knockdown completely abolished innate cytokine production in these cells, other PRRs, including RLRs or plasma membrane associated TLRs are likely involved in mediating innate immunity induced by these viruses. Lastly, asides from the differential IFN and inflammatory cytokine responses, IL-10 levels were higher in ZIKV- infected HTR8 cells compared to YFV and DENV- infected. It was reported that during another teratogen-Cytomegalovirus infection, enhanced IL-10 levels in placenta were correlated with reduced matrix metalloproteinase 9 activity, which then impaired cytotrophoblast remodeling of the uterine vasculature and ultimately restricted fetal growth in affected pregnancies (Yamamoto-Tabata et al., 2004). Whether IL-10 plays such a role in CZS remained to be defined.

In summary, although ZIKV has a similar tropism for placental trophoblasts compared to YFV and DENV, it induces differential innate immune responses, which may lead to brain damages and restriction of fetal growth. Our findings identify a potential link between virus-

induced MIA and ZIKV pathogenesis, which needs to be further explored in future animal studies. These results also suggest that blocking TLR-mediated inflammatory responses in the placenta is a potential therapeutic approach in treatment of CZS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Glossary

cDNA	Complementary DNA
CZS	congenital Zika syndrome
DENV	dengue virus
FBS	fetal bovine serum
FFA	Focus-forming assay
FFU	focus-forming unit
HTR-8	human extravillous trophoblast cells
IFN	interferon
IHC	immunohistochemical
ISG	interferon stimulated gene
JEV	Japanese encephalitis virus
KD	knockdown
MIA	Maternal immune activation
MOI	multiplicity of infection
NF	non-infected
PBMC	Peripheral blood mononuclear cell
PRR	pathogen recognition receptor
Q-PCR	Quantitative PCR
TLR	Toll-like receptor
WNV	West Nile virus

| **YFV** | Yellow fever virus |
| **ZIKV** | Zika virus |

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Highlights

- ZIKV, YFV and DENV have similar tropisms for human monocytic cells and first-trimester trophoblasts

- ZIKV induced strong anti-viral immune responses in human monocytes and peripheral blood mononuclear cells

- ZIKV infection in trophoblasts induced lower IFN and higher inflammatory immune Responses

- TLRs 3 and 8 mediate inflammatory cytokine responses in trophoblasts during ZIKV infection
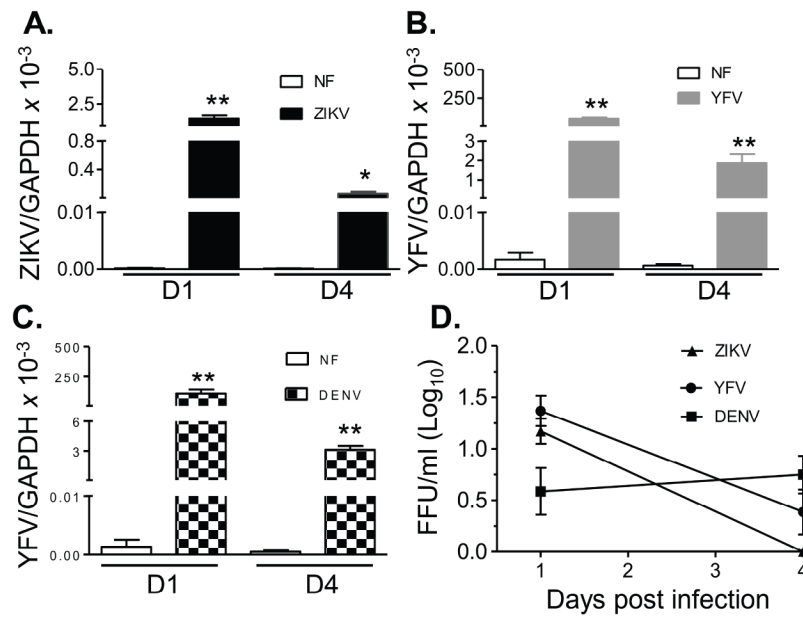
Figure 1. ZIKV, YFV and DENV infection in human monocytic cells
THP-1 cells were infected with ZIKV, YFV17D or DENV4 (MOI= 1). Viral load was measured at day 1 (D1) and day 4 (D4) pi by Q-PCR assay (*A–C*) and FFA (*D*). $n = 8$. *$P <$ 0.05 or **$P <$ 0.01 compared to non-infected (NF) cells.
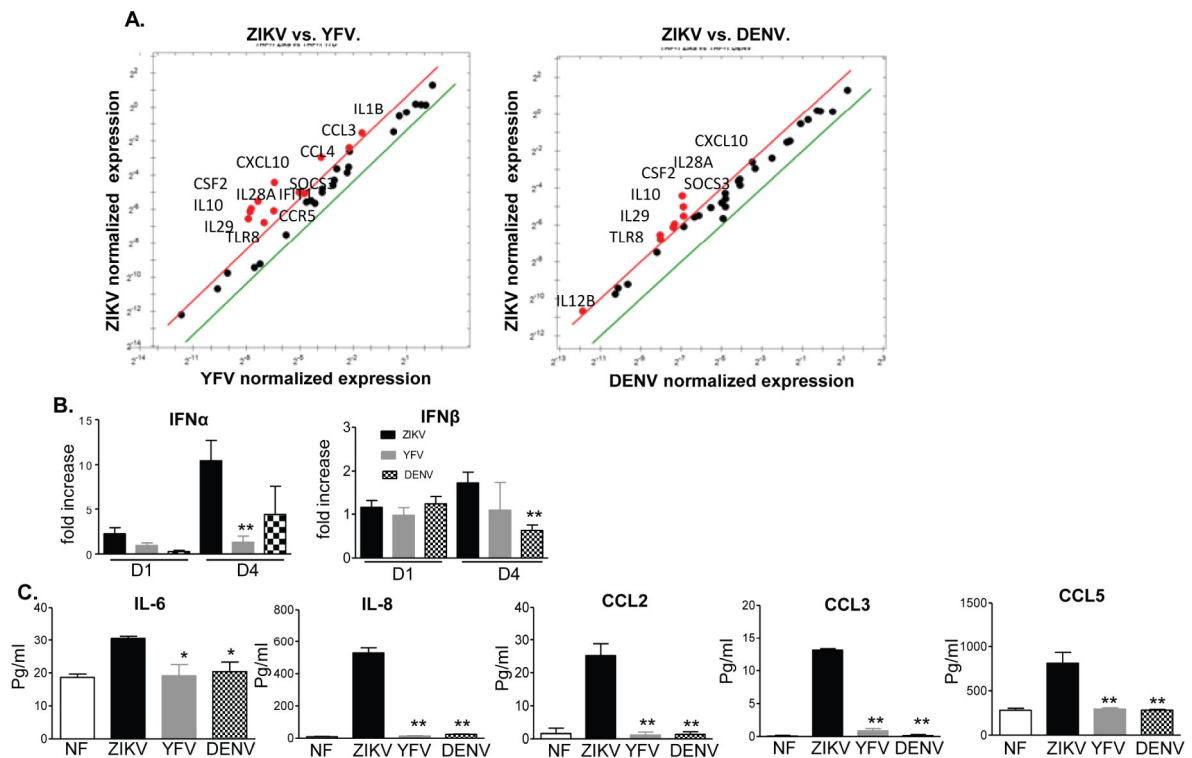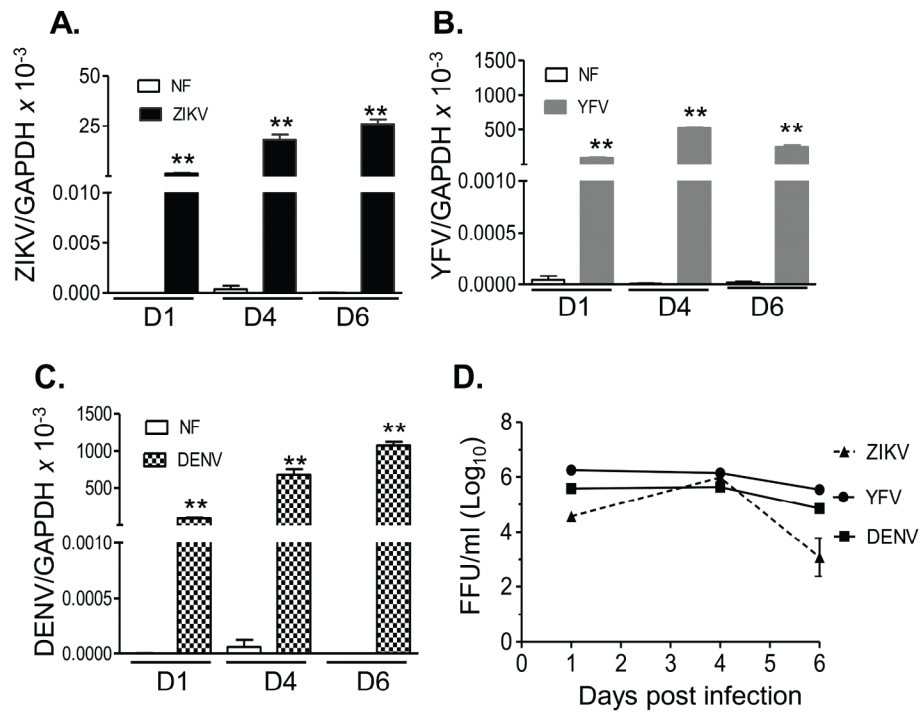
Figure 2. Immune response to ZIKV, YFV and DENV infection in human monocytic cells
THP-1 cells were infected with ZIKV, YFV17D or DENV-4 (MOI= 1). *A.* PrimePCR array.
The scatter plot shows the normalized expression of targets for ZIKV- infected THP-1 cells
versus YFV or DENV. The plot image shows the following changes in target expression
based on the threshold set of 2: upregulation as red circles, downregulation as green circles;
and no change as black circles. *B– C.* Cytokine or chemokine production was determined by
Q-PCR *or* by Bioplex. Data presented in panel B is the fold of increase compared to mock
infected. Results are representative of two experiments. $n = 5 –6$. ** $P < 0.01$ or * $P < 0.05$
compared to ZIKV-infected cells.
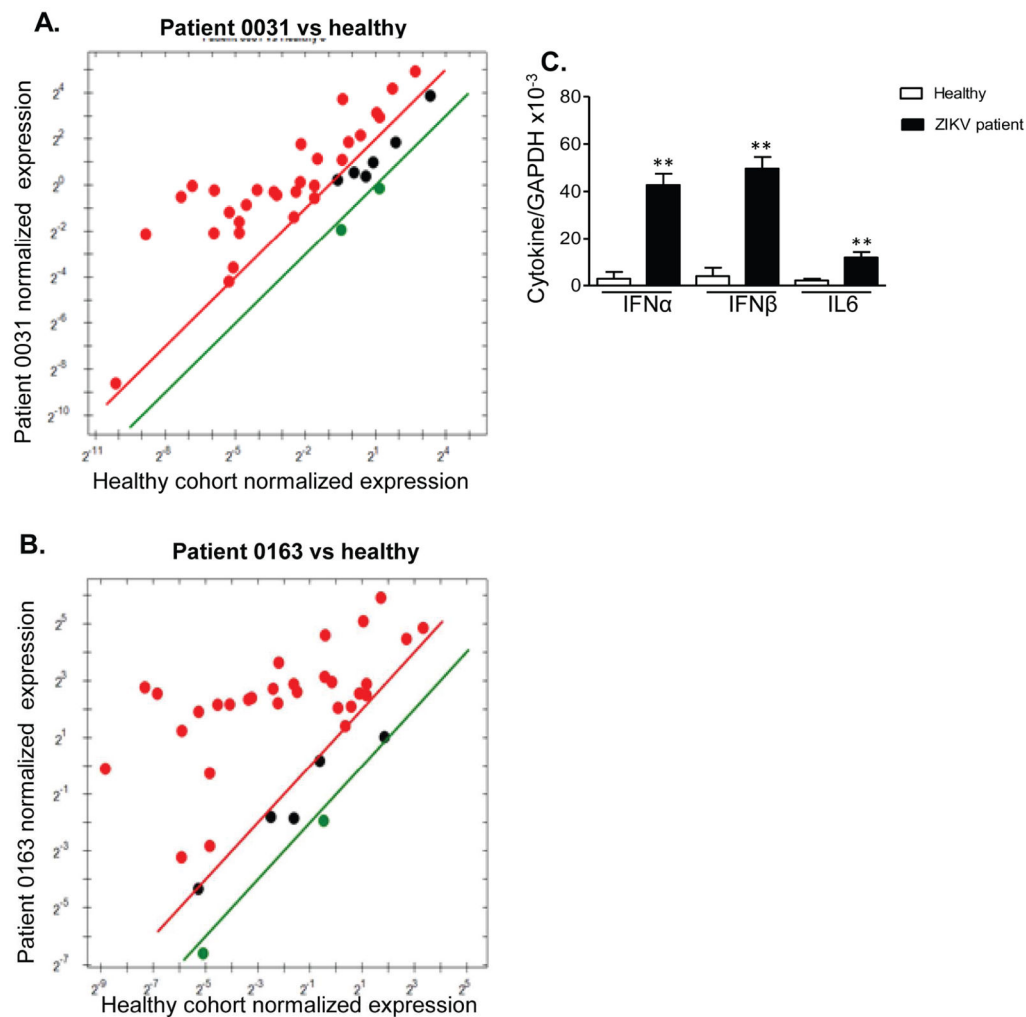
Figure 3. Antiviral cytokine production in ZIKV patients

A–B. PrimePCR array. The scatter plot shows the normalized expression of targets for two ZIKV patients versus matched healthy controls. The plot image shows the following changes in target expression based on the threshold set of 2: upregulation as red circles, downregulation as green circles; and no change as black circles. *C*. Cytokine production was determined by Q-PCR. $n = 5$. ** $P < 0.01$ or * $P < 0.05$ compared to healthy cohorts.

Figure 4. ZIKV, YFV and DENV infection in the first trimester human placental trophoblasts
HTR8 cells were infected with ZIKV, YFV-17D or DENV-4 (MOI= 1). Viral load was measured at indicated time points by Q-PCR assay (A– C) and FFA (D). $n = 9$. *$P < 0.05$ or **$P < 0.01$ compared to non-infected cells (NF).
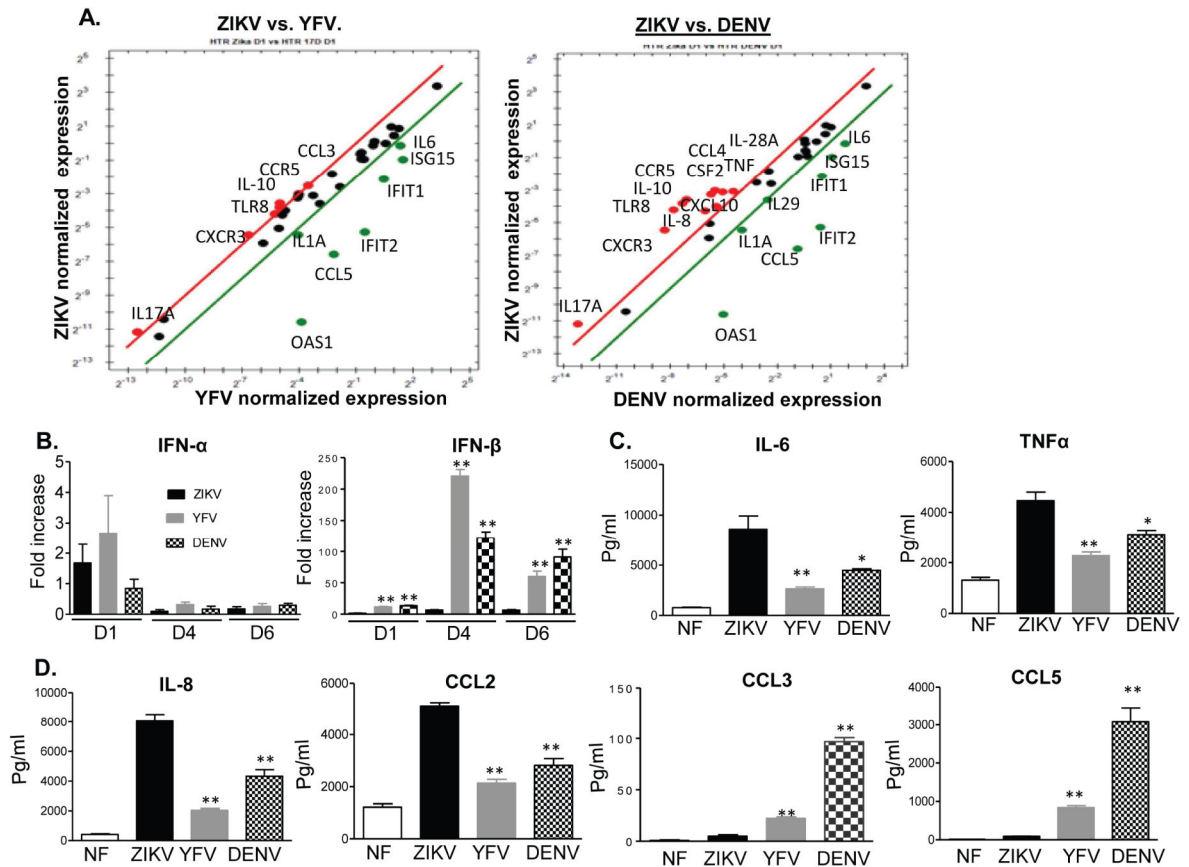
Figure 5. Immune responses to ZIKV, YFV and DENV infection in first trimester human placental trophoblasts

HTR8 cells were infected with ZIKV, YFV-17D or DENV-4 (MOI= 1). *A*. PrimePCR array. The scatter plot shows the normalized expression of targets for ZIKV- infected HTR8 cells versus YFV or DENV. The plot image shows the following changes in target expression based on the threshold set of 2: upregulation as red circles, downregulation as green circles; and no change as black circles. *B– D*. Cytokine or chemokine production was determined by Q-PCR (*B*) *or* by Bioplex (*C– D*). Data presented in panel B is the fold of increase compared to mock infected. Results are representative of two experiments. $n = 6 –9$. ** $P < 0.01$ or * $P < 0.05$ compared to ZIKV-infected cells.
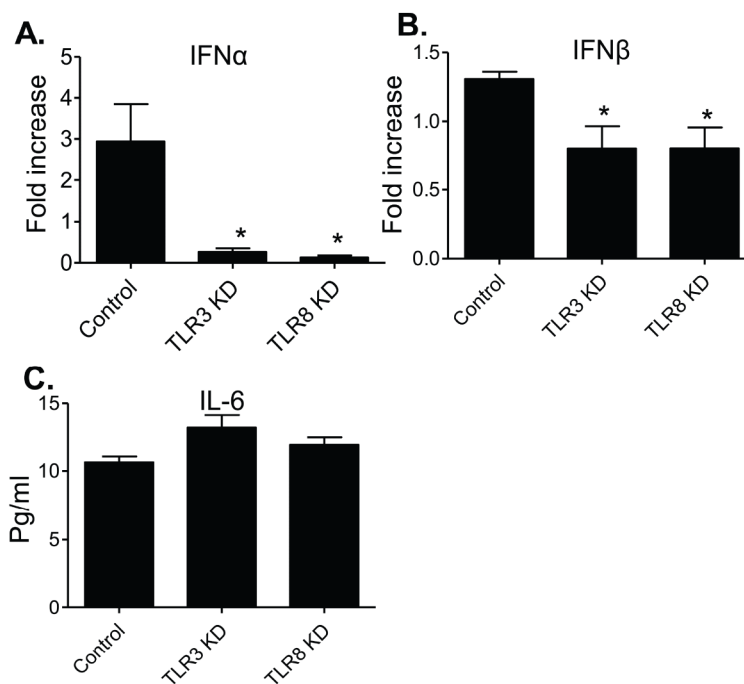
Figure 6. TLR3 and TLR8 are involved in induction of higher IFN and chemokine production in ZIKV -infected THP-1 cells

Cells were transfected with siRNAs to TLR3 (TLR3 KD), siRNA to TLR8 (TLR8 KD) or scrambled siRNAs (controls). Cells were harvested from mock and ZIKV-infected cells at day 4 pi to measure cytokine production by Q-PCR (*A– B*) or Bioplex (*C*). Fold increase compared to that of the mock-infected group was shown. Data are presented as means ± SEM, n = 3. ***P* < 0.01 or **P* < 0.05 compared to control vector-treated cells.
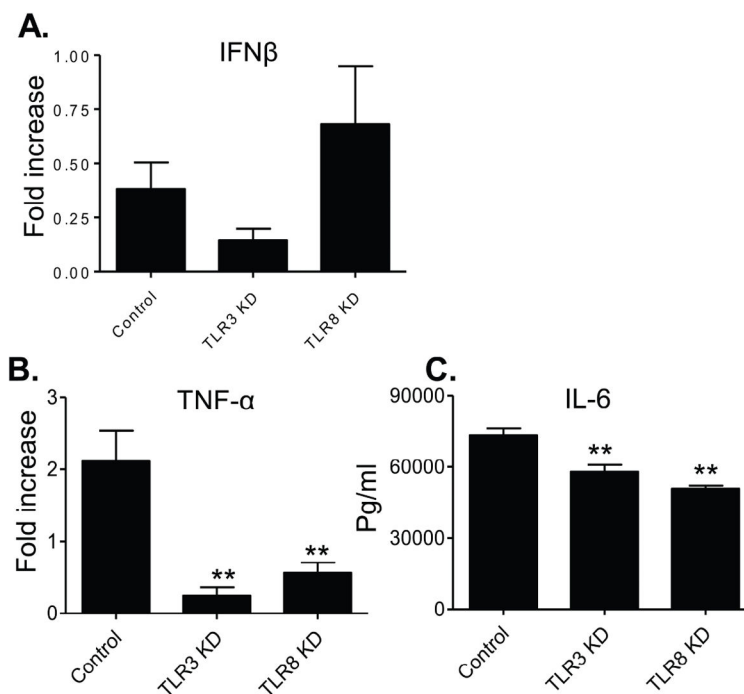
Figure 7. TLR3 and TLR8 are involved in induction of higher inflammatory cytokine response in ZIKV -infected HTR8 cells

Cells were transfected with siRNAs to TLR3 (TLR3 KD), or siRNA to TLR8 (TLR8 KD) or scrambled siRNA (control). Cells were harvested from mock- and ZIKV-infected cells at day 4 pi to measure cytokine production by Q-PCR (*A*– *B*) or Bioplex (*C*). Fold increase compared to that of the mock-infected group was shown. Data are presented as means ± SEM, n = 3. **$P < 0.01$ compared to control vector-treated cells.