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PREDICTION OF ANTIGENIC EPITOPES FROM *Tityus serrulatus* VENOM ALLERGEN 5: AN AID TO ANTITOXIC VACCINES

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Abstract- Tityus serrulatus venoms is composed of several toxins that may cause death in human. *Tityus serrulatus* Venom allergen 5 have significant immunomodulatory ability to enhance immune functions. In this assay we a have predicted potential epitopes from *Tityus serrulatus* scorpion Venom allergen 5 for peptide vaccine design against envenomation, based on cross protection phenomenon as, an ample immune response can be generated with a single epitope. We found MHC class II binding peptides of Venom allergen 5 are important determinant against the toxic effect. The analysis shows Venom allergen 5 having 212 amino acids, which shows 204 nonamers. In this assay, we have predicted MHC-I binding peptides for 8mer_H2_Db allele (optimal score is 17.016), 9mer_H2_Db allele (optimal score is 15.825), 10mer_H2_Db allele (optimal score is 11.305), 11mer_H2_Db allele (optimal score is 22.813). We also predicted the SVM based MHCII-IAb peptide regions, 123-WTQLYVCNY, 111-ITAVEIPDP, 185-FDETDFSNY (optimal score is 10.938); MHCII-IAd peptide regions, 158-CGSHCKKHN, 107-FTRGITAVE, 91-CDDCRKVEN (optimal score is 20.3); and MHCII-IAg7 peptide regions 36-NTIINLHNK, 13-HTFCKTKNQ, 194-IFNCDFKPE, 70-WDDELAQIA, 95-RKVENFDVG (optimal score is 14.595); which shows potential binders from *Tityus serrulatus* antigen 5. The method integrates prediction of MHC class I binding proteasomal C- terminal cleavage peptides and nine potential antigenic peptides at average propensity 1.021 having highest local hydrophilicity. Thus a small antigen fragment can induce immune response against whole antigen. This approach can be applied for designing subunit and synthetic peptide vaccines.
Keywords- envenomation, Antigenic peptides, MHC-Binders, Nonamers, synthetic peptide vaccines
Abbreviations- MHC-Major Histocompatibility Complex, SVM-Support Vector Machine, APC- Antigen Presenting Cell

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Introduction

Scorpion venoms consist of a complex of several toxins that exhibit a wide range of chemical compositions, biological characteristics and actions, toxicity, and pharmacokinetic and pharmacodynamic properties. *Tityus serrulatus* is considered the most dangerous scorpion in South America and responsible for most of the fatal cases, commonly known Brazilian yellow scorpion, belongs to the family Buthidae; its venom is extremely toxic. *Tityus serrulatus* scorpion Venom allergen 5 (antigen 5) is complex of molecules and having significant immunomodulatory capacities of stimulating immune functions. Because of its role in the peripheral nervous system (PNS) and enhancing the neurotransmitters secretion, antigen 5 is capable of exerting a several effects on excitable tissues [1,2].

Strategy

This approach is based on the phenomenon of cross-protection [3] hereby an individual affected with a mild toxin possess immunity against similar toxin. Body proteins are necessary for production of immunity in or on all food commodities. Relief from the requirement of a tolerance is established for residues of the drugs or chemicals.

MHC Class Binding Peptides

The new paradigm in vaccine design is emerging, following essential discoveries in immunology and development of new MHC class I binding peptides prediction tools [4-6]. MHC molecules are cell surface glycoproteins, which take active part in host immune reactions. The involvement of MHC class I in response to almost all antigens and the variable length of interacting peptides make the study of MHC class I molecules very interesting. MHC molecules have been well characterized in terms of their role in immune reactions. They bind to some of the peptide fragments generated after proteolytic cleavage of antigen [7]. This binding act like red flags for specific antigen and to generate immune response against the parent antigen, thus an antigen subunit can induce immune response against complete venom activities. Antigenic peptides are most suitable for subunit vaccine development because an epitope,

can generate ample immune response in large population. MHC-Peptide complexes will be translocated on the surface of antigen presenting cells (APCs). This theme is implemented in designing subunit and synthetic peptide vaccines [8-11]. One of the important problems in subunit vaccine design is to search for antigenic regions in toxin protein [12] that can stimulate T-cells called T-cell epitopes. Fortunately, in literature a large amount of data about such peptides is available. Pastly and presently, a number of databases have been developed to provide comprehensive information related to T-cell epitopes [13-17].

Materials and Methods

Protein Sequence Analysis

The antigenic protein sequence of *Tityus serrulatus* Venom allergen 5 (antigen 5) was analyzed to study the antigenicity [18], solvent accessible regions and MHC class binding peptides, which allows potential drug targets to identify active sites against venomous activity of the toxin protein.

Antigenicity Prediction

Antigenicity prediction program results those segments from *Tityus serrulatus* Venom allergen 5 that are likely to be antigenic by eliciting an antibody response. Antigenic epitopes are determined using the Gomase (2007), Hopp and Woods (1981), Welling (1985), Parker (1986), BepiPred Server (2006) and Kolaskar and Tongaonkar Antigenicity (1990) methods [19-24].

Protein Secondary Structure Prediction

The important concepts in secondary structure prediction are identified as: residue conformational propensities, sequence edge effects, moments of hydrophobicity, position of insertions and deletions in aligned homologous sequence, moments of conservation, auto-correlation, residue ratios, secondary structure feedback effects and filtering [25, 26].

MHC Binding Peptide Prediction

The MHC binding peptides are predicted by using neural networks trained on C terminals of known epitopes. In this work predicted MHC-Peptide binding is a log-transformed value related to the IC50 values in nM units. RankPep predicts peptide binders to MHC-I and MHC-II molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method has been used for prediction of promiscuous MHC class II binding peptides. The average accuracy of SVM based method for 42 alleles is ~80%. For determination of potential MHC binders, an elegant machine learning technique SVM has been applied. SVM has been trained on the binary input of single amino acid sequence. In addition, we predicts those MHC -I ligands whose C-terminal end is likely to be the result of proteosomal cleavage [27-33].

Result and Interpretation

A *Tityus serrulatus* Venom allergen 5 antigenic sequence (gi-193806572) is 212 residues long as-

ECPALYRRYSKEHTFCKTKNQKCNIKRWGVSQDDRNTI-
INLHNKVRNNIALGQDQSGRLPAAGDMLEMEWDDELAQIAQKLA
DQCVFKHDCDDCRKVENFDVGQNIFTRGITAVEIPDPFKSWTQL
YVCNYGPAGNLDDSELYKVDKPCEKCPSNTCCGSHCKKHNKST
SYLGLCDVLNGSGPDFDETDFSNYIFNCDFKPESDCNNKVEGS

Antigenic Peptides Prediction

In this assay we predicted the antigenic determinants by finding the area of highest local hydrophilicity. We studied methods Kolaskar and Tongaonkar antigenicity, BepiPred Server, Parker, Emini Surface Accessibility methods [Fig-1], [Fig-2], [Fig-3], [Fig-4], [Table-1] .

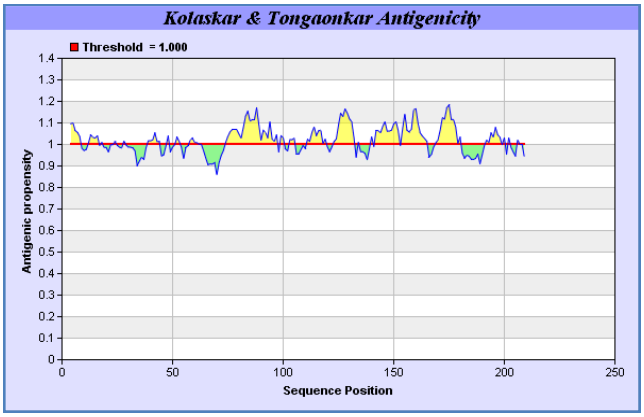

Fig. 1- Kolaskar and Tongaonkar antigenicity plot showing antibody recognized antigenicity for the *T. serrulatus* antien 5.

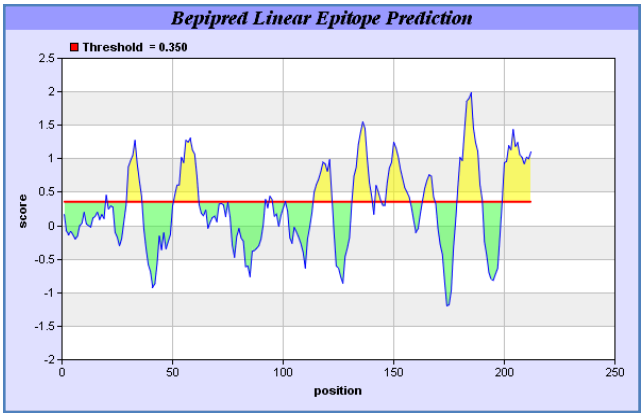

Fig. 2- Bepipred Linear Epitope Prediction plot showing antibody recognized B-cell epitopes of the *T. serrulatus* Venom allergen 5

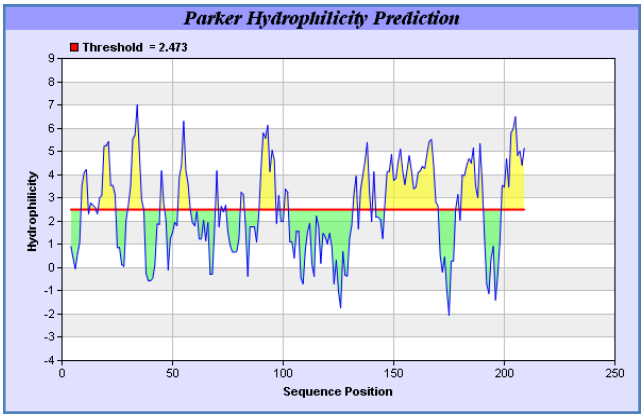

Fig. 3- HPLC / Parker et al. (1986) hydrophobicity plot of *T. serrulatus* Venom allergen 5 (antigen 5)

Hopp & Woods hydrophobicity method which predict the locations of antigenic determinants in antigen protein, assuming that the antigenic determinants would be exposed on the protein surface and thus would be located in hydrophilic regions [Fig-5], its values

are derived from the transfer-free energies for amino acid side chains between ethanol and water.

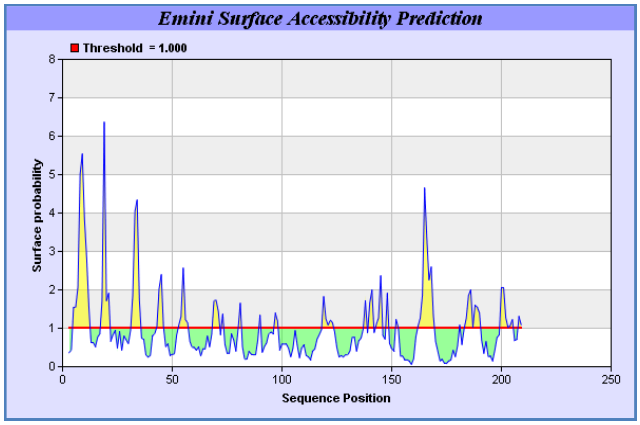

Fig. 4- Emini Surface Accessibility Prediction plot of *T. serrulatus* Venom allergen 5 (antigen 5)

Table 1- Antigenic epitopes of T. serrulatus Venom allergen 5

No.	Start Position	End Position	Peptide	Peptide Length
1	39	44	INLHNK	6
2	58	63	RLPAAG	6
3	74	97	LAQIAQKLADQCVFKHDCDDCRKV	24
4	111	119	ITAVEIPDP	9
5	123	132	WTQLYVCNYG	10
6	142	152	LYKVDKPCEKC	11
7	154	165	SNTCCGSHCKKH	12
8	169	180	TSYLGLCDVLNG	12
9	192	198	NYIFNCD	7

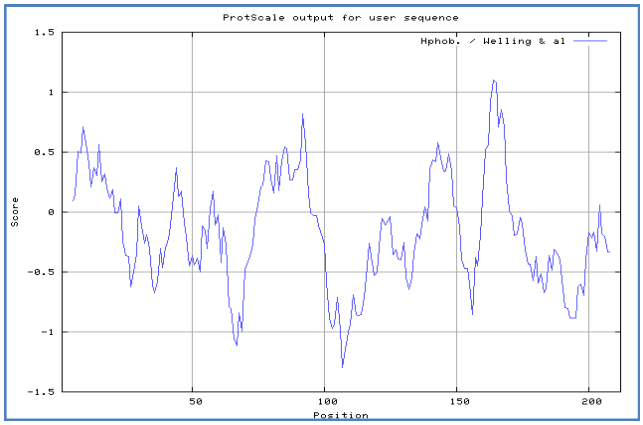

Fig. 5- Hopp and Woods (1981) hydrophobicity plot of *T. serrulatus* Venom allergen 5 (antigen 5)

Welling hydrophobicity plot gives value as the log of the quotient between percentage in a sample of known antigenic regions and percentage in average proteins [Fig-6]. The predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design.

Secondary Alignment

The Robson and Garnier method has been applied for the prediction of *T. serrulatus* Venom allergen 5 (antigen 5) secondary structure. Each residue is assigned values for alpha helix (*Shown in Red*), beta sheet (*Shown in Blue*) and coils (*Shown in Pink*) using a window of 7 residues [Fig-7]. Using these information parameters, the likelihood of a given residue assuming each of the four possible conformations alpha, beta, reverse turn, or coils calculated, and the conformation with the largest likelihood is assigned to the residue.

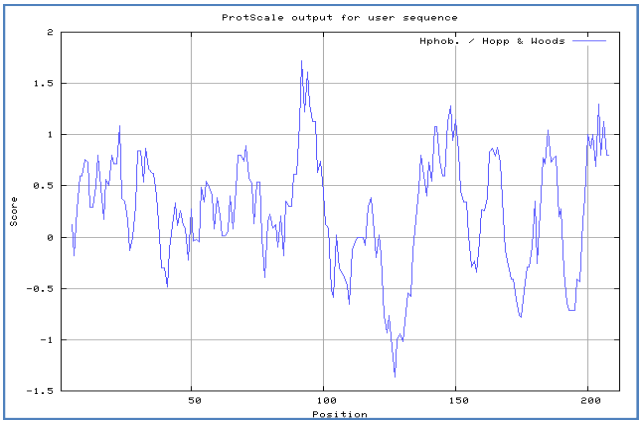

Fig. 6- Welling et al. (1985) hydrophobicity plot of *T. serrulatus* Venom allergen 5 (antigen 5)

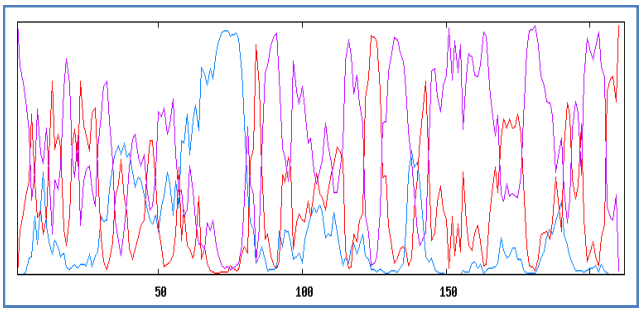

Fig. 7- Secondary structure plot of the *T. serrulatus* Venom allergen 5 (antigen 5)
**Red: helix, Blue: Sheet, Pink: Coil*

Prediction of MHC Binding Peptides

These MHC binding peptides are sufficient for producing the desired immune response. The prediction is based on support vector machine, using amino acids sequence. In this test, we found the MHC-I and MHC-II binding regions [Table-2], [Table-3]. MHC molecules are cell surface glycoproteins, which actively take part in host immune reactions and involvement of MHC-I and MHC-II in response to almost all antigens. In this study we predicted the binding affinity of *T. serrulatus* Venom allergen 5, having 212 amino acids, which show several potential nonamers [Table-2], [Table-3]. For development of MHC binding prediction method, an elegant machine learning technique Support Vector Machine (SVM) has been used. SVM has been trained on the binary input of single amino acid sequence. In this assay we predicted the binding affinity of *T. serrulatus* Venom allergen 5 sequence having 212 amino acids, which shows 204 nonamers.

We Predicted the SVM based MHCII-IAb peptide regions, 123-WTQLYVCNY, 111-ITAVEIPDP, 185-FDETDFSNY (optimal score is 10.938); MHCII-IAd peptide regions, 158-CGSHCKKHN, 107-FTRGITAVE, 91-CDDCRKVEN (optimal score is 20.3); and MHCII-IAg7 peptide regions 36-NTIINLHNK, 13-HTFCKTKNQ, 194-IFNCDFKPE, 70-WDDELAQIA, 95-RKVENFDVG (optimal score is 14.595); which shows predicted binders from antigen 5 [Table-3].

The predicted binding affinity is normalized by the 1% fractil. The MHC-Peptide binding is predicted using neural networks trained on C terminals of known epitopes. In this assay predicted MHC-Peptide binding is a log-transformed value related to the IC50 values in nM units. These MHC binding peptides can decently elicit the proper immune response. Predicted MHC binding regions in an toxin sequence and these are directly associated with immune reactions, we determined the MHC-I and MHC-II binding region.

Table 2- Prediction of MHC class I peptides, from T. serrulatus antigen 5 having C-terminal ends are proteosomal cleavage sites

MHC-I Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
8mer_H2_Db	121	DPF	KSWTQLYV	CNY	983.16	17.016	32.42%
8mer_H2_Db	104	DVG	QNIFTRGI	TAV	930.07	12.968	24.70%
8mer_H2_Db	31	WGV	SQDDRNTI	INL	929.94	11.89	22.65%
8mer_H2_Db	90	FKH	DCDDCRKV	ENF	935.04	8.788	16.74%
8mer_H2_Db	102	NFD	VGQNIFTR	GIT	916.04	8.646	16.47%
8mer_H2_Db	18	FCK	TKNQKCNI	KRW	930.07	8.219	15.66%
8mer_H2_Db	178	CDV	LNGSGPDF	DET	787.83	7.439	14.17%
8mer_H2_Db	109	IFT	RGITAVEI	PDP	839.99	7.239	13.79%
8mer_H2_Db	114	ITA	VEIPDPFK	SWT	926.09	5.083	9.68%
8mer_H2_Db	144	ELY	KVDKPCEK	CPS	928.11	4.999	9.52%
8mer_H2_Db	32	GVS	QDDRNTII	NLH	956.02	4.419	8.42%
8mer_H2_Db	135	GPA	GNLDDSEL	YKV	843.85	3.7	7.05%
8mer_H2_Db	124	KSW	TQLYVCNY	GPA	985.12	3.213	6.12%
8mer_H2_Db	80	IAQ	KLADQCVF	KHD	905.08	2.528	4.82%
8mer_H2_Db	19	CKT	KNQKCNIK	RWG	957.14	1.856	3.54%
8mer_H2_Db	165	CKK	HNKSTSYL	GLC	931.01	1.733	3.30%
8mer_H2_Db	116	AVE	IPDPFKSW	TQL	948.13	1.692	3.22%
8mer_H2_Db	61	RLP	AAGDMLEM	EWD	818.96	1.52	2.90%
8mer_H2_Db	193	FSN	YIFNCDFK	PES	1031.2	1.314	2.50%
8mer_H2_Db	186	PDF	DETDFSNY	IFN	971.94	0.926	1.76%
8mer_H2_Db	44	LHN	KVRNNIAL	GQD	909.09	0.19	0.36%
8mer_H2_Db	2	E	CPALYRRY	SKE	1023.24	0.033	0.06%
9mer_H2_Db	192	DFS	NYIFNCDFK	PES	1145.3	15.825	31.42%
9mer_H2_Db	87	QCV	FKHDCDDCR	KVE	1120.23	9.331	18.53%
9mer_H2_Db	43	NLH	NKVRNNIAL	GQD	1023.19	8.086	16.05%
9mer_H2_Db	170	KST	SYLGLCDVL	NGS	964.15	8.076	16.03%
9mer_H2_Db	101	ENF	DVGQNIFTR	GIT	1031.13	7.479	14.85%
9mer_H2_Db	58	QSG	RLPAAGDML	EME	925.12	7.361	14.62%
9mer_H2_Db	120	PDP	FKSWTQLYV	CNY	1130.34	5.075	10.08%
9mer_H2_Db	164	HCK	KHNKSTSYL	GLC	1059.18	3.583	7.11%
9mer_H2_Db	11	RYS	KEHTFCKTK	NQK	1103.29	2.407	4.78%
9mer_H2_Db	103	FDV	GQNIFTRGI	TAV	987.12	1.884	3.74%
9mer_H2_Db	60	GRL	PAAGDMLEM	EWD	916.08	1.673	3.32%
9mer_H2_Db	37	DRN	TIINLHNKV	RNN	1033.22	1.163	2.31%
10mer_H2_Db	36	DDR	NTIINLHNKV	RNN	1147.32	11.305	19.21%
10mer_H2_Db	163	SHC	KKHNKSTSYL	GLC	1187.35	8.905	15.13%
10mer_H2_Db	72	EWD	DELAQIAQKL	ADQ	1110.28	7.984	13.56%
10mer_H2_Db	181	LNG	SGPDFDETDF	SNY	1111.1	4.183	7.11%
10mer_H2_Db	169	NKS	TSYLGLCDVL	NGS	1065.25	3.783	6.43%
10mer_H2_Db	102	NFD	VGQNIFTRGI	TAV	1086.25	2.203	3.74%
10mer_H2_Db	154	KCP	SNTCCGSHCK	KHN	1021.14	2.054	3.49%
11mer_H2_Db	162	GSH	CKKHNKSTSYL	GLC	1290.49	22.813	28.70%
11mer_H2_Db	175	LGL	CDVLNGSGPDF	DET	1105.19	14.924	18.77%
11mer_H2_Db	101	ENF	DVGQNIFTRGI	TAV	1201.34	13.908	17.50%
11mer_H2_Db	121	DPF	KSWTQLYVCNY	GPA	1363.58	9.812	12.34%
11mer_H2_Db	16	HTF	CKTKNQKCNIK	RWG	1289.55	4.869	6.12%
11mer_H2_Db	164	HCK	KHNKSTSYLGL	CDV	1229.39	4.582	5.76%
11mer_H2_Db	39	NTI	INLHNKVRNNI	ALG	1316.51	4.261	5.36%
11mer_H2_Db	56	QDQ	SGRLPAAGDML	EME	1069.25	3.855	4.85%
11mer_H2_Db	36	DDR	NTIINLHNKVR	NNI	1303.51	0.563	0.71%

**Optimal Score for given MHC-I peptide binder in Mouse.*

Discussion and Conclusion

Gomase method (2007), BepiPred Server, Hopp and Woods, Welling, Parker, Kolaskar and Tongaonkar antigenicity scales were designed to predict the locations of antigenic determinants in *S. haematobium* 23-kDa transmembrane protein. It shows beta sheets regions, which have higher antigenic response than helical region of this peptide and shows high antigenicity [Fig-1], [Fig-2], [Fig-3], [Fig-4], [Fig-5], [Fig-6]. In this assay we predicted the binding affinity of *T. serrulatus* Venom allergen 5 having 212 amino acids, which shows 204 nonamers. We predicted MHC-I binding peptides for 8mer_H2_Db allele (optimal score is 17.016), 9mer_H2_Db allele (optimal score is 15.825), 10mer_H2_Db allele (optimal score is 11.305), 11mer_H2_Db allele (optimal score is 22.813) [Table-2]. MHC molecules are cell surface glycoproteins, which take active part in host immune reactions and involvement of MHC I and MHC II in response to almost all antigens [Table-2], [Table-3]. Kolaskar and Tongaonkar antigenicity predicted epitopes are the sites of molecules those are recognized by the immune system antibodies for the *T. serrulatus* Venom allergen 5 (antigen 5), analysis shows antigen 5 epitopes are able to induce desired immune response against envenomation. The region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because C- terminal regions of venom allergen 5 are solvent accessible and unstructured; antibodies against those regions are also likely to recognize the native protein. During prediction of antigenic determinant site of antigen 5, we found nine antigenic determinant sites in the sequence. The average propensity for the *T. serrulatus* Venom allergen 5 found is 1.021 [Fig-2]. All residues having above 1.0 propensity are always potentially antigenic [Table-1]. The predicted segments in toxin protein are 39-INLHNK-44, 58-RLPAAG-63, 74-LAQIAQKLADQCVFKHDCDDCRKV-97, 111-ITAVEIPDP-119, 123-WTQLYVCNYG-132, 142-LYKVDKPCEKC-152, 154-SNTCCGSHCKKH-165, 169-TSYLGLCDVLNG-180, 192-NYIFNCD-198. Fragments identified through this approach supposed to be high-efficiency binders, which is a much percentage of their molecules are directly involved in binding as compared to larger molecules.

Table 3- Peptide binders to MHCII molecules of T. serrulatus Venom allergen 5 (antigen 5)

MHC-I Allele	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
I_Ab	123	FKS	WTQLYVCNY	GPA	1148.33	10.938	30.70%
I_Ab	111	TRG	ITAVEIPDP	FKS	936.08	10.412	29.22%
I_Ab	185	GPD	FDETDFSNY	IFN	1119.12	10.204	28.64%
I_Ad	158	NTC	CGSHCKKHN	KST	995.13	20.3	38.20%
I_Ad	107	QNI	FTRGITAVE	IPD	975.11	8.454	15.91%
I_Ad	91	KHD	CDDCRKVEN	FDV	1063.17	7.129	13.41%
I_Ag7	36	DDR	NTIINLHNK	VRN	1048.19	14.595	35.71%
I_Ag7	13	SKE	HTFCKTKNQ	KCN	1088.23	13.54	33.13%
I_Ag7	194	SNY	IFNCDFKPE	SDC	1094.26	9.771	23.91%
I_Ag7	70	EME	WDDELAQIA	QKL	1019.12	8.305	20.32%
I_Ag7	95	DDC	RKVENFDVG	QNI	1045.16	7.685	18.80%

**Optimal Score for given MHC-II peptide binder in Mouse.*

Future Perspectives

This method will be applicable in cellular immunology, Vaccine design, immunodiagnostics, immunotherapeutics and molecular understanding of autoimmune susceptibility. *T. serrulatus* Venom allergen 5 (antigen 5) sequence contains multiple antigenic components to direct and empower the immune system to protect the host against envenomation. MHC molecules are cell surface proteins, which take active part in host immune reactions and involvement of MHC class in response to almost all antigens and it give impacts on specific sites. Predicted MHC binding regions acts like

red flags for specific antigen and generate immune response against complete venom toxin. The method integrates prediction of peptide MHC class binding; proteosomal C terminal cleavage and potential antigenic epitope prediction. This theme is implemented in designing subunit and synthetic peptide vaccines.

References

[1] Vera L. Petricevich and Ivo Lebrun (2005) *Mediators Inflamm*., (1), 39-49.
[2] Freire-Maia L., Campos J.A. (1989) *Natural Toxins: Characterization, Pharmacology and Therapeutics*, Oxford: Pergamon Press., 139-159.
[3] Valkonen J.P., Rajamäki M.L., Kekarainen T. (2002) *Mol. Plant Microbe Interact.*, 15, 683-92.
[4] Reche P.A., et al. (2002) *Human Immunology*, 63, 701-709.
[5] Reche P.A., et al. (2004) *Immunogenetics*, 56, 405-419.
[6] Reche P.A., Reinherz, E.L. (2007) *Methods Mol. Biol*., 409, 185 -200.
[7] Kumar M., Gromiha M.M., Raghava G.P. (2007) *BMC Bioinformatics,* 8, 463.
[8] Gomase V.S., Chitlange N.R. (2012) *Drug Designing*, 1(1), doi./10.4172/ddo.1000101.
[9] Gomase V.S., Chitlange N.R. (2012) *Vaccines & Vaccination*, 1 (1), doi./10.4172/2157-7560.1000131.
[10] Gomase V.S., Chitlange N.R. (2011) *Bioinfo Journal of Proteomics,* 1(1), 06-10.
[11] Gomase V.S., Kale K.V., Chikhale N.J., Changbhale S.S. (2007) *Curr. Drug Discov. Technol.*, 4*,* 117-1215.
[12] Schirle M., Weinschenk T., Stevanovic S. (2001) J. Immunol. Methods, 257, 1-16.
[13] Gomase V.S., Changbhale S.S., Kale K.V. (2008) *Advancements in Information Technology and Internet Security*, 370-378.
[14] Gomase V.S., Changbhale S.S., Patil S.A. and Kale K.V. (2008) *Current Drug Metabolism*, 9(1), 88-98.
[15] Rammensee H., Bachmann J., Emmerich N.P., Bachor O.A., Stevanovic S. (1999) *Immunogenetics,* 50, 213-9.
[16] Blythe M.J., Doytchinova I.A., Flower D.R. (2002) *Bioinformatics,* 18, 434-9.
[17] Schonbach C., Koh J.L., Flower D.R., Wong L., Brusic V. (2002) *Nucleic Acids Res.,* 30, 226-9.
[18] Korber T.M.B., Brander C., Haynes B.F., Koup R., Kuiken C., et al. (2001) *Theoretical Biology and Biophysics*, Los Alamos, New Mexico. LA-UR 02-4663.
[19] Saritha R.K., Jain R.K. (2007) *Arch. Virol*., 152(6), 1195-1200.
[20] Gomase V.S. (2006) *Curr. Drug Discov. Technol.,* 3, 225-229.
[21] Hopp T.P., Woods K.R. (1981) *Proc. Natl. Acad. Sci.* USA, 78, 3824-3828.
[22] Welling G.W., Weijer W.J., van der Zee R., Welling-Wester S. (1985) FEBS Lett., 188, 215-218.
[23] Jens E.P.L., Ole L., Morten N. (2006) *Immunome. Res.,* 2(2).
[24] Parker J.M.R., Guo D., Hodges R.S. (1986) *Biochemistry,* 25, 5425- 5431.
[25] Kolaskar A.S., Tongaonkar P.C. (1990) *FEBS Lett*., 276, 172-174.
[26] Garnier J., Osguthorpe D.J., Robson B. (1978) J. Mol. Biol., 120, 97-120.
[27] Robson B., Garnier J. (1993) *Nature,* 361(506).
[28] Wilkins M.R., Gasteiger E., Bairoch A., Sanchez J.C., Williams K.L., et al. (1999) *Methods Mol. Biol*., 112, 531-52.
[29] Brusic V., Rudy G., Honeyman G., Hammer J., Harrison L. (1998) *Bioinformatics*, 14, 121-30.
[30] Bhasin M., Raghava G.P. (2005) *Nucleic Acids Res*., 33, W202 -207.
[31] Gomase V.S., Changbhale S.S., Patil S.A., Kale K.V. (2008) *Current Drug Metabolism*, 9(1), 88-98.
[32] Gomase V.S., Chitlange N.R. (2010) *Int'l. J. of Pharma and Bio Sciences*, 1(3), 01-07.
[33] Gomase V.S. and Chitlange N.R. (2010) *Int'l. Journal of Immunology Research*, 1(1), 01-06.
[34] Gomase V.S. and Chitlange N.R. (2010) *International Journal of Drug Design and Discovery*, 1(4), 310-313.