In Silico Approach To Design a B-cell Epitope Based Vaccine Target Against Yellow Fever Virus

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Yellow fever virus is a prototype member of the Flaviviridae family causing high fever and jaundice. Though YF 17D vaccine is administered to yellow fever patients, however it can produce adverse effects in immunocompromised, older people and young infants. The aim of this study is to design an epitope-based peptide vaccine by targeting envelope (E) protein of Yellow Fever Virus. Thirty sequences of E protein of Yellow Fever Virus strains were retrieved from NCBI database. E protein was found to be mostly conserved among all the sequences with little variability and also was identified as a probable antigen. Different epitope prediction tools predicted 4 common epitopes, 3 of which were found to be antigenic. A peptide VKNPTDTGin E protein was predicted to have surface accessibility which overlaps with the VKNPTDTGHGT epitope. So, the whole VKNPTDTGHGT epitope was taken for further analysis. The VKNPTDTGHGT epitope showed 96.67% conservancy and also possesses flexibility, hydrophilicity and non-toxicity. Therefore, VKNPTDTGHGT can be regarded as a potential vaccine candidate against Yellow fever virus with further in vitro and in vivo validation.

Key words: Yellow fever virus, epitopes, peptide vaccine

Introduction

Yellow fever virus (YFV) belongs to the family Flaviviridae, genus Flavivirus. It is an arthropod-borne virus that can be transmitted by vectors such as ticks or mosquitoes, especially by *Aedes aegypti* mosquitoes in humans. Yellow fever is an acute multisystem disease causing hepato-renal failure, profound jaundice and a bleeding diathesis. The mortality rate of yellow fever is even higher than dengue causing death in 20-50% of these cases1. There are 200,000 cases reported annually, which includes 30,000 deaths2. Yellow fever virus originated in Africa and 150 yellow fever outbreaks in 26 African countries were reported to World Health Organization (WHO) in between 1980 and 2012. Though yellow fever was not reported in Asia earlier, in 2016, 10 confirmed patients of yellow fever were detected in China who travelled from Angola, Africa. The abundance of primary mosquito vector *Aedes aegypti*, 20,000 Chinese workers along with many Indians and other Asians currently working in Angola, air travel through continents are conducive to the yellow fever introduction into Asia. This is also supported by the evidence that after a chikungunya virus outbreak in 2004 in Kenya which spreads via the same mosquito vector, India and Southeast Asia experienced an explosive epidemic of chikungunya transmitted to these regions via the Indian ocean basin1.

The YFV genome is single stranded, positive sense RNA encoding for a polyprotein which undergoes further processing in the endoplasmic reticulum and is broken down into three structural proteins (capsid, pM and E) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The E protein is the most studied one due to its high antigenicity. It is crucial for various events such as viral attachment with host, penetration, fusion, host range and cell tropism3.

There is no clinically available antiviral drug against yellow fever virus, as a result prevention mainly depends on vaccination. Currently, a live attenuated vaccine known as 17D is in the market for yellow fever virus. But it can produce adverse effects in immunocompromised, older people and young infants. The aim of this study is to design an epitope-based peptide vaccine by targeting envelope (E) protein of Yellow Fever Virus. Thirty sequences of E protein of Yellow Fever Virus strains were retrieved from NCBI database. E protein was found to be mostly conserved among all the sequences with little variability and also was identified as a probable antigen. Different epitope prediction tools predicted 4 common epitopes, 3 of which were found to be antigenic. A peptide VKNPTDTG in E protein was predicted to have surface accessibility which overlaps with the VKNPTDTGHGT epitope. So, the whole VKNPTDTGHGT epitope was taken for further analysis. The VKNPTDTGHGT epitope showed 96.67% conservancy and also possesses flexibility, hydrophilicity and non-toxicity. Therefore, VKNPTDTGHGT can be regarded as a potential vaccine candidate against Yellow fever virus with further in vitro and in vivo validation.

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vaccines against particular pathogens. This has been in turn facilitated by the availability of huge sequence data. The process will reduce the workload of wet lab studies by narrowing down the number of epitopes to be tested in vitro and in vivo, thus reducing time also. In this study, we predicted one B-cell peptide epitope in E protein of yellow fever virus using various bioinformatic tools that may serve as a universal vaccine against all yellow fever virus strains.

Materials and Methods

Protein Sequence retrieval

Thirty sequences of E protein of Yellow Fever Virus were retrieved from NCBI database and downloaded in the FASTA format. The wild type isolates were found from various geographical locations and the size of E protein was found to be 493 amino acid residues.

Variability analysis of E protein

To find out the degree of conservation, all the sequences were aligned by EBI-Clustal Omega program and multiple sequence alignment of the retrieved sequences was generated. Jalview was used to visualize the MSA and their absolute site variability was calculated by Shannon entropy analysis by using Protein Variability server (PVS). Several variability metrics are utilized by PVS to calculate the sequence variability in a multiple sequence alignment.

Prediction of antigenicity

In order to develop an epitope-based vaccine, it is indispensable to check the antigenicity of E protein that would elicit an immune response. A reference E protein sequence with an accession number AAA92692.1 was tested for its antigenicity. The antigenicity was checked by using Vaxijen v2.0 server and Kolaskar&Tongaonkar method. Vaxijen is the first server for alignment-independent prediction of protective antigens. It was developed to allow antigen classification solely based on the physicochemical properties of proteins without recourse to sequence alignment.

Linear B cell prediction

B cell epitope predictions can be done with various servers each having their own strengths and weaknesses. Therefore, two most popular epitope prediction tools were used such as BepiPred 1.0 and BCPREDS to minimize false positive predictions. BepiPred 1.0 server uses the Hidden Markov Model to predict the epitope along with the propensity scale, with a threshold for the prediction set at 0.35 for viruses. BCPREDS was used to predict non-overlapping and fixed length epitopes of 12 amino acids with a specificity of 75%. The predicted epitopes from both servers were compared manually and the common epitopes were taken for further analysis.

Conservancy analysis of B cell epitope

Conservancy of the epitopes was analyzed by the IEDB conservancy analysis tool. For calculating the conservancy score the sequence identity threshold was kept at 80%.

Surface accessible regions prediction

In order to determine the surface accessible regions of E protein, Emini surface accessibility prediction tool of IEDB was used. The accessible regions were later compared to the conserved B cell epitopes. Common peptides from both of the servers were selected for further investigation.

Prediction of flexibility and hydrophilicity of B cell epitopes

The conserved epitopes were subjected to Karplus and Schulz (KS) flexibility prediction, and Parker hydrophilicity prediction tools for flexibility and hydrophilicity analyses respectively. For predicting protein flexibility, the KS method uses normalized B-values of C±-atoms in protein structures. Due to its robustness, it has been widely used for the analysis of protein flexibility.

Prediction of toxicity of the B-cell epitopes

An ideal epitope should have no or less toxicity. Hence, to determine the toxicity of the selected B-cell epitopes, Toxin Pred web server was used.

Prediction of the tertiary structure of E protein using in silico approach

The tertiary structure of the E protein was predicted using SWISS MODEL server. Template selection, target template alignment, model building and evaluation are the steps of this homology-modeling method. The model was then evaluated by ROCHECK software which constructed Ramachandran plot for the E protein. Ramachandran plot analyzes residue by residue geometry and overall structure geometry, thus assesses the stereochemical quality of the 3-D structure. For further quality evaluation of the 3-D structure, ProSA web tool was used which provides a Z-score measuring the quality of the 3-D model.

Validation of the workflow

Since the work flow used here includes various computational tools developed by different platforms, so the validation of the workflow was required. A conserved B-cell epitope, SVQYHPL, was identified from the envelope protein of the reticuloendotheliosis virus through wet lab screening. The sequence of that envelope protein was retrieved from the NCBI data base and fed into the work flow to check whether the work flow can identify and qualify that as a B-cell epitope.

Results

E protein is well conserved in all pathogenic Yellow Fever virus strains

In order to determine the conserved region, multiple sequence alignment (MSA) using Clustal Omega and Protein variability analysis were performed. MSA (Figure 1) and protein variability index (Figure 2) of these proteins identified that amino acids positioned from 108 to 445 are conserved among all the sequences.
E protein is antigenic

In order to become a vaccine candidate, a protein sequence must be antigenic to mount an immune response. The antigenicity of E protein sequence with accession number AAA92692.1 was found to be 0.5794 by using Vaxijen v2.0 server. A threshold of 0.4 was taken as a default for viruses. Kolaskar&Tongaonkar antigenicity prediction tool predicted 341 amino acids to be above the threshold of 1.0. The maximum and minimum scores were 1.238 (at position 62) and 0.830 (at position 470) respectively with an average of 1.028. The window size was kept at 7 with a central position of 4 (Figure 3).

Four epitopes are found to be common by all the prediction tools employed

Two widely used epitope prediction tools Bepipred and BCPREDS were used for epitope prediction. Bepipred predicted ten epitopes with a size range taken to be equal or above six amino acids, whereas BCPREDS predicted 14 epitopes, each having a length of twelve amino acids. Four common epitopes were found between them and their antigenicity was checked by Vaxijen v2.0 server with a threshold 0.4 (Table 1). The positions of these epitopes in the E protein are: 146-154; 249-255; 263-271; 307-317.

One B-cell epitope is highly conserved among the E protein sequences of Yellow Fever Virus

Conservancy is a percentile measure of our epitope’s availability in all the retrieved sequences. Among four common predicted epitopes, three were antigenic and one was not predicted to have antigenicity by Vaxijen v2.0 server. So only those three antigenic epitopes, GANQENWNA, TKDTNGS and VKNPTDTGHGT were analyzed for conservancy by IEDB conservancy analysis tool. Among those three, only one epitope was found to be conserved with an identity of 96.67%. It is 100% conserved in 29 out of 30 sequences.(Table 2)
Eleven surface accessible peptides are predicted

Emini surface accessibility prediction tool\textsuperscript{22} was used to determine the surface accessible peptides within the E protein above a threshold cut off 1.0 (Figure 4). Eleven such peptides above the threshold were detected and compared with the predicted epitopes (Table 3). The maximum and minimum scores were 5.814 (at position 95) and 0.065 (at position 480) respectively. The window size was kept at 6 with a central position of 3. Among these eleven peptides VKNPTDTG overlaps with the BepiPred and BCPREDS predicted consensus and conserved epitope VKNPTDTGHGT. As a result, the whole VKNPTDTGHGT epitope was taken for further analysis.

\textit{VKNPTDTGHGT} is flexible and hydrophilic

Flexibility and hydrophilicity are the properties of being an ideal epitope. VKNPTDTGHGT was found to be highly flexible by Karplus and Schulz prediction analysis\textsuperscript{23}(Fig 5). It was also found to be hydrophilic in nature by IEDB Parker hydrophilicity analysis\textsuperscript{24}(Fig 6). The maximum and minimum scores were 1.067 and 1.045 for flexibility prediction and 5.843 and 4.5 for hydrophilicity prediction respectively. The window size was kept at 7 with a central position of 4 for both of the analyses. Hence, VKNPTDTGHGT epitope fulfills all the main properties of being an ideal epitope candidate.
VKNPTDTGHGT is non-toxic
The toxicity of the VKNPTDTGHGT epitope was analyzed by the ToxinPred server\textsuperscript{29} and was found to be non-toxic to cell proving its potential as a candidate vaccine.

Tertiary structure of E protein was predicted and validated
As the full experimental tertiary structure of the E protein of Yellow fever virus is not available, in this study a tertiary structure was modelled by homology modelling\textsuperscript{30}. Two models were generated by SWISS MODEL server. Among those the best model was selected based on GMQE and QMEAN score, for which the template was 4fg0.1 (Fig 7). When the model was validated by Ramachandran plot generated by PROCHECK software, it was found that 87.2\% residues were in the favourable region and the average G-factor was -0.1 (Fig 8A). Z-score by ProSA was calculated to check whether the input structure is within the range of scores typically found for a native protein of similar size\textsuperscript{33}. Predicted Z-score was -8.66 which indicated the overall good quality of the model (Fig 8B). Along with the analysis of surface accessibility, flexibility and hydrophilicity, teritary structure also predicted that the VKNPTDTGHGT epitope is present on the surface of the E protein of Yellow fever virus (marked as blue colour in the 3D model).

Designed workflow concords with the experimental results
The workflow, designed to predict epitopes, used here was validated by positive controls. Xue et al., in 2012, mapped a linear B-cell epitope, SVQYHPL, located in the envelope protein of Reticuloendotheliosis virus\textsuperscript{34}. When the sequence of the envelope protein was fed into the workflow for the B-cell epitope, the same B-cell epitope, SVQYHPL, was successfully identified.


table

| No. | Peptide       | Length | Position in the E protein |
|-----|---------------|--------|--------------------------|
| 1   | APDKPS        | 6      | 35-40                    |
| 2   | HLAENNEG      | 8      | 81-88                    |
| 3   | ACKRTYSDRG    | 10     | 91-100                   |
| 4   | VDQTKI        | 6      | 130-135                  |
| 5   | ANQENW        | 6      | 147-152                  |
| 6   | GSQEAEE       | 6      | 166-171                  |
| 7   | GNQEGS        | 6      | 248-253                  |
| 8   | RVTKDNTGNSLY  | 12     | 263-274                  |
| 9   | VKNPTDTG      | 8      | 307-314                  |
| 10  | GDSRLTYWQKHKG | 13     | 381-393                  |
| 11  | QTMKGA        | 6      | 402-407                  |

Figure 4. Surface accessibility of the E protein. The threshold was kept at 1.0 indicated by the red line. The surface accessible residues are indicated by the yellow regions above the threshold cutoff.

Table 3. Predicted surface accessible antigenic sites by using Emini surface accessibility prediction analysis

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Figure 5. Flexibility of VKNPTDTGHGT epitope. Most of the residues of the desired VKNPTDTGHGT epitope were found to be flexible in nature (in the yellow colored region). The residues which are below the cut off 1.058 (red line) are in the green region.

Figure 6. Hydrophilicity of VKNPTDTGHGT epitope. Most of the residues of the desired VKNPTDTGHGT epitope were found to be hydrophilic in nature (in the yellow colored region). The residues which are below the cut off 5.280 (red line) are in the green region.

Figure 7. 3D structure of the E protein. Predicted conserved VKNPTDTGHGT epitope was mapped on the E protein using SWISS-PdbViewer\textsuperscript{47}. The blue coloured peptide region of the 3D model represents the conserved VKNPTDTGHGT epitope.
Discussion

Yellow fever has been a major threat to human health since 18th Century with repeated epidemics and dispersion from the endemic areas to coastal towns and cities. In 2016 and 2017, 576 confirmed cases of yellow fever including 184 deaths were reported in Brazil. In 2017 and 2018, the number of confirmed cases increased to 723 with 237 deaths in the same region. Though effective 17D and 17DD vaccines prevail against yellow fever viruses, those showed fatal side effects in some cases. The lethality of the adverse events of 17D vaccine associated viscerotropic disease is higher than that of the wild-type yellow fever. Moreover, the vaccine is not recommended for pregnant women, infants and immunocompromised people.

In the U.S an inactivated 17D vaccine was produced and tested clinically but failed to go for commercialization. Two non-viral DNA based antigens were also evaluated as vaccine candidates and revealed potency but further development studies are required. Considering these factors, the idea of designing an alternative vaccine for yellow fever virus to replace or complement the existing live attenuated one can be pursued.

With the ever-progressing knowledge of antigen recognition by antibodies at the molecular level, a new window for vaccine designing and development has opened, which overcomes the drawbacks of the traditional vaccine development processes. Though several T-cell epitope regions have been discovered in E protein, however no B-cell epitope has been reported so far. In this study, we aimed to design potential B-cell epitopes which can be used universally against all the strains of yellow fever viruses. The main benefit of immunization with an epitope-based vaccine is its ability to immunize with a minimal structure that is able to stimulate an effective specific immune response, while avoiding potential undesirable effects.

The first challenge to design an epitope-based vaccine is to confirm the conservancy of the candidate protein and find out the low variability regions in the sequences. In addition to that, the envelope protein has a tendency to undergo frequent mutations to evade host defense. Hence, we determined the conservancy of E protein by MSA and protein variability server that identified almost 70% residues of E protein to be conserved. We also found 70% of the amino acids in E protein are antigenic. For predicting B-cell epitopes, we used two B-cell epitope prediction tools to

Figure 8. Ramachandran plot along with statistics showing residues in the most favorable, additionally allowed, generously allowed and disallowed regions (A). Z-score for quality of the 3D structure E protein by ProSA (B).
avoid false positive results. This is because, though several B-cell epitope prediction methods are available, their efficacy to identify epitopes may far from ideal\textsuperscript{43}. Keeping this in mind, we only considered four common epitopes predicted by both of the servers, but only three of them proved to be antigenic in further evaluation. When conservancy was checked for the individual epitopes, it was revealed that only VKNPTDTGHGT epitope among the three is well conserved among the strains of yellow fever virus. To allow an antibody to bind with an epitope and induce immune response, the epitope should be accessible for the antibody\textsuperscript{44}. We identified 11 surface accessible peptides in E protein, among which the surface accessible VKNPTDTG peptide and the previously predicted epitope VKNPTDTGHGT were found to be located in the same region of the E protein. So, the whole VKNPTDTGHGT peptide was taken for consideration. The three-dimensional validated structure of E protein also showed that the epitope is present on the surface of the protein, hence it should be more accessible. This epitope also revealed to have good flexibility and hydrophilicity which strengthens its potency as a good vaccine candidate as flexibility and hydrophilicity are two characteristic features of an ideal epitope\textsuperscript{45}. An added value of the VKNPTDTGHGT epitope as a vaccine is; it is predicted to be a non-toxic peptide, thus having no possibility to generate adverse reactions inside the body.

All the data retrieved from our analysis hold promises to use the VKNPTDTGHGT epitope as a universal vaccine against yellow fever virus. Adjuvants might be added to increase its immunogenicity and stability\textsuperscript{46}. As this vaccine has been designed by \textit{in silico} analyses, the actual immunogenicity, efficacy, stability and delivery strategies in humans should be checked by \textit{in vitro} and \textit{in vivo} studies.

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