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Immunoinformatic Approach for Epitope-Based Peptide Vaccine against Lagos Rabies Virus Glycoprotein G

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Abstract

Background: Lagos rabies virus belongs to *lyssavirus* genus responsible for meningoencephalomyelitis in mammals that affect millions of people around the world and causes thousands of human deaths every year, to the best of our knowledge there is no peptide vaccine designed for Lagos rabies virus. The resulting peptide vaccine is expected to be more immunogenic and less allergic than conventional biochemical vaccines. The aim of this study was to design an Insilco peptide vaccine for Lagos rabies virus using Immunoinformatic tools.

Methods and Materials: Sequences of glycoprotein G of Lagos rabies virus was retrieved from NCBI, the retrieved sequences were then treated using different Immunoinformatic tools for B cell to find out the most conserved, surface and antigenic epitopes, and for T cell to find conserved peptides and to test their binding affinity to different MHC1 and MHC11 alleles. Then population coverage analysis and homology modeling was performed for most promising epitopes to show their structural positions in glycoprotein G.

Results and Conclusions: B cell tests were conducted for Bepipred with 22 conserved epitopes, Emini surface accessibility prediction with 12 conserved surface epitopes and Kolaskar and Tongaonkar antigenicity test with only three conserved epitopes being antigenic. 23 conserved epitopes were interacted with different MHC-1 alleles with (IC_{50}) ≤ 500 while 39 conserved epitopes interacted with MHC-II alleles with IC50 ≤ 1000. Among all the tested epitopes for world population coverage the epitope **FVGYVTTTF** binding to both MHC1 and MHC11 alleles was 97.30% and it was found to bind 13 different alleles that indicate strong potential to formulate peptide vaccine for Lagos rabies virus.

Keywords: Immunoinformatic; Lagos rabies virus; Glycoprotein G; *Lyssavirus*; Peptide vaccine; Epitope

Introduction

Rabies is zoonotic disease distributed globally and caused by 11 viral species belonging to single-stranded RNA virus from the *Lyssavirus* genus related to the family Rhabdoviridae [1,2]. The genus *Lyssavirus* includes classical rabies virus (Rabies virus (RABV), Lagos bat virus (LBV), Duvenhage virus (DUVV), Mokola virus (MOKV) and Shimoni bat virus (SHIBV) were isolated in Africa, while LBV, MOKV, DUVV and SHIBV exist exclusively in Africa [3]. Complex phylogeny of Lagos bat virus was demonstrated in recent studies [4]. The original isolate of LBV in Nigeria since 1956 was genetically distant from other LBV isolates found up to date. The two viruses originating from Senegal (1985) and found in France (was introduced *via* Togo or Egypt; 1999) makes another phylogenetic lineage and they were found similar to each other, where a third lineage is formed by isolates from Ethiopia, Zimbabwe, the Central African Republic and South Africa from 1974 to 2006 and the genetic distances between these lineages are greater than those found with other *lyssavirus* species [4]. This virus is able to infect all mammals and the disease causes thousands of human deaths every year mainly in Asia and Africa where the virus circulates endemically in domestic dogs (*Canis lupus familiaris*) [2]. The majority of these deaths (99%) are caused by bites from rabid dogs, causing continuous fear and threat to the suspected communities because the disease is invariably fatal but also preventable if managed earlier after infection, the role of domestic dogs as main vectors of rabies is recognized with little data about the dog-associated RABV variant than wild life variants such as skunk and raccoon RABVs circulating in North America [5]. These viruses are responsible for a meningo encephalomyelitis in mammals, transmission of the viruses to a healthy mammal occurs mainly through bite or scratch by an infected mammal (the saliva is the infectious material), bats are considered as the natural hosts of 10 of these viral species, however dogs are the main source of infection in humans with an estimate of 55,000 deaths per annum worldwide among which about 56% occurs in Asia and 44% in Africa [6]. The importance of geographical factors are well documented but are often neglected when measuring the economic load of the disease [7,8]. Since 2005 more data has become available and the global disease situation changed markedly with good control efforts in some parts of the world that leads to rabies-free countries but increased number of cases in other parts of the world was recorded [9-12]. Although there is no effective treatment after declaration of the disease, there is an effective management against RABV and related lyssaviruses when applied as soon as possible after exposure, it consists of local treatment of the wound, administration of rabies immunoglobulin (if indicated) and vaccinations against rabies that is able to prevent the onset of symptom and death [13]. Moreover, even with well-studied and documented epidemic expansions of wildlife

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RABV, there is little to know about the spread of rabies in endemic areas [14]. The replication potentials of various lyssaviruses looks different in a given host and the genome organization is highly conserved [15,16]. While the envelope components matrix protein M and glycoprotein G are required for virus release and virus infectivity, respectively [17]. Based on receptor binding and the apoptosis-inducing properties of the sole RABV surface antigen, Glycoprotein G has been designated as the major pathogenicity determinant of genotype 1 lyssaviruses [18,19]. However, many examples of glycoprotein G-independent mechanisms of virus-cell interaction exist, among which those involved in escape from antiviral innate immunity and to be important for *in vivo* virus replication [20-25]. The nucleotide substitution rate of lyssaviruses is estimated to be around 10^{-4} per site per year [26]. The RNA-dependent RNA-polymerase together with phosphoprotein (P), functions as the transcriptase and replicase complex, the glycoprotein G is the only outer membrane protein responsible for virus entry and inducing protective immune responses [27]. The role of vaccines is to generate an immune response in order to protect the vaccinated individual upon future exposures to the disease [28]. There is considerable differences in Individuals immune systems that in some cases individual's immune system will not respond adequately to protect against second exposure and this is one of the reasons explaining the failure of immunization [28]. Vaccine production that depends on biochemical experiments are time consuming, expensive and not always work, furthermore this type of vaccines might constitutes a few hundred of unnecessary proteins for the induction of immunity causing many reactogenic or allergic responses [29,30]. Therefore, Insilco prediction of epitopes of appropriate protein residues would help in production of peptide based vaccines with powerful immunogenic and minimal allergenic effect [29]. Our aim is to design a vaccine for Lagos Rabies virus using peptides of its glycoprotein G as an immunogen to stimulate protective immune response.

Materials and Methods

Protein sequence retrieval

A total of 26 strains of rabies Lagos virus strains' glycoprotein were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/protein). Database in November 2016. These 26 strains sequences retrieved are from different areas around the world (include 10 collected from South Africa, 6 from Nigeria, 3 from Senegal, 3 from Kenya, one from Zimbabwe, one from central Africa public and two samples were collected from collaborative laboratories).

Retrieved glycoprotein sequences with their accession number, date of collection and areas of collection were listed in Table 1.

Conserved regions determination

The retrieved sequences were aligned to obtain conserved regions using multiple sequence alignment (MSA), sequences aligned using Clustal-W as Applied in the Bio-Edit program, version 7.0.9.1 (Hall, 1999) to find out the conserved regions in all 26 retrieved Rabies glycoprotein G sequences [31]. Then the candidate epitopes were analyzed by different prediction tools from Immune Epitope Database IEDB analysis resource (http://www.iedb.org/) [32].

B-cell epitope prediction

The reference sequence of glycoprotein was subjected to different B cell tests [33].

Prediction of linear B-cell epitopes: Bepipred test from immune epitope database (http://tools.iedb.org/bcell/result/) [34], was used as linear B-cell epitopes prediction from the conserved region with a default threshold value of 0.148.

Accession Number	Date of collection	Country
***YP_007641390.1**	1985	Senegal
ABZ81170.1	1956	Nigeria
ABZ81160.1	1985	Senegal
AMR44684.1	1986	Nigeria
ADD84515.1	2009	Kenya
ABU87631.1	1985	Senegal
ABU87630.1	1999	South Africa
ABU87629.1	1956	Nigeria
ABU87628.1	1947	Central Africa Republic
ABU87627.1	1986	Zimbabwe
ABU87626.1	2004	South Africa
ABU87625.1	1980	South Africa
ABU87624.1	1980	South Africa
ABU87623.1	1982	South Africa
ABU87622.1	1980	South Africa
ABU87621.1	2004	South Africa
ABU87620.1	2006	South Africa
ABU87619.1	2003	South Africa
AAN63563.1	1956	Nigeria
AEE36616.1	2008	South Africa
AFW16649.1	2010	Kenya
ADQ01807.1	1956	Nigeria
ABY86624.1	2010	Kenya
Q8BDV6.1	1956	Nigeria
AAK97864.1	**	**
AAK97863.1	**	**

* Ref sequence.

** Collaborative Laboratories.

Table 1: Virus strains retrieved, their Accession numbers, date of collection and area of collection.

Prediction of surface accessibility: By using Emini surface accessibility prediction tool of the immune epitope database (IEDB) (http://tools.iedb.org/bcell/result/) [35]. The surface epitopes were predicted from the conserved region with the default threshold value 1.0.

Prediction of epitopes antigenicity: Using Kolaskar and Tongaonkar antigenicity method to determine the antigenic sites with a default threshold value of 1.042, (http://tools.iedb.org/bcell/result/) [36].

MHC class I binding predictions

Analysis of peptide binding to MHC1 molecules was assessed by the IEDB MHC I prediction tool at http://tools.iedb.org/mhc1. The attachment of cleaved peptides to MHC molecules was predicted using artificial neural network (ANN) method [37,38] . Prior to prediction, all epitope lengths were set as 9 mers, all conserved epitopes that bind to MHC1 alleles at score equal or less than 500 half-maximal inhibitory concentrations (IC_{50}) were selected for further analysis [37].

MHC class II binding predictions

Analysis of peptide binding to MHC II molecules was assessed by the IEDB MHC II prediction tool at (http://tools.iedb.org/mhcii/result/ [37]. For MHC II binding predication, human allele references set were used. MHC II groove has the ability to bind different lengths

peptides that makes prediction more difficult and less accurate [38]. We used artificial neural networks to identify both the binding affinity and MHC II binding core epitopes. All conserved epitopes that bind to many alleles at score equal or less than 1000 half-maximal inhibitory concentration (IC_{50}) were selected for further analysis [39].

Population coverage calculation

All proposed MHC I and MHC II epitopes from Lagos rabies virus glycoprotein G was assessed for population coverage to the whole world population with the selected MHC I and MHC II binding alleles using IEDB population coverage calculation tool at http://tools.iedb.org/tools/population/iedb_input [40].

Homology modeling

The reference sequence of Lagos rabies glycoprotein to Raptor X on 21/12/2016, the 3D structure of glycoprotein was received on 22/12/2016 and then treated with chimera software to show the position of proposed peptides [41-45].

Results

B-cell epitope prediction

The reference sequence of Lagos rabies virus glycoprotein (GP) was subjected to Bepipred linear epitope prediction, Emini surface accessibility and Kolaskar and Tongaonkar antigenicity methods in IEDB, to determine the epitope linearity, being in the surface and to test the immunogenicity respectively Tables 2,3 and Figures 1-4.

Prediction of Cytotoxic T-lymphocyte epitopes and interaction with MHC

The reference sequence of glycoprotein G of Lagos rabies virus was analyzed using IEDB MHC-1 binding prediction tool to predict T cell epitopes suggested to interact with different types of MHC I alleles based

Peptide	Start	End	Length	Emini Score	Antigenicity Score
LYTIPE	23	28	6	0.908	1.065
QKV	73	75	3	1.288	1.109
GFTCT	77	81	5	0.309	1.039
EAVT	86	89	4	0.754	1.052
TTFKRKHFKPT	99	109	11	6.55	0.976
KPT	112	114	3	2.236	0.986
SGDPRYEESLHTPYP	122	137	15	9.442	1.008
SWLRTVTTTK	139	148	10	1.614	0.998
RTLHSPMFP	166	174	9	1.181	1.022
FYPS	181	184	4	1.131	1.082
TNHD	190	193	4	2.121	0.914
LWLP	196	199	4	0.445	1.114
MNGS	220	223	4	0.849	0.872
MCGFTDERG	225	233	9	0.499	0.953
GLRL	251	254	4	0.53	1.062
QLVN	275	278	4	0.686	1.106
NNR	281	283	3	2.538	0.808
VPGYGKAYT	327	335	9	1.091	1.047
VHY	346	348	3	0.793	1.216
DIL	356	358	3	0.358	1.089
IKG	383	385	3	0.693	0.985
KDGDAD	427	432	6	2.451	0.911

Table 2: List of conserved peptides with their surface accessibility score and antigenicity score.

Epitope	Start	End	Length	Surface accessibility score	Antigenicity score
VPGYGKAYT	327	335	9	1.091	1.047
FYPS	181	184	4	1.131	1.082
QKV	73	75	3	1.288	1.109

Table 3: List of conserved peptide that passes Bepipred, Emini surface and Koalaskar tests with their length and scores.

Peptide	Start	End	Alleles	IC 50	Percentile rank
EAVTYTNFV	86	94	HLA-A*68:02	3.47	0.2
VTYTNFVGY	88	96	HLA-A*11:01	36.63	0.4
			HLA-A*29:02	14.58	0.2
			HLA-A*30:02	43.58	0.2
TYTNFVGYV	89	97	HLA-C*14:02	96.3	0.3
FVGYVTTTF	93	101	HLA-B*15:01	99.41	0.2
			HLA-B*35:01	18.33	0.2
			HLA-C*12:03	87.09	0.2
VGYVTTTFK	94	102	HLA-A*03:01	46.59	0.2
			HLA-A*11:01	82.1	0.4
GYVTTTFKR	95	103	HLA-A*31:01	47.13	0.4
TTFKRKHFK	99	107	HLA-A*03:01	17.37	0.2
			HLA-A*11:01	11.73	0.3
			HLA-A*30:01	53.78	0.5
			HLA-A*68:01	7.01	0.1
			HLA-A*31:01	15.2	0.3
FKRKHFKPT	101	109	HLA-B*08:01	99.49	0.2
YEESLHTPY	127	135	HLA-B*18:01	3.45	0.1
WLRTVTTTK	140	148	HLA-A*03:01	79.59	0.2
RTLHSPMFP	166	174	HLA-A*30:01	51.89	0.5
HFRKLVPGY	322	330	HLA-A*30:01	55.25	0.5
LVPGYGKAY	326	334	HLA-C*14:02	62.74	0.2

Table 4: List of conserved epitopes and their corresponding MHC-1 alleles along with their position in glycoprotein, IC50 and percentile rank.

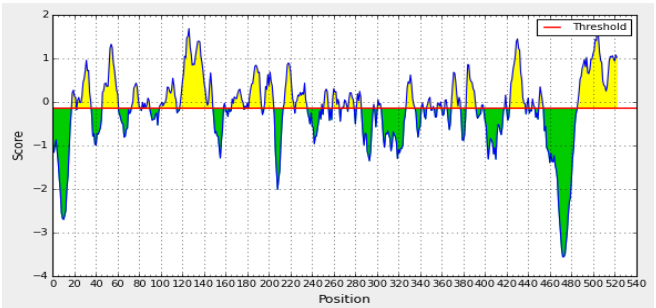

Figure 1: Bepipred Linear Epitope Prediction; Yellow areas above threshold (red line) are proposed to be a part of B cell epitopes and the green areas are not.

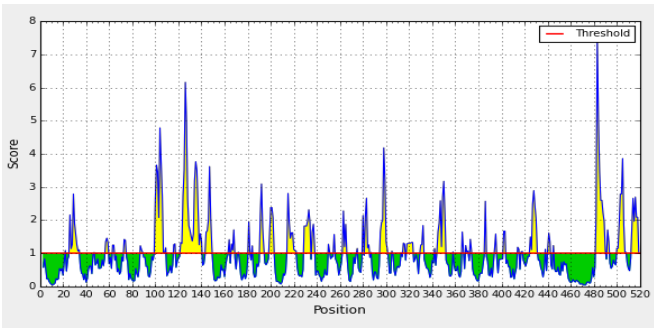

Figure 2: Emini surface accessibility prediction; Yellow areas above threshold (red line) are proposed to be a part of B cell epitopes and the green areas are not.

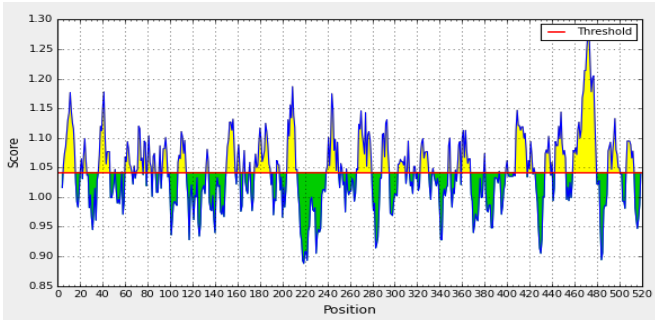

Figure 3: Kolaskar and Tongaonkar antigenicity prediction; Yellow areas above threshold (red line) are proposed to be a part of B cell epitope while green areas are not.

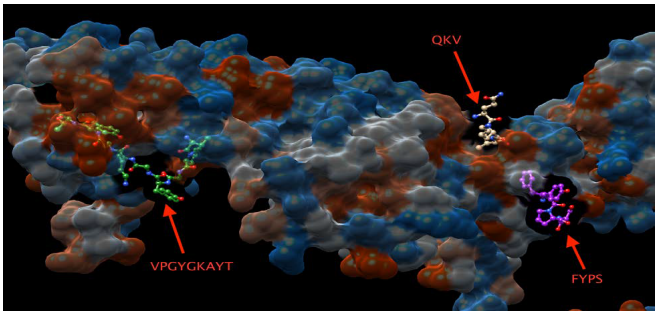

Figure 4: Structural position of the most promising conserved B cell epitopes of glycoprotein G of Lagos rabies virus.

on ANN- align with (IC_{50}) ≤ 500, the list of conserved epitopes and their corresponding MHC-1 alleles are shown in Table 4 and Figure 5.

Prediction of T helper cell epitopes and interaction with MHC II alleles

The reference sequence of glycoprotein G of Lagos rabies virus was analyzed using IEDB MHC II binding prediction tool based on NN-align with half-maximal inhibitory concentration (IC_{50}) ≤ 1000; the list of all epitopes and their correspondent binding MHC II alleles were shown in (supplementary Table 1) while the list most promising three epitopes that had Binding affinity with MHC II alleles along with their positions in the glycoprotein G of Lagos rabies virus were shown in Table 5 and Figure 6.

Population coverage analysis

Population coverage test was performed to detect the world coverage of all epitopes binds to MHC1 alleles, MHC11 alleles and combined MHC1 and MHC11 as well as selected most promising epitopes for each test.

Population coverage for MHC1: Population coverage results for all epitopes binding to MHC1 alleles of Lagos rabies virus was shown in supplementary Table (II) while the population coverage results of the most promising three peptides was shown on Table 6.

Population coverage for MHC11: The population coverage results of all peptides binding to MHC11 alleles with their coverage and total HLA hits were shown in supplementary Table (III) and the results of most promising three epitopes were shown on Table 6.

Population coverage for both MHC1 and MHC11 alleles: This test was performed to the most promising epitope alone (FVGYVTTTF) and it was found to bind 13 alleles with population coverage of 97.30% Figure 7.

Discussion

In the present study we proposed for the first time different selected peptides that could be recognized by B cell and T cell to produce antibodies against Lagos rabies virus. The resulting peptide vaccine is expected to be more antigenic and less allergic than the conventional biochemical vaccine.

The reference sequence of Lagos rabies virus glycoprotein G was subjected to Bepipred linear epitope prediction test, Emini surface accessibility test and Kolaskar and Tongaonkar antigenicity test in IEDB, to determine the binding to B cell, being in the surface and to test the immunogenicity respectively.

For Bepipred test there was 22 conserved epitopes that have the binding affinity to B cell while there are 11 epitopes predicted on the

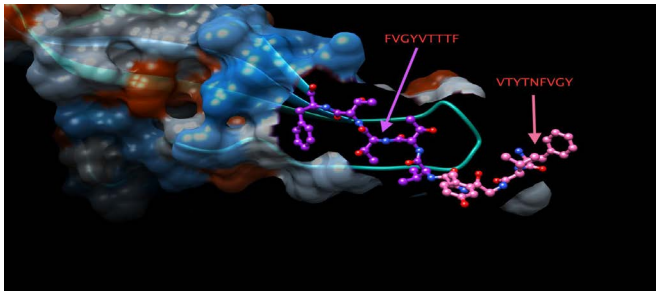

Figure 5: Structural Position of the two most promising conserved epitopes at glycoprotein G of Lagos rabies virus that interact with MHC I alleles.

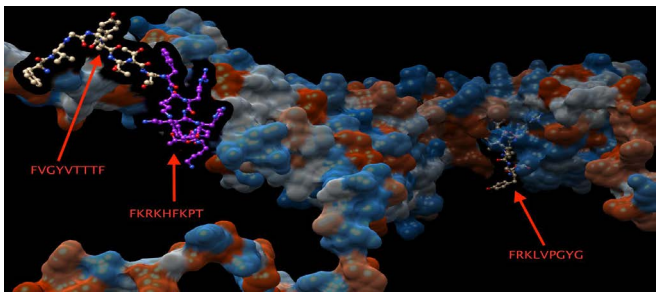

Figure 6: Molecular position of the most promising three epitopes of Lagos rabies virus that binds MHC II alleles.

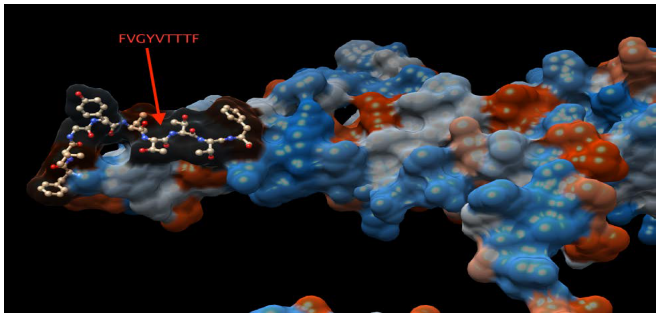

Figure 7: Molecular position of the most promising epitope for population coverage that binds both MHC1 and MHC11 alleles.

Core Sequence	Start	End	Peptide Sequence	Allele	IC50	Rank
FVGYVTTTF	93	101	TYTNFVGYVTTTFKR	HLA-DPA1*01:03/DPB1*02:01	52.4	5.33
			TYTNFVGYVTTTFKR	HLA-DPA1*02:01/DPB1*01:01	335.6	24.65
			VTYTNFVGYVTTTFK		429.2	28.33
			VTYTNFVGYVTTTFK	HLA-DQA1*05:01/DQB1*02:01	821.8	17.42
			AVTYTNFVGYVTTTF		912.6	18.98
			YTNFVGYVTTTFKRK	HLA-DRB1*04:01	26.2	1.54
			TYTNFVGYVTTTFKR		27.7	1.68
			VTYTNFVGYVTTTFK		32.3	2.12
			AVTYTNFVGYVTTTF		36.8	2.55
			TNFVGYVTTTFKRKH		39	2.76
			NFVGYVTTTFKRKHF		52.8	4.08
			FVGYVTTTFKRKHFK		83	6.73
			YTNFVGYVTTTFKRK	HLA-DRB1*01:01	89.4	28.65
			TNFVGYVTTTFKRKH		198.1	40.73
			NFVGYVTTTFKRKHF		314.7	48.97
			VTYTNFVGYVTTTFK	HLA-DRB1*07:01	47.4	8.32
			TYTNFVGYVTTTFKR		61.4	10.11
			AVTYTNFVGYVTTTF		62	10.19
			YTNFVGYVTTTFKRK	HLA-DRB1*04:04	62.4	7.41
			TYTNFVGYVTTTFKR		66.9	8.02
			YTNFVGYVTTTFKRK	HLA-DRB1*04:05	82.1	8.06
			TYTNFVGYVTTTFKR		83.9	8.22
			VTYTNFVGYVTTTFK		94.1	9.12
			TNFVGYVTTTFKRKH		94.5	9.16
			TNFVGYVTTTFKRKH	HLA-DRB1*08:02	112	1.93
			YTNFVGYVTTTFKRK		112.5	1.94
			AVTYTNFVGYVTTTF	HLA-DRB1*04:05	119.7	11.21
			TYTNFVGYVTTTFKR	HLA-DRB1*09:01	131.2	8.94
			AVTYTNFVGYVTTTF	HLA-DRB1*04:04	133.1	15.1
			NFVGYVTTTFKRKHF	HLA-DRB1*08:02	136.2	2.61
			YTNFVGYVTTTFKRK	HLA-DRB1*09:01	137.6	9.33
			VTYTNFVGYVTTTFK		143.6	9.71
			TYTNFVGYVTTTFKR	HLA-DRB1*08:02	146.2	2.89
			NFVGYVTTTFKRKHF	HLA-DRB1*07:01	148.7	18.1
			NFVGYVTTTFKRKHF	HLA-DRB1*04:05	153.6	13.63
			AVTYTNFVGYVTTTF	HLA-DRB1*09:01	159.3	10.64
			TNFVGYVTTTFKRKH		172.5	11.44
			FVGYVTTTFKRKHFK	HLA-DRB1*07:01	214.7	22.18
			VTYTNFVGYVTTTFK	HLA-DRB1*08:02	216.1	4.82
			FVGYVTTTFKRKHFK	HLA-DRB1*04:05	243.7	18.96
			NFVGYVTTTFKRKHF	HLA-DRB1*09:01	269.1	16.34
			FVGYVTTTFKRKHFK	HLA-DRB1*04:04	316.5	27.12
			AVTYTNFVGYVTTTF	HLA-DRB1*08:02	331	7.92
			FVGYVTTTFKRKHFK		347.1	8.33
			FVGYVTTTFKRKHFK	HLA-DRB1*09:01	393.5	21.68
			TNFVGYVTTTFKRKH	HLA-DRB1*15:01	134.1	12.67
			TYTNFVGYVTTTFKR		135	12.73
			NFVGYVTTTFKRKHF		148.7	13.7
			YTNFVGYVTTTFKRK		151.2	13.87
			VTYTNFVGYVTTTFK		163.8	14.72
			FVGYVTTTFKRKHFK		188.4	16.28
			AVTYTNFVGYVTTTF		198.6	16.88
			AVTYTNFVGYVTTTF	HLA-DRB3*01:01	423.1	10.64
			VTYTNFVGYVTTTFK		495.5	11.68
			AVTYTNFVGYVTTTF		552.6	34.42
			TYTNFVGYVTTTFKR		590.5	12.96

			YTNFVGYVTTTFKRK		753.5	14.99
FRKLVPGYG	323	331	RLSHFRKLVPGYGKA	HLA-DQA1*05:01/DQB1*03:01	368.2	31.01
			RRLSHFRKLVPGYGK	HLA-DQA1*05:01/DQB1*03:01	513.7	36.21
			FRRLSHFRKLVPGYG		584.4	38.4
			LSHFRKLVPGYGKAY	HLA-DRB1*01:01	5.1	0.88
			RLSHFRKLVPGYGKA		6.5	2.18
			SHFRKLVPGYGKAYT		6.7	2.37
			HFRKLVPGYGKAYTI		8.8	4.22
			RRLSHFRKLVPGYGK		9	4.39
			FRKLVPGYGKAYTIL		12.1	6.74
			FRRLSHFRKLVPGYG		14	7.99
			LSHFRKLVPGYGKAY	HLA-DRB1*09:01	26.3	1.28
			SHFRKLVPGYGKAYT		31.3	1.66
			RLSHFRKLVPGYGKA		34.8	1.94
			FRRLSHFRKLVPGYG		41.6	2.55
			HFRKLVPGYGKAYTI		42.8	2.66
			RRLSHFRKLVPGYGK		45.4	2.87
			FRKLVPGYGKAYTIL		56.4	3.74
			RRLSHFRKLVPGYGK	HLA-DRB1*04:04	155.3	16.91
			RLSHFRKLVPGYGKA		160.4	17.31
			FRRLSHFRKLVPGYG		169.4	18.01
			LSHFRKLVPGYGKAY		173.8	18.38
			SHFRKLVPGYGKAYT		274.6	24.92
			HFRKLVPGYGKAYTI		328.5	27.74
			FRRLSHFRKLVPGYG	HLA-DRB1*08:02	567.4	13.75
			RRLSHFRKLVPGYGK	HLA-DRB1*07:01	592.9	35.83
			RRLSHFRKLVPGYGK	HLA-DRB1*08:02	598.9	14.46
			RLSHFRKLVPGYGKA		662.8	15.81
			FRKLVPGYGKAYTIL	HLA-DRB1*04:04	714.7	41.07
			RLSHFRKLVPGYGKA	HLA-DRB1*07:01	858.8	41.76
			RRLSHFRKLVPGYGK	HLA-DRB1*04:05	890.7	39.23
			LSHFRKLVPGYGKAY	HLA-DRB1*07:01	905.7	42.64
			LSHFRKLVPGYGKAY	HLA-DRB1*11:01	11.6	1.7
			RLSHFRKLVPGYGKA		15.9	2.68
			SHFRKLVPGYGKAYT		16.3	2.77
			RRLSHFRKLVPGYGK		21.7	3.93
			HFRKLVPGYGKAYTI		23.9	4.37
			FRKLVPGYGKAYTIL		38.7	6.92
			SHFRKLVPGYGKAYT	HLA-DRB1*15:01	62.5	6.43
			LSHFRKLVPGYGKAY		116.8	11.32
			RRLSHFRKLVPGYGK		127.2	12.14
			RLSHFRKLVPGYGKA		130.4	12.38
			FRRLSHFRKLVPGYG		139.6	13.07
			LSHFRKLVPGYGKAY	HLA-DRB5*01:01	6.5	1.31
			SHFRKLVPGYGKAYT		7.1	1.5
			RLSHFRKLVPGYGKA		9.7	2.28
			HFRKLVPGYGKAYTI		10	2.37
			RRLSHFRKLVPGYGK		15.3	3.79
			FRKLVPGYGKAYTIL		16	3.96
FKRKHFKPT	101	109	TTTFKRKHFKPTVSA	HLA-DPA1*01/DPB1*04:01	222.9	9.14
			TTFKRKHFKPTVSAC		299	10.97
			TFKRKHFKPTVSACR		850	19.98
			VTTTFKRKHFKPTVS	HLA-DPA1*01:03/DPB1*02:01	237.2	14.23
			TTTFKRKHFKPTVSA		289.8	15.95
			TTFKRKHFKPTVSAC		364.2	18.14
			TTTFKRKHFKPTVSA	HLA-DRB1*01:01	545.1	58.11
			TTFKRKHFKPTVSAC		793.1	63.84

			FKRKHFKPTVSACRD	HLA-DRB1*04:04	110.8	12.98
			TFKRKHFKPTVSACR		154.3	16.84
			TTFKRKHFKPTVSAC		318.2	27.22
			TTTFKRKHFKPTVSA		531	35.77
			VTTTFKRKHFKPTVS		733.7	41.54
			YVTTTFKRKHFKPTV	HLA-DRB1*09:01	939.6	37.6
			TTTFKRKHFKPTVSA	HLA-DRB1*11:01	73.7	11.28
			TTFKRKHFKPTVSAC		124.8	15.68
			TFKRKHFKPTVSACR		162.3	18.16
			FKRKHFKPTVSACRD		218.7	21.23
			TTTFKRKHFKPTVSA	HLA-DRB5*01:01	124.5	17.25
			TTFKRKHFKPTVSAC		210.6	22.55

Table 5: List of most promising three epitopes that had Binding affinity with MHC II alleles along with their positions in the glycoprotein G of Lagos rabies virus, peptide sequence, binding alleles, IC50 and rank.

MHC1 peptides		MHC11 Peptides	
Epitope	**Coverage**	**Epitope**	**Coverage**
FVGYVTTTF	35.42%	FVGYVTTTF	97.30%
VTYTNFVGY	32.98%	FRKLVPGYG	95.31%
VTYTNFVGY	32.98%	FKRKHFKPT	95.31%
Epitope set	**56.71%**		**99.68%**

Table 6: Population coverage of most promising 3 epitopes binds to MHC1 alleles and MHC11 alleles of Lagos rabies virus along with their world population coverage.

surface according to Emini surface accessibility test and 10 epitopes being antigenic as detected by Tongaonkar antigenicity test while only three epitopes (VPGYGKAYT, FYPS and QKV) found to overlap all performed B Cell tests.

Also the reference sequence of Lagos rabies virus glycoprotein G was analyzed using IEDB MHC1 binding prediction tool to predict T cell epitopes interacting with different types of MHC I alleles. Based on Artificial neural network (ANN) with half-maximal inhibitory concentration (IC_{50}) ≤ 500; 23 conserved peptides were predicted to interact with different MHC-1 alleles. The peptide FVGYVTTTF from 93 to 101 had higher affinity to interact with 9 alleles (HLA-A*03:01, HLA-A*03:01, HLA-A*03:01, HLA-A*03:01, HLA-A*03:01, HLA-A*11:01, HLA-A*11:01, HLA-A*11:01, HLA-A*23:01), followed by VTYTNFVGY from 88 to 96 that binds 7 alleles (HLA-B*27:05, HLA-B*15:02, HLA-B*35:01, HLA-B*40:01, HLA-B*44:03, HLA-B*53:01, HLA-B*58:01). These two peptides could be thought about as a possible peptide vaccine for Lagos rabies virus.

The reference glycoprotein (GP) strain was analyzed using IEDB MHC II binding prediction tool based on NN-align with half-maximal inhibitory concentration (IC_{50}) ≤ 1000; there were 39 conserved predicted epitopes found to interact with MHC-II alleles. The peptides FVGYVTTTF, FKRKHFKPT and FRKLVPGYG had the affinity to bind the highest number of MHC11 alleles.

World population coverage results for total epitopes binding to MHC1 alleles was 88,42% and the most promising peptides was FVGYVTTTF, VTYTNFVGY and VTYTNFVGY with world population coverage 56.71% and total HLA hits 3,7 and 7 respectively.

The world population coverage results for all epitopes that have binding affinity to MHC11 alleles was 99.97% while world population coverage of the most promising three epitopes FKRKHFKPT, FRKLVPGYG and FVGYVTTTF was 99.68 % with HLA hits 7,7 and 13 respectively.

The peptide FVGYVTTTF exhibits exceptional population coverage results for both MHC1 and MHC11 alleles of 97.3% with total HLA hits 13 different alleles.

Conclusion and Recommendations

Conclusion

We conclude that to our knowledge this was the first study to propose peptide vaccine for Lagos rabies virus which is expected to be more immunogenic and less allergic than traditional biochemical vaccines, among the different peptides tested for both B cell and T cell this study proposed an interesting T cell epitope (FVGYVTTTF) that have very strong binding affinity to both MHC1 and MHC11 alleles and it was found to bind 13 different alleles with world population coverage 97.3%, which indicates strong potential to formulate peptide vaccine for Lagos Rabies virus.

Recommendations

We recommend further studies to propose a peptide vaccines for the other strains of rabies virus especially Rabies *lyssavirus*, there will be possibility to find common conserved promising epitopes for multiple strains. Also we recommend *in vitro* and *in vivo* evaluation of the most promising peptides.

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