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Original Article

Immunoinformatics Evaluation of a Fusion Protein Composed of *Leishmania infantum* LiHyV and *Phlebotomus kandelakii* Apyrase as a Vaccine Candidate against Visceral Leishmaniasis

Shima Fayaz [1,2], Fariborz Bahrami [2], Pezhman Fard-Esfahani [1], Parviz Parvizi [3], *Golnaz Bahramali [4], *Soheila Ajdary [2]

1. *Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran*
2. *Department of Immunology, Pasteur Institute of Iran, Tehran, Iran*
3. *Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran*
4. *Department of AIDS and Bloodborne Diseases, Pasteur Institute of Iran, Tehran, Iran*

Abstract
Background: Visceral leishmaniasis (VL) is a lethal parasitic disease, transmitted by sand fly vectors. Immunomodulatory properties of sand fly saliva proteins and their protective effects against *Leishmania* infection in pre-exposed animals suggest that a combination of an antigenic salivary protein along with a *Leishmania* antigen can be considered for designing a vaccine against leishmaniasis
Methods: Three different fusion forms of *L. infantum* hypothetical protein (LiHyV) in combination with *Phlebotomus kandelakii* salivary apyrase (PkanAp) were subjected to *in-silico* analyses. Major Histocompatibility Complex (MHC) class I and II epitopes in both humans and BALB/c mice were predicted. Antigenicity, immunogenicity, epitope conservancy, toxicity, and population coverage were also evaluated.
Results: Highly antigenic promiscuous epitopes consisting of truncated LiHyV (10-285) and full-length PkanAp (21-329) were identified in human and was named Model 1. This model contained 25 MHC-I and 141 MHC-II antigenic peptides which among them, MPANSDIRI and AQSLFDFSGLALDSN were fully conserved. LALDSNATV, RCSSALVSI, ALVSINVPL, SAVESGALF of MHC-I epitopes, and 28 MHC-II binding epitopes showed 60% conservancy among various clades. A population coverage with a rate of >75% in the Iranian population and >70% in the whole world was also identified.
Conclusion: Based on this *in-silico* approach, the predicted Model 1 could potentially be used as a vaccine candidate against VL.

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Introduction

*L*eishmaniases are a set of vector-borne diseases caused by intracellular parasites of the *Leishmania* genus, transmitted to vertebrate hosts by infected female Phlebotomine sand fly bites during blood-feeding (1, 2). Visceral Leishmaniasis (VL), also known as kala-azar, is the most serious form of leishmaniases and is fatal if left untreated (3). *L. (L.) infantum* is the main causative agent of VL in Iran (4). Many VL control policies such as limiting the vectors and reservoirs are proven ineffective (5, 6). Hence, vaccination remains the most effective approach to provide long-lasting immunity against the infection (6). Despite extensive studies, there is still no reliable VL vaccine (7). Recently, few antigenic proteins specific to *Leishmania* genus (annotated as hypothetical proteins in genome databases), have been revealed by proteomic studies (8). Effective antigen candidates against leishmaniases should be shared by different *Leishmania* species and induce immune responses against all or most of the species (9). Among them is *L. infantum* hypothetical protein V (LiHyV), present in both promastigote and amastigote stages of the parasite (10). LiHyV has a high homology at the amino acid level (> 85 %) among *L. major*, *L. amazonensis*, and *L. infantum* (11). The recombinant LiHyV protein (rLiHyV) is recognized by antibodies of dogs affected by VL. Moreover, the prophylactic efficacy of rLiHyV protein in a murine model has been reported (11).

Sand fly salivary proteins are immunomodulatory and have important roles in the establishment of *Leishmania* infection as well as the immune responses of the host (12, 13). Salivary apyrases of various sand fly species are recognized among the most antigenic salivary proteins, detectable by sera of repeatedly-bitten hosts (14-19). *Phlebotomus (P.) kandelakii* is a widespread vector of *L. infantum* in Iran

(20). Recently, we have characterized the full sequence of salivary apyrase of this vector (PkanAp; NCBI accession number QNG40038).

Assuming that the combination of a *Leishmania* antigen with sand fly salivary antigenic proteins could elevate the potential immunological responses (21), here we used immunoinformatics analyses of three different fusion constructs of LiHyV and PkanAp with a rigid linker (PQDPP), using *in-silico* methods. We then aimed to identify the potentially common immunogenic T-cell epitopes in mice and humans and predicted the best fusion construct, based on the predicted conservancy, antigenicity, physicochemical properties, and tertiary structures.

Materials and Methods

Study plan
A schematic plan of the methodology is demonstrated in Fig. 1.

Amino acid sequence retrieval, multiple sequence alignment, phylogenetic analysis and signal peptide predictions
The amino acid sequences of hypothetical proteins, conserved in 5 *Leishmania* species, and also salivary apyrases in various *Phlebotomus* and *Lutzomyia* species were retrieved from NCBI database. The Accession numbers were as follows: Hypothetical protein XP_001462854.1 (reference sequence), XP_003858079.1, XP_888524.1, XP_001561708.1 and XP_010703666.1; Salivary apyrase in *P. kandelakii* QNG40038 (reference sequence), AGT96454.1, AAG17637.1, ACS93497.1, ABB00907.1, AAX56357.1, ABI20151.1, ABA12135.1, ADJ54111.1, ADJ54077.1, AAD33513.1, AFP99246.1 and BAM69107.1.
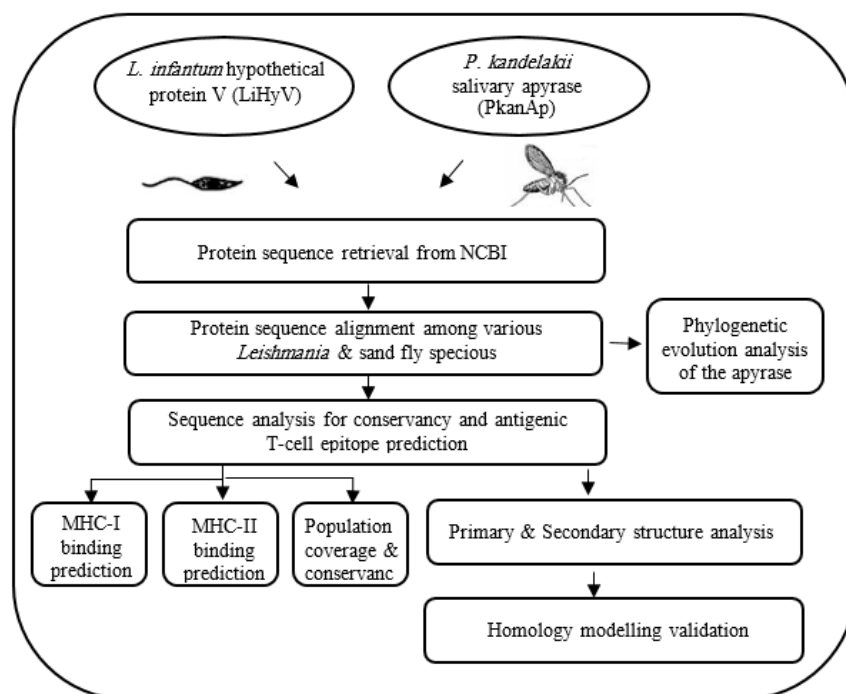
Fig. 1: Schematic representation of the immunoinformatics predictions

Conserved regions were obtained by multiple alignments of the hypothetical proteins, as well as the salivary apyrases by ClustalW (22). To infer the evolutionary history of salivary apyrase families, phylogenetic analysis was performed by the maximum likelihood method tested with Jones-Taylor-Thornton (JTT) model by MEGA software v. 6.0 (22). The presence and location of the putative signal peptide of the apyrase were analyzed by Signal P-5.0 server (23).

T-cell epitope prediction in BALB/c mice and humans

The Major Histocompatibility Complex (MHC) class I and II epitopes of the three arrangements of LiHyV-PQDPP-PkanAp were predicted by IEDB < http://tools.iedb.org/main/tcell/>. The most common Human Leukocyte Antigens (HLAs) in the Iranian population were selected according to <http://www.allelefrequencies.net> website and previously-published papers which in-

cluded Iranians from different regions (24, 25), Lur and Kurd ethnicities (26) as well as people from the following provinces of Khorasan in North-East (27), Fars in South (28) and Markazi in Center (29) of Iran. Regarding, BALB/c mice, H2-Dd, H2-Kd, and H2-Ld as MHC-I alleles and H2-IAd, H2-IEd as MHC-II alleles were considered for evaluation.

Predictions of antigenicity, immunogenicity, population coverage, and epitope conservancy

ANTIGENpro was used for the prediction of antigenicity <http://scratch.proteomics.ics.uci.edu/> and VaxiJen v2.0 for antigenic scores of the peptides <http://www.ddg-pharmfac.net/vaxijen/> (30) with 0.45 threshold. Immunogenic epitopes capable of eliciting cell-mediated immunity were predicted by IEDB MHC-I immunogenicity prediction module <http://tools.iedb.org/immunogenicity/>

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where higher scores indicate greater probabilities of eliciting an immune response. Population coverage analysis was done by submitting putative T-cell epitopes from the models to <http://tools.immuneepitope.org/tools/population/iedb_input> while conservancy or variability of the epitopes was evaluated by IEDB conservancy analysis tool <http://tools.iedb.org/conservancy/> (31).

Toxicity and allergenicity analyses

The predicted epitopes were evaluated by ToxinPred <http://crdd.osdd.net/raghava/toxinpred/> (32). AllerTOP v.2.0 was used to analyze the allergenicity <https://www.ddg-pharmfac.net/AllerTOP/method.html> (33).

Primary and secondary structure analyses

Physicochemical properties (Mw, amino acid composition, aliphatic index, theoretical Isoelectric point (pI), Grand Average of Hydropathicity index (GRAVY), estimated half-life, and extinction coefficient) were characterized by ProtParam webserver <https://web.expasy.org/protparam/> (34). The secondary structure elements (the number of α-helices, β-sheets, and random coils) of the selected models were determined by SOPMA alignment tool <https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html> (35, 36). DiANNA webserver was used to predict cysteine classification and disulfide connectivity (37).

Homology modeling validation

The 3D structures of the proteins were modeled using I-TASSER online server (38) and visualized by PyMol v1.2. The highest confidence score (c-score) signified the best model. The overall model quality was validated by ProSA web tool (39). The stereochemical quality was evaluated by Ramachandran's map from RAMPAGE online server < https://zlab.umassmed.edu/bu/rama/>.

Results

Multiple alignment and phylogenetic analysis

Based on the protein sequence alignment, two conserved regions (10- 285 and 350- 522 residues) of LiHyV protein were selected. Since *P. kandelakii* is considered as one of the endemic vectors of VL in Iran, a full-length sequence of PkanAp protein was used as the second part of the predicted constructs. The cleavage site of the signal peptide of PkanAp protein sequence was predicted between residues 20 and 21 with a 0.95 probability. Accordingly, 3 possible arrangements of LiHyV-PQDPP-PkanAp fusion protein were designated for further assessments (Table 1).

Table 1: Number of the antigenic peptides of the 3 fusion models

Fusion Model*	BALB/c mice		Human	
	CTL epitopes	HTL epitopes	CTL epitopes	HTL epitopes
Model 1 LiHyV (10-285)- PQDPP- PkanAp (21-329)	6	2	25	141
Model 2 LiHyV (1-528)- PQDPP- PkanAp (21-329)	7	8	34	153
Model 3 LiHyV (350-522)- PQDPP- PkanAp (21-329)	5	6	22	70

Antigenicity score ≥0.45
*Predicted fusion models from *L. infantum* hypothetical protein (LiHyV) and *P. kandelakii* salivary apyrase (PkanAp) with the linker (PQDPP). (The first 20 amino acids of PkanAp detected as a signal peptide were excluded)

Phylogenetic tree analyses of the apyrase family of *Phlebotomus* and *Lutzomyia* salivary proteins indicated a very close relationship between *P. kandelakii* and *P. orientalis* salivary apyrases (Fig. 2).
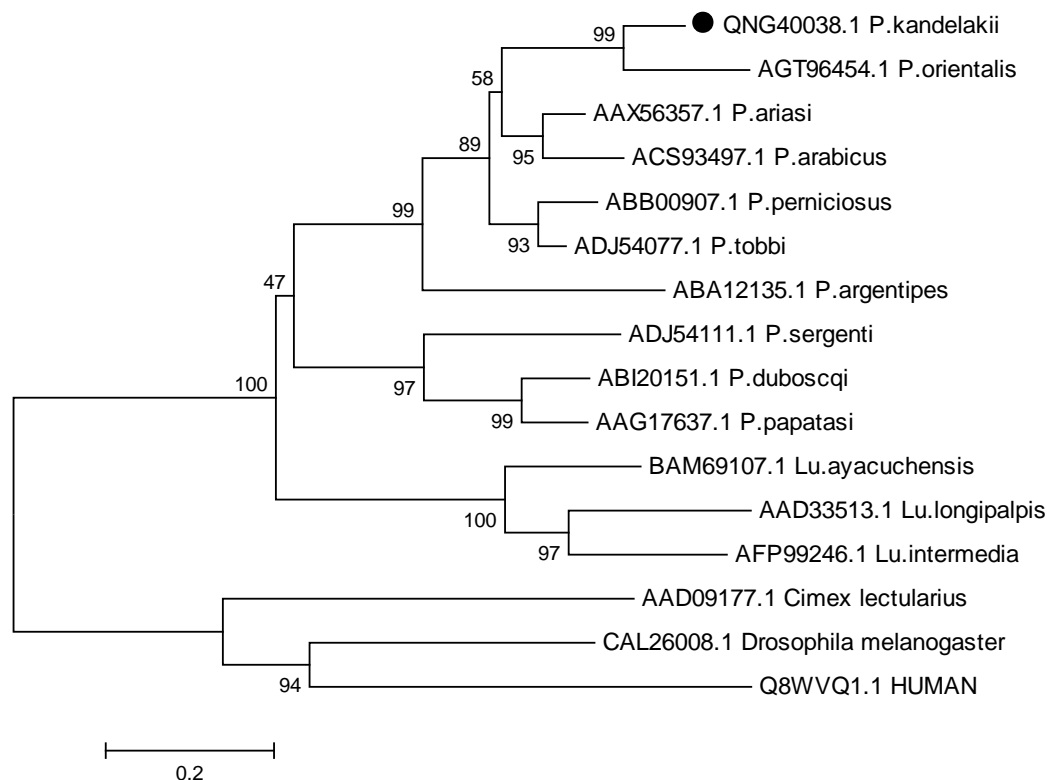

Fig. 2: Phylogenetic tree analysis comparing *P. kandelakii* salivary apyrase (PkanAp) (black circle) with the other apyrases family. Amino acid sequences were compared based on the maximum likelihood method with 1000 bootstrap replicates

Prediction of CTL and HTL epitopes

The frequencies of high-affinity antigenic peptides (percentile rank ≤ 1) of the 3 models are listed in Table 1. Model 2 contained the most peptides with a high antigenicity score. However, in vaccine design, using a short-length conserved model with a high antigenicity score is preferable. A comparison of the 2 truncated models revealed that Model 1 with more antigenic regions had a greater chance to induce a cellular immune response. The differences were not significant for BALB/c mice.

The antigenic Cytotoxic T-Lymphocyte (CTL) epitopes in Model 1 are indicated in Table 2. Since Model 1 had more human Helper T-lymphocyte (HTL) epitopes and the second part of all the models were the same, the conserved and antigenic promiscuous epitopes of truncated LiHyV in Model 1 were reported in Table 3. Altogether, the three fusion protein models were found to be antigenic, according to ANTIGENpro predicted scores of 0.88, 0.92, and 0.94.

(b)

Table 2: Antigenic epitopes of Model 1 interacting with human HLA-I

Peptide	Position	HLA-I	Percentile Rank	Antigenicity	Immunogenicity
ARLSMNMAI	78-86	HLA-B*27:02	0.08	0.4780	-0.4725
		HLA-B*27:05	0.8		
RCSSALVSI*	211-219	HLA-A*32:01	0.6	0.5634	-0.25433
LALDSNATV*	127-135	HLA-B*51:01	0.4	1.0046	-0.09277
ALLCAVVVL	3-11	HLA-A*02:01	0.9	0.5302	0.07807
SAVESGALF*	265-273	HLA-A*26:01	0.59	0.6385	0.01149
DVTMSDASF	26-34	HLA-A*26:01	0.58	0.7252	-0.36796
ALVSINVPL*	215-223	HLA-A*02:01	0.9	0.8812	-0.0012
CERCSSALV	209-217	HLA-B*50:01	0.1	0.932	-0.32774
MSDASFDDY	29-37	HLA-A*01:01	0.11	1.0868	0.02735
DASFDDYTM	31-39	HLA-B*35:01	0.5	1.2381	0.12614
MPANSDIRI**	100-108	HLA-B*51:01	0.2	1.4794	0.00853
ELIYFNGKL	351-359	HLA-A*26:01	0.66	0.9218	0.04999
ERNGQTVTY	549-557	HLA-B*27:02	0.86	1.2339	0.01326
ESGHITNIY	436-444	HLA-A*01:01	0.65	0.6599	0.28199
FTQNSYHGL	337-345	HLA-B*35:03	0.51	0.9496	-0.16159
GAELSELIY	346-354	HLA-A*01:01	0.73	0.9649	0.02289
IERNGQTVT	548-556	HLA-B*50:01	0.29	1.3453	-0.00887
KEISESGHI	432-440	HLA-B*50:01	0.33	1.6263	-0.134
NIYWENQYK	442-450	HLA-A*03:01	0.67	0.7244	0.21318
		HLA-A*11:01	0.79		
NRFTSIVKY	307-315	HLA-B*27:02	0.17	0.5829	-0.04992
		HLA-B*27:05	0.6		
SESGHITNI	435-443	HLA-B*50:01	0.29	1.2185	0.16616
SGHITNIYW	437-445	HLA-B*57:01	1	1.5017	0.28629
SIVKYGELK	311-319	HLA-A*11:01	0.44	0.9789	-0.09728
SPRKNIWVF	471-479	HLA-B*35:01	0.37	1.1449	0.12984
YFNGKLYTI	354-362	HLA-A*24:02	0.43	2.8865	-0.16888

Model 1 [LiHyV (10-285)-PQDPP-PkanAp (21-329)].
*epitopes with 60% conservancy, **fully conserved epitopes.
Antigenicity score ≥0.45.
 Positions of peptides: 1-276 aa of LiHyV, 277-281 aa of linker, 282-590 aa of PkanAp

Population coverage and conservancy analysis of Model 1

MHC-binding peptides analyzed for population coverage revealed acceptable coverage of 87.76% for MHC- I and 77.63% for MHC-II in the Iranian population. The results for other populations are indicated in Table 4. Two fully conserved epitopes including MPANS-DIRI and AQSLFDFSGLALDSN were indicated while four CTL epitopes including LALDSNATV, RCSSALVSI, ALVSINVPL, and SAVESGALF showed 60% conservancy (Table 2). Furthermore, 28 HTL epitopes were 60% preserved among the various clades (Table 3).

Table 3: Conserved and antigenic promiscuous T-cell epitopes of Model 1 interacting with human HLA-II

Peptide	*Position	HLA-II	Antigenicity
ADVVTVQLINS-QVSG	48-62	HLA-DRB1*03:06, HLA-DRB1*03:07, HLA-DRB1*03:08	0.5197
ITLSGVMPANSDIRI	94-108	HLA-DRB1*11:07	1.3287
MPANSDIRIVATTGS	100-114	HLA-DRB1*03:06, HLA-DRB1*03:07, HLA-DRB1*03:05, HLA-DRB1*03:08, HLA-DRB1*03:09, HLA-DRB1*04:02,	0.8680
PANSDIRIVATTGSL	101-115		0.7589
ANSDIRIVATTGSLA	102-116		0.8213
NSDIRIVATTGSLAP	103-117	HLA-DRB1*04:08, HLA-DRB1*04:10, HLA-DRB1*04:23, HLA-DRB1*04:26, HLA-DRB1*04:21, HLA-DRB1*11:07,	0.8890
SDIRIVATTGSLAPA	104-118		0.7071
DIRIVATTGSLAPAQ	105-119		0.8234
IRIVATTGSLAPAQS	106-120	HLA-DRB1*11:04, HLA-DRB1*11:06, HLA-DRB1*11:02, HLA-DRB1*11:21, HLA-DRB1*11:28, HLA-DRB1*13:07, HLA-DRB1*13:05, HLA-DRB1*13:01, HLA-DRB1*13:11, HLA-DRB1*13:22, HLA-DRB1*13:28, HLA-DRB1*13:27	0.4904
AQSLFDFSGLALDSN	118-132	HLA-DRB1*15:06	0.5132
QSLFDFSGLALDSNA	119-133		0.5365
SLFDFSGLALDSNAT	120-134		0.4744
LFDFSGLALDSNATV	121-135		0.5750
FDFSGLALDSNATVM	122-136	HLA-DRB1*04:01, HLA-DRB1*13:41	0.8913
DFSGLALDSNATVMV	123-137	HLA-DRB1*11:86, HLA-DRB1*13:16, HLA-DRB1*13:41	1.2197
FSGLALDSNATVMVE	124-138		1.4112
SGLALDSNATVMVEN	125-139	HLA-DRB1*13:38, HLA-DRB1*13:36, HLA-DRB1*13:65	1.3744
GLALDSNATVMVENT	126-140	HLA-DRB1*13:76, HLA-DRB1*13:96	1.3111
VDY-GRCERCSSALVS	204-218	HLA-DRB1*03:05, HLA-DRB1*11:14, HLA-DRB1*11:20	0.8439
DYGRCERCSSALVSI	205-219		0.7467
YGRCERCSSALVSIN	206-220	HLA-DRB1*11:28, HLA-DRB1*13:07, HLA-DRB1*13:05	0.7573
ERCSSALVSINVPLV		HLA-DRB1*13:23	
RCSSALVSINVPLVV	210-224	HLA-DRB1*03:05, HLA-DRB1*03:09, HLA-DRB1*11:07	0.6017
CSSALVSINVPLVVD	211-225		0.7988
SSALVSINVPLVVDA	212-226	HLA-DRB1*11:04, HLA-DRB1*11:06, HLA-DRB1*11:28	0.7077
SALVSINVPLVVDAS	213-227		0.8872
ALVSINVPLVVDASS	214-228	HLA-DRB1*13:05, HLA-DRB1*13:11	0.7955
	215-229		0.6058
LVSINVPLVVDASSL	216-230		0.5107
VSINVPLVVDASSLF	217-231	HLA-DRB1*03:05, HLA-DRB1*03:09, HLA-DRB1*11:07	0.6508

Model 1 [LiHyV (10-285)-PQDPP-PkanAp (21-329)].
*Positions of peptides: 1-276 aa of LiHyV, 277-281 aa of linker, 282-590 aa of PkanAp

Table 4: Population coverage of Model 1

Location	MHC-I *PPC(%)	Average of Epitope Hits	MHC-II PPC(%)	Average of Epitope Hits
Iran	87.76	8.07	77.63	30.01
Southwest Asia	80.9	6.76	94.07	27.99
Europe	96.65	8.68	79.07	32.48
North America	89.28	6.98	76.73	30.03
South America	64.96	3.47	58.8	15.83
North Africa	73.54	5.63	81.61	35.12
World	91.08	7.15	73.0	26.98

*PPC: Percent of Population Coverage

Toxicity and allergenicity appraisal of Model 1

Toxicity prediction of the epitopes confirmed that all 9-mer peptides were non-toxic. Except for TVDYGRCERCSSALV epitope, the rest of the 15-mer peptides were also identified as non-toxic. The allergenicity of this vaccine candidate was nonallergic and safe.

Primary and secondary structure analysis of Model 1

This Model is composed of 596 amino acids containing 55 negatively-charged and 57 positively-charged residues with a pI value of 8.11. The predicted Mw of the protein was 64.6 kDa. The protein model was estimated to be stable due to its high aliphatic index of 90.1 and appropriate instability index of 22.60. Moreover, the model is expected to be hydrophilic (GRAVY: -0.043), consisting of 16.44% alpha-helices, 10.57% beta turns, 40.60% ran-

dom coils and 32.38% extended strands. Three disulfide bonds were predicted at 6 – 209 (LiHyV: ALALLCAVVVL – VDYGRCERCSS), 212 – 499 (LiHyV: GRCERCSSALV – PkanAp: EENTGCNQIIT), and 236 – 484 (LiHyV: FRVANCKAVGA – PkanAp: FMPRKCSNQQF) locations.

Tertiary structure prediction and validation of Model 1

The generated C-score by I-TASSER was within an acceptable confidence range (-0.94). Ramachandran Plot results indicated that 61.1% of the residues were in the favored region and most of them were in the allowed regions (Fig. 3). The protein image is shown in Fig. 4A. ProSA z-score was -4.61 that indicating the acceptable quality of the generated model (Fig. 4B).
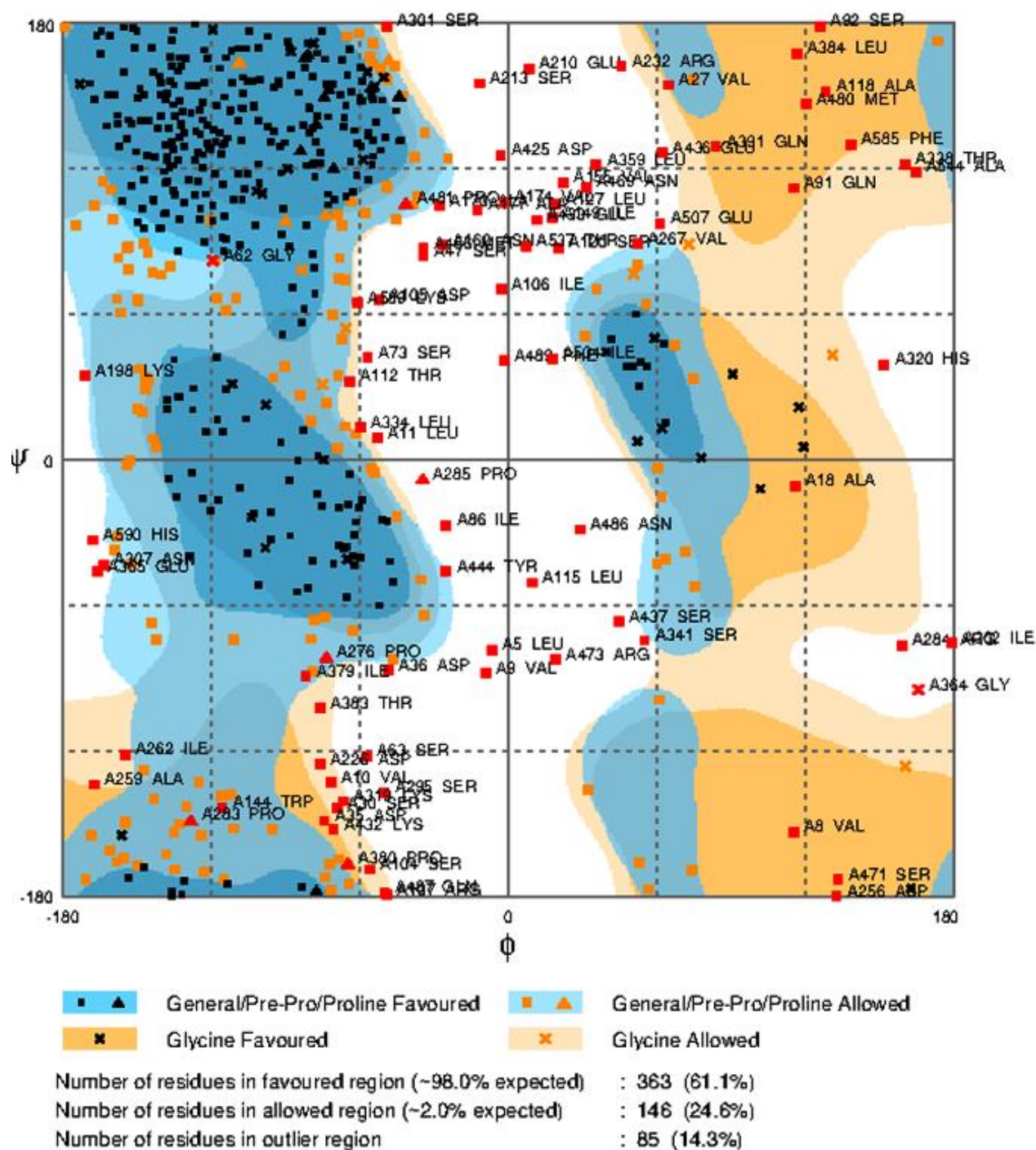
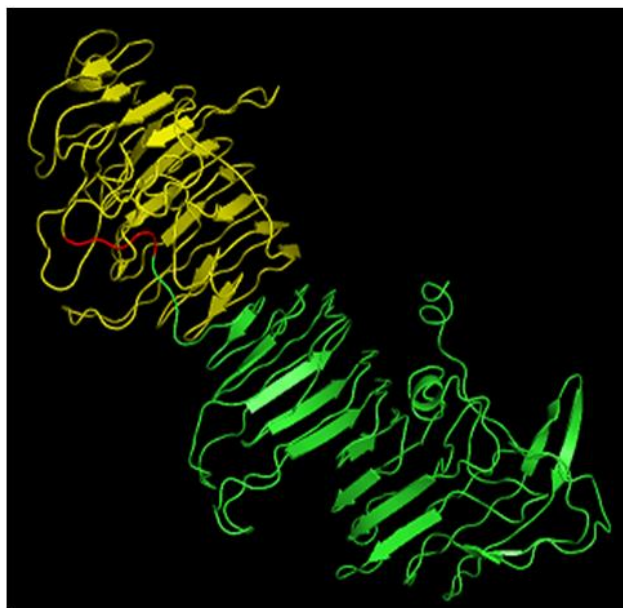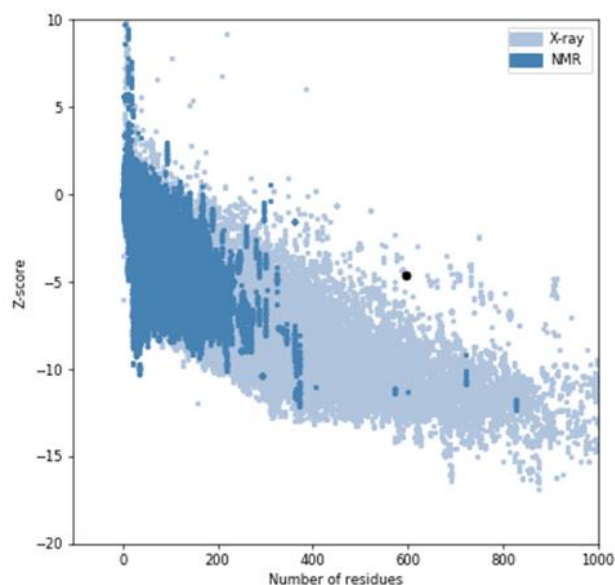**Fig. 3:** Ramachandran plot indicating the percentages of the residues in the favored and allowed regions

Fig. 4: Structural analyses of Model 1. **(A)** Tertiary structure of protein Model 1: yellow, red, and green colors indicates LiHyV (10-285), PQDPP (rigid linker), and PkanAp (21-329), respectively **(B)** ProSA plot in which the black spot represents the overall quality of the final model compared to the structure of proteins with a similar size that was determined by X-ray and NMR

Discussion

Many studies (reviewed by Ratnapriya et al) on vaccine development against VL have been conducted over the last decade; however, no appropriate VL vaccine is available so far (40). Since *L. infantum* is an intracellular parasite, the Th1 immune response plays a major role in controlling VL while the humoral response seems less important. Accordingly, T-cell epitopes-based vaccines are more efficient against VL (41). Among major considerations in designing vaccines is to overcome the discrepancy in the immune response in a genetically heterogeneous population. Therefore, prediction and conservancy analyses of promiscuous T-cell-binding epitopes to HLA-I and II molecules that drive CD8+ and CD4+ T-cell responses in a target population would be of utmost importance. Herein, we predicted for the first time three fusion protein models, incorporating LiHyV and PkanAp to de-

sign a subunit vaccine for the prevention of VL in humans. Furthermore, we evaluated potential T-cell epitopes, antigenicity, immunogenicity, epitope conservancy, toxicity, and population coverage of these models.

Immunization of BALB/c mice with a recombinant LiHyV (rLiHyV) and two of its CD8+ T-cell epitopes indicated that mice vaccinated with rLiHyV/saponin exhibited a Th1 cellular response with high production of IFN-γ and reduced parasite burden compared (11). Since the two aforementioned epitopes showed poor immunogenicity alone, it appears that selecting a larger portion of the protein with more immunogenic epitopes would make a better vaccine candidate. Computational vaccinology methods have been used in another study where using multiple peptides was assumed to improve the protective efficacy of a VL vaccine, in which potential immunodominant epitopes of LiHyV along with antigenic proteins were selected (42). Immunization of

BALB/c mice with this construct has caused robust Th1 response and significantly reduced Th2 response and parasite load (43).

It is known that sand fly salivary components are highly antigenic and the hosts repeatedly bitten by sand fly or immunized with sand fly salivary proteins, become protected against *Leishmania* infections (18, 19). Hence, recombinant proteins based on the antigens found in sand fly saliva are currently under investigation as vaccines against leishmaniases (18, 19). Interestingly, yellow related proteins and apyrase of *P. papatasi* saliva have been shown to induce significant CD4+ proliferation and IFN-γ production in the immunized individuals. Moreover, multiplex cytokine analysis has revealed that a Th1-polarized response could be prompted by such proteins (44).

Here, based on multiple sequences alignment of available LiHyV proteins in the database, two regions were selected as the first parts of fusion Models 1 and 3. In Model 2, full lengths of LiHyV and PkanAp were fused. The immunoinformatics analyses of the models indicated that the full lengths of both proteins contained more MHC-binding regions; however, they were not all conserved and epitopic. Consequently, we focused on Models 1 and 3 with shorter lengths, based on the T-cell epitopes localization in conserved regions and their binding abilities to BALB/c mice and human MHC-I and II, as well as their antigenicity and immunogenicity. The toxicity scores of the predicted T-cell epitopes of all the models were also examined. The BALB/c mice MHC-I binding regions with high antigenicity scores were comparatively alike in all 3 models. Considering MHC-II binding, Model 3 disclosed more antigenic properties than Model 1 for BALB/c mice. To verify whether the obtained results were also compatible with humans, human MHC molecules were also examined for the 3 models. When the most common HLA with the most frequent alleles in the Iranian population was selected, Model 1 contained 25 antigenic peptides with MHC-I affinity. Among them, MPANSDIRI showed a high Vaxigen score (1.4794), positive immunogenicity score, and 100% conservancy among different clades. Also, four epitopes of Model 1, showed a high antigenicity score with 60% conservancy (Table 2). We identified, 22 antigenic peptides with MHC-I affinity in Model 3 which only 3 epitopes were 60% conserved.

Considerable differences were also observed over HLA-II binding antigenic epitopes, between Models 1 and 3. In Mode 1, we detected 141 HLA-II binding peptides with high antigenicity scores which were similar to full-length Model 2 with 153 antigenic binding epitopes. However, the antigenic peptides were reduced to 70 in Model 3. In Model 1, from 141 HLA-II peptides, 76 epitopes were derived from LiHyV and 65 were from PkanAp. Since the second part of the models was the same, in Model 3 only 5 antigenic epitopes belonged to LiHyV.

Epitope conservancy has a principal role in the efficiency of a vaccine. While HLA-II antigenic epitopes of Model 1 were fully conserved and had 28 antigenic epitopes with 60% conservancy, no HLA-II antigenic epitopes with ≥ 60% conservancy could be revealed in Model 3. Altogether, fusion Model 1 with more antigenic regions, especially concerning HLA-II promiscuous epitopes, presented a greater possibility to induce a cellular immune response. The predicted peptides of Model 1 with affinity to human MHC-I, demonstrated 87.76% coverage in the Iranian population and 91.08% in the whole world whereas MHC-II peptides exhibited 77.63% in the Iranian population and 73.03% worldwide coverage. Moreover, high population coverage in Europe, America, Africa, and South-West Asia for both MHC classes was observed (Table 4).

Conclusion

Our proposed fusion construct of LiHyV-PkanAp incorporates highly promiscuous HLA-I and HLA-II restricted epitopes, as well as immune-dominant regions. Model 1 is envisaged to stimulate both CD4+ and CD8+ T-cell responses which could potentially contribute to the pathogen elimination inside the infected cells. Further in-vitro and in-vivo assessments are required to confirm the efficacy of this construct as a protective vaccine against VL.

Acknowledgements

Conflict of interest

The authors declare that they have no conflict of interest.

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