Aedes aegypti salivary extracts exert immunomodulatory effects onmacrophages, dendritic cells, and T lymphocytes and increase Zikavirus production in vitro.

Gabriel Hilario
Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA
Alison Haubert
Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA
Gilson Dorneles
Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA
Deise Nascimento Freitas
Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA
Onilda Santos Silva
Universidade Federal do Rio Grande do Sul
Josiane Somariva Prophiro
Universidade do Sul de Santa Catarina
Tiago Fazolo
Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA
Simone Gonçalves Fonseca
Universidade Federal de Goiás
Pedro Roosevelt Torres Romão
Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA
Luiz Rodrigues Junior
luizcrj@ufcspa.edu.br

Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA

Research Article

Keywords: Aedes aegypti, Zika Virus Infection, Salivary Glands, Cytokines, Immunomodulation, Arbovirus Pathogenesis, Oxidative Stress

Posted Date: March 1st, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3995109/v1

Additional Declarations: No competing interests reported.

Abstract

Background

This study delves into the complex interplay between *Aedes aegypti* salivary gland extract and immunological cells during Zika virus (ZIKV) infection. Focusing on key immune cells, including macrophages, dendritic cells, and mononuclear cells from human peripheral blood (PBMCs), it aims to unravel the intricate mechanisms through which mosquito saliva possibly modulates the immunological landscape, influencing ZIKV transmission, host susceptibility, and disease progression. The research sheds light on the role of mosquito saliva in enhancing viral replication and impairing cells involved in host defenses, offering new insights into arbovirus pathogenesis and potential intervention strategies.

Methods

Using the FIOCRUZ-PE243|2015 ZIKV strain, the research evaluates the *Aedes aegypt* salivary extracts (SGE's) impact on cytokine production in dendritic and macrophage cell lines, as well as its influence on the redox state and cytokine responses in human PBMCs. Flow cytometry assesses immune cell profiling, aiming to understand SGE's role in ZIKV infectivity and immune modulation.

Results

SGE enhances pro-inflammatory cytokine production in both dendritic cells and macrophages, while also inducing a Th2 cytokine profile, evidenced by decreased IFN-γ and increased IL-4 levels in PBMCs. Furthermore, SGE exposure leads to increased dendritic cell frequencies, altered T lymphocyte dynamics, and elevated oxidative stress markers.

Conclusions

It highlights how SGE not only facilitates ZIKV infection by modulating cytokine production and oxidative stress but also alters immune cell dynamics.

Background

The worldwide epidemiological landscape is increasingly burdened by arthropod-borne diseases, among which the Zika virus (ZIKV) has assumed a prominent role. This virus has demonstrated a widespread geographical dispersion, affecting various continents including South America, Central America, the Caribbean, and parts of Africa and Asia (1). ZIKV has been responsible for significant outbreaks in these regions, emerging as a particularly concerning pathogen due to its association with severe neurological complications and congenital anomalies, such as microcephaly in neonates, and Guillain-Barré syndrome in adults, a serious immune response that leads to muscle weakness and paralysis (1, 2). The

transmission dynamics of ZIKV are intricately linked to the hematophagic activity of *Aedes aegypti*, a vector that administers immunomodulatory biomolecules through its saliva into the host integument; these salivary components, acting as critical mediators at the host-vector interface, are essential in enhancing the efficiency of viral transmission (3–5).

The immunomodulatory properties of mosquito saliva have garnered significant attention, especially in the context of their influence on viral pathogenesis (6–8). The salivary glands of *Ae. aegypti* harbor an assortment of bioactive constituents capable of shaping the host's immune responses, thereby orchestrating a complex interplay between the mosquito vector and the host immune system (5, 9–12). Specifically, the salivary glands of blood-feeding arthropods secrete over 55 bioactive molecules, encompassing protease inhibitors, serine proteases, nucleotidases, immunomodulatory proteins, and antioxidant agents (3, 5). Collectively, these molecules are integral to the pathogenesis and severity of various arboviral diseases, including ZIKV, dengue virus (DENV), West Nile Virus (WNV), Rift Valley fever virus (RVF), and Semliki Forest virus (SFV) (13–20). These salivary components are known to augment the recruitment of target cells for viral infection, thereby increasing the pool of infected cells. Concurrently, they mitigate pro-inflammatory responses, notably by inhibiting Nuclear factor kappa B (NF-κB) transcriptional activity, thus creating a microenvironment conducive to viral replication (19, 21, 22). Moreover, certain constituents of mosquito saliva have been identified as inducers of autophagy and inhibitors of T and B lymphocyte proliferation, and they can initiate apoptotic pathways or suppress the expression of type I interferons (23–25). These processes are crucial in the host's antiviral defense mechanisms, thereby highlighting the complex role of mosquito saliva in shaping the immunological landscape of viral infections (23–25).

Mosquito saliva has been linked to the enhancement of virus transmission, host susceptibility, disease progression, viremia levels, and mortality (22). Also, the saliva´s components can recruit dendritic cells and reduce the influx of lymphocytes to the site of injury caused by the mosquito (26). However, there is still much to be learned about the specific mechanisms by which mosquito’s saliva affects host immunity and more research is needed to fully understand their role in arbovirus transmission and infection. Given this complex interplay between mosquito saliva and the host immune response, our study aims to explore in detail how these interactions specifically affect key immune cells during ZIKV infection. By focusing on mouse macrophages, dendritic cells (DC), and human peripheral blood mononuclear cells (PBMCs), we seek to uncover the nuanced mechanisms through which *Ae. aegypti* saliva influences disease progression. To our knowledge, this is the first study to perform a comparative analysis of the immunomodulatory effect of *Ag aegypti* saliva on ZIKV infection on antigen-presenting cells important for viral infection, DCs, and macrophages, as well as PBMCs.

Material and methods

Zika Virus Strains

In the current study, the ZIKV strain, designated as FIOCRUZ-PE243|2015, was utilized for experimental purposes. This specific viral isolate was kindly provided by Dr. Marli Tenório, associated with the research facility Aggeu Magalhães/CPqAM, a constituent of the Oswaldo Cruz Foundation (FIOCRUZ) in Pernambuco, Brazil. This strain's utilization is integral to the study's virological investigations.

Zika Virus production

ZIKV stocks for this study were generated through serial propagation in Vero cells (African green monkey kidney epithelial cells, ATCC® CCL-81™). These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with high glucose content (Sigma-Aldrich, USA), enriched with 5% fetal bovine serum (FBS) (Sigma), 50 U/mL penicillin, and 50 µg/mL streptomycin, under controlled conditions at 37°C and 5% CO_2. The Vero cells were cultivated in a 75 cm² culture flask until they achieved approximately 75% confluency. After growth medium removal and Phosphate Buffered Saline (PBS) washing, the cells underwent infection with 200µL of ZIKV inoculum in 10 mL of serum-free DMEM, followed by incubation (37°C, 5% CO_2) for two hours. Post initial incubation, the infection medium was substituted with DMEM supplemented with 2% FBS, 50 U/mL penicillin, 50 µg/mL streptomycin, and 15 mM HEPES buffer. Cytopathic effects were observed in about 70% of the cell culture five days post-infection. At this phase, the cells were detached and centrifuged at 1500 rpm for 5 minutes. The supernatant was collected, and subsequently centrifugated at 5000 rpm for 15 minutes. The supernatant was aliquoted and stored at -80°C for future experiments. The virus title was determined by plate assay and the results were expressed in plate forming units per milliliters (PFU/mL).

Salivary gland extract (SGE)

Female *Aedes aegypti* mosquitoes from the Rockefeller strain, aged between 7 to 10 days, were rendered immobile via exposure to low temperatures. After immobilization, surface sterilization was achieved through brief immersion in 70% ethanol. The salivary glands were then meticulously dissected in a PBS solution, with the harvested glands being stored in aliquots at -80°C until further processing. To prepare the protein extracts, the glands underwent a series of three freeze-thaw cycles, a method employed to disrupt the glandular tissue. The resultant pooled saliva was then subjected to filtration using a 0.22 µm filter. Protein concentration was determined by NanoDrop 2000 (Thermo Fisher Scientific) (27–29).

Effect of SGE on cytokines production by mouse antigen-presenting cells

To determine the influence of SGE on cytokine production by antigen-present cells upon ZIKV infection, we used the mouse dendritic cell line JAWSII (ATCC® CRL-11904™) and the mouse macrophage cell line RAW 264.7 (ATCC® TIB-71™). Those two cells are present at the site of mosquito bite. Jaws II were maintained in alpha minimum essential medium (MEM) with ribonucleosides (Sigma), deoxyribonucleosides, 4 mM L-glutamine, 1 mM sodium pyruvate, and 5 ng/mL murine GM-CSF (Peptrotech), 20% FBS, and incubated at 37°C in 5% of CO_2 atmosphere. RAW 264.7 was maintained in DMEM 10% FBS. Each cell line was plated in 24 well plates (1×10^4/ well) and cultured under the

following conditions: medium (control), medium plus SGE (1 µg/mL), infected with ZIKV (MOI 0.1), or infected with ZIKV (MOI 0.1) and treated with different concentrations of SGE (0.5 µg/mL, 1 µg/mL, 2.5 µg/mL, and 5 µg/mL). After 90 min incubation at 37 ºC and 5% CO_2 with shaking every 15 min, FBS at the final concentration of 10% was added to each well. After 72 h of infection, the supernatants were separated and used to quantify IL-1β, TNF-α, IL-12, and IL-10 using commercial enzyme-linked immunosorbent assay following the manufacturer's instructions (Thermo Fisher/Peprotech).

Effect of SGE on the response of human peripheral mononuclear cells (PBMC)

To determine the influence of SGE on human cells, human PBMCs from healthy individuals who had no contact with ZIKV were used. A total of 10 mL of venous blood was collected from the antecubital vein of six healthy young individuals (n = 6) in tubes containing EDTA. PBMC were isolated using ficoll histopaque 1077 gradient (Sigma) (30). Cell viability was determined by the trypan blue exclusion method. PBMCs ($1x10^6$) were initially plated in 300 µL of RPMI-1640 (Gibco) culture medium without FBS. ZIKV was added in dilution of 1:8 (MOI 0.1) alone or combined with SGE 1 to 5 µg/mL (dissolved in sterile PBS). The final volume of each well was 900 µL/well. After 90 min of incubation at 37 ºC and 5% of CO_2, with shaking every 15 min, FBS was added to a final volume of 100 µL/well. After 48 h incubation, the plate was centrifuged (500g, 7 min), and the supernatant and cells were harvested and frozen at -80ºC. The cytokines IL-4 and IFN-γ in the supernatant were quantified by ELISA following the manufacturer's recommendations (Ebioscience / Peprotech, USA).

Cell redox state

To determine the cell redox state in PBMC upon ZIKV infection, cells were infected and treated or not with SGE 1 to 5 µg/mL as previously described. 48 h after incubation they were resuspended in PBS and submitted to two freeze-thaw cycles to promote cell lysis. The cell lysate was centrifuged (10,000 g, 15 min) and the supernatant was used to determine the redox state. Thiobarbituric acid reactive substance (TBARS) levels were determined according to the method described by Ohkawa et al. (1979) (31). The total content of glutathione (GSH) was analyzed by the method of recycling the 5,5`-dithiobis - [2-nitrobenzoic] acid, DTNB - GSSG proposed by Griffith (1980) (32). Nitrite levels were a reliable measure of nitric oxide and evaluated through the reaction of Griess's reagent with the sample, according to the method described by Miranda and collaborators (2001)(33). Plasma Advanced Oxidation Protein Products (AOPP) were determined spectrophotometrically according to the method previously described by Henke et al (2018) (34). The results were calculated using the standard curve as the chloramine equivalent and are shown as µmol/L.

Flow Cytometric Analysis of PBMCs

PBMCs were isolated from five healthy individuals as described above, and $1x10^5$ cells/well were cultured in RPMI medium 10% FBS, supplemented with IL-2 (5 ng/mL) to guarantee lymphocyte survival. Then, the cells were incubated with medium, medium plus SGE 5 µg/mL, or infected with ZIKV (MOI 0.1)

and incubated or not with SGE 5 µg/mL. After 72 h, the cells were prepared for flow cytometric analysis. The antibodies used for analysis were cell viability (amcyan; Cat 564406), HLA-DR (APCH7; Clone G46-6; Cat 561358), Lineage 2 (FITC; Cat 643397), CD141 (PE; Clone 1A4; Cat 559781), CD303 (BV421; Clone V24-785; Cat 566427), and CD11c (PercP-cy5.5; Clone B-Ly6; Cat 565227). Similarly, for the assessment of lymphocyte populations, the following markers were utilized: cell viability (amcyan; Cat 564406), CD3 (PE; Clone Okt3; Cat 317308), CD4 (Percpcy5; Clone rpat4; Cat 560650), CD8 (FITC; Clone H1T8a; Cat 555634), KI67 (APC; Clone b56; Cat 561126), and Granzyme B (BV421; Clone gb11; Cat: 563389). All antibodies used were from BD Biosciences, also the samples were analyzed using BD Biosciences - FACSCanto II and FlowJo 10.7.1 software.

Effect of Aedes SGE on ZIKV infection in Vero cells

To evaluate the effect of *Ae. aegypti* SGE on the propagation of the ZIKV, Vero cells ($1x10^4$/well) were seed into a 24-well plate until confluence and infected with ZIKV ($1x10^3$ PFU/mL) and treated or not with different concentrations of SGE (0.5 µg/mL, 1 µg/mL, 2.5 µg/mL and 5 µg/mL). The infection was performed according to the previously described standardized protocol in Vero cells. Cytopathic effects were observed daily, and at the end of 3 days, the plaque medium was removed, and the cells were stained with 1% violet crystal to determine the viral titer. The results were expressed in PFU/mL.

Statistical Analysis

Results with cell lines are expressed as mean ± standard error of the mean (SEM) from three replicates in three independent experiments and analyzed by one-way ANOVA, followed by Tukey. Statistical tests were performed using GraphPad Prism 6.01 software. The results with human cells were analyzed using the statistical program SPSS 22.0 (SPSS Inc., USA). Values were presented as mean ± standard deviation. A one-way analysis of variance (ANOVA) followed by a Bonferroni post-test was applied to assess the effect of treatments. A significance level of $p < 0.05$ was adopted for all analyses.

Results

Aedes aegypti salivary gland extract increases the production of inflammatory cytokines by antigen-presenting cells (APCs) infected by ZIKV

Within the framework of ZIKV transmission dynamics facilitated by *Aedes aegypti*, our first analyses focused on assessing the effects of *Ae. aegypti* SGE on cytokine secretion by antigen-presenting cells (APCs). Specifically, we observed the response of DC to ZIKV infection at a multiplicity of infection (MOI) of 0.1. Our results indicated a statistically significant ($p < 0.05$) elevation in the production of tumor necrosis factor-alpha (TNF-α) upon ZIKV infection, which was further augmented when exposed to SGE at a concentration of 5 µg/mL ($p < 0.001$) as depicted in Fig. 1A. Additionally, the incubation of ZIKV-infected JAWS II dendritic cells with SGE notably enhanced interleukin-1 beta (IL-1β) secretion ($p < 0.0001$), shown in Fig. 1B. However, the influence of *Ae. aegypti* SGE on interleukin-10 (IL-10) production in ZIKV-infected JAWS II cells was not statistically significant, as illustrated in Fig. 1C. In contrast, the

production of interleukin-12 (IL-12), a crucial cytokine involved in the induction of Th1 immune response, was significantly reduced ($p < 0.05$) in the presence of SGE at a concentration of 5 µg/mL, as shown in Fig. 1D.

In parallel, we investigated the immunomodulatory impact of *Ae. aegypti* SGE on a macrophage cell line, RAW 264.7. Similar to DC, exposure to *Ae. aegypti* SGE in ZIKV-infected macrophages led to a significant increase in TNF-α ($p < 0.0001$) and IL-1β ($p < 0.05$) production, as evidenced in Figs. 1E and 1F, respectively. However, IL-12 production in ZIKV-infected macrophages did not exhibit significant variation, whether treated with SGE or not (Fig. 1G). Intriguingly, at a concentration of 5 µg/mL, SGE notably elevated the production of IL-10 in ZIKV-infected macrophages ($p < 0.05$), particularly when compared to infected cells treated with 1 µg/mL SGE and the control group (Fig. 1H). These findings collectively underscore the modulatory effects of *Ae. aegypti* SGE on the production of pro-inflammatory, Th1, and immunoregulatory cytokines in the context of ZIKV infection in both DC and macrophages.

Aedes aegypti salivary glands induces a Th2 cytokine polarization upon ZIKV infection

In light of our observations about the modulatory effects of *Ae. aegypti* SGE on cytokine secretion by APCs during ZIKV infection, particularly the notable reduction of IL-12 in murine dendritic cells, we proceeded to assess the influence of SGE on Th1 and Th2 cytokine polarization in PBMCs obtained from healthy donors following *in vitro* ZIKV infection.

As shown in Fig. 2A, ZIKV infection induced a significant increase in interferon-gamma (IFN-γ) production by PBMCs. Conversely, treatment with SGE in vitro resulted in a pronounced decrease in IFN-γ levels ($p < 0.001$). It is important to emphasize this ability of SGE to attenuate the production of IFN-γ a Th1 cytokine, during ZIKV infection, it is in line with the reduction of IL-12 observed in DC. To further elucidate the impact of SGE on Th2 cytokine polarization, we examined the production of interleukin-4 (IL-4) in PBMCs infected with ZIKV and subsequently incubated with SGE. Figure 2B illustrates that SGE significantly elevated IL-4 production ($p < 0.0001$) in ZIKV-infected PBMCs and also in the absence of ZIKV infection.

To determine the cytokine polarization profile for Th1 or Th2, we calculated the IFN-γ:IL-4 ratio. As presented in Fig. 3C, treatment with SGE led to a substantial reduction in the IFN-γ/IL-4 ratio ($p < 0.001$), indicative of a shift in cytokine polarization favoring a Th2 over Th1 response pattern. This finding highlights the pivotal role of *Ae. aegypti* SGE in orchestrating the immune response dynamics during ZIKV infection.

Aedes aegypti salivary gland extracts increase the frequency of human dendritic cells

In this investigation, we explored the impact of *Ae. aegypti* SGE on the frequency of DC populations in the milieu of ZIKV infection. To perform the analysis we used the gate strategy presented in Fig. 3A. Our findings revealed a notable augmentation in the proportion of dendritic cells when PBMCs were incubated *in vitro* with ZIKV in the presence of SGE ($p < 0.05$), as depicted in Fig. 3B. This outcome underscores the significant influence exerted by SGE on dendritic cell differentiation during ZIKV infection.

Subsequent analysis was directed towards characterizing specific dendritic cell subsets, including conventional type 1 dendritic cells (DC1), plasmacytoid dendritic cells (pDC), and myeloid dendritic cells (mDC). Our results indicated a discernible decrease in the prevalence of the DC1 subset following PBMC incubation with ZIKV ($p < 0.05$), as shown in Fig. 3C. Moreover, a significant reduction in the frequency of the mDC subset was observed in PBMCs incubated with both ZIKV and SGE, compared to cells treated exclusively with SGE ($p < 0.01$), as illustrated in Fig. 3E. These observations collectively highlight the differential regulatory effects of SGE on various DC subsets in the context of ZIKV exposure.

Ae aegypti SGE increases the $CD4^+$ T cell frequency

To elucidate the effect of *Ae. aegypti* SGE on T cell-mediated immune responses, we undertook a detailed assessment of T cell frequency, proliferation, and granzyme B production following ZIKV infection and SGE treatment in PBMCs. The gate strategy for the analyze is presented in Fig. 4A

The incubation of PBMCs with SGE significantly increased the overall frequency of total lymphocytes as shown in Fig. 4B and 4C ($p < 0.05$). Additionally, the investigation into T cell subpopulations yielded differential responses. Notably, exposure to SGE resulted in an elevation in the frequency of CD4 + T cells ($p < 0.05$), as depicted in Fig. 4D. However, there was no significant alteration in the frequency of CD8 + T cells, as shown in Fig. 4G.

To investigate if the effects of SGE in lymphocyte frequency was mediated by any influence on cell proliferation, the Ki67 marker was quantified. Our findings indicated a significant reduction in the proliferation of both CD4 + and CD8 + T cell subsets in response to SGE (CD4 + T cells, $p < 0.05$), ZIKV infection (CD4 + T cells, $p < 0.05$; CD8 + T cells, $p < 0.001$), and the combined effect of virus and SGE (CD4 + T cells, $p < 0.01$; CD8 + T cells, $p < 0.001$) [Figures 4E and 4H]. In addition, ZIKV infection, but not SGE, diminished the proportion of CD8 + T cells expressing granzyme B, a key cytotoxic molecule ($p < 0.05$), as evidenced in Fig. 4I. Similar observation was observed in CD4 + T cells but not significant (Fig. 4F). These results collectively contribute to a more comprehensive understanding of the modulatory effects of *Ae. aegypti* SGE on T cell dynamics during ZIKV infection.

Aedes aegypti salivary glands increase the ROS production during ZIKV infection in vitro

Given the established role of oxidative stress in viral infections, which can influence viral replication, cellular functions, and disease pathogenesis, our study delved into the effects of *Ae. aegypti* SGE on various oxidative stress markers in PBMCs infected with ZIKV in vitro. Specifically, we quantified reactive

oxygen species (ROS) production, lipid peroxidation levels, total glutathione (GSH) content, advanced oxidant protein products (AOPP), and nitric oxide (NO) synthesis.

Our results demonstrated that ZIKV infection notably increased thiobarbituric acid reactive substances (TBARS) and AOPP levels while simultaneously reducing GSH content within PBMCs (Fig. 5A, 5B, 5C and 5D). Regarding the impact of SGE treatment, we observed that its presence in conjunction with ZIKV significantly attenuated TBARS ($p < 0.001$) and AOPP levels ($p < 0.001$). Additionally, SGE treatment in the context of viral infection was associated with an increase in GSH content ($p < 0.01$) and enhanced NO production ($p < 0.01$). These findings indicate the potential modulatory role of *Ae. aegypti* SGE on oxidative stress parameters within the cellular environment during ZIKV infection.

Ae. aegypti salivary glands improve ZIKV infection in vitro

To assess the potential impact of *Ae. aegypti* salivary glands on ZIKV infection and replication *in vitro*, we conducted a plate reduction assay utilizing the susceptible Vero host cell line. Remarkably, a dose-dependent increase in the number of Plaque-Forming Units per milliliter (PFU/ml) of ZIKV was observed in the infected Vero cells, directly correlating with the quantity of SGE administered, as illustrated in Fig. 6A.

In Fig. 6B, visual representations of the cytopathic effects of ZIKV on Vero cells are presented, delineating the discernible impact of escalating doses of *Ae. aegypti* salivary glands on the cell cultures. This visual evidence unequivocally demonstrates an augmentation of the cytopathic effects of ZIKV in direct association with increasing amounts of SGE.

Discussion

The ZIKV is known for its ability to evade host immune mechanisms, a trait that poses a significant challenge to the immune system. This evasion is achieved through various strategies, including impairing the induction and signaling pathways of the immune response at multiple steps (35). Recent advances in research have shed light on the intricate interplay between mosquito saliva and the host immune response, underscoring the pivotal role of mosquito saliva in shaping the outcomes of arboviral infections (3–5). Our study sought to delineate the immunomodulatory effects of *Ae. aegypti* salivary glands in the context of ZIKV infection using antigen-presenting T cell models and human PBMCs, juxtaposed against scenarios where saliva is not a contributing factor. The findings presented herein not only deepen our understanding of the immune evasion mechanisms employed by ZIKV but also offer critical insights into the nuanced interactions between *Ae. aegypti* salivary components and the host immune system. The observed profound influence of *Ae. aegypti* salivary glands on ZIKV infection dynamics signifies a crucial factor in the intricate web of host-mosquito-pathogen interactions.

The impact of *Ae. aegypti* SGE on APCs during ZIKV infection aligns with current research exploring the interaction between mosquito saliva and the host immune system. Studies have shown that mosquito saliva can modulate the immune response in various ways (29, 36–38). For example, it's been observed

that mosquito saliva has the capacity to alter leukocyte recruitment and cytokine signaling by APCs during arbovirus infections, such as with West Nile Virus (29). This includes effects on dendritic cells and macrophages, which are critical in early arbovirus infection, serving as primary sites of viral replication and key players in orchestrating the immune response to the virus. In the setting of ZIKV infection, we observe that the presence of *Ae. aegypti* SGE has been shown to increase the production of pro-inflammatory cytokines, such as TNF-α and IL-1β, while simultaneously reducing the levels of IL-12 in dendritic cells. This modulation of cytokine production suggests a critical role for mosquito saliva in influencing the host's immune response, potentially affecting the disease's progression and transmission. The increase in TNF-α and IL-1 modulated by SEG is a strategy that benefits arboviruses, as these cytokines are associated with increased edema, which helps the local retention of viruses and more APCs (DCs and macrophages) infection (39). Our data indicated that this can also be a phenomenon modulated in ZIKV infection. On the other hand the reduction in IL-12 production, an essential cytokine for Th1 antiviral response, further indicates a nuanced dysregulation of APC antiviral signaling by mosquito saliva (36). The IL-12 reduction can downregulate the function of NK and CD8 + T cells, both extremely relevant to controlling ZIKV infection (40).

Our study's outcomes align with previous research demonstrating that *Ae. aegypti* saliva can suppress innate immune responses, which can facilitate arbovirus infections. The observed alterations in cytokine profiles in DC and macrophages following exposure to *Ae. aegypti* SGE are consistent with the effects reported in other studies involving arboviral infections and mosquito saliva (29, 36–38). Furthermore, research has revealed that *Ae. aegypti* SGE can impact macrophage polarization, particularly influencing the M1/M2 macrophage profile, which is crucial for the inflammatory response. This polarization is essential in the host's response to pathogens, with M1 macrophages generally being pro-inflammatory and M2 macrophages being more associated with tissue repair and anti-inflammatory responses (38). Our study unveiled a distinct shift in the Th1/Th2 cytokine polarization in response to *Ae. aegypti* salivary glands during ZIKV infection. Salivary gland treatment was associated with decreased IFN-γ production and a concurrent increase in IL-4, indicative of a shift towards Th2 polarization. Our results firmly suggest that *Ae. aegypti* salivary glands drive a shift towards Th2 polarization, as evidenced by the observed changes in this ratio. The modulation of the Th1/Th2 cytokine balance by mosquito saliva has been investigated (41, 42). These studies suggest that mosquito saliva can influence the cytokine milieu of APCs and impact the polarization of T-cell responses (41, 42). Our study aligns with these findings, demonstrating that *Ae. aegypti* salivary glands can influence the Th1/Th2 cytokine polarization in PBMCs during ZIKV infection(42). The changes in IL-4 and IFN-γ levels suggest mosquito saliva could contribute to the modulation of T cell responses, potentially affecting the outcome of viral infections.

Bridging the findings on *Ae. aegypti* salivary glands' impact on cytokine polarization and DC profile, it becomes evident that ZIKV exploits these immune alterations to its advantage. The shift towards a Th2 cytokine profile, driven by mosquito saliva, may play a critical role in compromising the traditionally robust antiviral defense mechanisms of DC. This interplay showcases how ZIKV, along with the influence of mosquito saliva, can manipulate key immune cells, turning them from potent defenders into facilitators of viral replication. DC are specialized immune cells known for their unique functions,

including recognizing viral threats, presenting foreign antigens to both innate and adaptive immune cells, and initiating immune responses to combat viruses and protect the host. They also possess a remarkable ability to hinder different stages of viral replication by producing a wide range of antiviral molecules, activated in response to type I Interferons (43).Due to these robust characteristics, dendritic cells are traditionally viewed as hostile environments for viral replication, owing to their robust antiviral defense mechanisms. However, ZIKV's ability to infect immune cells, particularly myeloid dendritic cells (mDCs), despite their traditionally robust antiviral mechanisms, showcases a distinct immune escape tactic (44–47). By transforming mDCs into a supportive environment for viral replication, ZIKV effectively circumvents the typical barriers to infection (44–47). Furthermore, our investigation into immune cell populations reveals that mosquito saliva can alter the proportions of dendritic cells. These changes may affect immune surveillance, antigen presentation, and overall immune system function during ZIKV infection. Our findings corroborate the unexpected susceptibility of mDCs to ZIKV infection. Our results align with emerging evidence(44–47) suggesting that ZIKV combined with SGE spread the ability to transform mDCs into a cellular niche conducive to viral propagation, representing a decrease in this population because of death by viral infection and replication.

The link between the manipulation of dendritic cells and T-cell responses by *Ae. aegypti* SGE in ZIKV infection suggests a coordinated strategy of immune modulation. This strategy not only compromises the dendritic cell's antiviral defenses but also extends to altering T cell dynamics, further indicating the depth and complexity of the immune system manipulation by ZIKV in the presence of SGE. The findings from the investigation into the effects of *Ae. aegypti* SGE on T cell-mediated immune responses during ZIKV infection are intriguing and align with broader research in the field of immunology. The observed increase in CD4 + T cell frequency and the lack of significant alteration in CD8 + T cells, along with changes in cytokine production, suggest a nuanced modulatory role of SGE in T cell dynamics. It's possible that during ZIKV infection the SGE contributes to the differentiations of CD4 + T cell populations, mostly Th2, since others previously identified the protein SAAG-4 present in SEG that reduces CD4 expression, induces Th2 differentiation and IL-4 production (48).This modulation could potentially affect the immune response's balance, influencing the outcome of ZIKV infection. In particular, the significant reduction in the proliferation of both CD4 + and CD8 + T cell subsets in response to SGE, as well as the impact on granzyme B production in CD8 + T cells, highlights the complex interplay between the mosquito saliva and the host immune system. Previous studies using mouse cells have shown that A. *aegypti* SGE induces apoptosis in CD4 + and CD8 + T cells, and B cells through a mechanism involving caspase-3 and caspase-8 (25). Aligning with the findings, the results of a previous study indicate that *Ae. aegypti* SGE induces apoptosis in various lymphocyte subsets, including CD4 + and CD8 + T cells, as well as B cells. This apoptosis mechanism involves caspase-3 and caspase-8 activation, underscoring the intricacy of the immunomodulatory pathways exploited by mosquito saliva. (25). The results suggest that mosquito saliva, through components like LTRIN increases inflammatory cytokines and decreases the Th1 polarization mediated by of antigen-presenting cells and impact T-cell responses(21). Indeed, saliva products from other vectors such as sand flies reduces antigen presenting cells activation and negatively modulate the secretion of Th1 and Th2 cytokines by PBMCs (49).

Additionally, our investigation revealed a link between *Ae. aegypti* salivary gland components and oxidative stress responses. The alteration of ROS production, lipid peroxidation, and antioxidant levels in PBMCs indicates a multifaceted impact on cellular redox dynamics. The heightened nitric oxide production and the dynamic alterations in lipid peroxidation, GSH levels, and AOPP content suggest that the mosquito's salivary glands play a role in modulating the oxidative stress environment of host cells. To further understand the link between these oxidative stress-related findings and the broader immunomodulatory effects of *Ae. aegypti* salivary glands, additional studies are warranted to dissect the underlying mechanisms driving these observed changes. Such knowledge could offer promising avenues for therapeutic interventions to mitigate the detrimental effects of ZIKV infection. The impact of mosquito saliva on oxidative stress has been elucidated in studies that highlight the presence of defense-related proteins in mosquito saliva (50, 51). These proteins can decrease and increase the redox state within host cells, depending of the parameters analyzed. Our study extends this understanding by revealing that *Ae. aegypti* salivary glands have the potential to modulate oxidative stress in PBMCs during ZIKV infection. The elevation of NO levels and changes in GSH content observed in our study align with the findings from these earlier investigations. Therefore, previous study shows that SGE plays a protective role in septic animals, contributing to oxidative and inflammatory balance during sepsis, and this effect seems to be mediated by the control of inflammation and oxidative damage (12). These results underscore mosquito saliva's multifaceted role in impacting cellular oxidative stress and redox signaling pathways.

The SGE from these mosquitoes demonstrated the capacity to potentiate ZIKV infection *in vitro*, a phenomenon evidenced by the dose-dependent increase cytopathic effect within Vero cells. The dose-dependent relationship observed between the quantity of SGE and the PFU underscores the significant role played by salivary gland factors in influencing ZIKV infection dynamics. Similar to other studies, our results demonstrated that mosquito saliva could enhance viral infection and replication (52–55). The enhancement of ZIKV infection observed in Vero cells and DC treated with *Ae. aegypti* SGE was also observed by Styer et al. and Wasinpiyamongkol et al., which highlighted the ability of mosquito saliva to augment the infection of West Nile virus and dengue virus (8, 20). Furthermore, Vero cells do not produce type I interferon, an important element of antiviral immunity, which makes the role of SGE more significant in the spread of infection and its relationship with the immunological response. These data suggest a common mechanism by which mosquito saliva might promote the entry and propagation of arboviruses within host cells.

Collectively, the results presented here and insights from previous research emphasize the significance of mosquito saliva in shaping the immune response to arboviral infections. Our findings align with existing literature, illustrating the ability of *Ae. aegypti* salivary glands to enhance viral infection, manipulate cytokine production, and influence oxidative stress dynamics. These observations strengthen the notion that mosquito saliva is not just a mechanical facilitator of blood feeding but a sophisticated mediator that can tip the balance in favor of viral transmission and disease exacerbation. These findings offer novel insights into the intricate modulation of host immune responses by mosquito salivary gland factors, with potential sources for new therapeutic molecule(s).

Conclusion

The intricate interplay between *Ae. aegypti* SGE and host immune responses during ZIKV infection presents a significant area for further exploration and potential therapeutic interventions. This study and previous research highlight mosquito saliva's dual role in facilitating viral infection and modulating host immune responses. The observed shift towards Th2 polarization and the increasing on oxidative stress markers in PBMCs further illustrate the complex role of mosquito saliva in viral infection dynamics. The findings suggest that *Ae. aegypti* SGE can enhance ZIKV infection by modulation of cytokine production and impacting oxidative stress dynamics in host cells. These results underscore the importance of understanding vector-host interactions in the spread and impact of arboviral diseases.

Abbreviations

AOPP
Advanced Oxidation Protein Products
APCs
Antigen-Presenting Cells
DC
Dendritic Cells
DC1
Conventional Type 1 Dendritic Cells
DENV
Dengue Virus
DMEM
Dulbecco's Modified Eagle Medium
FBS
Fetal bovine serum
FIOCRUZ
Oswaldo Cruz Foundation
GSH
Glutathione
IFN-γ
Interferon Gamma
IL-10
Interleukin-10
IL-12
Interleukin-12
IL-1β
Interleukin-1 Beta
IL-4
Interleukin-4

mDC
MYELOID DENDRITIC CELLS
MOI
Multiplicity of infection
NF-κB
Nuclear factor kappa B
NO
Nitric Oxide
PBMCs
Peripheral blood mononuclear cells
PBS
Phosphate Buffered Saline
pDC
Plasmacytoid Dendritic Cells
PFU
Plaque-forming units
ROS
Reactive Oxygen Species
RVF
Rift Valley fever virus
SEM
Standard error of the mean
SFV
Semliki Forest virus
SGE
Salivary gland extract
TBARS
Thiobarbituric acid reactive substance
TNF-α
Tumor necrosis factor-alpha
WNV
West Nile Virus
ZIKV
Zika Virus.

Declarations

Acknowledgments

We thank Dra. Marli Tenório from Fundação Oswaldo Cuz (FIOCRUZ) for kindly providing the Zika virus strain.

Funding

This work was supported, in part, by funds from Fundação de Amparo a Pesquisa no Rio Grande do Sul (FAPERGS) e and scholarships from Coordenação de Aperfeiçoamento de Pessoa de Nível Superior (CAPES).

Availability of data and materials

All materials described in the manuscript are available to any scientist wishing to use them for non-commercial purposes.

Authors' contributions

GM, AH, GD, TF, and DF performed experiments and analyzed the data. LR and GM designed the work, and wrote and reviewed the manuscript. LR and PR obtaining and managing resources for work. OS and JS maintenance of mosquito colonies and obtaining salivary glands. GM, TF, and DF: flow cytometry experiments. PR and OS: manuscript review.

Ethics approval and consent to participate

The Ethics Committee of the Hospital e Maternidade Dona Iris, Goiânia, Coiás, Brazil, approved the protocol utilized in the present study, under the protocol CAAE: 62903216.6.0000.8058. All subjects participating in the study provided written informed consent and signed the Free and Informed Consent Terms.

Consent for publication

All the authors read and approved the final version of the manuscript. All the

people involved in the study gave their consent for its publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Ryan SJ, Carlson CJ, Mordecai EA, Johnson LR. Global expansion and redistribution of Aedes-borne virus transmission risk with climate change. PLoS Negl Trop Dis. 2019 Mar 28;13(3):e0007213.
2. Pielnaa P, Al-Saadawe M, Saro A, Dama MF, Zhou M, Huang Y, et al. Zika virus-spread, epidemiology, genome, transmission cycle, clinical manifestation, associated challenges, vaccine and antiviral drug development. Virology. 2020 Apr;543:34–42.
3. Ribeiro JM, Arcà B, Lombardo F, Calvo E, Van My Phan, Chandra PK, et al. An annotated catalogue of salivary gland transcripts in the adult female mosquito, Ædes ægypti*. BMC Genomics. 2007 Dec

4;8(1):6.

4. Ribeiro JMC, Mans BJ, Arcà B. An insight into the sialome of blood-feeding Nematocera. Insect Biochem Mol Biol [Internet]. 2010 Nov;40(11):767–84. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0965174810001700
5. Melo B, Silva N, Gomes R, Navegantes K, Oliveira A, Almeida L, et al. Bioactive Compounds of the Salivary Glands from Aedes aegypti with Anti-Hemostatic Action. Annu Res Rev Biol. 2015 Jan 10;8(2):1–17.
6. Sri-in C, Thontiravong A, Bartholomay LC, Tiawsirisup S. Effects of Aedes aegypti salivary protein on duck Tembusu virus replication and transmission in salivary glands. Acta Trop. 2022 Apr;228:106310.
7. Valenzuela-Leon PC, Shrivastava G, Martin-Martin I, Cardenas JC, Londono-Renteria B, Calvo E. Multiple Salivary Proteins from Aedes aegypti Mosquito Bind to the Zika Virus Envelope Protein. Viruses. 2022 Jan 24;14(2):221.
8. Chisenhall DM, Christofferson RC, McCracken MK, Johnson AMF, Londono-Renteria B, Mores CN. Infection with dengue-2 virus alters proteins in naturally expectorated saliva of Aedes aegypti mosquitoes. Parasit Vectors [Internet]. 2014 Dec 30;7(1):252. Available from: https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-7-252
9. Cerqueira ARA, Rodrigues L, Coavoy-Sánchez SA, Teixeira SA, Feitosa KB, Taniguchi EY, et al. Aedes aegypti salivary gland extract alleviates acute itching by blocking TRPA1 channels. Front Physiol. 2023 Jun 27;14.
10. Gavor E, Choong YK, Liu Y, Pompon J, Ooi EE, Mok YK, et al. Identification of Aedes aegypti salivary gland proteins interacting with human immune receptor proteins. Ribeiro JMC, editor. PLoS Negl Trop Dis. 2022 Sep 7;16(9):e0010743.
11. Sales-Campos H, de Souza PR, Basso PJ, Ramos AD, Nardini V, Chica JEL, et al. Aedes aegypti salivary gland extract ameliorates experimental inflammatory bowel disease. Int Immunopharmacol. 2015 May;26(1):13–22.
12. de Souza Gomes R, Navegantes-Lima K, Monteiro V, de Brito Oliveira A, Rodrigues D, Reis J, et al. Salivary Gland Extract from Aedes aegypti Improves Survival in Murine Polymicrobial Sepsis through Oxidative Mechanisms. Cells [Internet]. 2018 Oct 23;7(11):182. Available from: http://www.mdpi.com/2073-4409/7/11/182
13. Cox J, Mota J, Sukupolvi-Petty S, Diamond MS, Rico-Hesse R. Mosquito Bite Delivery of Dengue Virus Enhances Immunogenicity and Pathogenesis in Humanized Mice. J Virol. 2012 Jul 15;86(14):7637–49.
14. Garcia M, Alout H, Diop F, Damour A, Bengue M, Weill M, et al. Innate Immune Response of Primary Human Keratinocytes to West Nile Virus Infection and Its Modulation by Mosquito Saliva. Front Cell Infect Microbiol. 2018 Nov 2;8.
15. Le Coupanec A, Babin D, Fiette L, Jouvion G, Ave P, Misse D, et al. Aedes Mosquito Saliva Modulates Rift Valley Fever Virus Pathogenicity. Turell MJ, editor. PLoS Negl Trop Dis. 2013 Jun 13;7(6):e2237.

16. Pingen M, Bryden SR, Pondeville E, Schnettler E, Kohl A, Merits A, et al. Host Inflammatory Response to Mosquito Bites Enhances the Severity of Arbovirus Infection. Immunity. 2016 Jun;44(6):1455–69.
17. Surasombatpattana P, Hamel R, Patramool S, Luplertlop N, Thomas F, Desprès P, et al. Dengue virus replication in infected human keratinocytes leads to activation of antiviral innate immune responses. Infection, Genetics and Evolution. 2011 Oct;11(7):1664–73.
18. Wichit S, Diop F, Hamel R, Talignani L, Ferraris P, Cornelie S, et al. Aedes Aegypti saliva enhances chikungunya virus replication in human skin fibroblasts via inhibition of the type I interferon signaling pathway. Infection, Genetics and Evolution. 2017 Nov;55:68–70.
19. Salazar MI, Richardson JH, Sánchez-Vargas I, Olson KE, Beaty BJ. Dengue virus type 2: replication and tropisms in orally infected Aedes aegypti mosquitoes. BMC Microbiol [Internet]. 2007 Dec 30;7(1):9. Available from: https://bmcmicrobiol.biomedcentral.com/articles/10.1186/1471-2180-7-9
20. Styer LM, Lim PY, Louie KL, Albright RG, Kramer LD, Bernard KA. Mosquito Saliva Causes Enhancement of West Nile Virus Infection in Mice. J Virol [Internet]. 2011 Feb 15;85(4):1517–27. Available from: https://journals.asm.org/doi/10.1128/JVI.02142-10
21. Jin L, Guo X, Shen C, Hao X, Sun P, Li P, et al. Salivary factor LTRIN from Aedes aegypti facilitates the transmission of Zika virus by interfering with the lymphotoxin-β receptor. Nat Immunol [Internet]. 2018 Apr 5;19(4):342–53. Available from: https://www.nature.com/articles/s41590-018-0063-9
22. Guerrero D, Cantaert T, Missé D. Aedes Mosquito Salivary Components and Their Effect on the Immune Response to Arboviruses. Vol. 10, Frontiers in Cellular and Infection Microbiology. Frontiers Media S.A.; 2020.
23. Sun P, Nie K, Zhu Y, Liu Y, Wu P, Liu Z, et al. A mosquito salivary protein promotes flavivirus transmission by activation of autophagy. Nat Commun. 2020 Jan 14;11(1):260.
24. Surasombatpattana P, Ekchariyawat P, Hamel R, Patramool S, Thongrungkiat S, Denizot M, et al. Aedes aegypti Saliva Contains a Prominent 34-kDa Protein that Strongly Enhances Dengue Virus Replication in Human Keratinocytes. Journal of Investigative Dermatology. 2014 Jan;134(1):281–4.
25. Bizzarro B, Barros MS, Maciel C, Gueroni DI, Lino CN, Campopiano J, et al. Effects of Aedes aegypti salivary components on dendritic cell and lymphocyte biology. Parasit Vectors [Internet]. 2013 Dec 15;6(1):329. Available from: https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-6-329
26. Briant L, Desprès P, Choumet V, Missé D. Role of skin immune cells on the host susceptibility to mosquito-borne viruses. Virology. 2014 Sep;464–465:26–32.
27. CAVALCANTE RR, PEREIRA MH, GONTIJO NF. Anti-complement activity in the saliva of phlebotomine sand flies and other haematophagous insects. Parasitology. 2003 Jul;127(1):S0031182003003329.
28. SLÁMOVÁ M, SKALLOVÁ A, PÁLENÍKOVÁ J, KOPECKÝ J. Effect of tick saliva on immune interactions between Borrelia afzelii and murine dendritic cells. Parasite Immunol. 2011 Dec;33(12):654–60.
29. Schneider BS, Soong L, Coffey LL, Stevenson HL, McGee CE, Higgs S. Aedes aegypti Saliva Alters Leukocyte Recruitment and Cytokine Signaling by Antigen-Presenting Cells during West Nile Virus

Infection. PLoS One. 2010 Jul 22;5(7):e11704.

30. Dorneles GP, Boeira MCR, Schipper LL, Silva IRV, Elsner VR, Dal Lago P, et al. Acute Strenuous Exercise Induces an Imbalance on Histone H4 Acetylation/Histone Deacetylase 2 and Increases the Proinflammatory Profile of PBMC of Obese Individuals. Oxid Med Cell Longev. 2017;2017.
31. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351–8.
32. Griffith O. Determination of glutatione reductase and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem. 1980;106((1)):207–12.
33. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide. 2001;5(1):62–71.
34. Henke E, Oliveira VS, Silva IM da, Schipper L, Dorneles G, Elsner VR, et al. Acute and chronic effects of High Intensity Interval Training on inflammatory and oxidative stress markers of postmenopausal obese women. Transl Sports Med. 2018;1(6):257–64.
35. Serman TM, Gack MU. Evasion of Innate and Intrinsic Antiviral Pathways by the Zika Virus. Viruses. 2019 Oct 22;11(10):970.
36. Wanasen N, Nussenzveig RH, Champagne DE, Soong L, Higgs S. Differential modulation of murine host immune response by salivary gland extracts from the mosquitoes Aedes aegypti and Culex quinquefasciatus. Med Vet Entomol [Internet]. 2004 Jun;18(2):191–9. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2915.2004.00498.x
37. Lara PG, Esteves E, Sales-Campos H, Assis JB, Henrique MO, Barros MS, et al. AeMOPE-1, a Novel Salivary Peptide From Aedes aegypti, Selectively Modulates Activation of Murine Macrophages and Ameliorates Experimental Colitis. Front Immunol. 2021 Jul 19;12.
38. Barros MS, Lara PG, Fonseca MT, Moretti EH, Filgueiras LR, Martins JO, et al. Aedes aegypti saliva impairs M1-associated proinflammatory phenotype without promoting or affecting M2 polarization of murine macrophages. Parasit Vectors. 2019 Dec 16;12(1):239.
39. Pingen M, Bryden SR, Pondeville E, Schnettler E, Kohl A, Merits A, et al. Host Inflammatory Response to Mosquito Bites Enhances the Severity of Arbovirus Infection. Immunity [Internet]. 2016 Jun;44(6):1455–69. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1074761316302059
40. Maucourant C, Nonato Queiroz GA, Corneau A, Leandro Gois L, Meghraoui-Kheddar A, Tarantino N, et al. NK Cell Responses in Zika Virus Infection Are Biased towards Cytokine-Mediated Effector Functions. The Journal of Immunology. 2021 Sep 1;207(5):1333–43.
41. Arcà B, Lombardo F, Francischetti IMB, Pham VM, Mestres-Simon M, Andersen JF, et al. An insight into the sialome of the adult female mosquito Aedes albopictus. Insect Biochem Mol Biol. 2007 Feb;37(2):107–27.
42. Spencer Clinton JL, Vogt MB, Kneubehl AR, Hibl BM, Paust S, Rico-Hesse R. Sialokinin in mosquito saliva shifts human immune responses towards intracellular pathogens. PLoS Negl Trop Dis. 2023 Feb 3;17(2):e0011095.

43. Thiele RJ, Grünhagel B, Muenchhoff M, Pujantell-Graell M, Jocham L, Düsedau A, et al. Magnitude of Type I Interferon Responses by Plasmacytoid Dendritic Cells After TLR7 Stimulation Is Associated With Human Immunodeficiency Virus Type 1 (HIV-1) Reservoir Sizes in Cisgender Women With HIV-1 on Antiretroviral Therapy. J Infect Dis. 2024 Feb 22;
44. Sun X, Hua S, Gao C, Blackmer JE, Ouyang Z, Ard K, et al. Immune-profiling of ZIKV-infected patients identifies a distinct function of plasmacytoid dendritic cells for immune cross-regulation. Nat Commun. 2020 May 15;11(1):2421.
45. Sun X, Hua S, Chen HR, Ouyang Z, Einkauf K, Tse S, et al. Transcriptional Changes during Naturally Acquired Zika Virus Infection Render Dendritic Cells Highly Conducive to Viral Replication. Cell Rep. 2017 Dec;21(12):3471–82.
46. Bowen JR, Quicke KM, Maddur MS, O'Neal JT, McDonald CE, Fedorova NB, et al. Zika Virus Antagonizes Type I Interferon Responses during Infection of Human Dendritic Cells. PLoS Pathog. 2017 Feb 2;13(2):e1006164.
47. Michlmayr D, Andrade P, Gonzalez K, Balmaseda A, Harris E. CD14+CD16+ monocytes are the main target of Zika virus infection in peripheral blood mononuclear cells in a paediatric study in Nicaragua. Nat Microbiol. 2017 Oct 2;2(11):1462–70.
48. BOPPANA VD, THANGAMANI S, ADLER AJ, WIKEL SK. SAAG-4 is a novel mosquito salivary protein that programmes host CD4 $^{+}$ T cells to express IL-4. Parasite Immunol. 2009 Jun 26;31(6):287–95.
49. Souissi C, Marzouki S, Elbini-Dhouib I, Jebali J, Oliveira F, Valenzuela JG, et al. PpSP32, the Phlebotomus papatasi immunodominant salivary protein, exerts immunomodulatory effects on human monocytes, macrophages, and lymphocytes. Parasit Vectors. 2023 Jan 2;16(1):1.
50. Ribeiro JMC, Charlab R, Pham VM, Garfield M, Valenzuela JG. An insight into the salivary transcriptome and proteome of the adult female mosquito Culex pipiens quinquefasciatus. Insect Biochem Mol Biol. 2004 Jun;34(6):543–63.
51. Hillyer JF, Christensen BM. Characterization of hemocytes from the yellow fever mosquito, Aedes aegypti. Histochem Cell Biol [Internet]. 2002 May 26;117(5):431–40. Available from: http://link.springer.com/10.1007/s00418-002-0408-0
52. Cheang KW, Chen WY, Wu-Hsieh BA, Shiao SH. Infecting mosquitoes alters DENV-2 characteristics and enhances hemorrhage-induction potential in Stat1-/- mice. Murray KO, editor. PLoS Negl Trop Dis. 2021 Aug 27;15(8):e0009728.
53. Conway MJ, Londono-Renteria B, Troupin A, Watson AM, Klimstra WB, Fikrig E, et al. Aedes aegypti D7 Saliva Protein Inhibits Dengue Virus Infection. Dinglasan RR, editor. PLoS Negl Trop Dis. 2016 Sep 15;10(9):e0004941.
54. McCracken MK, Gromowski GD, Garver LS, Goupil BA, Walker KD, Friberg H, et al. Route of inoculation and mosquito vector exposure modulate dengue virus replication kinetics and immune responses in rhesus macaques. Brault AC, editor. PLoS Negl Trop Dis. 2020 Apr 8;14 4):e0008191.
55. Schneider BS, Higgs S. The enhancement of arbovirus transmission and disease by mosquito saliva is associated with modulation of the host immune response. Trans R Soc Trop Med Hyg. 2008

May;102 (5):400–8.

Figures

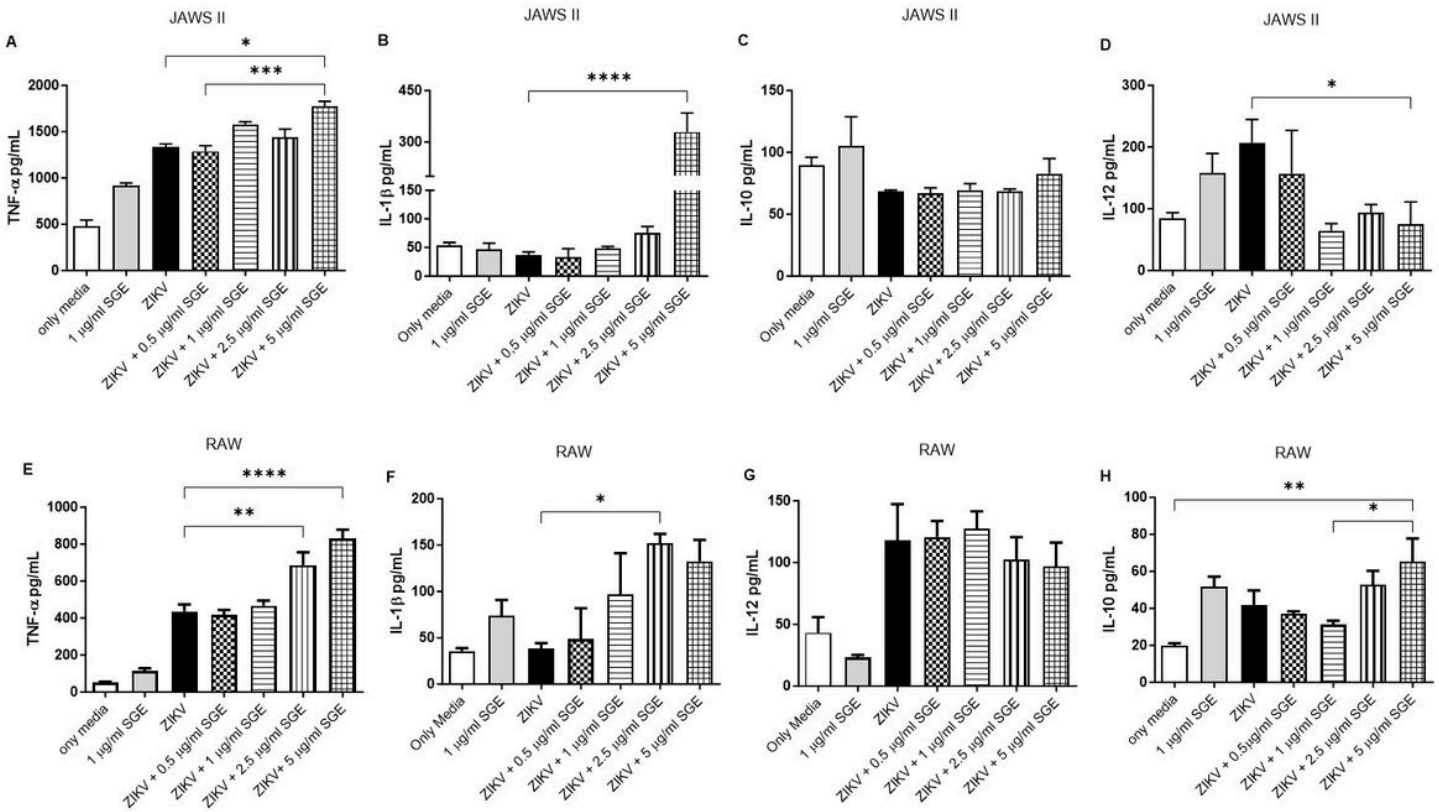

Figure 1

Effect of SGE on cytokines production by ZIKV-infected dendritic cells and macrophages: mouse dendritic cell line (JAWS II) and mouse macrophage cell line (RAW 264.7) were infected with ZIKV at 0.1 MOI in the presence of 0.5 µg/ mL, 1 µg/ mL, 2.5 µg/ mL or 5 µg/ mL SGE of *Ae aegypti*. After 72 h of incubation, the levels of TNF-α, IL-1β, IL-12, and IL-10 were quantified by ELISA. **A, B, C, and D (**JAWS II). **E, F, G, H** (RAW 264.7). * Denotes p<0.05.** Denotes p<0.01.*** Denotes p<0.001. **** Denotes p<0.0001. MOI: Multiplicity of infection.

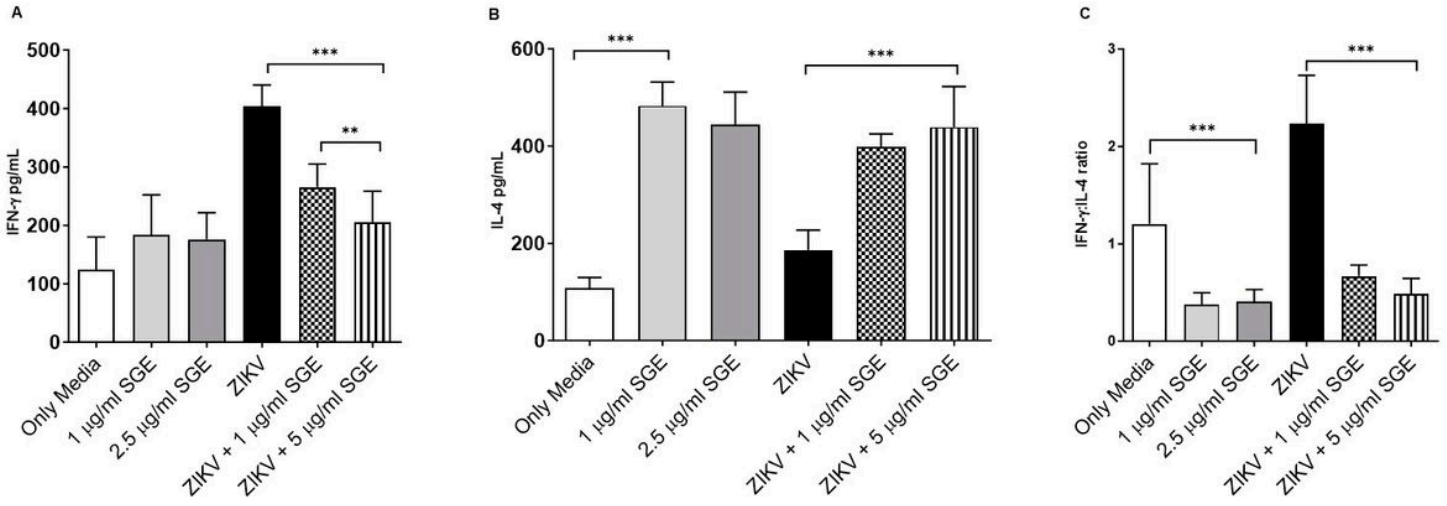

Figure 2

Cytokine profile in PBMCs infected with ZIKV in presence of SGE from *Ae. Aegypti*. PBMC were cultured with medium, SGE, ZIKV or ZIKV plus SGE and the production of IFN- γ (A) and IL-4 quantified after 72 h incubation using ELISA. C) IFN-γ/IL-4 ratio. * Denotes p<0.05.** Denotes p<0.01.*** Denotes p<0.001. ****Denotes p<0.0001.

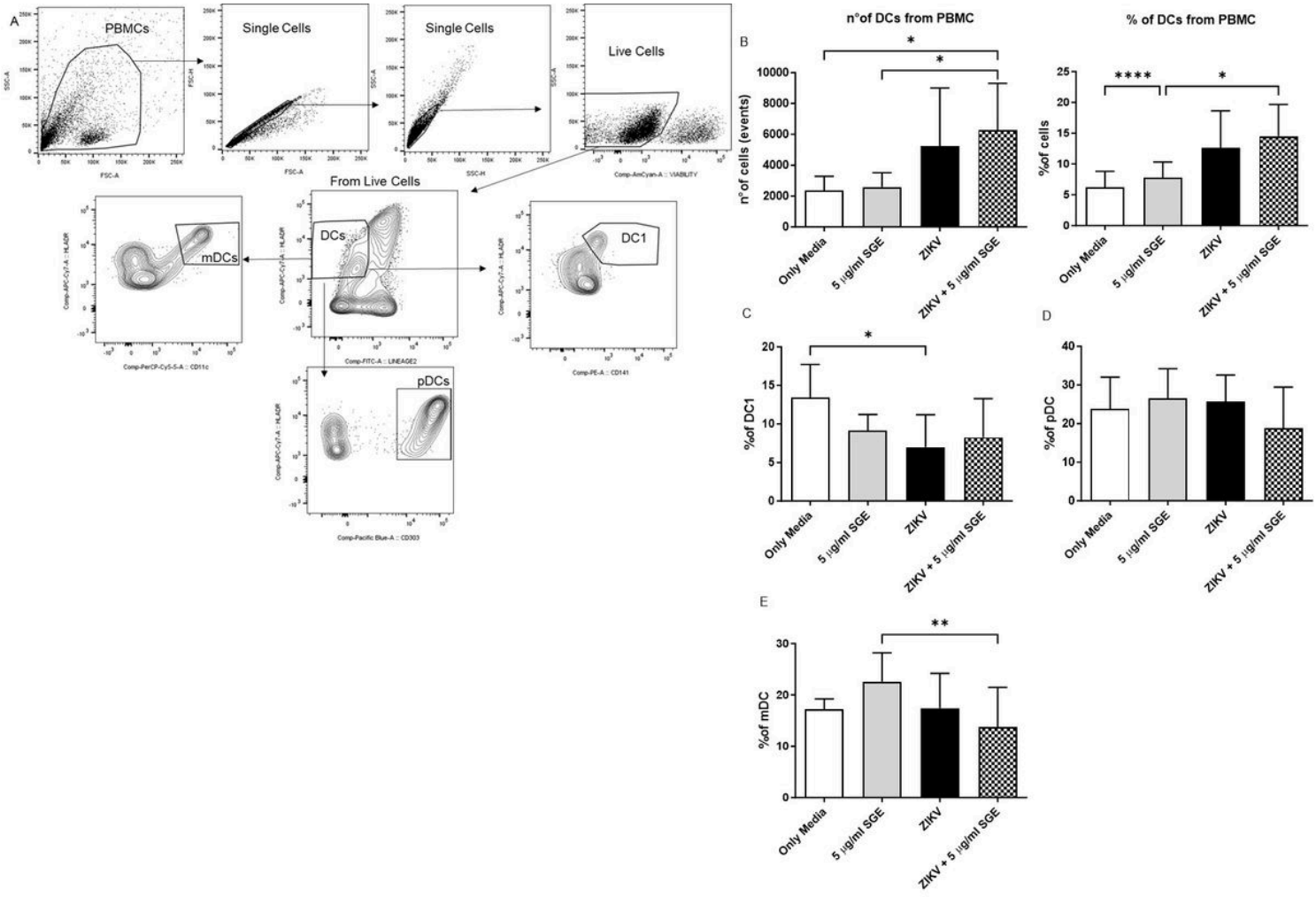

Figure 3

Effect of SGE in the frequency of DC subsets in PBMCs. Gating strategy (A), % of DCs (B), % of DC1s subset (C), % pDCs subset (D), % mDCs subset (E). * Denotes p<0.05.** Denotes p<0.01.*** Denotes p<0.001.****Denotes p<0.0001. DC: dendritic cells, DC1, pDC,.mDC.

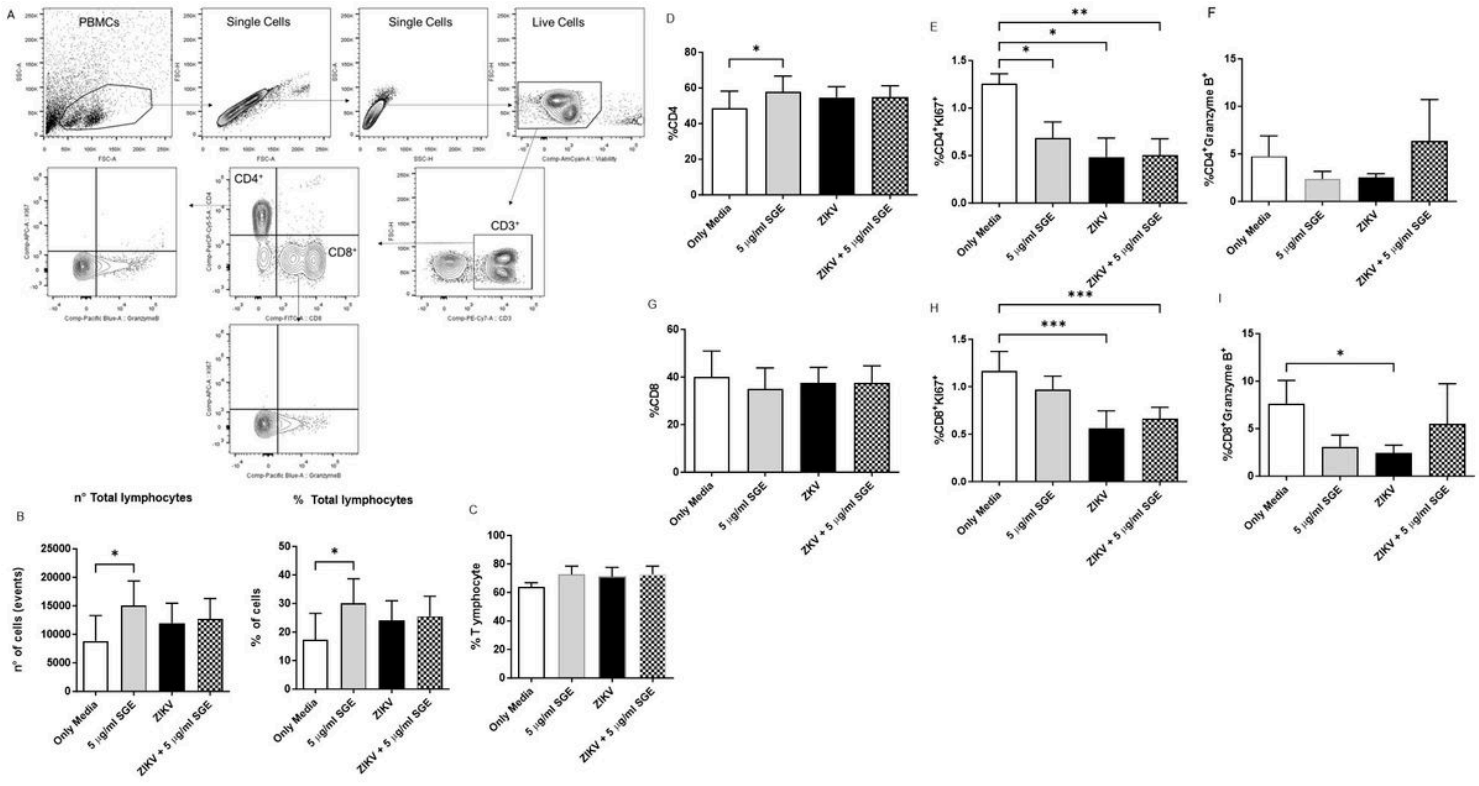

Figure 4

T lymphocytes from PBMC analysis. Gating strategy (A), % of PBMC (B), % of Lymphocyte (C), %CD4 (D), %CD4KI67 (E), %CD4 Gramzym B (F), %CD8 (G), %CD8 KI67 (H), %CD8 Granzyme B (I). * Denotes $p<0.05$.** Denotes $p<0.01$.*** Denotes $p<0.001$.

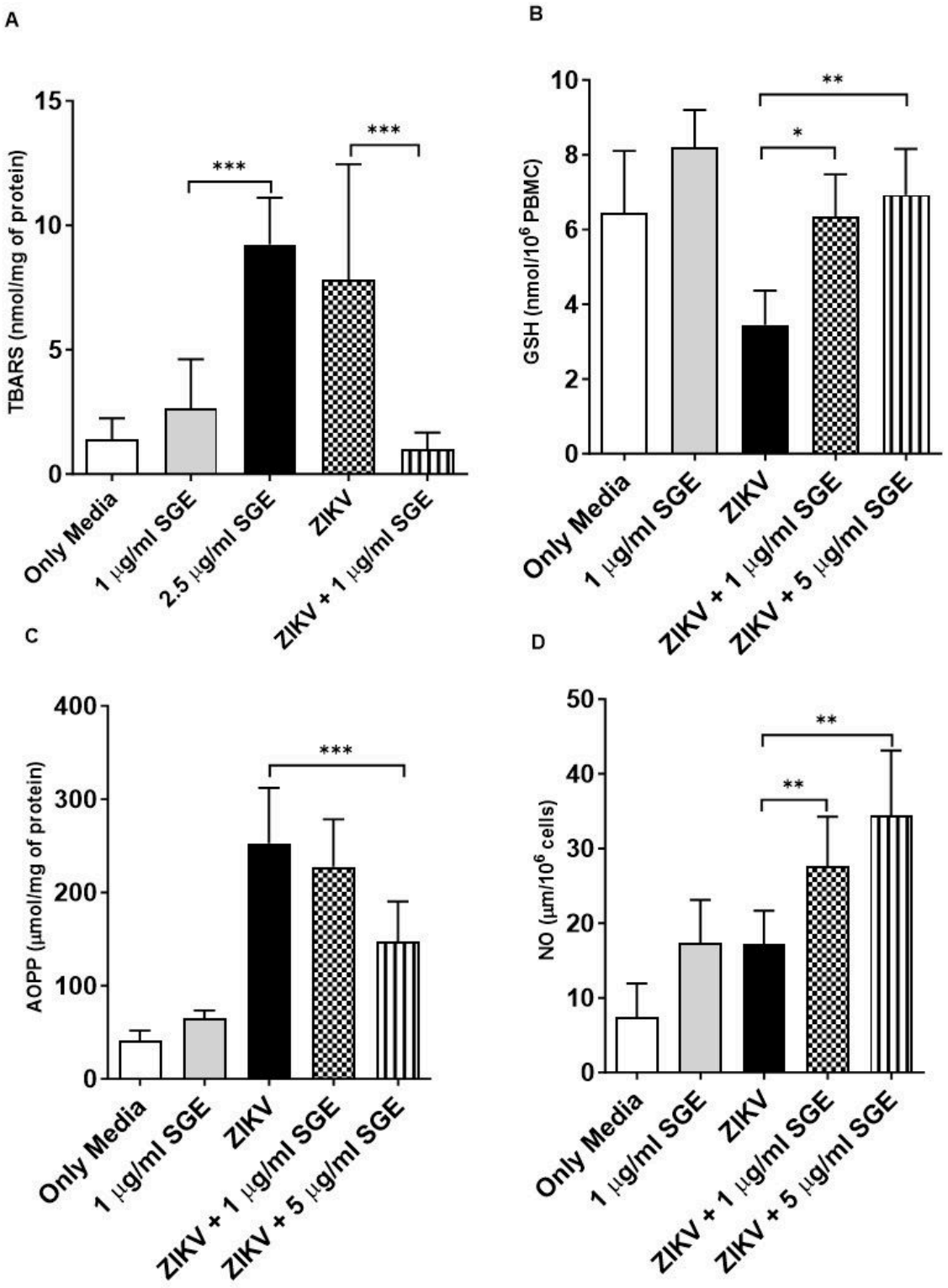

Figure 5

Redox state of PBMC stimulated with mosquito salivary gland and ZKV. The levels of lipid peroxidation TBARS (A), GSH (B), AOPP (C), and NOx (D) were evaluated after the treatments. * Denotes $p<0.05$.** Denotes $p<0.01$.*** Denotes $p<0.001$.

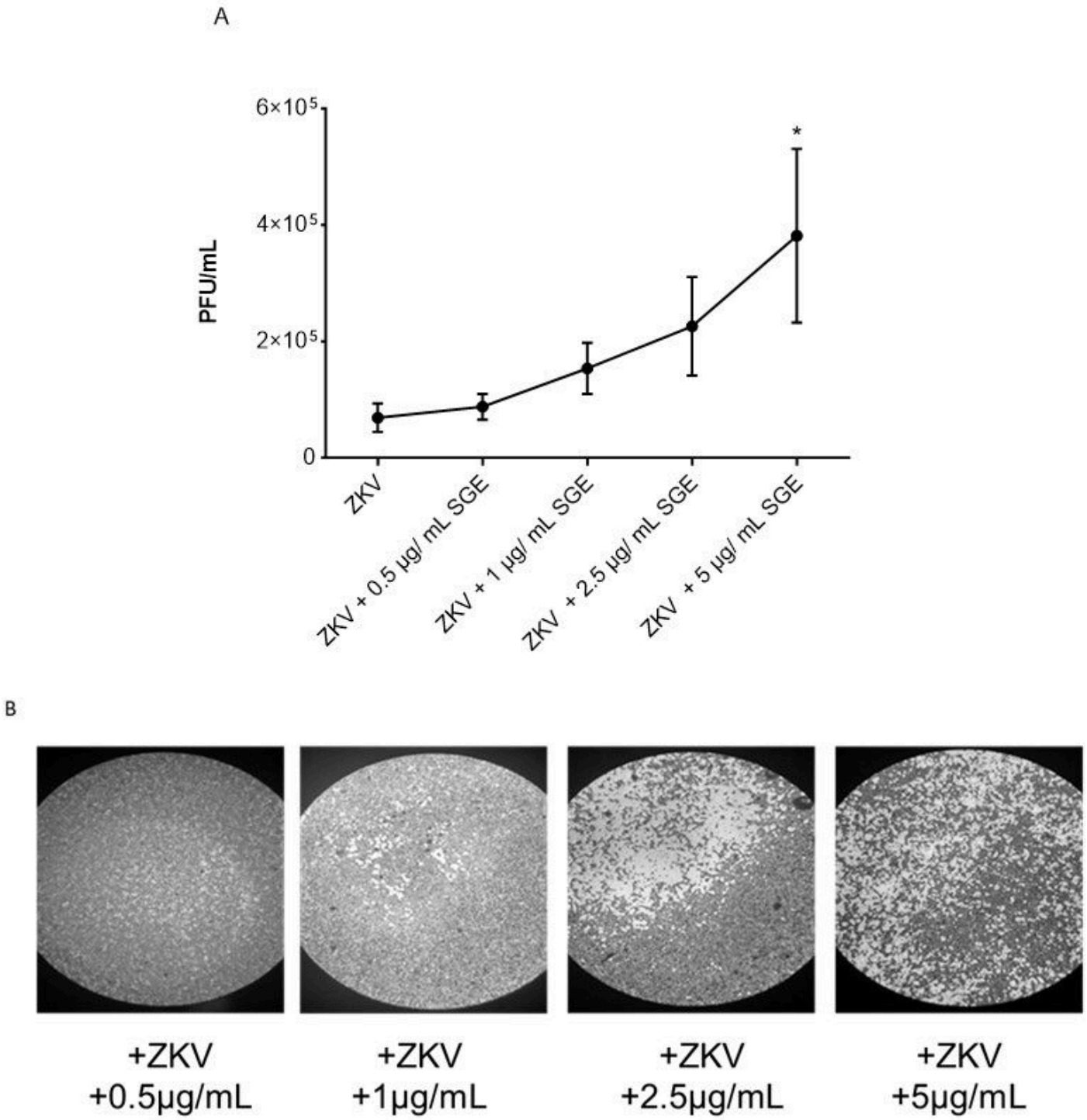

Figure 6

Effect of *Aedes aegypti* salivary gland in ZIKV replication: A) Dose depend curve of SGE (0.5 µg/ mL, 1 µg/ mL, 2.5 µg/ mL and 5 µg/ mL) from *Ae aegypti* vs PFU/ml recovery from Vero cells infected with 0.1MOI of ZIKV**. B)** Images from Vero cells infected with 0.1MOI of ZIKV in presence of 0.5 µg/ mL, 1 µg/ mL, 2.5 µg/ mL and 5 µg/ mL SGE.