BIO.04 - Linear B-cell epitopes on Zika virus´s Envelope protein: A rational approach to determination of highly specific targets against Zika virus

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Introduction: Zika virus (ZIKV) is an arbovirus and belongs to the *Flaviviridae* family, like the related dengue, yellow fever, West Nile, and Japanese encephalitis viruses. Although ZIKV usually causes an asymptomatic or mildly symptomatic disease in infected adults, it can lead to severe brain abnormalities in fetuses, who are infected *in utero* by vertical transmission of the virus through the placenta, and is associated with serious neurological complications such Guillain-Barre syndrome and meningoencephalitis. Despite this, there are no specific treatments or vaccines against ZIKV. On this context, considering that the Envelope protein of Flavivirus is essential to invasion of host cells and is the major target of neutralizing antibodies, the identification of its immunogenic regions is a crucial step to the development of immunotherapies based on monoclonal antibodies and novel vaccines.

Objective: The present study aimed to identify non-conserved B-cell epitopes on ZIKV's Envelope protein, which could be applied in novel vaccine formulations and development of monoclonal antibodies to immunotherapies against ZIKA.

Methodology: The combination of three prediction algorithms (BepiPred 1.0, BepiPred 2.0, EMINI Surface Accessibility Prediction) was used to predict linear B-cell epitopes on entire sequence of ZIKV Envelope protein. To evaluate the conservation degree of predicted epitopes, we aligned its sequences to Envelope proteins of other arbovirus and compared the similarity among them, using BioEdit Sequence Alignments Editor. Finally, for non-conserved sequences, specific epitopes were considered and synthetized as linear peptides and tested by their reactivity against samples from patients infected by ZIKV (n=21) and patients infected by Dengue virus in 2007 or 2008 (n=17), who never presented Zika episodes. Finally, to experimentally validate the predicted epitopes; we compared the magnitude of response against each epitope between ZIKV and Dengue patients.

Results: Firstly, we predicted three linear B-cell epitopes on Domain-III (E4, E5, E6) of Envelope protein, the main region target of neutralizing antibodies, two epitopes on Domain-II (E1, E3) and one in Domain-I (E2). Interestingly, epitopes located on Domain II were conserved epitopes, presenting more than 50% of similarity with Flavivirus. Based on the low conservation degree (similarity <50%), the epitopes located on Domain-III (E4, E5 and E6) and epitope located on Domain-I (E2) were selected to experimental validation. Remarkably, against all selected epitopes (E2, E4, E5 and E6), we observed responder individuals, which presented reactivity values higher than double of Dengue patients value. Moreover, the optical densities against epitopes E2, E4 and E5 were statistically higher in Zika patients than in Dengue patients ($p=0.037$, $p=0.037$ and $p=0.004$, respectively).

Conclusion: Our study allowed the identification of four promising linear B-cell epitopes on ZIKV's Envelope protein, which were non-conserved among Flavivirus and could be applied in the development of novel vaccines and monoclonal antibodies to immunotherapies against Zika virus.

Keywords: Zika virus; B-cell epitope; conservation degree