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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Ebola research

doi:10.1016/S2222-1808(15)61007-6

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Prediction and conservancy analysis of promiscuous T-cell binding epitopes of Ebola virus L protein: An in silico approach

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ARTICLE INFO

Article history:

Received 21 Jan 2016

Received in revised form 28 Jan 2016

Accepted 15 Feb 2016

Available online 3 Mar 2016

Keywords:

Ebola virus

L protein

Consensus sequence

B-cell and T- cell epitopes

Vaccine

ABSTRACT

Objective: To predict T-cell antigenic epitopes from the L protein of the *Zaire ebolavirus* strain, which will help in the development of an effective epitope based vaccine.

Methods: The L protein was selected due to complete absence of any epitope data for this particular protein up to date. We retrieved 101 full-length L protein sequences of *Zaire ebolavirus* species belonging to the 2014 outbreak from the NCBI database. A consensus sequence was then drawn and was used to predict the T cell epitopes that binded the human leukocyte antigen (HLA) Class I and Class II alleles using the ProPred and ProPred-I immunoinformatics algorithms. The predicted epitopes were also analyzed for conservation in the Immune Epitope Database in comparison with all other *Ebolavirus* species.

Results: A total of 34 epitopes were predicted for the HLA Class I molecules and 30 epitopes for the HLA Class II molecules. Nearly all the predicted epitopes were conserved at 70% of sequence identity across all the species of *Ebolavirus* under consideration.

Conclusions: The study revealed that the predicted antigenic epitopes can potentially be used in a future efficacious vaccine development and are anticipated to provoke a strong T cell mediated immune response against the lethal strains of the *Ebolavirus*.

1. Introduction

Filoviruses are responsible for complicated hemorrhagic fever in humans and primates with case fatality rates of up to 77%[1]. The Filovirus family belongs to the order Mononegavirales and is divided into two genera (*Ebolavirus* and *Marburgvirus*). The genus *Ebolavirus* comprising of five discrete species includes *Zaire ebolavirus* (ZEBOV) (EBOV), *Tai Forest ebolavirus*, *Reston ebolavirus* (RESTV), *Bundibugyo ebolavirus* and *Sudan ebolavirus* while the genus *Marburgvirus* consists of only one species *i.e.* *Marburg marburgvirus*. A third genus, *Cuevavirus* has also been proposed recently[2]. The genome of the ebolaviruses is a non-segmented, negative-sense and single-stranded RNA. The EBOV

genome is almost 19000 base pairs in length and contains seven open reading frames that code for seven structural proteins in the order (3'-NP-VP35-VP40-GP-VP30-VP24-L-5')[3].

The 2014 ZEBOV epidemic [now referred to as Ebola virus (EBOV)] in West Africa is by far the largest ever reported Ebola epidemic in history, gripping several West African countries with no signs of halting as yet. As of 14 Jan 2016, the total numbers of deaths achieve 11 315 persons while 28 638 suspected cases are reported by World Health Organization[4]. There are still no marketed antiviral drugs or vaccines for EBOV till this moment and the search for effective and safe antiviral drugs is in a dire need of time[5].

Although numerous EBOV vaccine candidates have been put forward in the last few years or so, none has made it to the clinical trials with most of them showing success in non-human primates only[6]. Keeping in view the current epidemic, there has been a fast-tracked concentrated effort to seek for potential vaccine candidates conferring adequate immunity in humans. Recently, two prospective vaccine candidates are selected to clinical trials and

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

showed encouraging results. Both are viral based recombinant vector vaccines designed to express the EBOV surface glycoprotein. The cAd3-EBOV vaccine uses a chimpanzee adenovirus serotype 3 or ChAd3 vector designed to express the *Sudan* and *Zaire* strain derived from surface glycoprotein while the vesicular stomatitis virus (VSV)-ZEBOV vaccine uses the VSV based expression of surface glycoprotein of *Zaire* strain[7,8]. The former vaccine conferred 100% protection in 16 macaques induced with the EBOV while the latter also conferred 100% protection in 20 macaques challenged with the EBOV in pre-clinical studies[9,10].

Developing an enhanced vaccine for Ebola, whether a replacement for current vaccines in times of unforeseen failure, an additional booster to the present vaccine candidates or antiviral drugs, or a vaccine that specifically targets the replication proteins, is of vital significance in the struggle to overcome the annihilating disease[11]. This cannot be accomplished until we fully understand the interaction of the viral proteins with the host immune system. A plethora of genes are involved in the whole range of immune responses; however, the major histocompatibility complex (MHC) genes also known as human lymphocyte antigen (HLA) are amongst the utmost essential and greatly varied genes. MHC molecules have been classified into two categories[12]. The function of both is to present the foreign antigenic peptides to T-cell lymphocytes for dissolution. MHC Class I proteins are responsible for the presentation of foreign antigenic peptides that are generated intra-cellularly to the cytotoxic CD⁸⁺ T cells (Class I) or cytotoxic lymphocytes for the matter while MHC Class II proteins are responsible for the presentation of extracellular foreign pathogens to the CD⁴⁺ T helper cells (Class II), thereby instigating a sequential immune response[13,14]. The recognition of the antigens by the specific T cell lymphocytes requires the association of the antigen with the specific MHC molecule or else the T cell mediated immune response is not generated. Of the 20 participants involved in the clinical experimental trial, the cAd3-EBOV vaccine was found to elicit CD⁸⁺ T cell response in 9 individuals while a CD⁴⁺ T cell response was observed in 13 individuals after 4 weeks of vaccine administration. An antibody response against the glycoproteins derived from either of the strains was also seen in all 20 participants with no major side-effects or adverse reactions reported, therefore deeming the vaccine safe[9]. Previous studies on primates challenged with the EBOV showed comparable T cell responses, thus highlighting the significance of the T cell mediated response in counteracting the EBOV pathogenicity. The VSV-ZEBOV vaccine has only recently entered the clinical trials and findings are still underway.

The current vaccines undergoing clinical trials all employ the surface glycoprotein. Another possible candidate could be a T cell epitope based vaccine. Epitopes are the diminutive immunologically active attachment parts of the viral proteins which are responsible for triggering an immune response in the host. Epitopes based subunit vaccines offer a much stronger and measured immune response as well as avoid the possible fatal consequences of employing entire viral proteins and peptides[15]. In an effort to contribute to the ongoing struggle, we aim to identify MHC Class I and Class II binding promiscuous epitopes that bind to the maximum number of HLA molecules within a given set of population in EBOV L protein by using in silico approach which is a prerequisite in the development of an epitope based vaccine design.

The L protein is 2212 amino acids in length and is classified

as the largest protein encoded by this virus. This protein does not function independently; instead it is the part of an RNA-dependent RNA polymerase complex, which plays an important role in the replication and transcription of the viral genome together with the nucleoprotein and viral transcription factors, VP30 and VP35. The L protein is thought to serve as the major catalytic subunit of this complex[16]. Much of the data available on the precise structure and function of the *Ebolavirus* L protein are theoretical and inadequate, heavily relying on comparative analysis and data incurred from the L proteins of other well studied non-segmented negative RNA viruses of the order Mononegavirales rather than on experimental observations. RNA-dependent RNA polymerase of other RNA viruses such as hepatitis c virus has been proven to be a successful target for the development of antiviral drug therapies and vaccines owing to the presence of several highly conserved motifs and/or residues[17,18].

The ZEBOV is currently responsible for the 2014 West African epidemic. Thus, the aim of the study was to evaluate immunological determinants by calculating conserved epitopes across the entire length of the Ebola L protein sequence by means of a range of integrated online software packages to aid in the designing of potentially new vaccine candidates along with current entrants.

2. Materials and methods

The complete full-length protein sequence of fully sequenced 101 EBOV L proteins from the 2014 outbreak was retrieved from NCBI for the prediction of T-lymphocyte epitopes. The sequences were then fed into the Jalview Desktop [multiple alignment editor (v.2.8.1)] to draw a representing consensus sequence[19]. The resulting consensus sequence was then aligned using the built in MAFFT multiple sequence alignment program (v.7.205) with the L-INS-i algorithm. Target epitopes for T-cell lymphocytes in the EBOV L protein sequence were identified for binding to both classes of the MHC molecules. ProPred-I was used to identify the MHC Class I or HLA Class I binding regions in the L protein for a total of 47 HLA Class I alleles at a default threshold value of 4% by using a threshold value of 5% for the proteasome and immunoproteasome filters, while ProPred was used to predict MHC Class II binding regions in the L protein for a total of 51 Class II HLA alleles by using a default value of 3%. The default threshold value compromises between the specificity and sensitivity of the predicted results. These algorithms were chosen due to a higher coverage of the HLA molecules when compared to alternate softwares. Promiscuous epitopes that had a score higher than the threshold value were chosen as predicted binding epitopes for that particular HLA Class.

The conservancy of each predicted T cell epitope in the L protein sequence was then analyzed using the epitope conservancy analysis tool across all EBOV L protein sequences known to date *i.e.* *Tai Forest ebolavirus*, *RESTV*, *Bundibugyo ebolavirus*, *Sudan ebolavirus* and *ZEBOV* using a threshold value of 70% sequence identity (the epitope must have 70% or more identical amino acid sequences in the query L protein)[20]. The epitopes that showed 77% to 100% conservancy were selected.

3. Results

The location and characterization of probable T cell lymphocyte HLA Class I and II epitopes were identified in the EBOV L protein

consensus sequence and the predicted epitopes were then scrutinized using the epitope conservation analysis database (Immune Epitope Database). In order to avoid an unforeseen autoimmune response, all the predicted HLA Class I and II-binding epitopes were investigated for homology with human proteome. It was found that none of the epitope was homologous with the human proteome.

For MHC Class I, a total of 34 antigenic peptides were predicted by ProPred-I using the full-length L protein sequence of the 2014 EBOV (Table 1). Epitopes number 1, 16, 27, 29 and 30 were the best binders and had the greatest MHC allele coverage. These promiscuous epitopes were well served as excellent targets for the development of a subunit based vaccine.

Table 1

Class I MHC T cell-specific predicted epitopes, their HLA alleles and percent conservation in all EBOV strains.

Peptides No.	Class I MHC-specific T-cell epitopes	Starting position	Peptide length	HLA alleles	Epitope conservancy in all EBOV (%)
1.	LSDVPVATL	63	9	43	77.78
2.	HTHIVSVST	225	9	22	88.89
3.	LYQSGDYLL	286	9	25	77.78
4.	LYQSGDYLL	290	9	19	77.78
5.	GRFLTQMH	317	9	23	100.00
6.	GHPVLHSET	376	9	35	88.89
7.	KVKKHATVL	388	9	29	88.89
8.	KHATVLKAL	391	9	36	88.89
9.	DLSIFIKDR	477	9	21	100.00
10.	ENFSIENVL	524	9	39	77.78
11.	SFSLKEKEL	549	9	28	100.00
12.	VQTLCEALL	574	9	24	100.00
13.	CAQISLVEI	721	9	20	100.00
14.	SAEDNAARV	764	9	33	88.89
15.	NAARVAASL	768	9	23	100.00
16.	KTATRMAPL	817	9	41	88.89
17.	IFDDLQGT	829	9	23	100.00
18.	AVPQVLGGL	904	9	25	100.00
19.	GSQDLTSFL	987	9	39	100.00
20.	SRTPSGKRL	1036	9	22	100.00
21.	YLEGTRILL	1049	9	38	100.00
22.	KPKCPSAAL	1207	9	26	88.89
23.	EAIELASRL	1221	9	38	88.89
24.	SDLLIKPFL	1236	9	23	77.78
25.	RLIVSTNTL	1291	9	20	88.89
26.	NVINYAVAL	1318	9	37	88.89
27.	IIEDDLIRL	1408	9	40	100.00
28.	NTILRTKKL	1478	9	32	77.78
29.	RGLSDAARL	1541	9	46	100.00
30.	LSDAARLFL	1543	9	43	100.00
31.	VKTLFFNTL	1850	9	28	77.78
32.	KAVVLKVFL	1957	9	27	77.78
33.	GFPSLEKVL	2060	9	35	100.00
34.	VNDYLFKFFL	2128	9	29	77.78

For MHC Class II, a total of 30 antigenic peptides were predicted by ProPred (Table 2). Of these, epitope numbers 5, 13, 15, 17, 18, 19, 21 and 25 were seen as good binders and had the greatest MHC allele coverage.

Through the application of conservation analysis, we found that the predicted epitopes for both the classes were conserved at 70% sequence identity across all the *Ebolavirus* species range to a varying degree. Since the antigenic epitopes were derived from the consensus sequence of the ZEBOV lineage, all the predicted epitopes were 100% similar to the protein level across the *Zaire* lineage. Thus, the conservation percentages in both tables were for the other strains e.g. the epitope 1 in Table 1 which had conservation percentage of 77.78. This showed that the epitope had 78% homology or identity with L protein sequences of the other 4 Ebola strains. The percentage

was only an average due to slight variation in conservancy for each of the 4 strains. Thus, epitopes 5, 9, 11, 12, 13 and so on showed 100% conservancy amongst all the lethal strains of EBOV when all L protein sequences were employed for conservancy analysis.

Table 2

Class II MHC T cell-specific predicted epitopes, their HLA alleles and percent conservation in all EBOV strains.

Peptide No.	Class II MHC-specific T-cell epitopes	Starting position	Peptide length	HLA alleles	Epitope conservancy in all EBOV (%)
1.	IVLDQCDELV	17	9	39	100.00
2.	LVTRACGL	24	8	29	100.00
3.	LRNCKLPKH	41	9	33	77.78
4.	YTMQDALFL	105	9	41	77.78
5.	LAILTRRGR	154	9	50	100.00
6.	YVFWKIPIS	187	9	38	77.78
7.	IVSVSTADV	227	9	22	77.78
8.	IKFLEPLCL	294	9	31	88.89
9.	VLHSETAIQ	378	9	19	88.89
10.	IVIFETYCV	402	9	33	88.89
11.	LNSYIKRNQ	438	9	36	88.89
12.	IFIKDRATA	479	9	41	100.00
13.	LLPQYRNFS	540	9	48	88.89
14.	FRYEFTAPV	638	9	36	88.89
15.	IKTGFKLRS	728	9	49	100.00
16.	LKPDETFVH	785	8	37	100.00
17.	FIYFGKKQY	797	9	53	100.00
18.	VRILQYHH	867	8	45	87.50
19.	VRILQYHH	933	8	48	87.50
20.	VRRTITLSA	987	9	42	88.89
21.	WLLSSTPVMS	1017	10	48	100.00
22.	LQILGYLEG	1044	9	42	100.00
23.	VVWLKPYEQ	1138	9	36	77.78
24.	IVSAWPNAS	1171	8	40	77.78
25.	LQMTSPHYS	1254	9	48	100.00
26.	WELAKTIMQ	1421	9	37	77.78
27.	WELAKTIMQ	1505	9	31	77.78
28.	LLLIQKYQV	1840	9	39	88.89
29.	ILNNSASQ	1891	8	23	100.00
30.	YHFIRTAKG	2112	9	24	77.78

4. Discussion

EBOV disease which was widespread in West Africa exhibits a very high mortality rate in humans. Lack of appropriate treatment and management practices along with the non-availability of an efficacious vaccine have made this deadly virus becoming a significant public health problem. Although number of vaccine candidates for EBOV has been reported in last few years, a single vaccine has not been successful to get the approval for use in human[21].

Epitopic or subunit based vaccines seems promising in next-generation vaccines. With the recent advances in the field of bioinformatics and synthetic biology, newer strategies are being devised to control and combat infectious diseases. The accessibility of a large number of sequence data has made the prospective T and B cell antigenic peptide epitope to identify a favorable method for developing effective vaccines as well as therapeutics in the fight against infective disease[22]. In this present time, the use of bioinformatics tools and computational algorithms has eased epitope prediction and cost effective efficacious vaccines candidates can be designed in short time span[23]. Subunit based vaccines against HIV, tuberculosis and malaria have already shown excellent results and will hopefully pave the way for future research in this zone[24]. Several studies have already been published on epitope predictions in the Filovirus family. According to an Immune Epitope Database

report published last year in August, a total of 142 experimental epitopes including 97 T cell epitopes and 46 B cell epitopes have been reported for the family Filovirus from a total of 29 research papers. Out of these, 23 papers are related to the ZEBOV epitope data. A total of 35 B cell epitopes including both conformational and linear epitopes along with 67 T lymphocyte (CD⁴⁺ and CD⁸⁺) epitopes from viruses within the Marburg and *Ebolavirus* genera have been reported. The *Ebolavirus* genera epitope data are a collective data of all five species. Nearly half of the T cell epitope data for the EBOV genome revolve around the glycoprotein (GP) followed by the nucleoprotein with a minute amount of data for the other proteins[25]. The B cell epitope data are even much lesser; only recently some progress has been made[26]. While no data are available for the L protein of the EBOV. Greater than 90% of all EBOV related epitope data is based on HLA binding assays. The UCSC Ebola Genome Portal contains a complete map of the EBOV sequences from previous as well as the 2014 West African epidemic. All of the reported epitopes are uploaded to the portal to help the scientific community in advancing a step further towards the development of an effective vaccine. A quick view at the UCSC Ebola Genome Browser reveals that T and B cell experimental epitope data are available; however, the L protein epitope data are completely absent. In an effort to bridge the existing knowledge gap, the EBOV GP MHC Class II T cell epitopes were predicted last year in August taking into account the 2014 EBOV sequences. A total of 38 epitopes were found to be conserved across all the EBOV species[27]. Our paper aims to add additional epitope data to the pool of existing knowledge and to speed up the analysis of the EBOV genome.

Several immune-bioinformatics tools and methods have already been developed to help the search for MHC binding peptides. For this study, we used full-length L protein sequences in order to increase the coverage and look out for epitopes in conserved regions. Most of the previous studies only focused on a single species and the genetic diversity of other virulent strains is ignored. In the *Ebolavirus* genus, the RESTV specie has not been documented to cause any disease in humans, however the case-fatality rates in the other 4 species have ranged from 20% to 80%[28]. In our study, most of the predicted peptides were also found to be conserved across other EBOV L protein sequences. Hence, choosing epitopes that work against a whole range of *Ebolavirus* species would be excellent. However, in most of the outbreaks, strains of the ZEBOV specie are responsible. Therefore, efforts are more concentrated in the confinement of this specific strain. A higher number of peptides were seen for MHC Class II as compared to Class I. For a vaccine to be efficacious, it must contain promiscuous epitopes that has a maximum coverage of different set of populations. In our case, many promiscuous epitopes were seen for HLA Class I. The advantage of this is the development of successful vaccine candidates that will not only work in the African population but rather across an entire range of diverse populations. Stretches of residues ranging in length from four to seven amino acids, clustered into six domains of strong conservation separated by variable region have been defined for the L protein across the entire *Ebolavirus* genus[29]. Most of the epitopes were seen to be clustered within these domains but none of these epitopes were exactly found in previously reported specific conserved motifs/residues; however, keeping in view the recently reported 360 mutations in the 2014 EBOV, it is no surprise that mutations pose a serious threat to the development of subunit peptide

vaccines. Another finding is the clustering of the epitopes[30]. T lymphocyte epitopes in most of the proteins tend to cluster or group into very specific regions. This accounts for most of the promiscuous overlapping epitope binding sites. The inclusion of these epitopes in any vaccine formulation will probably give excellent results.

In yet another recent study, a peptide region spanning nine amino acids of sequence TLASIGTAF was found as the most potential B and T cell epitopes, respectively. This peptide could interact with 12 HLAs and showed high population coverage up to 80.99%. Using molecular docking, the epitope was further appraised for binding against HLA molecules to verify the binding cleft interaction. In addition with this, the allergenicity of the epitopes was also evaluated[31]. Yet another study, targeting GP2 and viral protein 24 (VP24) of the EBOV that, respectively, facilitate attachment and fusion of EBOV with host cells were tested to predict probable epitopes using various tools. Binding analyses of the peptides with MHC Class I and Class II molecules, population coverage, and linear B cell epitope prediction were also performed. Predicted peptides were found to interact with with multiple MHC alleles and illustrated maximal population coverage for both GP2 and VP24 proteins, respectively. The predicted Class-I nonamers, FLYDRLAST, LFLRATTEL and NYNGLLSSI were found to cover the maximum number of MHC I alleles. The highest scoring Class II MHC binding peptides were EGAFFLYDRLASTVI and SPLWALRVILAAGIQ. Putative B cell epitopes were also found on 4 conserved regions in GP2 and two conserved regions in VP24[32].

One drawback of our study is the unavailability of *in vitro* and *in vivo* experimental studies in order to test whether these immunogenic epitopes will elicit any immune response unlike previous MHC binding experimental assays. As all of the epitopes have been predicted by making use of a computational approach. Henceforth, the determination of the actual effectiveness, stability, and immunogenicity of the immunogenic peptides needs to be done by additional *in vivo* and *in vitro* studies. As the current experimental vaccines undergo clinical trial in parallel, more data regarding T and B cell responses in the host will be revealed which will eventually help the scientific community in focusing on specific aspects of the immune response.

In general, development of therapeutics, diagnostic tool and effective vaccines against deadly viral diseases is augmented by the classification and characterization of immunogenic antigenic sites with the viral proteins. We have focused on MHC Class I and II T cell epitopes for the EBOV L 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. Our future work aims to address the additional EBOV proteins and B cell epitope prediction studies alongside mutational analysis of these epitopes and their implications in the future design of vaccines.

Conflict of interest statement

We declare that we have no conflict of interest.

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