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Research article

In-silico epitope based vaccine an excellent solution against Marburg virus

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Abstract

Marburg virus is the most deadly virus identified in the year 1967. MARV comprises a 19-KB noninfectious single-stranded RNA genome that encodes seven basic structural proteins. Right now; there is no accessible treatment or vaccine to cure the MARV disease since couples of immunizations are under clinical trial studies. Here the computational approach is used to predict multi-epitope vaccine candidates against this virus. Both T cell and B cell were checked for the peptides to confirm that they can bring both humoral and cell-mediated immunity. A 9mer T cell epitope ALSLTCAGI interacted with most of the MHC-I alleles and 10mer B cell epitope SLFVQAALYV was identified on antigenicity prediction. The regions; 7 – 12, 139 – 144 and 186 – 191 amino acid residues were more accessible as B cell epitopes. The predicted epitope docked with the human HLA:*A0201. On the basis of the computational analysis, the predicted epitope can be the best accessible solution to cure infections of Marburg virus.

Introduction

The family Filoviridae incorporates two known genera, Ebola virus, and Marburg virus. The genus Ebola virus incorporates five species, while the Marburg virus genus incorporates single species: *Marburg* virus, which has two individuals: Marburg virus (MARV) and Ravn virus (RAVV) [1]. About Forty years ago, in August 1967, the first filovirus ever identified as the Marburg virus appeared in Europe, bringing about extreme and deadly hemorrhagic fever among the laborers of laboratory settings in Marburg and Frankfurt [2, 3]. Marburg hemorrhagic fever (MHF) is described by systemic viral replication, strange inflammatory reactions, and immunosuppression, including various systemic dysfunctions; hemorrhages, multi-organ failure, coagulation variations, edema, and shock, that often results in death [4].

MARV comprises a 19-KB noninfectious single-stranded RNA genome that encodes seven basic structural proteins: Nucleoprotein (NP), Virion protein 24 (VP24),VP35, VP30, VP40, Glycoprotein (GP) and Polymerase L protein (L) [5]. Right now, there is no accessible treatment or vaccine to cure the MARV disease, whereas the couples of immunizations are under clinical trial studies. A novel method coordinating immunogenetics and immunogenomics with bioinformatics for the advancement of immunization vaccines is known as Vaccinomics [6]. This method is utilized for the discovery and development of new vaccines. The fast in-silico informatics-based method has received much recognition in the sequencing of numerous pathogenic genomes and protein sequence databases [7].

The "Vaccinomics" approach has sparingly turned out to be crucial for fighting infections, for example, multiple sclerosis [8], malaria [9], and tumors [10]. However, these techniques to develop vaccines work to determine the human leukocyte antigens (HLA) ligands and T cell epitopes [11] which determine the choice of the strong vaccine candidates concerned with the transporter of antigen presentation (TAP) molecules [12–15]. In the current study, an attempt is made to design an epitope-based peptide vaccine against the MARV strain of Marburg virus by means of the vaccinomics approach.

Experimental

Oany *et al.,* [16] used novel and detailed method to design an epitope based vaccine against corona virus, in this research work similar methodology is applied to design an

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epitope based vaccine against Marburg virus. The proper flowchart of applied method is shown in Figure 1.

Selection of viral strain

Viral Zone, a database of the ExPASy Bioinformatics Resource Portal was utilized to collect of MARV and its related data as well as the host, transmission, ailment, genus, family, genome, and proteome. The primary sequences of MARV proteins were retrieved from the UniProtKB database [17].

Determination of most antigenic protein

VaxiJen v2.0 [18] a server for the forecast of defensive antigens and subunit vaccines, was utilized to identify the most powerful antigenic protein.

T cell epitope Prediction

The NetCTL 1.2 servers were utilized to determine the T cell epitopes [19].

Prediction of MHC1 binding alleles

A tool of the Immune Epitope Database [20] was utilized to expect the MHC-I bindings. The stabilized matrix base method (SMM) [21] was utilized to find out the half-maximal inhibitory concentration (IC50) estimations of peptide bindings to MHC-I. For the binding examination, every one of the alleles was chosen, and the length was set at 9.0. For the chosen epitopes, an online server was utilized to anticipate proteasomal cleavage, TAP transport, and MHC-I [22].

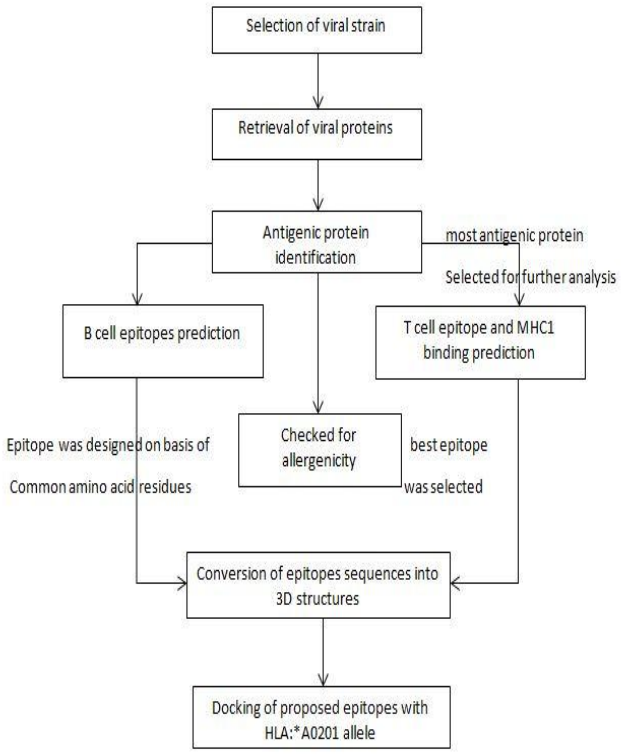


Figure 1. The method applied to design a vaccine against Marburg Virus

Allergen protein identification

The AllerHunter server [23] was utilized to predict the allergenicity of proposed epitope to develop the vaccine. The Aller Hunter predicts allergens and additionally nonallergens with high specificity. This makes Aller Hunter is an exceptionally valuable tool for cross-reactivity prediction of allergens [24, 25].

B cell epitopes prediction

The aim to predict the B cell epitope was to locate the potential antigen that would associate with B lymphocytes to start an immune response. Tools from IEDB were utilized to recognize the B cell antigenicity, including the Kolaskar and Tongaonkar antigenicity scale [26], Karplus and Schulz flexibility prediction [27], Emini surface accessibility prediction [28], The Chou and Fasman prediction tool [29] and Bepipred linear epitope prediction analysis [30].

Docking analysis of B cell and T cell epitopes

For the docking studies, the T cell epitope ALSLTCAGI and B cell epitope SLFVQAALYV were subjected to PEP-FOLD electronic server [31] for 3D structural transformation, To investigate the interactions with various HLAs, a docking study was performed utilizing Galaxy PEPP-DOCK server. The HLA-A0201 was chosen for docking on the premise of the accessible Protein Data Bank (PDB) structure stored in the database, which interacted with proposed epitope [32].

Results and Discussion

Results

A total of seven primary sequences of NP, VP24,VP35, VP30, VP40, GP and L proteins were retrieved from UniProtKb database. The VaxiJen server evaluated all of the retrieved protein sequences to discover the most powerful antigenic protein. UniProtKB id:VP30_MARVM was selected as the most effective antigenic protein, with a maximum total prediction score of 0.5694. Then, this protein was utilized for further analysis. The NetCTL server anticipated the six effective T cell epitopes from the selected protein sequence such as NLGHILSYL, KLDETSLRA, ALSLTCAGI, SLINTMTEL, FVQAALYVM and VQASYDHFI with a specificity score of 0.970 and a sensitivity score of 0.80. The MHC-I alleles for which the epitopes showed higher affinity (IC50 <200 nM) are shown in Table 1.

Amongst the six T cell epitopes, a 9mer epitope, ALSLTCAGI, interacted with most of the MHC-I alleles, including HLA:*H2Kb, HLA:*H2Kk, HLA:*A0203, HLA:* A0206, HLA:*A1101, HLA:* DRB0101, HLA:* DRB0701, HLA:*IAb, HLA:*IAs and TAP with higher affinity values of 0.75–1.0. The sequence-based allergenicity prediction tool specifically calculated by the use of the Aller Hunter tool, and the predicted queried epitope, allergenicity

score was 0.06 having a sensitivity of 98.4% and a specificity value of 13.1%. Different methods were used to predict potential B cell epitopes, the Kolaskar and Tongaonkar antigenicity prediction method examined antigenicity of B-cell epitope on the basis of the physiochemical properties of amino acids. The average antigenicity value obtained was 1.013. Nine epitopes represented to be a potential to express the B cell response shown in Table 2 and Figure 2.

A 10mer B cell epitope SLFVQAALYV was designed on the basis of common amino acid residues produced in peptide sequences of Kolaskar and Tongaonkar antigenicity prediction.

For a B cell to be a potential epitope, it must have proper surface accessibility. Therefore, Emini surface accessibility prediction was done. The regions from 7–12, 139–144 and 186 – 191 amino acid residues were more accessible. The accessible residues are shown in Figure 3.

Table 1. The six potential T-cell epitopes, along with their interacting HLA alleles and inhibitory concentration values

Epitope	Predicted IC50 Value (nM)	Interacting HLA alleles
N L G H I L S Y L	27.16, 53.06, 108.64, 179.06, 87.10, 10.79, 137.09	A0202, A0206, A0203, A0301 A1101, DRB0101, Iak
K L D E T S L R A	140.28, 83.18, 151.71, 1.58, 145.55, 41.02, 88.72	H2Kk, A1101, A6801, DRB0101, DRB0701, IAd, IAs,
A L S L T C A G I	39.99, 62.37, 9.73, 98.40, 62.23, 46.13, 152.76, 186.64, 14.16, 8.30	H2Kb, H2Kk, A0203,A0206, A1101, DRB0101, DRB0701, IAb, IAs, TAP
S L I N T M T E L	217.77, 27.86, 48.98, 22.80, 128.82, 110.92	A0202, A0203, A1101, DRB0101, IAs, TAP
F V Q A A L Y V M	43.55, 94.84, 21.73, 150.31, 1.16, 45.39, 71.61, 34.99	A1101, A6801, A6802, B3501, DRB0101, DRB0401, IAd, IAs,
V Q A S Y D H F I	59.16, 146.89, 97.27, 83.37	A0202, A0203, A1101, Iak

Table 2. B-cell epitopes based on Kolaskar and Tongaonkar antigenicity analysis

Position	Residue	Start	End	Peptide
249	**A**	246	252	VQAALYV
247	**Q**	244	250	LFVQAAL
159	**S**	156	162	HILSYLH
248	**A**	245	251	FVQAALY
245	**F**	242	248	ISLFVQA
179	**S**	176	182	AALSLTC
180	**L**	177	183	ALSLTCA
244	**L**	241	247	SISLFVQ
246	**V**	243	249	SLFVQAA

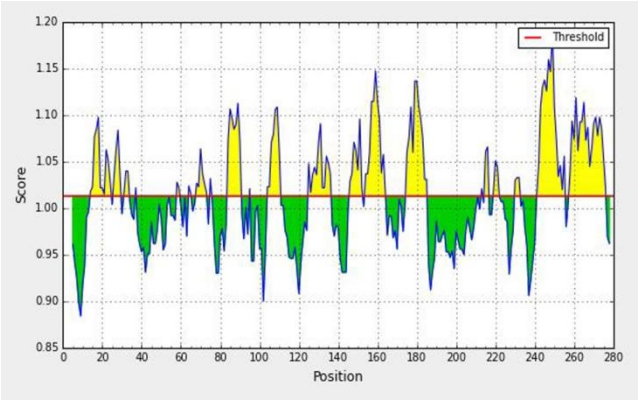


Figure 2. Kolashkar and Tongaonkar antigenicity prediction of the most antigenic protein VP30

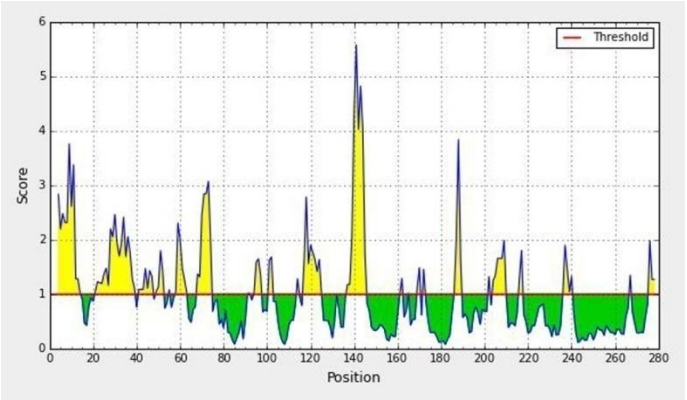


Figure 3. Emini surface accessibility prediction of the most antigenic protein VP30

Chou and Fasman beta-turn prediction method predicted regions from 68 – 76 and 124 – 133 as potential Beta-turn regions. From the experimental confirmation, it is evaluated that the flexibility of the peptide is associated to its antigenicity therefore, the Karplus and Schulz flexibility prediction method were used [39], this prediction method displayed the region of 273 – 280 amino acid residues as a most flexible region.

Table 3. Bepipred linear epitope prediction for B cell epitope

St	End	Peptide
1	16	MQQPRGRSRTRNHQVT
18	20	TIY
23	78	TQLPSKPHYTNYHPRARSMSSTRSSAESSPTNHIPRARPPSTFNLSKPPPPPKDMC
87	99	CADPTCNRDHDLD
115	146	LPNTDKTFRSPQDCGSPSLSKGLSKDKQEQTK
167	169	GKL
171	171	E
205	214	NLPQDQNGVI
224	224	D
226	229	GGQF
239	239	K
257	257	P
259	259	E
261	263	SIS

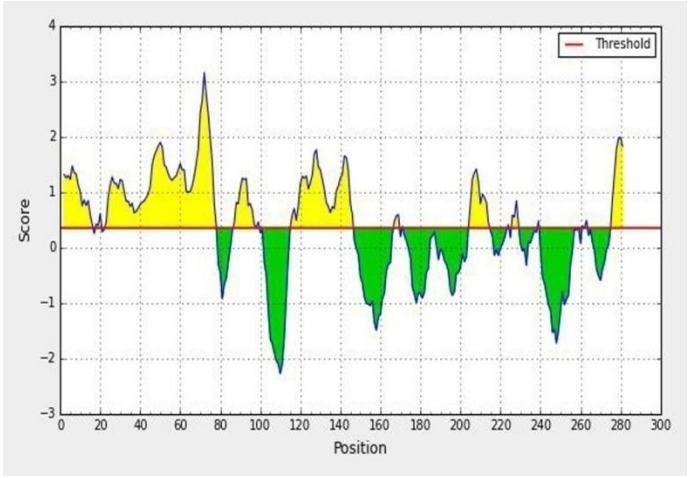


Figure 4. Bepipred linear epitope prediction of the most antigenic protein VP30

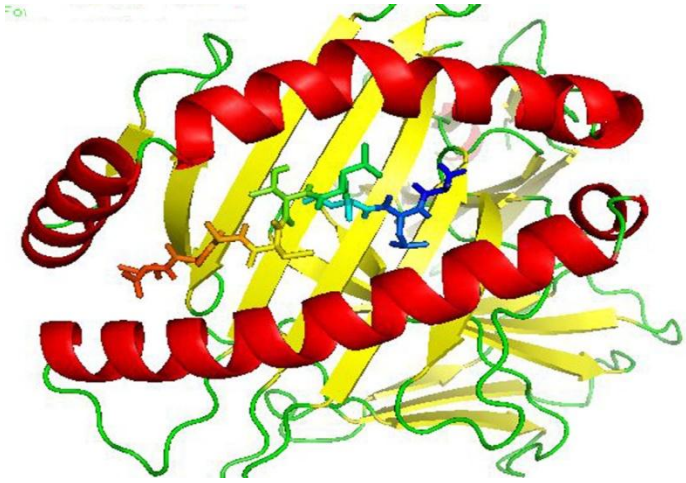


Figure 5. Docking results of HLA:*A0201 and proposed T cell epitope ALSLTCAGI.

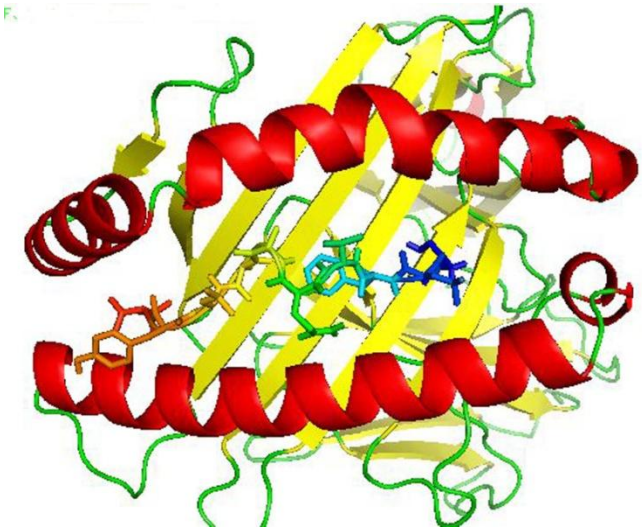


Figure 6. Docking results of HLA:*A0201 and proposed B cell epitope SLFVQAALVY

Bipred prediction method predicted that the peptide sequences from 68 – 76 and 124 – 133 amino acids are able to induce the preferred immune responses as B cell epitopes. These regions are shown in Table 3 and Figure 4.

The T cell epitope ALSLTCAGI was selected on the basis of its interactions with large number of alleles, while the B cell epitope SLFVQAALYV was selected on the basis of frequent occurance of common amino acid residues, predicted as B cell epitope. The T cell epitope ALSLTCAGI and B cell epitope SLFVQAALYV docked with the human HLA:*A0201, they fixed into the alpha helix groove of allele properly that demonstrated their stability and efficacy as a potential vaccine against Marburg virus. The docked complexes are shown in Figure 5 and Figure 6.

On the basis of all the computational analysis, it is suggested that the predicted epitope can be a best accessible solution to cure infections of Marburg virus.

Discussion

The development of novel vaccines in a shorter period of time is very important to cope up with the ever rising diseases [33-37]. Due to the advancement in the sequence-based technologies, now there is a lot of information available about the genomes and proteomes of several viruses. As a result, with the assistance of various bioinformatics tools, peptide-based vaccines can

be designed. Though, the concept of epitope-based vaccine design is getting recognition, but still, there is no work on epitope-based vaccine design for Marburg virus. In this work, an attempt is made to design Insilico epitope-based vaccine for Marburg virus. At present, vaccines are generally based B cell immunity. However, immunization taking into account T cell epitope has been energized, as the host can create a strong immune response by CD8+ T cell against the infected cell [38]. With time, because of antigenic drift, any foreign molecule can get away from the memory response of an antibody; however, the immune responses generated by T cell regularly give long lasting immunity.

There are a few criteria that should be satisfied by an epitope-based vaccine candidate, and the proposed epitope against Marburg virus satisfied each of the criteria. Basically; allergenicity is one of the most important obstacles to the development of vaccines. Therefore, today, most of the vaccines designed to stimulate the immune system are checked for unfavorable allergic reaction, first [39] through inducing the T helper cell type 2 (Th2) and immunoglobulin E (IgE).

The AllerHunter score is the likelihood that a specific sequence is a cross-receptive allergen. However, the limit for the prediction of allergen cross-reactivity is approximately greater than 0.08. Here, the proposed epitope's allergenicity score was 0.06, due to this, it was considered as a nonallergen. All these anticipated in silico results depend on the analysis of sequences and immune databases. This kind of study has gotten experimental validation [40] and hence, it is recommended that the proposed epitope would have the capacity to trigger a strong immune response as a peptide immunization in vivo.

Conclusion

In this research Insilico method was used to design the T cell and B cell epitope based peptide vaccine against Marburg virus. This study has revealed that combined computational approaches could be useful for predicting vaccine candidates against pathogens such as Marburg virus. In this way, in silico studies save both time and costs for development of vaccine candidates. They are helpful for researchers, and can guide the experimental work with higher possibilities of finding the desiredsolutions and with fewer clinical trials. In future this research work can be further utilized by the help of *in-vitro* experimental procetures to check its adequacy and efficacy

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Conflict of Interest

This research work is unique and has not been submitted to any journal yet. None of the authors have challenged conflicts of interest.

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