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Prediction of promiscuous T-cell epitopes in the Zika virus polyprotein: An *in silico* approach

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ABSTRACT

Objective: To predict immunogenic promiscuous T cell epitopes from the polyprotein of the Zika virus using a range of bioinformatics tools. To date, no epitope data are available for the Zika virus in the IEDB database.
Methods: We retrieved nearly 54 full length polyprotein sequences of the Zika virus from the NCBI database belonging to different outbreaks. A consensus sequence was then used to predict the promiscuous T cell epitopes that bind MHC 1 and MHC II alleles using PorPred1 and ProPred immunoinformatic algorithms respectively. The antigenicity predicted score was also calculated for each predicted epitope using the VaxiJen 2.0 tool.
Results: By using ProPred1, 23 antigenic epitopes for HLA class I and 48 antigenic epitopes for HLA class II were predicted from the consensus polyprotein sequence of Zika virus. The greatest number of MHC class I binding epitopes were projected within the NS5 (21%), followed by Envelope (17%). For MHC class II, greatest number of predicted epitopes were in NS5 (19%) followed by the Envelope, NS1 and NS2 (17% each). A variety of epitopes with good binding affinity, promiscuity and antigenicity were predicted for both the HLA classes.
Conclusion: The predicted conserved promiscuous T-cell epitopes examined in this study were reported for the first time and will contribute to the imminent design of Zika virus vaccine candidates, which will be able to induce a broad range of immune responses in a heterogeneous HLA population. However, our results can be verified and employed in future efficacious vaccine formulations only after successful experimental studies.

1. Introduction

Zika virus is a single stranded RNA virus belonged to Flaviviridae family [1]. The genome of the virus is 10 794 nucleotides long, which is translated into 3 410 amino acids [2]. The large polypeptide chain that is encoded by long and single ORF is cleaved into: Envelope, a membrane precursor, a capsid and non-structural proteins including NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B, and NS5. The envelope protein of the virus is involved in the process of fusion of the virus with the receptor of host cells and is also involved in the replication cycle of the virus. The NS5 protein has two terminals: N terminus and C terminus, the N terminus has a role in protection of RNA while the C terminus encodes RNA dependant RPA activity [3].

During 1947–2006, more than twenty cases of Zika virus infection were reported, but the research on them was not given prime importance because of its geographical spread limited to the countries in Africa and South Asia, and mild clinical signs and symptoms of the Zika virus infection [4]. After 2006, a sudden outbreak of Zika virus was reported in 2007 in the Yap Island, where 73% of the population was infected with Zika virus [5]. In 2013 a major outbreak of Zika virus was reported in the French Polynesia [6]. The infectious Zika virus then started spreading into the other islands of Pacific Ocean and in 2014 it arrived in Chile and Eastern island of Western Hemisphere [7] and in Latin America probably due to infected travellers. The virus is a mosquito borne virus, and mosquito

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plays a key role in the transmission of the Zika virus infection in humans, which is the primary host of Zika virus. The transmission of Zika virus is carried out by *Aedes* species that includes *Aedes albopictus (A. albopictus), Aedes aegypt (A. aegypt)* [8], *Aedes luteocephalus (A. luteocephalus), Aedes furcifer (A. furcifer), Aedes taylori (A. taylori), Aedes africanus (A. africanus),* and monkeys (Rhesus Macaques) [9]. The studies on the transmission of Zika virus show that the virus can be transmitted through sexual contact [10] due to its extended persistence in the semen [11], and also through blood transfusion [12]. Viral load is greater than other arboviruses and commences about ten days before the clinical manifestation of the disease [13]. The acute symptoms of Zika virus infections are arthralgia, maculopapular rash, myalgia, conjunctivitis, emesis, retro-orbital pain and headache; however, 80% of the patients are asymptomatic during the initial stages of infection. Recent reports about the outbreak of Zika virus in Brazil are linked to microcephaly and Guillain–Barre syndrome [14]. This association poses serious teratogenic and neuropathic risks to the health of fetus. A fatal Zika virus infection has also been reported that shows increased risk of disease and mortality in individual having compromised immune system [15].

The infection of Zika virus is fatal and can cause serious health threatening issues, so an antiviral vaccine or antiviral therapy needs to be designed in order to control the disease state. Antiviral therapies need to be designed by targeting enzymes that are involved in post translational packaging of viral protein [16] or by targeting enzymes that are essential for the replication of virus [17]. Development of vaccine for the treatment of Zika virus is extremely important in current situation as the virus has caused a great number of deaths in Brazil and is spreading in the other parts of world. Currently there is no prophylactic or therapeutic vaccine available in the market to curtail this infection.

Though the development of live attenuated YFV vaccine was a milestone but with the new advancements, epitope-based vaccine are gaining more importance, as the live attenuated vaccine may prove fatal in immunocompromised patients [18].

Advances in immunoinformatics research found that many conservative and highly immunogenic T/B cell epitopes (antigenic determinants that are recognized by host immune cells and can elicit both a humoral and cellular immune response) on the virus antigen could be used as potential vaccine targets. These epitopes can induce a protective immune response against a wide range of pathogenic microorganisms. After the artificial T-cell epitope is presented via the appropriate MHC molecule on the surface of the target cell to its corresponding T-cell, the epitope is recognized by T cells through TCR recognition, thereby activating the T-cell to proliferate and generate an appropriate immune response. Based on this scenario, the use of different pathogenic microorganisms and their corresponding T cell epitopes can be used to develop a CD4$^+$ T cell epitope vaccine (mostly for exogenous antigens that are degraded in the APCs after phagocytosis, thereafter binding to MHC II molecules, and finally presentation to CD4$^+$ T cells) or a CD8$^+$ T cell epitope vaccine (mostly for endogenous antigen that are digested following uptake by the APCs, and subsequent presentation to CD8$^+$ T cells via MHC-I molecules) [19].

Epitope vaccine or an epitope based subunit-vaccine has lesser side effects when compared to conventional vaccines, is easier to produce, is cheaper to manufacture, is easier to get rid of in the *in vitro* restriction cultures when compared to engineered subunit vaccines, does not contain any complete component of the pathogens, allows for the *in vitro* incorporation of sugar analogs which is difficult to achieve through engineered subunit vaccines and also takes less time to produce along with improved stability, specificity and sustainability [20]. However, due to the highly polymorphic nature of the HLA genes in the human population, the epitope specific HLA restricted vaccine is not normally expected to cause an immune response in all individuals within a given population. Thus, there is a need for the development of promiscuous epitopes that can bind to multiple HLA alleles within a heterogeneous population thereby catering to the need of a wide range of individuals [21].

The present study targets the near full length polyprotein of the Zika virus containing key structural and non-structural proteins, for prediction of promiscuous and antigenic epitopes using a range of online tools for the development of a safe and effective epitope based subunit vaccine.

2. Materials and methods

2.1. Sequence retrieval

54 Zika virus polyprotein sequences derived from 54 different genomes were retrieved from the NIAID Virus Pathogen Database and Analysis Resource (ViPR) through the web site at [22] as shown in S1 Table. The sequences were aligned and consensus sequence was generated using the multiple sequence alignment tool, Jalview [23].

2.2. Prediction of T cell epitopes

To determine the T cell epitopes, both HLA I and HLA II binding peptide sequences were required. ProPred I (www.imtech.res.in/raghava/ProPred1/) [24]was used to predict the HLA class I binding promiscuous epitopes in the consensus sequence. 4% default threshold value was selected and proteasome and immunoproteasome filters were enabled at 5% threshold value to maximize the efficiency of finding T cell epitopes. ProPred I determines epitopes that can bind to 47 HLA class I alleles. To predict epitopes for HLA class II alleles, ProPred [25] was used at a cut off value of 3% threshold. ProPred allows the prediction of antigenic epitopes for 51 HLA class II alleles.

2.3. Antigenic prediction

All the promiscuous T cell epitopes obtained from ProPred and ProPred1 tools were analysed for their antigenic properties using VaxiJen version 2.0 at [26]. Threshold value of 0.5 antigenic score was kept to filer probable non-antigenic sequences. Moreover, 87% accurate results are obtained for viruses at this default threshold. Vaxijen server performs alignment-independent prediction of protective antigens on the basis of their physicochemical properties.

2.4. Class I immunogenicity prediction

All the HLA 1 binding antigenic epitopes were scanned for MHC 1 immunogenicity using IEDB Analysis tool [27]. Default