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Durable cellular immune response against inactivated ZIKV and envelope proteins in ZIKV-infected women during pregnancy

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Introduction: Zika virus (ZIKV) infection has been associated to Guillain-Barré syndrome in adults and congenital malformations during pregnancy, leading to the manifestation of congenital Zika syndrome (CZS). The ZIKV envelope protein (E_{ZIKV}), prominently displayed on the virus surface, is a primary target for the humoral immune response. However, limited information exists regarding its capacity to induce cellular immunity, particularly in pregnant women with a history of ZIKV infection. The E_{ZIKV} protein comprises three domains: the central domain (EDI), a dimerization domain (EDII), and a domain responsible for binding to the cell surface receptor (EDIII). To examine the regions of E_{ZIKV} targeted by cellular immunity, we examined cellular immune responses in a cohort of mothers infected with ZIKV, whose infants exhibited microcephaly.

Methods: To assess the ZIKV-specific response, we used inactivated virus and different recombinant viral envelope proteins (E_{ZIKV}, EDI/II_{ZIKV} and $EDIII_{ZIKV}$). All women in the study contracted the infection during pregnancy, with 72% experiencing symptoms such as fever, rash, joint pain, and retro-orbital pain. Peripheral blood mononuclear cells (PMBC) were collected post- ZIKV diagnosis confirmation, with a median time of 18 months (IQR 13.5-19) after parturition. Using the ELISpot assay, we quantified specific interferon-gamma (IFNγ) producing cells by stimulating PBMC with either inactivated ZIKV particles or equimolar amounts of recombinant E_{ZIKV}, EDI/II_{ZIKV} and $EDIII_{ZIKV}$.

Results and discussion: Our findings demonstrate the induction of IFN-γ producing cells in PBMC from ZIKV-convalescent mothers, whose infants manifested

microcephaly, upon stimulation with both inactivated ZIKV particles and recombinant proteins. The identification of immunodominant regions within ZIKV can contribute for the development of targeted treatments and vaccine candidates tailored for pregnant women.

KEYWORDS

Zika virus, cellular immune response, T cells, pregnancy, microcephaly

Introduction

Zika virus (ZIKV), a mosquito-borne flavivirus closely related to yellow fever, dengue, and West Nile viruses (1) has undergone rapid global dissemination since 2015, with over 80 countries reporting local transmission (2). While primarily transmitted by *Aedes* mosquitos, non-vector transmission can also occur including sexual contact, transfusion, and vertical transmission from mother to child (3, 4).

Most ZIKV infections are asymptomatic, with a minority causing self-limited acute febrile illnesses characterized by fever, headache, arthralgia, myalgia, fatigue, and rash (5). However, in adults, ZIKV infection has been sporadically associated with Guillain-Barré syndrome (GBS) (6, 7). In pregnant women, the virus can persist for weeks in the reproductive tract (8, 9), and fetal infection has been associated with congenital malformations such as brain calcification, microcephaly, and spontaneous abortion, defining the Congenital Zika Syndrome (CZS) (10–12).

Studies suggest that approximately 20% of infants born to mothers exposed to Zika virus during pregnancy, who initially exhibited no signs of birth defects, later displayed impaired cognitive development and other neurological abnormalities (13, 14).

The rapid global spread of ZIKV and its association with neurological complications highlight the urgent need for an effective vaccine and specific treatment. Despite scientific efforts, no licensed therapeutic or prophylactic vaccines against ZIKV have been developed until now (15).

The ZIKV genome is a single-stranded positive-sense RNA (ssRNA) that encodes a polyprotein, subsequently cleaved into three structural proteins (Capsid (C), Premembrane/Membrane (prM/M) and Envelope (E)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5), vital for virus replication and assembly (16, 17). The Envelope (E) protein orchestrates viral assembly, binds to cell receptors, and is essential for the subsequent fusion of the membrane involved in virus entry into the target cell (18). Similar to other flaviviruses, the ZIKV E protein comprises three distinct domains: the central domain (EDI), the fusion peptide-containing dimerization domain (EDII), and the cell surface receptor-binding (EDIII) (19).

Several studies have demonstrated the highly antigenic structure of the E protein, serving as the primary target for host antibody responses, including several neutralizing antibodies (20, 21). While the humoral response is fundamental in protection against ZIKV infection, the involvement of $CD4^+$ and $CD8^+$ T cell responses is essential for complete virus elimination. Despite various animal studies highlighting the importance of $CD4^+$ and $CD8^+$ T cell responses against ZIKV (22–27), limited and conflicting data exist regarding the preferred target regions for human-specific cellular immune response to ZIKV (28, 29). Furthermore, despite ZIKV's unique impact on fetal health, few studies have investigated the immune response of pregnant women. A recent longitudinal study with 10 non-pregnant women with acute ZIKV infection revealed that $CD8^+$ T cell responses are directed more towards non-structural antigens, while $CD4^+$ T cell responses are more balanced between structural and non-structural antigens (30). Similarly, ZIKV-specific CD4+ memory T cell responses were observed in mothers infected with ZIKV during pregnancy, with no discernible differences in T cell responses between children affected or unaffected by CZS (31).

In this study, we investigated whether ZIKV inactivated particles and different ZIKV-envelope proteins induce cellular-mediated immunity after ZIKV infection in cells from convalescent mothers of newborns with microcephaly *in vitro*.

Materials and methods

Participants

A dual-center study was conducted at the Hospital Universitário da Universidade Federal de Sergipe and Universidade Federal de São Paulo. Cryopreserved peripheral blood mononuclear cells (PBMC) from 32 ZIKV-infected women, who delivered babies with microcephaly, were used. Participant characteristics are detailed in Table 1. PBMCs were collected post ZIKV-positive diagnosis via ELISA (IgG Euroimunn) approximately 18 months (IQR 13.5-19) after parturition. Additionally, 10 healthy ZIKV-seronegative participants were recruited at Universidade Federal de São Paulo and utilized as controls. All participants provided written informed consent, and the study received approval from the local ethics committee (CAAE: 54835916.2.0000.5546 and CAAE: 80487717.7.0000.5505).

10.3389/fitd.2024.1369608

TABLE 1 Cohort of participants exposed to Zika virus during pregnancy that gave birth to babies affected by microcephaly.

ID	Age (years)	Period of pregnancy which symptoms occur	Symptoms
001	20	not specified	not specified
004	36	not specified	Skin rash
005	29	first and second trimester of pregnancy	Fever, skin rash
006	28	not specified	Arthralgia, skin rash, myalgia, retro-orbital pain
009	26	not specified	not specified
012	30	second trimester of pregnancy	Fever, arthralgia, skin rash, retro-orbital pain, myalgia
013	41	not specified	Fever, arthralgia, skin rash, conjunctivitis, retro-orbital pain, myalgia
015	40	first trimester of pregnancy	Fever, arthralgia, skin rash, conjunctivitis, retro-orbital pain, myalgia, lymphadenopathy
019	24	not specified	not specified
021	26	not specified	Skin rash
025	17	not specified	not specified
026	21	not specified	not specified
031	17	not specified	Skin rash
033	28	not specified	Fever, arthralgia, myalgia, skin rash
035	37	not specified	Fever
037	19	not specified	Fever, arthralgia, myalgia, skin rash
038	25	eighth month of pregnancy	Fever, arthralgia, skin rash, myalgia, retro-orbital pain
040	19	not specified	Fever, rash skin
041	27	not specified	not specified
044	15	not specified	Fever, skin rash
047	23	not specified	arthralgia, myalgia, skin rash, retro-orbital pain
048	19	not specified	not specified
051	17	not specified	not specified
053	38	third month of pregnancy	Fever, arthralgia, skin rash, myalgia, retro-orbital pain
054	40	third trimester of pregnancy	Skin rash, myalgia
055	20	third month of pregnancy	Arthralgia, skin rash, retro-orbital pain
056	28	second month of pregnancy	Arthralgia, skin rash, retro-orbital pain, myalgia
057	19	not specified	Fever
060	N/S	sixth month of pregnancy	Skin rash
062	21	first month of pregnancy	Arthralgia, skin rash, retro-orbital pain
067	29	second month of pregnancy	Fever, arthralgia, skin rash, retro-orbital pain
068	19	not specified	not specified

Sample collection

PBMC were isolated from participant blood using Ficoll-Paque (GE Healthcare) density-gradient sedimentation. Subsequently, PBMC were washed twice in Hank's balanced salt solution (Gibco) and cryopreserved in 90% fetal bovine serum (FBS; Gibco) and 10% dimethyl sulfoxide (DMSO; Sigma). Cryopreserved cells were stored in liquid nitrogen until use.

Inactivated Zika virus particles

ZIKV-Br (GenBank accession number MH882531.1) was provided by Dr. Danielle Bruna Leal de Oliveira (Laboratório de Virologia Clínica e Molecular, University of São Paulo, Brazil). Virus propagation was performed using *Aedes albopictus* mosquito cells (clone C6/36), as previously described (32). The virus was precipitated with 50% polyethylene glycol (Synth), resuspended in DMEM (Gibco) plus 25 mM HEPES (Gibco), and stored at -80°C until use. For inactivation, we exposed virus preparations to UV light for up to 60 minutes (33).

Expression and purification of recombinant E_{ZIKV} protein and its ectodomains EDI/II_{ZIKV} and $EDIII_{ZIKV}$

The *E. coli* BL21 (DE3) RIL strain, harboring the plasmids pET21a-E, pET21a-EDI/II and pET21a-EDIII (34, 35), were cultivated in LB medium containing 100 µg/mL ampicillin exactly as described by Lunardelli et al., 2022. After induction with 0.01mM Isopropyl β-D-thiogalactoside (IPTG, Sigma), bacterial pellets were suspended and lysed in the APLAB-10 homogenizer (ARTEPEÇAS, Brazil). The inclusion bodies were solubilized in a urea-containing solution, and the recombinant protein was refolded and purified using nickel affinity chromatography with a HisPur™ Ni-NTA Superflow Agarose column (Thermo Scientific™), as recommended by the manufacturer. Purified E_{ZIKV}, EDI/II_{ZIKV} and $EDIII_{ZIKV}$ were assessed by 15% SDS-PAGE gel under reducing conditions.

Dot blot

Approximately 10 µL containing $2x10^5$ PFU of inactivated ZIKV or 10 µL containing 3 µg of BSA (negative control) were added to nitrocellulose membranes (Hybond-C extra nitrocellulose – GE Healthcare). After drying, the membranes were blocked with PBS Tween 20 (PBST) (0.02% v/v), non-fat milk (5% w/v) and BSA (2.5% w/v) or PBS BSA (5% w/v, for human sample), for 2 h at room temperature (rt). Next, the membranes were washed three times with PBST (0,05% v/v) and incubated with monoclonal pan-flavivirus antibody 4G2 (1 µg/mL) or human serum (ZIKV-infected patient or healthy individual, 1:500) for 2 h at rt. After 3 washes with PBST (0.05% v/v) the membranes were incubated with horseradish peroxidase-labeled goat anti-mouse IgG (1:5000; KPL) or alkaline phosphatase AffinePure goat anti-human IgG (1:2000; Jackson ImmunoResearch) for 1 h. After 3 washes with PBST, the reaction was developed with a chemiluminescence detection system ECL (GE Healthcare) or NBT/BCIP (Thermo Fisher Scientific) according to manufacturer's instructions and analyzed by Alliance 4.7 software (Uvitec; Cambridge).

Western blot

Approximately 1 µg of recombinant E_{ZIKV}, EDI/II_{ZIKV} or $EDIII_{ZIKV}$ proteins were subjected to SDS-PAGE gel electrophoresis under reducing conditions and transferred to nitrocellulose membranes (Hybond-C extra nitrocellulose – GE Healthcare). Next, the membrane was blocked with PBS BSA (5% w/v), overnight at 4°C. The membrane was washed three times with PBST (0,05% v/v) and incubated human serum (ZIKV-infected patient, 1:500) for 2 h. After 3 washes with PBST (0.05% v/v) the membrane was incubated with alkaline phosphatase AffinePure goat anti-human IgG (1:2000; Jackson ImmunoResearch) for 1 h. After 3 washes with PBST, the reaction was developed with NBT/BCIP (Thermo Fisher Scientific) according to manufacturer's instructions and analyzed by Alliance 4.7 software (Uvitec; Cambridge).

Detection of IFN-γ producing cells by ELISPOT

The ELISPOT assay was performed using human IFN-γ ELISPOT Ready-SET-Go! (eBiosciences) according to manufacturer's instructions. At the time of the assay, PBMC were rapidly thawed in a 37°C water bath, washed and transferred to tubes containing R10 (RPMI supplemented with 10% of fetal bovine serum, 2 mM l-glutamine, 1% v/v vitamin solution, 1 mM sodium pyruvate, 1% v/v non-essential amino acids solution, 40 µg/mL of gentamicin, 2-mercaptoethanol (all from Gibco), 20 µg/mL of Ciprofloxacin (Ciprobacter, Isofarma) and 30 U/mL of recombinant IL-2 (Zodiac) and incubated in a 5% CO_2 chamber at 37°C for 20 hours. After this period, cells were counted, checked for viability by Trypan blue dye exclusion (only samples with 80% or more viable cells were used) and resuspended (concentration $2x10^6$ cells/mL; 100 µl/well – $2x10^5$ cells/well) in R10. Then the cells were stimulated with inactivated ZIKV particles ($1x10^5$ PFU/well), equimolar amounts of recombinant E_{ZIKV} (10 µg/mL) protein and its ectodomains EDI/II_{ZIKV} (7.78 µg/mL) and $EDIII_{ZIKV}$ (2.44 µg/mL), medium alone as negative control or phorbol 12-myristate 13-acetate (PMA) and ionomycin (50 ng/mL and 1 µg/mL, respectively) as positive control. Spots were counted using an AID ELISPOT Reader System (Autoimmun Diagnostika GmbH, Germany). The number of IFN-γ producing cells/10^6 PBMC was calculated after subtracting the negative control values and the cutoff was 22 spots for inactivated ZIKV and 64; 65; 96 spots per million cells for E_{ZIKV}, EDI/II_{ZIKV} and $EDIII_{ZIKV}$, respectively.

Data analysis

Statistical significance (*p*-values) was calculated by Kruskal-Wallis followed by Dunn's *post hoc* test for multiple comparisons (ZIKV vs E_{ZIKV} and ectodomains) or Mann-Whitney test. Statistical analysis and graphical representation were conducted using GraphPad Prism version 9.0 software.

Results

Characteristics of convalescent samples

All 32 mothers delivered babies with microcephaly attributed to ZIKV infection. The majority (21/32 or 65.62%) were unable to specify the exact onset of ZIKV-related symptoms. Seven (21.87%) participants reported symptom initiation during the first trimester of pregnancy, and the remaining (4/32 or 12.5%) during the second or third trimester. Seventy-two percent (72%) of the participants presented one or more symptoms, including fever (18.3%), rash (29.5%), arthralgia (16.9%), conjunctivitis (2.8%), retro-orbital pain (15.4%), myalgia (15.49%) and lymphadenopathy (1.4%) (Figure 1A). Blood samples were collected post ZIKV diagnosis confirmation, with a median time of 18 months (IQR 13.5-19) after delivery. Serum samples were tested for IgG detection for both ZIKV and DENV infection, following the Brazilian Ministry of Health recommendations. All participants tested positive for ZIKV and DENV, with significantly higher reactivity against ZIKV (Figure 1B).

Antigenicity of the recombinant proteins and inactivated virus

E_{ZIKV} (50kDa), EDI/II_{ZIKV} (36kDa) and $EDIII_{ZIKV}$ (11kDa) were purified by affinity chromatography. To assess whether the recombinant proteins retained their antigenicity, a western blot analysis was conducted using serum from a convalescent individual infected with ZIKV. The serum antibodies recognized all E_{ZIKV} recombinant proteins indicating that the recombinant proteins retained their antigenic properties (Supplementary Figure 1A). Subsequently, serum from a ZIKV-infected individual and the monoclonal antibody 4G2 (pan flavivirus) recognized the

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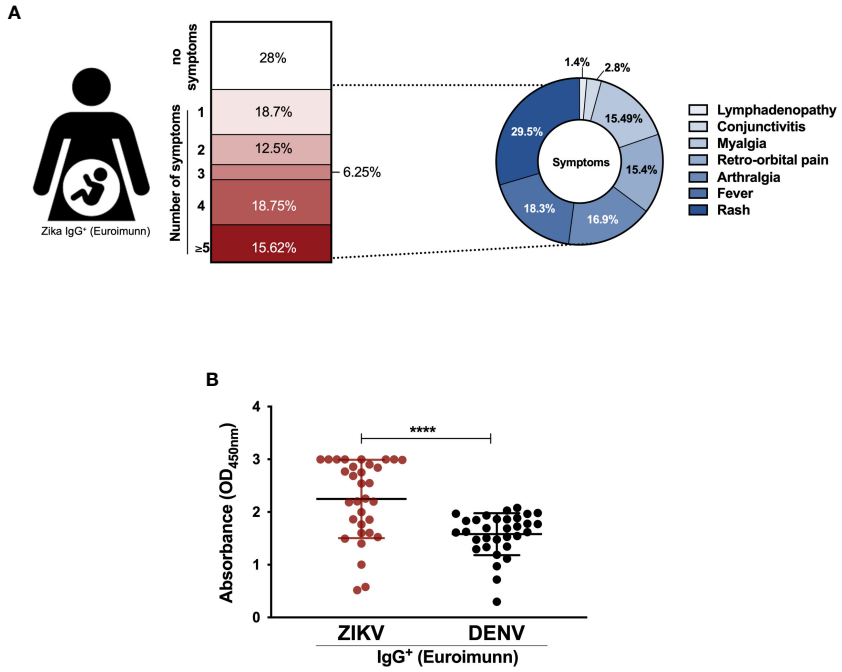

FIGURE 1
Cohort characteristics. **(A)** Schematic diagram of symptoms from convalescent mothers of newborns with microcephaly; **(B)** Antibody response against dengue virus (DENV) and Zika virus (ZIKV) by ELISA (IgG Euroimunn). Statistical significance was tested by Mann-Whitney test. ****p<0.0001.

inactivated ZIKV by dot blot analysis (Supplementary Figure 1B), confirming that it retained conformational and antigenic properties post inactivation. In contrast, no recognition of the inactivated ZIKV was observed by the control serum (ZIKV$^-$).

Inactivated ZIKV and different E-proteins induce specific IFNγ-secreting cells from convalescent mothers

To analyze whether PBMC from convalescent mothers could produce specific IFN-γ against inactivated virus or different ZIKV-envelope proteins, an ELISpot assay was performed. Initially, PBMC from all participants produced IFN-γ when stimulated with inactivated ZIKV particles (Figure 2), with most (31/32) displaying a higher number of IFN-γ-producing cells compared to PBMC from healthy controls. Subsequently, the response against the recombinant E_{ZIKV} protein and its ectodomains was evaluated. The majority of PBMC from infected participants produced IFN-γ when stimulated with recombinant E_{ZIKV} protein (90.6% of positivity, Figure 3A), and its ectodomains EDI/II_{ZIKV} (96.9% of positivity) and $EDIII_{ZIKV}$ (90.6% of positivity) (Figures 3B, C, respectively). Only one participant (#068) presented values below the cutoff (22 spots). In contrast, PBMC from healthy controls

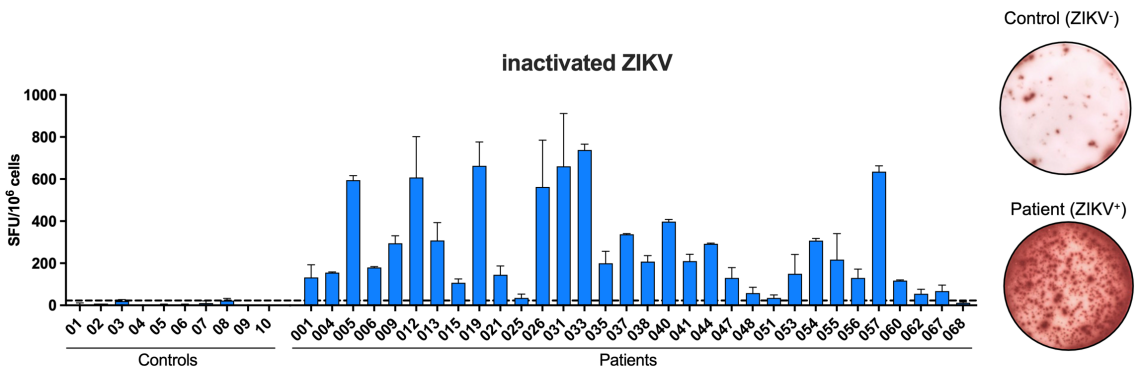

FIGURE 2
Peripheral blood mononuclear cells (PBMC) of convalescent mothers of newborns with microcephaly produce ZIKV-specific IFN-γ. PBMC were cultured in the presence of inactivated ZIKV (10^5 PFU) for 24 h to evaluate the number of IFNγ-producing cells by ELISpot assay. SFU, spot-forming units. Cutoff = 22.63 SFU/10^6 cells.

exhibited a low number of specific IFN-γ producing cells when stimulated with inactivated ZIKV particles (Figure 2), E_{ZIKV} protein, or its ectodomains (Figure 3). Notably, PBMC of participant #068 failed to produce IFN-γ against all stimuli tested, despite the high antibody titers against ZIKV in the serum. A comparison of the number of IFN-γ producing cells among all stimuli (Figure 4A) revealed a difference in the magnitude of the response. In general, when a sample was positive for the E_{ZIKV} protein, it also responded to its ectodomains, suggesting that the ectodomains are as antigenic as the entire E_{ZIKV} protein. Samples from healthy controls showed numbers of IFN-γ-producing cells below their respective cutoffs (Supplementary Figure 2).

Figure 4B displays the magnitude of the IFN-γ response for each participant. Overall, a high specific cellular response was observed, with patient #057 presenting the highest magnitude against the three recombinant proteins tested. Additional analysis (Figure 5) revealed a significant correlation of IFN-γ production between ZIKV virus particles and all recombinant proteins, suggesting that the recombinant proteins preserved their structures similarly to the native virus. Furthermore, no significant correlation was observed between the number of symptoms and cellular immunity against the virus nor to recombinant proteins (Supplementary Figure 3).

Discussion

The recent global spread of ZIKV infection, together with its association with neurologic morbidity in neonates and adults,

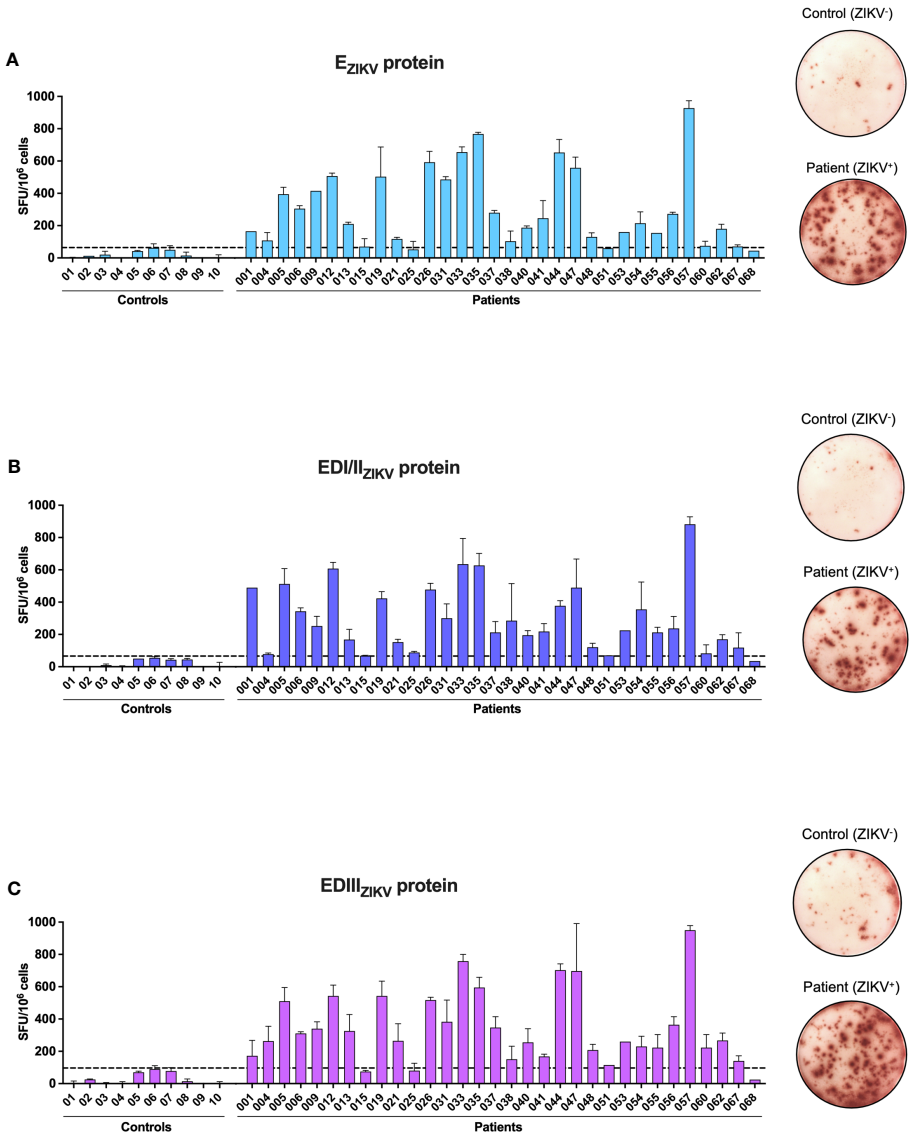

FIGURE 3
Convalescent PBMC produce IFN-γ against recombinant E_{ZIKV} and its ectodomains. PBMC were cultured in the presence of recombinant E_{ZIKV} protein **(A)** or its ectodomains EDI/EDII$_{ZIKV}$ **(B)** and EDIII$_{ZIKV}$ **(C)** for 24 h to evaluate the number of IFN-γ-producing cells by ELISpot assay. Cells were cultured with equimolar amounts of recombinant E_{ZIKV} protein or its ectodomains. SFU, spot-forming units. Cutoff E_{ZIKV} = 64 SFU/10^6 cells; EDI/EDII$_{ZIKV}$ = 65.86 SFU/10^6 cells and EDIII$_{ZIKV}$ = 96.51 SFU/10^6 cells.

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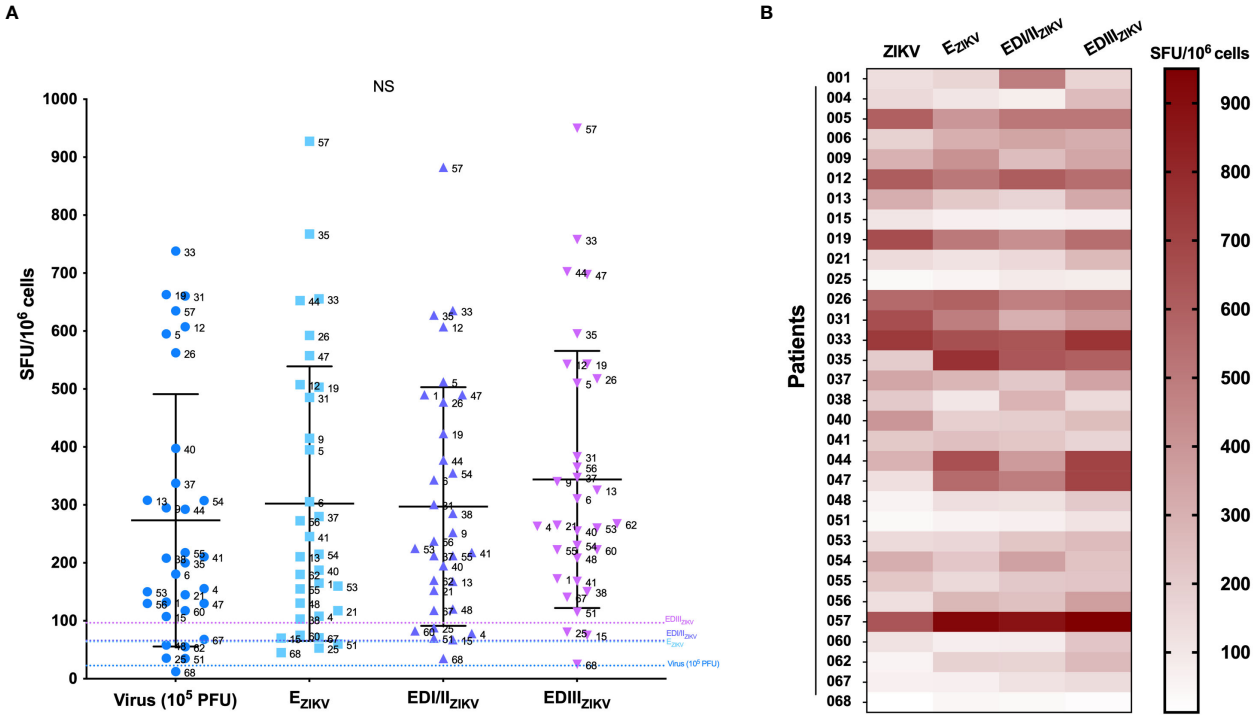

FIGURE 4
Comparison of the number of IFN-γ producing cells. **(A)** PBMC were cultured in the presence of inactivated ZIKV (10^5 PFU), E_{ZIKV} protein or its ectodomains EDI/EDII$_{ZIKV}$ and EDIII$_{ZIKV}$ (equimolar amounts) for 24 h to evaluate the number of IFN-γ-producing cells by ELISpot assay. SFU, spot forming units. Cutoff E_{ZIKV} = 64 SFU/10^6 cells; EDI/EDII$_{ZIKV}$ = 65.86 SFU/10^6 cells and EDIII$_{ZIKV}$ = 96.51 SFU/10^6 cells. Statistical significance was tested using the Kruskal-Wallis and Dunn's *post hoc* tests for multiple comparisons. **(B)** Heat map showing the number of IFN-γ-producing cells (SFU) for each stimulus. NS, Non-significant.

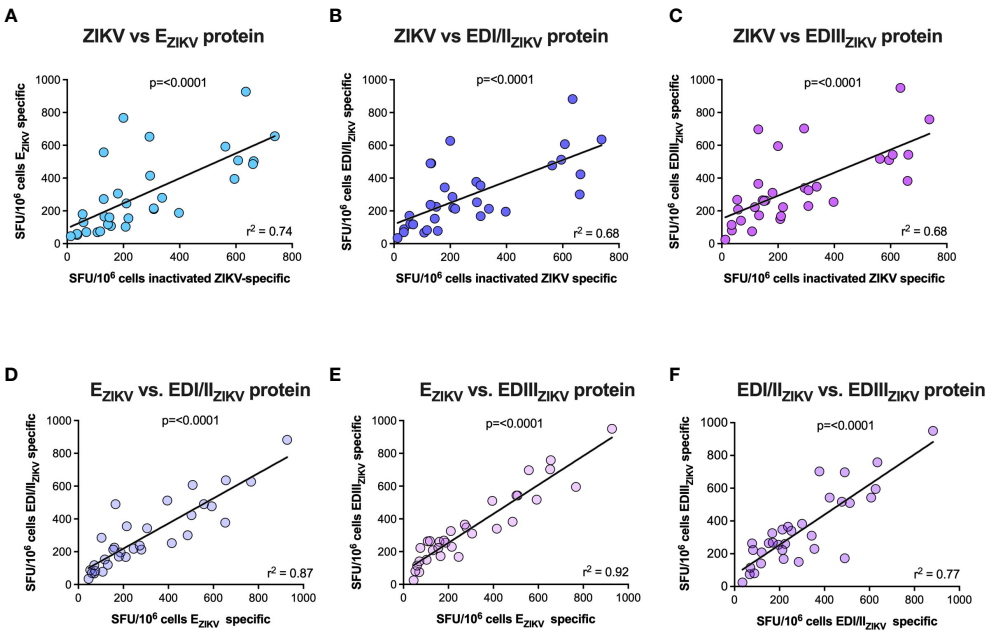

FIGURE 5
Correlation of IFN-γ response. Spearman correlation was used to evaluate the correlation between the number of IFN-γ-producing cells when stimulated with ZIKV inactivated versus E_{ZIKV} **(A)**, ZIKV inactivated versus EDI/II$_{ZIKV}$ **(B)**, ZIKV inactivated versus EDIII$_{ZIKV}$ **(C)**, E_{ZIKV} versus EDI/II$_{ZIKV}$ **(D)**, E_{ZIKV} versus EDIII$_{ZIKV}$ **(E)**, and EDI/II$_{ZIKV}$ versus EDIII$_{ZIKV}$ **(F)**.

underscores the urgent need for a safe and effective vaccine against this virus. Success in developing vaccines for flavivirus, such as the yellow fever and Japanese encephalitis viruses, demonstrated the feasibility of vaccine-induced immunity (36, 37). However, existing vaccines based on live attenuated viruses have limitations, with contraindications for certain populations, such as children (<6months of age), pregnant women, and immunocompromised individuals (38, 39). In this study, we aimed to better understand the specific regions of E_{ZIKV} targeted by cellular immunity, with a focus on understanding T cell responses in PBMC from women infected with ZIKV during pregnancy. All mothers in the cohort delivered babies with microcephaly and tested seropositive for both ZIKV and DENV.

The ZIKV envelope protein, essential for virus entry, is the main antigen that triggers the host immune response (18). The $EDIII_{ZIKV}$ domain, in particular, has shown promise as a vaccine candidate, inducing protection against ZIKV challenge in mice (40–42). Furthermore, antibodies generated against EDIII persist for a long period of time (43). Our study revealed that not only the entire envelope protein (E_{ZIKV}) but also its structural domains, EDI/II_{ZIKV} and $EDIII_{ZIKV,}$ induced robust adaptive immune responses.

Characterization of the immune response against the ZIKV in animal models has been extensively investigated by various studies (22, 23, 27, 44), including our group, revealing increased recognition of envelope regions by the adaptive immune response, presenting promising implications for the ZIKV diagnosis and vaccine development (35, 45). However, elucidating the immune response against ZIKV in humans poses challenges, primarily attributable to a substantial decline in the reported cases.

A recent study, using PBMC from ZIKV-infected pregnant women, analyzed the immune response 2-3 years post-infection. This study demonstrated sustained $CD4^+$ T cell immunity, but the same was not observed for $CD8^+$ T cells. Intriguingly, the T cell response of the mothers against ZIKV did not exhibit a clear correlation with the clinical outcomes of their children (31). Using PBMC from convalescent ZIKV patients, we detected a specific cellular immune response 1.5 years post-infection, suggesting that natural infection induces the generation of memory T cells capable of producing IFN-γ against the inactivated virus and various E_{ZIKV} proteins.

A longitudinal study involving 10 nonpregnant ZIKV-infected women observed the presence of ZIKV antibodies and virus-specific $CD4^+$ and $CD8^+$ T cells (30). Indeed, structural proteins of ZIKV, including E, prM, and C, emerged as major targets for both $CD4^+$ and $CD8^+$ T cell responses (28). Notably, the immune response against ZIKV is detectable during acute infection, with $CD4^+$ T cell responses primarily targeting E, prM, C and NS5 (46). Prior exposure to DENV was suggested to influence the T cell response to subsequent ZIKV infection (28), with cross-reactive T cells potentially expanded via stimulation with ZIKV peptides (47).

Considering the co-circulation of DENV and ZIKV in Brazil (48), it is probable that our cohort was exposed to DENV before ZIKV, supported by the positive serology for DENV. However, the chronological sequence of DENV exposure and ZIKV infection remains uncertain and could potentially influence or alter the observed responses. We cannot exclude the possibility that DENV exposure could have occurred after ZIKV infection and may have skewed or altered the responses in some way. Although several studies evaluated T cell immune responses against ZIKV proteins in DENV immune or non-immune participants (27, 49–51), the precise influence of a previous DENV exposure on ZIKV response modulation remains unclear. A study indicated that acute ZIKV infection proceeded by DENV infection had limited effects on T cells (30, 52). In DENV-naïve/ZIKV-infected patients, $CD4^+$ and $CD8^+$ T cell responses targeted envelope proteins (53). Thus, even without being able to state whether previous exposure to the DENV virus can interfere with the modulation of the response against ZIKV, all mothers presented strong immune response against envelope proteins when compared to control individuals, thus demonstrating that the E_{ZIKV}-immune response is long-term and specific.

Finally, interpretation of our findings must consider certain limitations. A longitudinal study, following volunteers during the acute phase rather than just convalescence, would provide a more comprehensive understanding of acquired immunity to ZIKV. Additionally, the sample size was limited, and it would be valuable to compare immune responses from mothers who were infected and did not have babies with microcephaly. Unfortunately, such cases were not available and further studies are required to address these issues.

In summary, our findings highlight the recognition of ZIKV envelope protein regions by memory T cells in mothers 1.5 years post-infection. These results provide important insights into vulnerable regions of viral proteins, contributing to the development of specific treatments (monoclonal antibodies) and an effective and safe vaccine suitable for administration to pregnant women.

Conclusion

Taken together, our data reveal that distinct E_{ZIKV} proteins elicited specific IFN-γ production in PBMC derived from women previously infected with ZIKV. This feature holds significant promise for helping the design of safe ZIKV vaccines tailored for pregnant women.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by ethics committee from Federal University of Sergipe and Federal University of São Paulo committee (CAAE: 54835916.2.0000.5546 and CAAE: 80487717.7.0000.5505). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JA: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. VL: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. SB: Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing. VF: Resources, Writing – original draft. RMA: Resources, Writing – original draft. JK: Conceptualization, Funding acquisition, Methodology, Resources, Writing – original draft. RPA: Resources, Writing – original draft. EC-N: Conceptualization, Funding acquisition, Methodology, Resources, Writing – original draft. DR: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fitd.2024.1369608/full#supplementary-material

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