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Prediction of promiscuous T cell epitopes in RNA dependent RNA polymerase of Chikungunya virus

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ABSTRACT

Objective: To explore RNA dependent RNA polymerase of Chikungunya virus (CHIKV) and develop T cell based epitopes with high antigenicity and good binding affinity for the human leukocyte antigen (HLA) classes as targets for epitopes based CHIKV vaccine.

Methods: In this study we downloaded 371 non-structural protein 4 protein sequences of CHIKV belonging to different regions of the world from the US National Institute of Allergy and Infectious Diseases (NIAID) virus pathogen resource database. All the sequences were aligned by using CLUSTALW software and a consensus sequence was developed by using Uni Pro U Gene Software version 1.2.1. Propred I and Propred software were used to predict HLA I and HLA II binding promiscuous epitopes from the consensus sequence of non-structural protein 4 protein. The predicted epitopes were analyzed to determine their antigenicity through Vaxijen server version 2.0. All the HLA I binding epitopes were scanned to determine their immunogenic potential through the Immune Epitope Database (IEDB). All the predicted epitopes of our study were fed to IEDB database to determine whether they had been tested earlier.

Results: Twenty two HLA class II epitopes and eight HLA class I epitopes were predicted. The promiscuous epitopes WMNMEVKII at position 486–494 and VRRLNAVLL at 331–339 were found to bind with 37 and 36 of the 51 HLA class II alleles respectively. Epitope MANRSRYQS at position 58–66 and epitopes YQSRKVENM at positions 64–72 were predicted to bind with 12 and 9 HLA II alleles with antigenicity scores of 0.754 9 and 1.013 0 respectively. Epitope YSPPINVRL was predicted to bind 18 HLA I alleles and its antigenicity score was 1.425 9 and immunogenicity score was 0.173 83. This epitope is very useful in the preparation of a universal vaccine against CHIKV infection.

Conclusions: Epitopes reported in this study showed promiscuity, antigenicity as well as good binding affinity for the HLA classes. These epitopes will provide the baseline for development of efficacious vaccine for CHIKV.

1. Introduction

Chikungunya virus (CHIKV) is an alpha virus found in tropical and subtropical African areas, in the Indian Islands of the Indian Ocean, and in south/southeast Asia. The virus was isolated first time in 1952–1953 from a febrile patient in the course of an outbreak on the Makonde Plateau located in the Tanzanian southern province [1]. The virus was named Chikungunya, which describes both virus and the resulting disease. The word "Chikungunya" is rooted from a Makonde or Swahili word Kun qunwala, which translates into "to become contorted" or "that which bends up".

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Chikungunya shares some of its symptoms with Dengue such as the onset of sudden fever often followed by joint pain. Additional mutual signs and indications consist of muscle pain, nausea, fatigue, headache and rashes [2]. The joint pain usually leads to weakness and persists for a few days or in other cases to weeks. Consequently, the viral infection may lead to acute, subacute or chronic forms of the disease. In majority of the cases, patients completely recover. However, in some situations, joint pain may extend up to many months or even years. Few cases of neurological, ocular, and cardiac and gastrointestinal complications have been recognized. Serious complications are rare, but deaths have been described in older people subsequent to the viral infection. Often infected individuals show mild symptoms and due to similar symptoms with dengue, the disease can be misdiagnosed in regions where dengue arises [2].

CHIKV belongs to the *Alphavirus* genus of the family Togaviridae [3]. Alphaviruses are enveloped viruses and their genome comprises of a positive-sense, single-stranded RNA of nearly 12 000 bp. The 5′ terminal of the genome is capped with a 7-methylguanosine while a poly adenine tail is attached at the 3′ end. The non-structural proteins are directly translated from 5′ 2/3 of the single-stranded RNA. Transcription of a part of the genome positive-strand RNA acknowledged as 26S RNA (similar to the 3′ 1/3 of the RNA) occurs through an intermediate negative-stranded RNA. This viral RNA takes part as the messenger RNA for the synthesis of all the structural proteins [4–6]. When viewed in light of the genomic organization of other alphaviruses, the CHIKV genome organization is proposed as: 5′ cap-nsP1-nsP2-nsP3-nsP4-(junction region)-C-E3-E2-6K-E1-poly(A) 3′. In this study, nsP4 (non-structural protein 4) has been focused since it contains RNA dependent RNA polymerase and hence is involved in the crucial viral replication [7].

Phylogenetic analyses have revealed the presence of three separate lineages of CHIKV strains: West Africa (*Aedes furcifer*, *Aedes taylori* and *Aedes luteocephalus*), Asia [*Aedes aegypti* (*Ae. aegypti*) and *Aedes albopictus* (*Ae. albopictus*)], and East/South/Central Africa (ESCA) (*Aedes cordellieri* and *Aedes furcifer*). Strains from India and Indian Ocean are separated into two independent sub-lineages that are possibly derived from an East African ancestral genotype [8]. Of late, the *Ae. aegypti* mosquito species was believed as the chief urban vector of the CHIKV. However, during the 2005–2006 outbreak in Réunion Island, the *Ae. albopictus* mosquito was found to be majorly involved in Chikungunya transmission. Intercontinental travel and worldwide expansion of the two Chikungunya urban arthropod vectors (*Ae. albopictus* and *Ae. aegypti*) have facilitated the various virus strains to expand to novel areas where ecological conditions are favorable for viral spread [9].

Recently, a synthetic DNA vaccine based on the component of CHIKV envelope glycoprotein was able to generate protective immune responses in mouse as well as non-human primate models [10]. However, there is no licensed vaccine or approved antiviral drug in the market against CHIKV infection. Moreover, the various virus strains have not been characterized and laboratory studies are not sufficiently available. Hence, limited knowledge is present in the literature against the viral infection that is spreading globally at an increasing pace.

Vaccinomics is an innovative and unique strategy that integrates immunogenomics and immunogenetics with bioinformatics [11]. Some *in-vitro* culture experiments show that some antigens fail to express and may not be remarkable candidates for the development of vaccines, however using *in-silico* approach for determining antigens is gaining much importance because of the advancement of protein sequence, human leukocyte antigen (HLA) and pathogen sequencing databases [12]. The advancement in bioinformatics and vaccinomics has led to the development of effective vaccines against tumors, malaria, and multiple sclerosis [13–15]. Realizing the pathogenicity and health problems caused by CHIKV there is a requirement of a conserved epitope-based vaccine that can initiate an inflammatory response to fight infection. Our study focuses on the development and identification of effective T cell-based HLA I and HLA II epitopes that were predicted to be antigenic and immunogenic. Our study also highlights the important common epitopes in HLA I and HLA II.

In some cases, the immune responses triggered by the natural infection are not adequate and need to be further improved. Hence, components of the pathogens are isolated and incorporated in vaccine to achieve the desired response [16]. T-cell binding epitopes are identified accordingly and different strategies are employed later to deliver the antigenic components. Epitope based vaccines are safe, can be rationally designed to increase potency value and breadth, and can be directed to focus immune responses against specific antigenic sequences. Epitope vaccine has lesser side effects when compared to conventional vaccines, is cheap, is easier to produce, is easier to be removed from the *in vitro* restriction cultures, and does not contain the whole pathogen (which can be harmful). Moreover, *in vitro* incorporation of sugar analogs can be achieved and it takes less time to produce along with improved specificity, stability and sustainability. However, due to the diversity and polymorphism of *HLA* genes in humans, the epitope based HLA restricted vaccine needs to be checked to determine whether it has the ability to cause immune responses in all the individuals of a given population or not [17]. Hence, there exists a need to identify promiscuous HLA epitopes that have the ability to bind multiple alleles within a diverse population and thus cater to their requirements.

The present study targets the polymerase protein of CHIKV, for prediction of antigenic and promiscuous epitopes. Different online tools were used for the future development of an effective and safe epitope based vaccine.

2. Materials and methods

2.1. Sequence retrieval

A total of 371 nsP4 protein sequences of CHIKV belonging to various areas of the world were downloaded from the US National Institute of Allergy and Infectious Diseases (NIAID) Virus Pathogen Resource database [18]. Multiple sequence alignment was generated by using default settings of CLUSTALW at http://www.genome.jp/tools/clustalw/ [19]. The aligned sequences were visualized and consensus sequence was generated from the Uni Pro U Gene Software version 1.2.1 using default settings [20].

2.2. Prediction of T cell binding 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/) was used to predict the HLA class I binding promiscuous epitopes in the consensus sequence [21]. Default threshold was selected and both proteasome and immunoproteasome filters were kept on at 5% value to maximize the efficiency of finding T cell epitopes. ProPred I determines epitopes that can bind to 47 HLA class I alleles. ProPred (http://www.imtech.res.in/raghava/propred/) was used at default conditions to predict peptides that can bind HLA class II alleles [22]. ProPred allows the prediction of promiscuous epitopes for 51 HLA class II alleles.

2.3. Antigenicity analysis

The predicted epitopes were analyzed to determine their antigenicity through Vaxijen server version 2.0 [23]. Threshold was kept at 0.4 to filter probable non-antigenic sequences. VaxiJen performs the prediction of protective antigens on the basis of amino acid properties without resorting to sequence alignment. Accuracy of VaxiJen ranges from 70% to 89%, hence it is an important tool in reverse vaccinology.

2.4. MHC I immunogenicity prediction

All the HLA I binding epitopes were scanned to determine their immunogenic potential through the Immune Epitope Database (IEDB) [24]. The IEDB tool used was only validated for 9-mer peptide which was provided by Propred I.

2.5. Validation of predicted epitopes

All the predicted epitopes of our study were fed to IEDB database to determine whether they had been tested earlier. The IEDB database contains experimental data regarding T cell epitopes and antibody in humans, non-human primates and various other animal species.

3. Results

3.1. Prediction of HLA class II binding T cell epitopes

By using ProPred and other tools, 22 antigenic epitopes for HLA class II were predicted from the consensus sequence of CHIKV RNA Polymerase (Table 1). Since epitopes were derived from the consensus sequence, hence they are mostly conserved across all the strains of CHIKV. Notably, the promiscuous epitopes WMNMEVKII at position 486–494 and VRRLNAVLL at 331–339 were found to bind to 37 and 36 of the 51 HLA class II alleles respectively.

Epitope MANRSRYQS at position 58–66 was predicted to bind 12 HLA II alleles and its antigenicity score as analyzed from Vaxijen was 0.754 9. Epitopes YQSRKVENM was predicted to bind nine HLA II alleles while its antigenicity was projected to be comparatively high, *i.e.*, 1.01 3. Similarly, VENMKAAII was predicted to bind alleles while antigenic score was found to be 0.619 3. Epitopes IIQRLKRGC and IQRLKRGCR were predicted to bind 17 and 13 HLA II alleles and their antigenic score was found to be related, *i.e.*, 1.632 4 and 1.672 3 respectively. Other notable epitopes having significant results were also obtained. For example, YRTTYPAPV at position 98–106 was found to bind 13 HLA II alleles while its antigenicity value was analyzed to be 1.032 9. Epitope VNTLLNITI at 438–446 position was indicated to bind 14 HLA II alleles and its antigenicity value was found to be 0.774 4. Likewise, epitopes LLNITIASR and VVSLKAPYF were predicted to bind 10 and 19 alleles, their antigenic score being 2.387 2 and 1.070 5 respectively. Epitope IRWQRTGLI spanning position 555–563 was predicted to bind 19 alleles and its Vaxijen score was found be quite high (1.774 8). Comparatively, another epitope VQGISVVVM was proposed to bind 34 HLA II alleles while its antigenic score was found to be 0.662 0.

Table 1
T-cell class II MHC-specific predicted epitopes of the Chikungunya virus polymerase protein, their number of alleles and antigenicity prediction score.

Position	Epitope sequence	No. of HLA II binding alleles	Antigenicity score
58–66	MANRSRYQS	12	0.754 9
64–72	YQSRKVENM	9	1.013 0
69–77	VENMKAAII	7	0.619 3
76–84	IIQRLKRGC	17	1.632 4
77–85	IQRLKRGCR	13	1.672 3
98–106	YRTTYPAPV	13	1.032 9
111–119	INVRLSNPE	5	0.953 4
199–207	LAAATKRNC	5	1.213 8
290–298	MKRDVKVTP	8	1.828 9
330–338	LVRRLNAVL	9	0.506 8
331–339	VRRLNAVLL	36	0.872 0
422–430	FKFGAMMKS	5	0.942 9
437–445	FVNTLLNIT	5	0.635 0
438–446	VNTLLNITI	14	0.774 4
441–449	LLNITIASR	10	2.387 2
486–494	WMNMEVKII	37	1.165 0
497–505	VVSLKAPYF	19	1.070 5
498–506	VSLKAPYFC	7	1.503 5
510–518	ILHDTVTGT	8	1.125 1
555–563	IRWQRTGLI	19	1.774 8
571–579	YSRYEVQGI	9	1.264 1
576–584	VQGISVVVM	34	0.662 0

3.2. Prediction of HLA class I binding T cell epitopes

By using ProPred1 and other tools, eight antigenic and immune-provoking epitopes for HLA class I were finally identified from the consensus polymerase sequence of CHIKV (Table 2). Notably, the promiscuous epitope YSPPINVRL was predicted to bind 18 HLA I alleles.

Table 2
MHC class I-specific predicted T cell epitopes of the Chikungunya virus polymerase protein, their number of binding alleles, antigenicity score and immunogenicity evaluation.

Position	Epitope sequence	No. of HLA I binding alleles	Antigenicity score	Immunogenicity score
107–115	YSPPINVRL	18	1.425 9	0.173 83
262–270	GPKAAALFA	8	0.645 0	0.103 34
330–338	LVRRLNAVL	8	0.506 8	0.109 13
331–339	VRRLNAVLL	8	0.872 0	0.064 53
380–388	DDSLALTAL	6	0.898 1	0.018 42
392–400	EDLGVDHSL	7	0.524 4	0.022 22
558–566	QRTGLIDEL	7	0.519 9	0.238 40
492–500	KIIDAVVSL	17	0.412 8	0.080 66

Epitope YSPPINVRL as already discussed, was predicted to bind 18 HLA I alleles and its antigenicity score (1.425 9) was calculated to be quite significant. Moreover, its immunogenicity score (0.173 83) was also found to be high. Hence, this epitope can be quite useful in the preparation of a universal vaccine against CHIKV infection. Epitopes GPKAAALFA, LVRRLNAVL and VRRLNAVLL were all predicted to bind eight HLA I alleles, with antigenicity scores 0.645 0, 0.506 8 and 0.872 0 respectively. Similarly, epitopes EDLGVDHSL and QRTGLIDEL were predicted to bind seven HLA I alleles, their antigenic score being 0.524 4 and 0.519 9 respectively. Likewise, KIIDAVVSL at position 492–500 was predicted to bind a significant number (17) of HLA I alleles and its antigenicity as found by Vaxijen was 0.412 8.

4. Discussion

Dispersal pattern of the spread of CHIKV infection has been reported from Africa to Europe as well as the Indian Ocean. There are chances that the disease will further spread in the area if proper measures are not taken to prevent or control it. Moreover, no FDA approved drug or license vaccine is available against the viral infection that is spreading with an increased pace. Paracetamol/acetaminophen and non-steroidal anti-inflammatory drugs are currently in use to control the symptoms and provide temporary relief to the patients [25].

In silico prediction of T cell epitopes has been performed for the envelope proteins of CHIKV by other groups [26,27]. Notably, both structural and non-structural proteins have been targeted by Kori *et al* to find the antigenic epitopes that could be incorporated in vaccine formulation [28].

A variety of online immunoinformatic tools are available to aid in the search for HLA binding epitopes. In the present study, all the available sequences of the nsP4 protein of CHIKV have been analyzed to find the epitopes with good binding affinity, promuscousity and antigenicity. Most of the previous studies were based on a single or a few viral strains. nsP4 which is proposes to be the RNA dependent RNA polymerase was chosen because it is crucial for the replication of the virus and is therefore an excellent target for vaccine formulations. For this study, all the CHIKV nsP4 sequences belonging to different outbreaks of CHIKV infection have been looked into in detail through alignment and other *in silico* approaches. A consensus sequence was drawn and T cell epitopes were chosen to ultimately find the antigenic sequences in nsP4 that are mostly conserved across all the strains of CHIKV. In the study, a larger number of epitopes were projected for HLA class II as compared to HLA class I. The epitopes are proposed to be incorporated in a vaccine to meet the demands of diverse and heterogeneous human populations. Specifically, epitopes LVRRLNAVL and VRRLNAVLL have been predicted to bind both HLA class I and HLA class II alleles and thus should be considered for further experimental studies. Epitope LVRRLNAVL at position 330–338 has been predicted to bind nine HLA II and eight HLA I alleles respectively while its antigenicity and immunogenicity scores were found to be significant, *i.e.*, 0.506 8 and 0.109 13, respectively. The results of second epitope VRRLNAVLL at position 331–339 was found to be more significant comparatively. VRRLNAVLL was found to bind 36 HLA II and 8 HLA I alleles, and its antigenicity and immunogenicity score was found to be quite high, *i.e.*, 0.872 0 and 0.064 53 respectively.

Advancements in genomics and proteomics have enabled researchers to understand the evolutionary and epidemiological aspects of the emerging pathogens. Tong *et al* indicated that selective pressure operates and plays an important part in CHIKV evolution [29]. CHIKV was also shown to undergo positive selection particularly due to the host-driven immune pressure. Particularly, in response to immune pressure, specific mutations were observed leading to the loss of HLA I restricting elements. However, more such studies are needed to apprehend the in depth evolutionary aspects of the virus and to analyze the influence of host immune responses on pathogen evolution.

One limitation of the present study is the unavailability of *in vivo* as well as *in vitro* experimental studies to practically establish whether these antigenic and immune-provoking epitopes will elicit any immune response. Since all the epitopes have been predicted by *in silico* approaches, hence the determination of the actual immunogenicity, robustness, effectiveness and stability of the peptides needs to be performed by further wet lab studies. In the future, more data regarding T and B cell responses in the host is likely to be available which will ultimately help the researchers in focusing on specific aspects of the antiviral immunity. Development of robust diagnostic procedures, therapeutics, and vaccines against viral infections is augmented by the description and annotation of antigenic regions within the viral proteins. Our group has focused on MHC class I and II derived T cell epitopes for CHIKV nsP4 protein sequence. Prediction of epitope immunogenicity and characterization on the basis of peptide sequences is a significant milestone in developing a potent peptide vaccine for EBOV. In the future, our team would like to address other CHIKV proteins responsible for the invasion and pathogenicity of the virus. Moreover, effects of mutations of these epitopes and their implications in the future design of vaccines will be performed to gain an in-depth understanding of the virus that is spreading globally at an alarming pace.

Conflict of interest statement

The authors declare no conflict of interests.

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