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A unique immune signature of serum cytokine and chemokine dynamics in patients with Zika virus infection from a tropical region in Southern Mexico

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ABSTRACT

Objectives: To describe the kinetics of circulating cytokines and chemokines in humans with ZIKAV infection.
Methods: Serum levels of different immune mediators in patients with ZIKAV infection were measured at distinct stages of the disease, as well as in culture supernatants from human monocytes infected with a clinical ZIKAV isolate. We also looked for clinical features associated with specific immune signatures among symptomatic patients.
Results: We evaluated 23 ZIKAV-infected patients. Their mean age was 32 ± 8.3 years and 65% were female. ZIKAV patients showed elevated IL-9, IL-17A, and CXCL10 levels at acute stages of the disease. At day 28, levels of CCL4 and CCL5 were increased, whereas IL-1RA, CXCL8 and CCL2 were decreased. At baseline, IL-7 was increased among patients with headache, whereas CCL2, and CCL3 were decreased in patients with bleeding and rash, respectively. Our clinical ZIKAV isolate induced a broad immune response in monocytes that did not resemble the signature observed in ZIKAV patients.

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Conclusions: We showed a unique immune signature in our cohort of ZIKAV-infected patients. Our study may provide valuable evidence helpful to identify immune correlates of protection against ZIKAV.

Background

Zika virus (ZIKAV) infection is a mosquito-borne reemerging disease caused by a single-stranded RNA arbovirus. Recently, ZIKAV disease has become an international health problem, particularly following two major outbreaks occurring at Yap Island in the Federal States of Micronesia and at French Polynesia in 2007 and 2013, respectively (Duffy et al., 2009; Cao-Leormeau et al., 2014). Also, in 2015, novel unrecognized severe complications of ZIKAV infection, such as microcephaly and Guillain-Barré syndrome, were documented during a major epidemic outbreak in Brazil (de Araújo et al., 2016; Ladhani et al., 2016). Since then, at least 86 other countries have reported sustained transmission of ZIKAV (WHO, 2016a).

Despite its epidemiological relevance, little is known about the human immune response against ZIKAV infection. Currently, only a few studies have profiled the cytokine and chemokine response to the virus among different populations of patients with ZIKAV disease (Tappe et al., 2016; Galliez et al., 2016; Kam et al., 2017; Wang et al., 2018; Lum et al., 2018). However, the immune signatures that have been observed in such studies do not fully coincide, suggesting that host and pathogen-specific factors may influence the quality of immune responses elicited by ZIKAV. Therefore, more studies addressing the dynamics of cytokine and chemokine production among different populations of patients with ZIKAV disease are needed.

In the current study, we characterized the kinetics of circulating immune mediators during acute and convalescence stages of ZIKAV disease in patients from a tropical region of Southern Mexico. We also profiled the cytokine response of healthy monocytes to the infection with a clinical ZIKAV isolate. Our results show a unique immune signature in our cohort of ZIKAV infected patients that was not fully recapitulated by healthy monocytes exposed to the clinical ZIKAV isolate *in vitro*. Thus, our study provides a valuable description of the cytokine dynamics induced by ZIKAV in humans that may be helpful to identify immune mechanisms associated with protective immunity against this emerging disease.

Subjects, materials, and methods

Participants

This was a substudy of the first 23 consecutive patients with ZIKAV infection enrolled in the Zik01 study during 2016 (ClinicalTrials.gov Identifier: NCT02831699; Gouel-Cheron et al., 2019; Ravichandran et al., 2019). Zik01 was a longitudinal, observational study of individuals in Tapachula, Chiapas, an endemic area of mosquito-spread diseases at southern Mexico, who sought medical attention between June 2016 and August 2018 (Gouel-Cheron et al., 2019; Ravichandran et al., 2019). Patients with any two of the WHO/PAHO case definition symptoms for probable ZIKAV infection were enrolled (PAHO/WHO, 2016). The eligibility symptoms included rash, elevated body temperature (>37.2 °C), arthralgia, myalgia, non-purulent conjunctivitis or conjunctival hyperemia, headache or malaise. All symptoms must have occurred within 7 days of the initial visit with no obvious alternative diagnosis to explain the symptoms. Participants were evaluated by clinical interview and medical examination at the day of enrollment (day 0), and subsequently at days 3, 7 and 28. Also, serum samples were collected at each time point for determination of soluble immune mediators and detection of ZIKAV by reverse transcription polymerase chain reaction (RT-PCR), as previously described (Gouel-Cheron et al., 2019; Lanciotti et al., 2008; WHO, 2016). Serum samples from day 0 were collected on the day of enrollment, which occurred 1–8 days after the onset of symptoms. Participants were also tested for dengue virus (DENV) and chikungunya virus (CHIKV) infection by RT-PCR (Gouel-Cheron et al., 2019; Ravichandran et al., 2019). Individuals with concomitant DENV or CHIKV infection, as well as pregnant women, were not included for this analysis. A group of 13 age- and gender-matched healthy volunteers with negative test results for ZIKAV infection from a distinct geographic area were also recruited and considered as controls.

ZIKAV isolation

We obtained and expanded a clinical ZIKAV isolate from the serum sample of a participant with acute febrile illness caused by ZIKAV infection confirmed by RT-PCR. Briefly, Vero E6 cells (ATCC® CCL-81™) were exposed to the serum sample containing ZIKAV and maintained in tissue culture flasks with DMEM medium (Invitrogen/Thermo Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen/Thermo Scientific, Waltham, MA, USA). Virus titers in culture supernatants were determined by plaque assay. For this purpose, confluent Vero E6 cells were exposed to serial dilutions of ZIKAV (10^{-2} to 10^{-7}) for 12, 24, 48 and 72 h in 24-well plates. After such time points, ZIKAV/Vero E6 cell cultures were treated with 1.5% carboxyl-methyl-cellulose solution and incubated for another 6 days. Then, Vero E6 cells were fixed with a 50%/50% acetone/ethanol solution for 15 min and stained with 2% crystal violet solution for 15 min. Plaques were counted and titers expressed as plaque-forming units per volume (PFU/ml). Aliquots of the isolated virus were tested by RT-PCR before use to confirm the identity of the ZIKAV and to rule out contamination with other viruses.

In vitro infection of human CD14+ monocytes with ZIKV

Buffy coats from 5 healthy volunteer donors were obtained from the Blood Bank of the National Institute of Respiratory Diseases (INER) in Mexico City, and used for peripheral blood mononuclear cells (PBMCs) isolation by density gradient centrifugation using Ficoll (Lymphoprep, Axis-Shield, Oslo, Norway). Human CD14+ monocytes were purified from total PBMCs by magnetic cell sorting using a commercial kit of magnetic microbeads (Milteny, Auburn, CA, USA). Purity of isolated monocytes was assessed by flow cytometry using anti-human CD14 (HC14, BioLegend, San Diego, CA, USA) and anti-human CD3 antibodies (OKT3, BioLegend, San Diego, CA, USA), obtaining a purity of ~99%. Isolated monocytes were cultured in RPMI-1640 medium (Gibco BRL, Life Technologies, Waltham, MA, USA) supplemented with 10% FBS, 1% L-glutamine, penicillin (0.6 mg/mL), and streptomycin (60 mg/mL; Gibco BRL, Life Technologies, Waltham, MA, USA) at a concentration of 5×10^5 cells per well onto 24-well plates, during 2 days at 37 °C and 5% CO2. After this time, monocytes were infected with the clinical ZIKAV isolate at a multiplicity of infection (MOI) of 4 for 12, 24, 48 and 72 h. Some

cells received virus-free culture medium and were considered as controls. After each incubation period, supernatants were collected and stored at −80 °C for posterior analysis. All *in vitro* assays were performed at least by triplicate.

Cytokine and chemokine quantitation in serum samples and culture supernatants

The levels of 23 different cytokines, chemokines and growth factors (Supplementary Table S1) in serum samples from patients with ZIKAV infection and in culture supernatants from infected monocytes were assessed by a 23-plex Luminex assay using the Luminex platform Bio-Plex Multiplex 200 (Bio-Rad Laboratories, Inc., Hercules, CA, USA), as previously described (Zuniga et al., 2013).

Statistical analysis

Descriptive statistics were used to clinically characterize the study population. Frequencies and proportions were calculated for categorical data. Means, medians, standard deviations (SD), 95% confidence intervals (CI), and interquartile ranges (IQR) were used for continuous data. T-tests on $\log_{10}$ values of cytokines were used for comparisons. P-values were adjusted for multiple comparisons in the following ways. For comparisons of the levels of cytokines between ZIKAV patients and healthy donors we first controlled for the 4 time point comparisons by multiplying each *p*-value by 4 and then performing a Holm's procedure at each time point (Aickin and Gensler, 1996). For the comparisons of ZIKAV infected monocytes to noninfected monocytes across time, we multiplied each of the *p*-values by 4 (to control for the comparisons across time) and then performed a Holm's procedure with 23 comparisons to account for 23 cytokines. For the comparison of the 23 cytokines between those with a symptom and those without a symptom at baseline, we used the Holm's procedure to control for 23 cytokine comparisons for each of the 23 symptoms. Values of $p < 0.05$ were considered statistically significant.

Ethics statement

The study was sponsored by the Mexican Emerging Infectious Disease Clinical Research Network, Mexico (La Red), and conducted in accordance with the applicable regulatory and International Conference on Harmonization—Good Clinical Practice requirements. The study protocol was approved by an institutional review board for each participant site of the Zik01 study. All participants or their legal guardians provided written informed consent in accordance with the Declaration of Helsinki for Human Research.

Results

Participants' characteristics

The data in this substudy is from the first 23 consecutive patients with RT-PCR confirmed ZIKAV infection from the Zik01 study. Their mean age was 32 ± 8.3 years and 65% were female. From these, 83% had positive RT-PCR results for ZIKAV detection at day 3, and 70% remained viremic at day 7. However, at day 28 no participant had virus detectable by PCR. Inclusion criteria symptoms at baseline occurred at the following rates: rash (87%), conjunctivitis (74%), myalgia (83%), arthralgia (57%) and fever (65%). Other symptoms that were observed at enrollment included malaise, rash, headache, periorbital pain, photophobia, back pain, fatigue, paresthesia, sore throat, and nausea. A third of the participants remain with arthralgia and myalgia at day 28. No severe acute complications occurred in the enrolled patients during the follow-up period. Clinical characteristics of study participants are summarized in Table 1.

Circulating levels of cytokines, chemokines and growth factors during acute and convalescence stages of ZIKAV infection

As compared with healthy controls, patients with ZIKAV infection showed an early immune response characterized by elevated serum levels of interleukin 9 (IL-9), interleukin 17 (IL-17A), and C–X–C motif chemokine ligand 10 (CXCL10) across all time points (Figure 1a–c). Interleukin 1 receptor antagonist (IL-1RA) and C–X–C motif chemokine ligand 8 (CXCL8) were significantly lower than control at day 28 but not early at day 0, 3 or 7 (Figure 1d–e), whereas C–C motif chemokine ligand 4 (CCL4) and C–C motif chemokine ligand 5 (CCL5) were elevated at day 28 (Figure 1f–g). C–C motif chemokine ligand 2 (CCL2) was significantly lower at day 7 and 28 (Figure 1h). Elevated levels of IL-9 remain unchanged at day 28 (Figure 1a), whereas CXCL10 peaked at days 3–7 and then slightly declined but remained elevated during convalescence (Figure 1c). Of note, IL-17A levels continued rising over time and were maximum at day 28 (Figure 1b), similar to CCL4 and CCL5 (Figure 1f–g). No significant changes in the levels of all other evaluated cytokines, chemokines, and growth factors were observed (Supplementary Figure S1).

Immune signatures associated with clinical symptoms in ZIKAV patients

We screened for clinical features associated with increased or diminished serum levels of immune mediators at baseline. Notably, we found that patients with headache had higher levels of interleukin 7 (IL-7) than patients without headache. CCL2 was decreased in patients with bleeding, whereas C–C motif chemokine ligand 3 (CCL3) was decreased in patients with rash (Table 2 and Supplementary Figure S2).

ZIKAV induces a polyfunctional immune activation in human monocytes

Human CD14+ monocytes strongly responded to the *in vitro* exposure to a clinical ZIKAV isolate by producing a broad spectrum of inflammatory mediators including tumor necrosis factor alfa (TNF-α), interferon gamma (IFN-γ), interleukin 1 beta (IL-1β), interleukin 4 (IL-4), IL-6, IL-9, interleukin 10 (IL-10), and IL-17A (Figure 2a–h), as well as CXCL10, CCL2, CCL3, CCL4, CCL5, C–C motif chemokine ligand 11 (CCL11; Figure 3a–f), basic fibroblast growth factor (bFGF), granulocyte colony-stimulating factor (G-CSF), and vascular endothelial growth factor (VEGF) (Figure 3h–i and k). A poor induction of IL-7, interleukin 12 p70 subunit (IL-12p70), interleukin 13 (IL-13; Figure 2i–k), CXCL8, and platelet derived growth factor (PDGF-BB; Figure 3g and j) was observed along the time points of the *in vitro* infection.

Discussion

ZIKAV infection remained an obscure mosquito-borne disease until it was recently associated with severe neurological and congenital complications (de Araújo et al., 2016; Ladhani et al., 2016; Cao-Lormeau et al., 2014). Hence, little is known about the immune response elicited by ZIKAV. Recent studies in humans have described a broad signature of cytokines, chemokines and growth factors that are overregulated during ZIKAV infection (Tappe et al., 2016; Galliez et al., 2016; Kam et al., 2017; Wang et al., 2018; Lum et al., 2018). Yet, it remains unclear whether this polyfunctional immune induction represents the true general

Table 1
Clinical characteristics of patients with Zika virus infection in Tapachula Chiapas, Mexico (2016).

Characteristic	(n = 23)	Day 0	Day 3	Day 7	Day 28
Age (years), mean (SD)	32(8.3)				
Gender					
Male, n (%)	8(34.8)				
Female, n (%)	15(65.2)				
Positive ZIKAV RT-PCR test, n (%)		23(100)	19(83)	16(70)	0(0)

Case definition symptoms	Day 0	Day 3	Day 7	Day 28
Rash, n (%)	20 (87)	20 (87)	9 (40)	2 (9)
Fever, n (%)	15 (65)	1(4.3)	0 (0)	0 (0)
Conjunctivitis, n (%)	17 (74)	13 (57)	5 (22)	2 (9)
Arthralgia, n (%)	13 (57)	11 (48)	4 (18)	9 (40)
Myalgia, n (%)	19 (83)	14 (61)	10 (44)	8 (35)
Other symptoms				
Malaise, n (%)	19 (83)	19 (83)	9 (40)	5 (22)
Pruritus, n (%)	14 (61)	14 (61)	10 (44)	1 (5)
Headache, n (%)	18 (78)	11 (48)	8 (35)	5 (22)
Periorbital pain, n (%)	7 (30)	7 (31)	5 (22)	5 (22)
Photophobia, n (%)	14 (61)	14 (61)	7 (31)	6 (26)
Back pain, n (%)	12 (52)	12 (53)	6 (26)	7 (31)
Fatigue, n (%)	14 (61)	14 (61)	10 (44)	6 (26)
Paresthesia, n (%)	10 (43)	10 (44)	6 (26)	3 (13)
Sore throat, n (%)	7 (30)	6 (26)	5 (22)	5 (22)
Nausea, n (%)	7 (30)	7 (31)	8 (35)	3 (13)
Disorientation, n (%)	2(9)	2 (9)	1 (5)	1 (5)
Diarrhea, n (%)	1 (4)	1 (5)	4 (18)	4 (18)
Mouth ulcers, n (%)	2 (9)	2 (9)	0 (0)	0 (0)
Cough, n (%)	3 (13)	3 (13)	3 (13)	3 (13)
Bleeding, n (%)	2 (9)	2 (9)	1 (5)	1 (5)
Vomiting, n (%)	2 (9)	2 (9)	3 (13)	0 (0)
Petechiae, n (%)	0 (0)	1 (5)	1 (5)	0 (0)
Altered behavior, n (%)	0 (0)	0 (0)	0 (0)	1 (5)

RT-PCR, reverse transcription polymerase chain reaction; SD, standard deviation; ZIKAV, Zika virus.

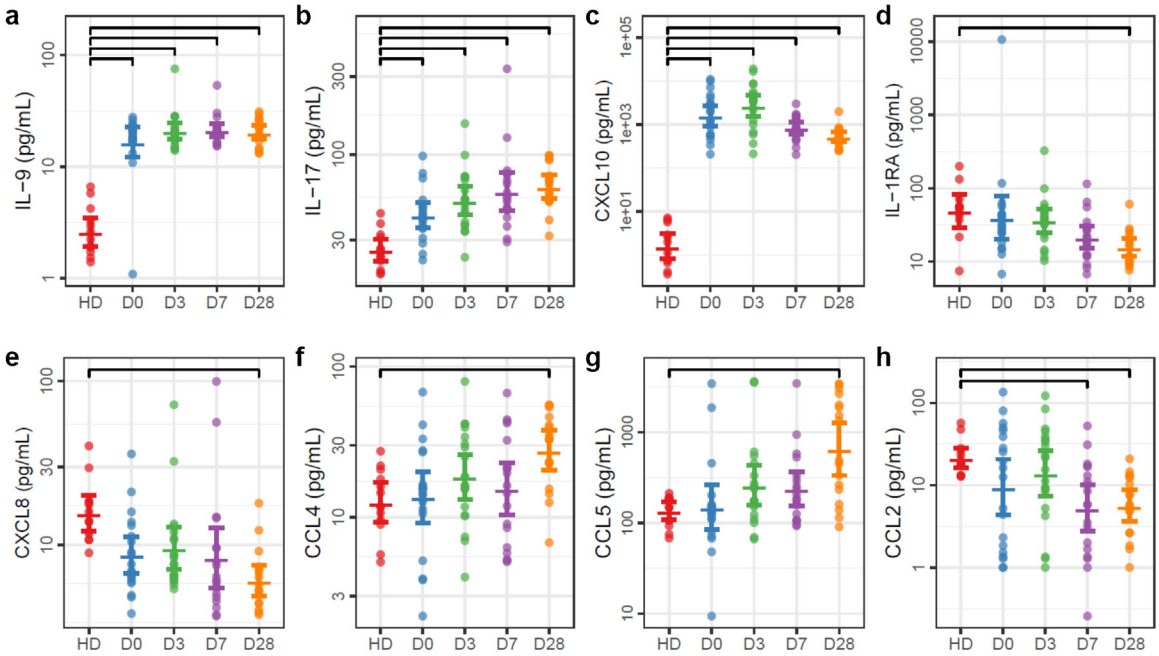

Figure 1. Kinetics of circulating cytokines and chemokines in patients with ZIKAV infection. Levels of cytokines and chemokines in serum samples from patients with ZIKAV infection collected along the acute and convalescence stages of disease, as well as in samples from volunteer healthy donors assessed by Luminex assay. Geometric means and 95% confidence intervals are presented. Significant differences from healthy donor group ($p \leq 0.05$) are noted by bars. (a) IL-9, interleukin 9; (b) IL-17, interleukin 17A; (c) CXCL10, C–X–C Motif Chemokine Ligand 10; (d) IL-1RA, interleukin 1 receptor antagonist; (e) CXCL8, C–X–C Motif Chemokine Ligand 8; (f) CCL4, C–C Motif Chemokine Ligand 4; (g) CCL5, C–C Motif Chemokine Ligand 5; (h) CCL2, C–C Motif Chemokine Ligand 2. D0, day 0; D3, day 3; D7, day 7; D28, day 28; HD, healthy donors; ZIKAV, Zika virus.

Table 2
Cytokine levels by symptom presence at baseline.

Symptom	Cytokine/Chemokine	Yes	No	*p*-Value
Headache	IL-7	3.53 (2.24–5.58)	1.16 (0.83–1.60)	0.027
Bleeding	CCL2	1.33 (1.27–1.39)	11.96 (5.74–24.94)	0.0005
Rash	CCL3	0.44 (0.29–0.69)	1.04 (0.88–1.23)	0.049

95% CI, 95% confidence interval.

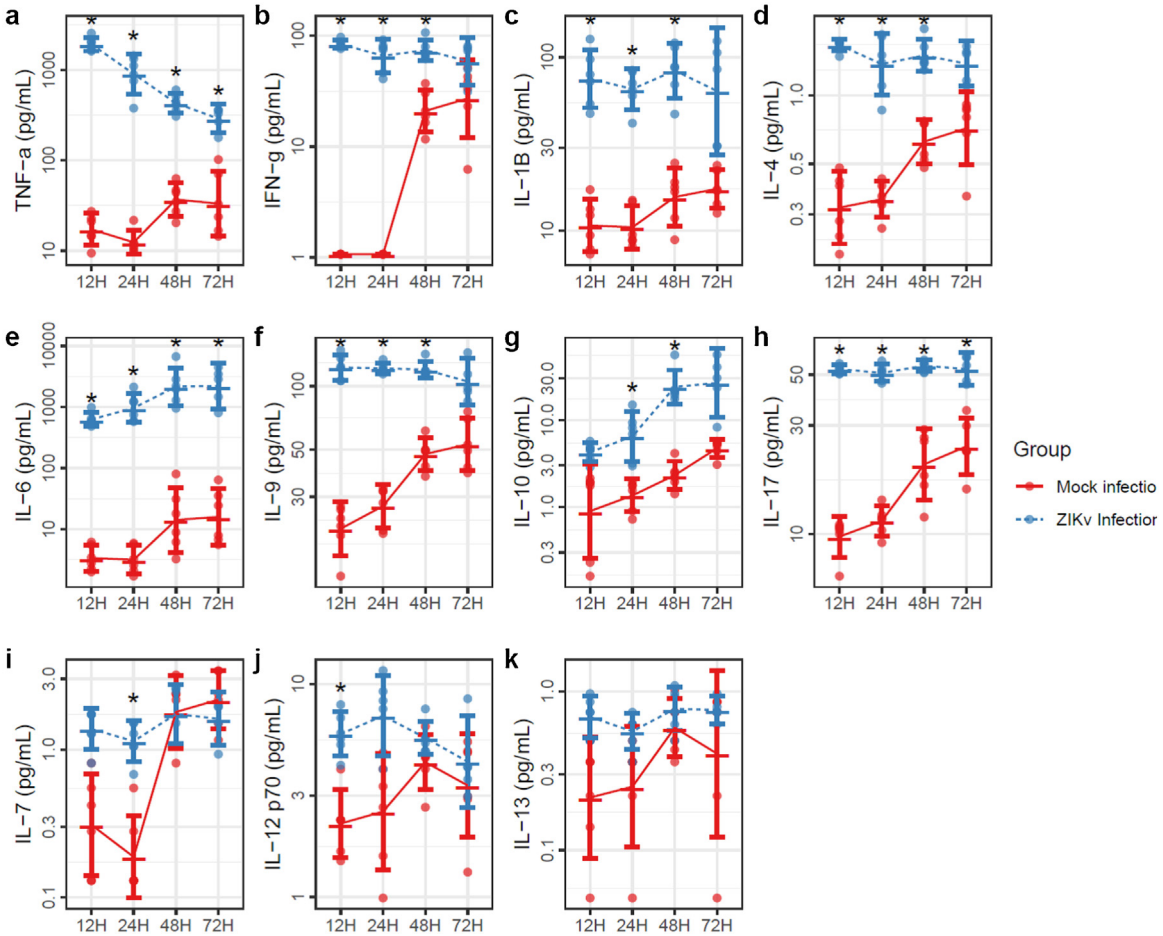

Figure 2. Cytokine production by human CD14+ monocytes after *in vitro* infection with a clinical ZIKAV isolate. Purified human CD14+ monocytes were exposed *in vitro* to a clinical ZIKAV isolate during different intervals (blue line). Non-infected CD14+ monocytes were cultured during the same intervals of time and considered as controls (red line). At given time points, cells were harvested and the levels of cytokines in supernatants were assessed by Luminex assay. Significant differences are denoted by *. Geometric means and 95% confidence intervals are displayed. (a) TNF-α, tumor necrosis factor alpha; (b) IFN-γ, interferon gamma; (c) IL-1β, interleukin 1 beta; (d) IL-4, interleukin 4; (e) IL-6, interleukin 6; (f) IL-9, interleukin 9; (g) IL-10, interleukin 10; (h) IL-17, interleukin 17A; (i) IL-7, interleukin 7; (j) IL-12p70, interleukin 12 p70 subunit; (k) IL-13, interleukin 13.

host-response elicited by ZIKAV infection, or if specific immune factors are responsible for mediating protective immunity. Furthermore, immune signatures observed among ZIKAV patients from different geographic regions do not fully coincide. Therefore, more data sets from cohorts of other populations are needed.

In this study, we analyzed the serum levels of immune mediators in patients with ZIKV infection from a tropical region in Chiapas State located in the Southeast of Mexico. Notably, our results showed increased circulating levels of IL-9, IL-17A, and CXCL10 during the acute stage of ZIKAV disease, as well as late production of CCL4 and CCL5 during the recovery phase. These latter chemokines participate in the recruitment of NK cells, monocytes, T cells and eosinophils to inflamed tissues acting via the receptors C–C motif chemokine receptor 1 (CCR1), C–C motif chemokine receptor 3 (CCR3), and C–C motif chemokine receptor 5 (CCR5; Schall et al., 1990). Thus, our findings suggest a role for these cellular subsets and chemokine axes during the recovery stages of the disease. Our findings also reveal a possible involvement of Th9 responses during acute ZIKAV infection, but the pathogenic/protective nature of this phenomenon is unknown. In other human virus infections, the magnitude of Th9 responses has been associated with the severity of disease (Geevarghese and Weinberg, 2014). Thus, future investigations should evaluate the effect of IL-9 on the clinical outcome of ZIKAV infection. The current study also suggests an active participation of IL-17A in the immune response against ZIKAV. Downstream targets of IL-17A include CXCL10 (Gaffen, 2008), whose levels peaked early after onset of ZIKAV disease in our population, coinciding with increased early production of IL-17A. However, although CXCL10 remained elevated at recovery phases, it showed a slight decrease over time despite persistent increased production of IL-17A. This may reflect

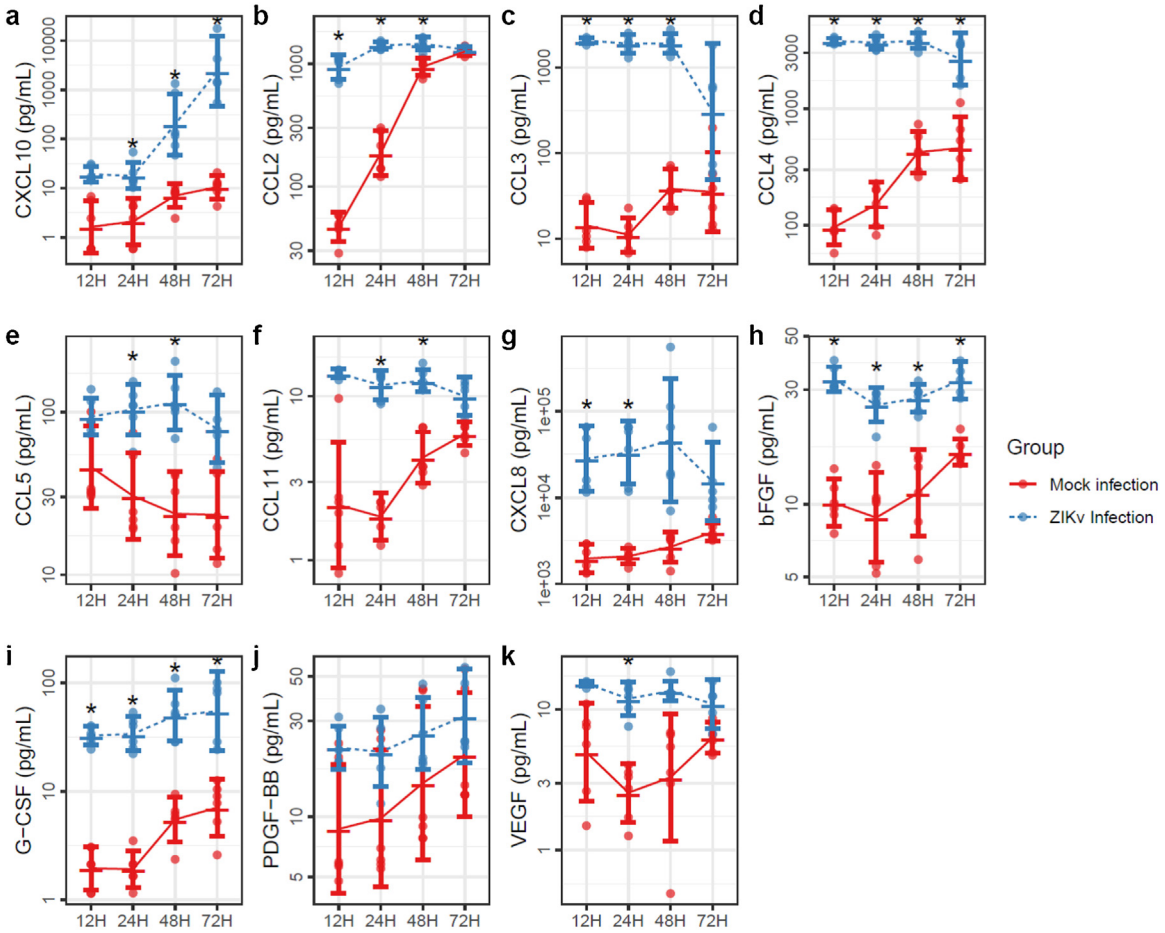

Figure 3. Spectrum of chemokines and growth factors produced by human CD14+ monocytes in response to ZIKAV infection. Purified human CD14+ monocytes were exposed *in vitro* to a clinical ZIKAV isolate during different intervals (blue line). Non-infected CD14+ monocytes were cultured in parallel and considered as controls (red line). At given time points, cells were harvested and the levels of chemokines and growth factors in supernatants were assessed by Luminex assay. Significant differences are denoted by *. Geometric means and 95% confidence intervals are displayed. (a) CXCL10, C–X–C Motif Chemokine Ligand 10; (b) CCL2, C–C Motif Chemokine Ligand 2; (c) CCL3, C–C Motif Chemokine Ligand 3; (d) CCL4, C–C Motif Chemokine Ligand 4; (e) CCL5, C–C Motif Chemokine Ligand 5; (f) CCL11, Motif Chemokine Ligand 11; (g) CXCL8, C–X–C Motif Chemokine Ligand 8; (h) bFGF, basic fibroblast growth factor; (i) G-CSF, granulocyte colony-stimulating factor; (j) PDGF-BB, platelet-derived growth factor; (k) VEGF, vascular endothelial growth factor.

an important role of CXCL10 for early antiviral responses that might be regulated or replaced by other Th17 effector mechanisms as disease progresses.

CXCL10 exerts its biological effects by binding to the C–X–C motif chemokine receptor 3 (CXCR3), which is expressed on macrophages, activated T-cells, B lymphocytes, NK cells, and dendritic cells (DCs) (Groom and Luster, 2011). Recently, an important antiviral activity of CXCL10 has been described against other arboviruses, such as the DENV (Ip and Liao, 2010; Hsieh et al., 2014). In fact, CXCR3 and CXCL10 knockout mice showed an increased susceptibility to DENV (Ip and Liao, 2010). High levels of CXCL10 have also been found in a group of CHIKV-infected patients (Kelvin et al., 2011). Interestingly, CXCL10 was associated with the presence of moderate symptoms in another cohort of ZIKAV infected patients and this chemokine has been found elevated in individuals that developed neurological or congenital complications (de Araújo et al., 2016; Galliez et al., 2016; Lum et al., 2018). Moreover, CXCL10 is associated with arthralgia and myalgia in CHIKV–infected patients (Lohachanakul et al., 2015; Chen et al., 2015). This suggests an important role of CXCL10 in the induction of inflammation at epithelial barriers and joints during viral infections. Indeed, CXCL10 is involved in homing of T-cells to inflammatory lesions occurring at skin and conjunctiva (Enríquez-de-Salamanca et al., 2008; Fernández et al., 2009). Taken together, our results and data from previous studies suggest a role for CXCL10 in the immune response against ZIKAV infection and possibly against other arboviruses.

Contrary to the cytokine profiles observed in other cohorts (Kam et al., 2017; Lum et al., 2018), we did not find increased levels of circulating TNF-α, IFN-γ, IL-1β, IL-1RA, IL-7, IL-10, IL-12p70, IL-13, CXCL8, CCL2, CCL3, and CCL11. The unique signature of our population could result from the activity of distinctive host factors differentially influencing immunity to ZIKAV. To address such a possibility, we used a clinical ZIKAV isolate to experimentally infect monocytes from healthy donors and characterized their cytokine/chemokine/growth factor production. Remarkably, we found a strong response in infected monocytes that did not fully resemble the immune signature observed in our cohort of patients, as monocyte supernatants were all significantly different at some time point except for IL-13 and PDGF-BB. Monocyte supernatants contained high levels of TNF-α, IFN-γ, IL-1β, IL-4, IL-6, IL-10, CCL2, CCL3, CCL11, CXCL18, bFGF, G-CSF, and VEGF, which were not increased in serum of ZIKAV patients. These findings suggest that several immune factors absent in our monocyte cultures actively shape the response to ZIKAV infection inside the host, which may also explain the discrepancies in immune responses observed *in vivo* and *in vitro*, as well as the distinctive signatures found in different studies (Tappe et al., 2016; Galliez et al., 2016; Kam et al., 2017; Wang et al., 2018; Lum et al., 2018).

The virulence of different ZIKAV strains could also modulate immunity *in vivo*. In this regard, we could not sequence the genome of our clinical ZIKAV isolate, but other studies showed that the ZIKAV strains circulating in Mexico at the time when we recruited our cohort belong to an Asian lineage (Díaz-Quiñonez et al., 2016). Indeed, the profile of immune factors produced by monocytes in response to our clinical isolate was similar to the response observed in PBMCs infected with a strain of the Asian lineage (Colavita et al., 2018). It also resembled the cytokine signature of monocytes infected with a South American strain, although the latter did not induce TNF-α and IFN-γ production (Khaiboullina et al., 2017). Such subtle differences may reflect recent mutations acquired by ZIKAV strains isolated in Mexico (Díaz-Quiñonez et al., 2016). Collectively, our data and findings of previous studies suggest that immune profiles of patients with ZIKAV infection differ among individuals with distinct ethnic background. Future studies looking at immune signatures conserved across several populations of ZIKAV patients may identify the correlates of protection needed for vaccine development.

The current study has some limitations that must be taken into consideration when interpreting our findings. First, the number of enrolled ZIKAV-infected patients and controls was low due to the careful selection of participants to avoid possible confounding factors such as concomitant infection with CHIKV and DENV. Secondly, we could not accurately investigate the status of a previous DENV infection in our study participants, which would have been useful to adjust our analyses of circulating cytokine dynamics as there is certain antigenic proximity between ZIKAV and DENV. Thus, an earlier DENV infection in our participants could have impacted on their immune responses to a secondary infection by ZIKAV. Finally, it would have been relevant to complete this study by measuring the *in vitro* response of patients' monocytes to the clinical ZIKAV isolate, in order to address possible differences with respect to the immune signature observed in monocytes from healthy volunteers. However, we were unable to obtain higher volumes of blood from ZIKAV-infected patients to isolate circulating immune cells for *in vitro* experiments.

In summary, we showed a unique immune signature characterized by high serum levels of IL-9, IL-17A, and CXCL10 as well as CCL4 and CCL5 during the acute and recovery phases of ZIKAV infection. We also found lower levels of IL-RA, CXCL8 and CCL2 during recovery. Finally, we propose that host and pathogen factors influence the dynamics of cytokine responses elicited by ZIKAV. The current study may provide evidence useful for future investigations aimed to identify correlates of protection and targets for vaccine development against this emerging disease.

Author contribution

Collected clinical data and biological samples for the study: HVC, AEG, SF, PFBZ, SPP, LG, JR, GNC, SCS, HRL, GRP. Performed the Luminex assays: LJA, ACL, EM, GRM, EHM, AFL, MFCC AG. Isolation of ZIKAV: CC. Performed *in vitro* experiments: LJA, JACP, ACL, GRM, AG, AH, NERZ, CMM. Analyzed the data: MC, JACP, MS, AMH, MFCC, AFL, AM, SH, JB, JR, JZ. Contributed to the writing of the paper: JACP, SH, JB, JZ. Conception and design of the study: JZ, LJZ,JR, PFBZ, SPP, LG, GRP. All the authors reviewed and approved the final version of the manuscript.

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Ethical approval

The current study was reviewed and approved by the Institutional Review Boards of the National Institute of Medical Sciences and Nutrition "Salvador Zubirán" (INCMNSZ), the National Institute of Respiratory Diseases (INER), and the Instituto Mexicano del Seguro Social (IMSS). All participants or their legal guardians provided written informed consent in accordance with the Declaration of Helsinki for Human Research. All samples were processed and stored according to the Mexican Constitution law NOM-012-SSA3-2012, that establishes criteria for the execution of clinical research projects in humans.

Conflicts of interest

The authors declare no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.02.014.

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