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Computational Prediction of Leishmania Infantum Epitopes: A Bioinformatic-based Step to Leishmaniasis Vaccine Design

Amirhossein Larijani[1,2], Ali Kia-Karimi[3], Mohammad Rahmati*[4,5], Roya Derakhshan[6]

1. *Student Research Committee, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran*
2. *Regenerative Medicine Research Center, Guilan University of Medical Sciences, Rasht, Iran*
3. *Student Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
4. *Department of Medical Biotechnology, School of Paramedicine, Guilan University of Medical Sciences, Rasht, Iran*
5. *Medical Biotechnology Research Center, School of Paramedicine, Guilan University of Medical Sciences, Rasht, Iran*
6. *Department of Oncology, Endometriosis Research Center, Iran University of Medical Sciences, Tehran, Iran*

ABSTRACT

Leishmaniasis, a significant public health concern in resource-limited areas, is caused by the parasitic protozoan Leishmania. The insufficiency of current treatments underscores the urgent need for effective vaccines. Researchers have pinpointed promising vaccine targets through comprehensive antigen screening methods, showing their ability to trigger protective immune responses against Leishmania. The delicate balance between pro-inflammatory (Th1) and anti-inflammatory (Th2) responses in Leishmaniasis underscores the immune regulation complexity vital for fighting the infection. Leveraging bioinformatics tools epitope prediction targeting KMP11, GP63, and LACK antigens aims to induce both humoral and cellular responses.

Keywords: Leishmaniasis, Bioinformatics, Vaccine, Epitope-prediction

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Corresponding Information: **Mohammad Rahmati**, *Department of Medical Biotechnology, School of Paramedicine, Guilan University of Medical Sciences, Rasht, Iran* & Email: mrahmati.ch@gmail.com

1. Introduction

Leishmaniasis, a neglected tropical disease caused by the protozoan parasite Leishmania, affects millions globally, particularly in resource-limited regions **(1)**. The intricate life cycle of Leishmania involves transmission via infected sandfly bites, resulting in diverse clinical manifestations ranging from cutaneous to visceral forms of the disease (Figure 1). The inadequacy of current treatment options underscores the urgent requirement for efficacious and safe vaccines to manage and prevent Leishmaniasis **(2)**.

Researchers have directed their efforts towards identifying antigenic proteins expressed at various stages of the parasite's life cycle to uncover novel vaccine targets through high-throughput and highly accurate methods **(3, 4)**. This pursuit has uncovered several promising vaccine candidates, such as glycoprotein 46 (gp46), cathepsin L-like and B-like

proteases, histone H2A, glucose-regulated protein 78 (grp78), and stress-inducible protein 1 (STI-1) **(5)**. These antigens have demonstrated the ability to provoke protective immune responses against Leishmania infection in preclinical investigations.

The immune response to Leishmania infection is intricate, involving a delicate equilibrium between pro-inflammatory (Th1) and anti-inflammatory (Th2) cytokines **(6, 7)**.

Th1-mediated responses, characterized by IFN-γ production, play a pivotal role in controlling parasite growth by activating macrophages to eliminate intracellular parasites **(4, 7)**. Conversely, Th2-mediated responses can support parasite survival and disease progression. Understanding the interplay between these immune responses is vital for designing effective vaccines against Leishmaniasis.

The field of bioinformatics and computational biology has transformed vaccine development by facilitating the prediction of immunodominant epitopes and antigenic proteins. *In silico* methodologies enable swift screening of potential vaccine candidates, reducing the time and resources needed for experimental validation **(3)**. Leveraging these computational tools, researchers can craft epitope prediction that target specific immune pathways to bolster protective immunity against Leishmania. In a recent study, epitopes were devised for KMP11, GP63, and LACK antigens using specific bioinformatic tools.

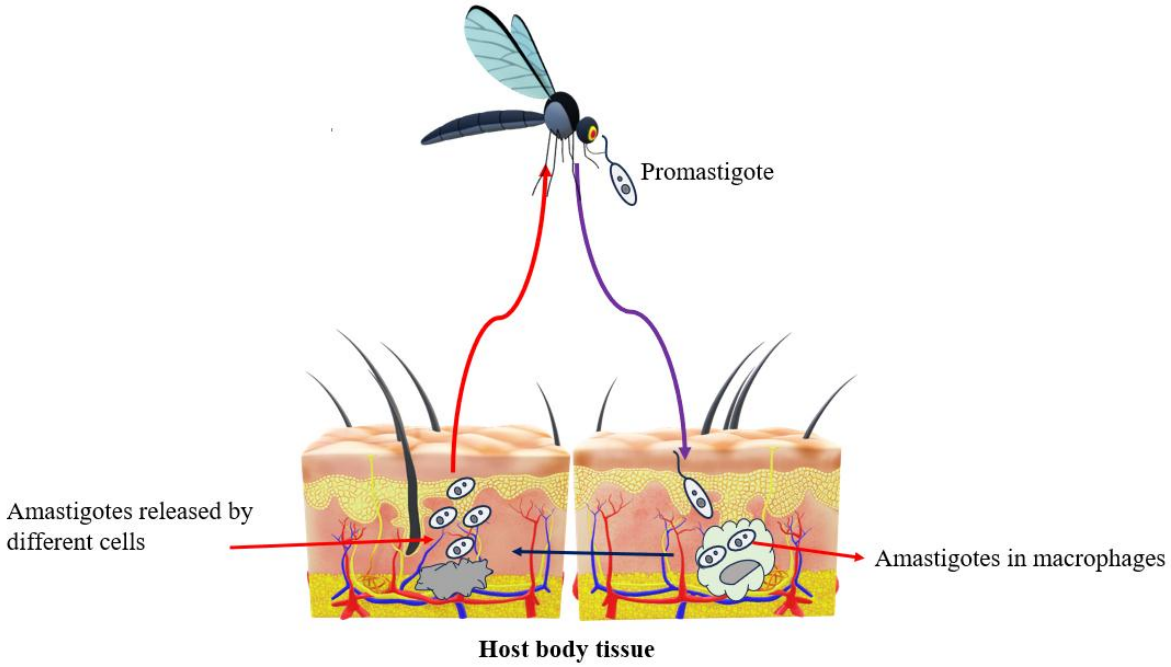

Figure 1. Transmission and life cycle of Leishmania infantum in the vector and host body. (Design by Authors, 2024)

2. Protocol

2-1- Obtain the amino acid sequence for the target proteins

The FASTA-formatted amino acid sequences for L. infantum GP63 (Accession no. QJF54184), KMP11 (Accession no. AGV77135), and LACK (Accession no. UQI50440) were collected from the National Center for Biotechnology Information (NCBI) website located at **http://www.ncbi.nlm.nih.gov (8).**

2-2- Forecasting the location of antigenic epitopes

In this step we opted to predict CTL epitopes, linear B cell epitopes, and MHC class I and II epitopes. To do this, different bioinformatics servers capable of identifying these sorts of epitopes were used.

a. Prediction and screening of linear B cell epitope: Linear B cell epitopes were predicted utilizing the (B cell epitope prediction) section of the IEDB server which located at http://tools.iedb.org/bcell/ . The mentioned server method was also set to Bepipred Linear Epitope Prediction 2.0. BepiPred is a predictive method that identifies the location of linear B-cell epitopes. It achieves this by utilizing a hidden Markov model and propensity scale method, which allow it to determine which residues are part of an epitope based on their scores. Residues with scores higher than the threshold value of 0.35 are colored yellow on the graph and labeled with "E" in the output table. The accuracy of the prediction method for epitope/non-epitope predictions is determined by a table that

summarizes data from a large benchmark calculation that included almost 85 B cell epitopes **(9)**.

b. Cytotoxic T lymphocyte (CTL) epitope prediction and screening: In this section we used CTLPRED server to predict the CTL epitopes which available at http://crdd.osdd.net/raghava/ctlpred/ . The approach of this server based on two form of machine learning techniques such as Artificial Neural network (ANN) and support vector machine (SVM). Based on these two processes of server, the methods also allow for consensus and combined prediction **(10)**.

c. Prediction and selection approach for MHC class 1 and MHC class 2 epitopes: To predict MHC class 1 epitopes, we utilized the T cell class I tool provided on the IEDB website at https://nextgen-tools.iedb.org/. The tool employs Artificial Neural Networks (ANNs) and utilizes data on 177 MHC molecules from various species such as humans, mice, cattle, primates, pigs, horses, and dogs. The IEDB recommends using percentile rank as the primary metric for ranking binding predictions, with a percentile rank of less than or equal to 1% covering 80% of the immune response for many alleles **(11)**. In the following to anticipate MHC class 2 epitopes, the MHC2PRED at http://crdd.osdd.net/raghava/mhc2pred/ address was used. In the algorithm of this server, Support Vector Machine (SVM), a machine learning approach, was used to construct a prediction strategy for MHC binding. Individual amino acid sequences represented by binary input were used to train SVM. Each amino acid in a 9-mer peptide was turned into a 20-dimensional vector, giving each peptide a 180-dimensional vector. Non-binders were classified as -1, whereas binders were designated as +1. Experimentation was used to identify the best kernel type for data categorization, such as RBF, Polynomial, Linear, and Sigmoid. Finally, by methodically modifying the parameters and analyzing prediction performance, the kernel features and regulatory parameter C were then tuned **(12)**.

3. Results and Discussion

3-1- Predicted epitopes

a. B-Cell Epitope Prediction: The high-scored linear and conformational B-cell epitopes that were predicted within the full-length of the designed vaccine by the IEDB, also in combination with BepiPred linear epitope prediction 2.0, respectively (Table 1).

Table 1. B-cell epitopes predicted by the IEDB

Antigen	Start	End	Peptide	Length
KMP11	7	7	E	1
	9	10	SA	2
	13	14	KR	2
	16	76	DEEFNRKMQALNAKFFADKPDESTLSPEMKE HYEKFERMIKEHTEKFNKKMHEHSEHFKQK	61
	78	89	AELLEQQKAAQY	12
LACK	29	48	NPDRHSVDSDYGLPSHRLEG	20
	81	90	NGQCQRKFLK	10
	125	135	CMHEFLRDGHE	11
	168	178	GGKCERTLKGH	11
GP63	16	20	QLHTE	5
	23	56	KVRQVQDKWNATGMVDEICGDFKVPPAHITEGFS	34
	83	86	FSDG	4
	100	106	IASRYDQ	7
	126	144	FFEGARILESISNVRHKDF	19
	172	191	IEDQGGAGSAGSHIKMRNAQ	20
	217	219	FYQ	3

b. CTL Epitopes Prediction: The high-ranked CTL epitopes (9-mer length) with a binding affinity score were selected as final CTL epitopes in three antigens (Table 2).

Table 2. Cytotoxic T-lymphocyte (CTL) epitopes of selected antigens prediction using CTLpred server (Combined approach; Cutoff Score=0.51).

Antigen	Position	Sequence	Score (ANN/SVM)
KMP11	7	FFADKPDES	0.990
	9	KFFADKPDE	0.980
	13	RLDEEFNRK	0.950
Lack	16	SHRLEGHTG	1.000
	78	KFLKHTKDV	1.000
	29	FVSCVSLAH	0.990
GP63	81	RILESISNV	1.000
	125	PQALQLHTE	0.990
	168	VRQVQDKWN	0.990

c. MHC Peptide Prediction: To predict the binding epitopes for Major Histocompatibility Complex (MHC) class I, we utilized the next-generation tools of Immune Epitope Database (IEDB, available at https://nextgen-tools.iedb.org/tc1) and focused on 9-mer length peptides and human HLAs. Specifically, based on IEDB recommended method 2020.09 (NetMHCpan EL 4.1) **(13)**, most reference HLA allele set used for prediction; e.g. 16 class A alleles (01:01, 02:01, 02:03, 02:06, 03:01, 11:01, 23:01, 24:02, 26:01, 30:01, 30:02, 31:01, 32:01, 33:01, 68:01 and 68:02) and 11 class B alleles (07:02, 08:01, 15:01, 35:01, 40:01,44:02, 44:03, 51:01, 53:01, 57 l:01 and 58:01) (Table 3-5).

Table 3. *L. infantum* KMP11 binding to MHC-I alleles with highest score which obtained by the IEDB web server

Peptide	Length	Start	End	Allele	Peptide index	Core	Icore	Score
KMHEHSEHF	9	65	73	HLA-B*15:01	1577	KMHEHSEHF	KMHEHSEHF	0.960679
HFKQKFAEL	9	72	80	HLA-B*08:01	1500	HFKQKFAEL	HFKQKFAEL	0.955418
LEQQKAAQY	9	81	89	HLA-B*44:03	1929	LEQQKAAQY	LEQQKAAQY	0.907147
LEQQKAAQY	9	81	89	HLA-B*44:02	1845	LEQQKAAQY	LEQQKAAQY	0.901512
MIKEHTEKF	9	54	62	HLA-B*15:01	1566	MIKEHTEKF	MIKEHTEKF	0.889601
RMIKEHTEK	9	53	61	HLA-A*03:01	389	RMIKEHTEK	RMIKEHTEK	0.864294
EFNRKMQAL	9	18	26	HLA-B*08:01	1446	EFNRKMQAL	EFNRKMQAL	0.828076

Table 4. *L. infantum* LACK binding to MHC-I alleles with highest score which obtained by the IEDB web server

Peptide	Length	Start	End	Allele	Peptide index	Core	Icore	Score
HPIVVSGSW	9	149	157	HLA-B*53:01	5093	HPIVVSGSW	HPIVVSGSW	0.942054
RTLKGHSNY	9	173	181	HLA-A*30:02	2233	RTLKGHSNY	RTLKGHSNY	0.91673
TLKGHSNYV	9	174	182	HLA-A*02:03	586	TLKGHSNYV	TLKGHSNYV	0.913724
RTLKGHSNY	9	173	181	HLA-B*57:01	5323	RTLKGHSNY	RTLKGHSNY	0.856624
KVWNVNGGK	9	162	170	HLA-A*03:01	986	KVWNVNGGK	KVWNVNGGK	0.83114

Table 5. *L. infantum* GP63 binding to MHC-I alleles with highest score which obtained by the IEDB web server

Peptide	Length	Start	End	Allele	Peptide index	Core	Icore	Score
KVRQVQDKW	9	23	31	HLA-B*57:01	5398	KVRQVQDKW	KVRQVQDKW	0.991967
YLIPQALQL	9	9	17	HLA-A*02:01	224	YLIPQALQL	YLIPQALQL	0.989888
YLIPQALQL	9	9	17	HLA-A*02:03	439	YLIPQALQL	YLIPQALQL	0.967609
YLIPQALQL	9	9	17	HLA-A*02:06	654	YLIPQALQL	YLIPQALQL	0.966576
KVRQVQDKW	9	23	31	HLA-B*58:01	5613	KVRQVQDKW	KVRQVQDKW	0.963771
RILESISNV	9	131	139	HLA-A*02:06	776	RILESISNV	RILESISNV	0.956299
HEMAHALGF	9	114	122	HLA-B*44:03	4844	HEMAHALGF	HEMAHALGF	0.938416
RILESISNV	9	131	139	HLA-A*02:01	346	RILESISNV	RILESISNV	0.923092
FSNTDFVMY	9	55	63	HLA-A*01:01	55	FSNTDFVMY	FSNTDFVMY	0.911191
HEMAHALGF	9	114	122	HLA-B*44:02	4629	HEMAHALGF	HEMAHALGF	0.910567

For MHC class II binding epitopes, we employed the MHC2PRED server and selected several peptides for each antigen, ensuring that they had a percentile rank of ≤1 and an IC50 value of ≤50. These stringent criteria were used to identify peptides with high binding scores for MHC class I and II. The specific peptides and their corresponding binding scores can be found in Table 6-8.

Table 6. *L. infantum* KMP11 binding to MHC-II alleles obtained by MHCPred v2.0 server (Prediction method: SVM).

Allele	Sequence	Residue No.	Peptide Score
HLA-DR9	RTEINLEIS	24	1.245
	KFERMIKEH	95	1.185
	EPRTEINLE	22	1.182
	FNRKMQALN	64	1.176
HLA-DR3	EEFSAPFKR	51	1.888
	RLDEEFNRK	59	1.546
	HEHSEHFKQ	112	1.325
	TEKFNKKMH	104	1.26
HLA-DQ7	GVKINETPL	2	1.439
	EMRANEPRT	17	1.413
	EFSAPFKRL	52	1.375
	NIAINFANT	36	1.084
HLA-DQ8	RKMQALNAK	66	2.077
	EPRTEINLE	22	1.736
	ISHMANIAI	31	1.675
	KQKFAELLE	119	1.618
HLA-DRB1*0901	PRTEINLEI	23	1.452
	STLSPEMKE	83	1.432
	HEHSEHFKQ	112	1.415
	DESTLSPEM	81	1.353
	FANTMMATT	41	1.556

HLA-DRB1*0401	GVKINETPL	2	1.136
	YEKFERMIK	93	1.099
	FFADKPDES	75	1.024

Table 7. *L. infantum* LACK binding to MHC-II alleles obtained by MHCPred v2.0 server (Prediction method: SVM).

MHC-II Alleles	Predicted Epitopes
IAb	VYDLESKAV, VTSLACPQQ, ATDYALTAS, GAALLWDLS, HKDNLIRVW
IAd	TAISWKANP, VATERSLSV, WVTSLACPQ, DRLIVSAGR, FSPDDRLIV
IAs	FVSCVSLAH, DGNTLYSGH, ICFSPSLEH, ATDYALTAS, RGWVTSLAC
IEd	RVWNVAGEC, RGWVTSLAC, FSPNRFWMC, RLIVSAGRD, VSCVSLAHA
MHC-II Alleles	Predicted Epitopes

Table 8. *L. infantum* GP63 binding to MHC-II alleles obtained by MHCPred v2.0 server (Prediction method: SVM).

Allele	Sequence	Residue No.	Peptide Score
HLA-DR9	KDFDVPVIN	178	1.297
	KRDILVKYL	38	1.272
	SVPSEEGVL	102	1.261
	VDEICGDFK	73	1.246
HLA-DRB1*0101	NIAINFANT	27	2.215
	VINIPAANI	128	1.671
	AINFANTMK	29	1.52
	LIPQALQLH	46	1.507
HLA-DQ7	SSTAVAKAR	187	1.632
	IPQALQLHT	47	1.415
	IPAANIASR	131	1.213
	PVINSSTAV	183	1.163
HLA-DQ8	CDTLEYLEI	200	1.921
	MKKRDILVK	36	1.864
	ISHMANIAI	22	1.675
	KKRDILVKY	37	1.574
HLA-DR13	VINIPAANI	128	1.468
	HPAVGVINI	123	1.271
	VGFFEGARI	160	1.269
	VGVINIPAA	126	1.267
I-Ag7	MAPAAAAGY	231	1.903
	ELMAPAAAA	229	1.857
	NIPAANIAS	130	1.814
	GFFEGARIL	161	1.785

In summary, this process involved predicting and selecting potential binding epitopes for both MHC class I and II, using different servers and criteria to identify peptides with strong binding affinity to human HLAs.

Leishmaniasis is widespread in subtropical regions, causing a significant burden annually **(14)**. In spite of various chemotherapy and drug therapy against leishmaniasis which have some side effects, recent advances in development of efficacious vaccines seems to be an appropriate outstanding preventive strategy for improvement of the public health and infectious diseases control **(15-17)**.

Epitope-prediction is the first and most basic step in the design of multi-epitopic vaccines. Today, with the advancement of new methods in epitope mapping, computer tools have increased the accuracy and speed of this process.

In order to make these predictions as accurate as possible, after determining the immunogenic proteins in Leishmania infantum, all three prediction modes of linear B-cell epitopes, CTL epitopes and epitopes of MHC class 1 and 2 should be determined.

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Ethical Considerations

Not applicable.

Conflict of Interest

The authors declared no conflict of interest.

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