IN-SILICO APPROACH FOR PREDICTION OF VACCINE POTENTIAL ANTIGENIC PEPTIDES FROM 23-kDa TRANSMEMBRANE ANTIGEN PROTEIN OF Schistosoma haematobium

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Abstract- Urogenital schistosomiasis is frequently occurring parasitic disease in tropical countries, S. haematobium is main causative agent responsible for urogenital schistosomiasis; till date no effective invention made to against urogenital schistosomiasis. In this analysis we have predicted suitable antigenic peptides from Schistosoma haematobium 23-kDa transmembrane protein for peptide vaccine design against urogenital schistosomiasis based on cross protection phenomenon as, an ample immune response can be generated with a single protein subunit. We found MHC class II binding peptides of S. haematobium 23-kDa are important determinant against the diseased condition. The analysis shows S. haematobium 23-kDa transmembrane protein having 216 amino acids, which shows 210 nonamers. In this assay, we have predicted MHC-I binding peptides for 8mer_H2_Db allele (optimal score is 14.128), 9mer_H2_Db allele (optimal score is 20.065), 10mer_H2_Db allele (optimal score is 13.776), 11mer_H2_Db allele (optimal score is 31.213). We also predicted the SVM based MHC-II-Iab peptide regions, 152-DYGPNIPAS, 51-WQAAAPIAI, 50-WQAAAPIAI, 142-FHCCGAKGP, 97-AELAAIVA (optimal score is 14.911); MHCII-IaD peptide regions, 100-AAIAVAVVY, 71-LGCCGAIKE, 192-FGVCFFQLL, 186-IVACVAFGV (optimal score is 13.112); and MHCII-IAg peptides regions 42-QYGDNLHKV, 101-AAIVAIVAVYK, 28-VLIGAGAYV, 103-IVAVYKDR, 203-VIACCLGRQ (optimal score is 11.605) which shows potential binders from S. haematobium 23-kDa transmembrane protein. The method integrates prediction of MHC class I binding proteasomal C- terminal cleavage peptides and six potential antigenic peptides at average propensity 1.094 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- Urogenital schistosomiasis, parasitic disease, Antigenic peptides, MHC-Binders, Nonamers, synthetic peptide vaccines

Abbreviations- MHC-Major Histocompatibility Complex, SVM-Support Vector Machine, APC-Antigen Presenting Cell

Introduction

Schistosomiasis is one of the neglected tropical disease caused by a parasitic worm of Schistosoma spp. that primarily lives in the blood. The parasite is transmitted to humans by penetration of the skin in fresh water and cause severe damage including bleeding and cancer, which may cause the death of an individual. Schistosomiasis infection estimated in ~240 million people in the world. The classic sign of urogenital schistosomiasis is haematuria. Fibrosis of the bladder and ureter and kidney damage are common findings. S. haematobium is one of the major causative agents of urogenital schistosomiasis in tropical and sub-tropical countries [1].

Pathogenesis

The free living cercarial form of the S. haematobium penetrates human skin in fresh water. The cercariae travel through the tissue to the blood stream. Mature male and female worms over mating in the veins of the liver before moving to their final destination, the veins that drain to the bladder. The female adult worm produces 200-2000 eggs per day. Eggs circulate in the blood vessels until they become lodged in various organs. The accumulation of eggs in the various tissues and organs of the body can cause severe damage including bleeding and cancer. An accumulated damage caused by the eggs rather than the parasites themselves that caus-
es the majority of mortality and morbidity associated with the disease [2].

Strategy

This approach is based on the phenomenon of cross-protection [3] whereby an individual infected with a mild strain of pathogen possess immunity against more severe strain of the same pathogen. 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 whole antigen. Antigenic peptides are most suitable for subunit vaccine development because with single epitope, the immune response can be generated 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 an antigen 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 Schistosoma haematobium 23-kDa transmembrane protein 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 lymphatic filariasis.

Antigenicity Prediction

Antigenicity prediction program results those segments from Schistosoma haematobium 23-kDa transmembrane protein 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 Schistosoma haematobium 23-kDa antigenic sequence (gi-2501225) is 218 residues long as-

MATLGTGMRCLKSCVFVLNIICLCCSLVLIGAGAYVEVKFSOYGDNLHKVKQAPIAVIIVGVLISVFLGCGAIEKVNCMLMYAFLIIJELAALAAVAVYKDRSIDEIDALMTGALDKPTEITEMDLIQS SFHCCGAKGPQDYGPNIAPSCRGGETTYHHECGVPFGAFKLRLNVIACVAFGVCFFQLSIVIACCLGRQIKEYENV

Antigenic Peptides Prediction

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

![Bepipred Linear Epitope Prediction](image)

**Fig. 1-** Bepipred Linear Epitope Prediction plot showing antibody recognized B-cell epitopes of the S. haematobium 23-kDa
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.

Fig. 2- Kolaskar and Tongaonkar antigenicity plot showing antibody recognized antigenicity for the *S. haematobium* 23-kDa.

Fig. 3- HPLC / Parker et al. (1986) hydrophobicity plot of *S. haematobium* 23-kDa

Fig. 4- Emini Surface Accessibility Prediction plot of *S. haematobium* 23-kDa

Fig. 5- Hopp and Woods (1981) hydrophobicity plot of *S. haematobium* 23-kDa

Fig. 6- Welling et al. (1985) hydrophobicity plot of *S. haematobium* 23-kDa

**Secondary Alignment**

The Robson and Garnier method has been applied for the prediction of *Schistosoma haematobium* 23-kDa transmembrane protein 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. 7- Secondary structure plot of the *S. haematobium* 23-kDa transmembrane protein.

*Red: helix, Blue: Sheet, Pink: Coil*
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### Table 1- Antigenic epitopes of S. haematobium 23-kDa transmembrane protein

| No. | Start Position | End Position | Peptide | Peptide Length |
|-----|----------------|--------------|---------|----------------|
| 1   | 71             | 111          | GMIRCLKSCVFLVNLICLCSLVILGAGAYVEVKFS | 35 |
| 2   | 47             | 110          | LHKVWQAPAIIVGIVILVFSLLGCAGKENVLVMAYFAFFILUAAALAAAIVAVVKD | 64 |
| 3   | 136            | 148          | DLQGSSHHCCGAK | 13 |
| 4   | 156            | 161          | NIPASC | 6 |
| 5   | 166            | 179          | TVYHEGCVPVFQAF | 14 |
| 6   | 181            | 210          | KRNLVIVACFGVFCQOLLSSIVIACGLQR | 30 |

### 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 Schistosoma haematobium 23-kDa protein, having 218 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 23-kDa protein sequence having 218 amino acids, which shows 210 nonamers.

Table 2- Prediction of MHC class I peptides, from S. haematobium 23-kDa transmembrane protein having C-terminal ends are proteosomal cleavage sites

| MHC-I Allele | POS | N | SEQUENCE | C | MW (Da) | SCORE | % OPT. |
|--------------|-----|---|----------|---|---------|-------|-------|
| 8mer_H2_Db   | 50  | AKG | PGQYGDPI | 84.95 | 14.128 | 26.91% |
| 8mer_H2_Db   | 61  | TGA | LDKTPPTEI | 89.04 | 11.679 | 22.25% |
| 8mer_H2_Db   | 52  | KKV | QAAPAIIV | 777.97 | 9.638 | 18.36% |
| 8mer_H2_Db   | 51  | HKV | WQAAPAIIV | 828.02 | 8.25 | 15.72% |
| 8mer_H2_Db   | 60  | All | VVVIIVF | 807.06 | 6.37 | 12.13% |
| 8mer_H2_Db   | 173 | EGC | VPVFQGAFL | 831.03 | 6.357 | 12.11% |
| 8mer_H2_Db   | 78  | GAY | KENOMY | 981.19 | 6.245 | 11.90% |
| 8mer_H2_Db   | 29  | SLV | LIGAVY | 744.89 | 6.162 | 11.74% |
| 8mer_H2_Db   | 49  | NH | KWWQAPI | 871.08 | 5.716 | 10.89% |
| 8mer_H2_Db   | 190 | VAC | VAFQGCFF | 871.07 | 5.767 | 10.81% |
| 8mer_H2_Db   | 146 | HCC | KGQPGQYD | 861.87 | 5.4 | 10.29% |
| 8mer_H2_Db   | 31  | VLI | GAGAYVEF | 746.82 | 4.94 | 9.41% |
| 8mer_H2_Db   | 77  | CCA | INEKMVL | 931.77 | 4.036 | 7.69% |
| 8mer_H2_Db   | 54  | WQA | APAIIIV | 772.02 | 3.746 | 7.14% |
| 8mer_H2_Db   | 85  | CML | YMYAFFLI | 1049.31 | 3.215 | 6.12% |
| 8mer_H2_Db   | 205 | IVI | ACALLQRI | 874.05 | 2.062 | 3.93% |
| 8mer_H2_Db   | 36  | GAY | VEVKFSQY | 981.12 | 1.98 | 3.77% |
| 8mer_H2_Db   | 185 | RNL | VIVACAF | 803.03 | 1.535 | 2.92% |
| 8mer_H2_Db   | 18  | VFV | LNYLICLCL | 886.18 | 1.467 | 2.79% |
| 8mer_H2_Db   | 193 | VAF | VGCFQOLL | 908.13 | 1.134 | 2.16% |
| 8mer_H2_Db   | 17  | CVF | LWNCLLC | 862.17 | 1.044 | 1.99% |
| 8mer_H2_Db   | 70  | IVS | FLGCAGKENV | 706.94 | 0.48 | 0.91% |
| 8mer_H2_Db   | 192 | CVA | FGVGFQOLL | 810.21 | 0.08 | 0.16% |
| 8mer_H2_Db   | 172 | HEG | CVPQFQAF | 934.17 | 13.666 | 27.13% |
| 8mer_H2_Db   | 16  | SCV | FVLNIICL | 1029.35 | 12.346 | 24.51% |
| 8mer_H2_Db   | 70  | IVS | FLGCAGKEN | 893.13 | 10.577 | 21.00% |
| 8mer_H2_Db   | 179 | FGA | FLKRNLI | 1083.38 | 10.175 | 20.20% |
| 8mer_H2_Db   | 22  | NII | CCLSLVLG | 958.29 | 9.086 | 20.03% |
| 8mer_H2_Db   | 3  | MA | TLTLTMGRCL | 933.14 | 9.834 | 19.53% |
| 8mer_H2_Db   | 19  | FVL | NIIILCLSL | 973.26 | 9.563 | 18.99% |
| 8mer_H2_Db   | 191 | ACG | AFVGFQOLL | 1013.23 | 9.532 | 18.93% |
| 8mer_H2_Db   | 189 | IVA | CAVFQGQOLL | 974.21 | 9.521 | 18.90% |
| 8mer_H2_Db   | 76  | CCG | AKENVCMY | 1002.25 | 9.469 | 18.80% |
| 8mer_H2_Db   | 10  | GMR | CLKSCVFLVL | 993.29 | 9.12 | 18.11% |

*Optional Score for given MHC binder in Mouse

We Predicted the SVM based MHC-IaAb peptide regions, 152-DYGPINPAS, 51-WQAAPAIIV, 50-WQVAPAIIV, 142-FHCAGGAK, 97-AEELAAYI (optimal score is 14.911); MHC-IIaAb peptide regions, 100-AAAIVAVYY, 71-LQCGCAGKE, 192-FGVCQFQOLL, 186-

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IVACVAFGV (optimal score is 13.112); and MHCII-IaG7 peptide regions 42-QYGDNLHKV, 101-AAIAVVYK, 28-VLGAGAYV, 103-IVAVYKDR, 203-VIACCLGRQ (optimal score is 11.605); which represents predicted binders for S. haematobium 23-kDa transmembrane protein [Table-3]. The predicted binding affinity is normalized by the 1% fractile. The MHC-Peptide binding is predicted using neural networks trained on C terminals of known epitopes. In this analysis predicted MHC-Peptide binding is a log-transformed value related to the IC50 values in nM units. These MHC binding peptides can decently elicited the desired immune response. Predicted MHC binding regions in an antigen sequence and these are directly associated with immune reactions, in analysis we found the MHC-I and MHC-II binding region.

Table 3 - Peptide binders to MHCII molecules of S. haematobium 23-kDa transmembrane protein

| MHC-II Allele | POS. | N | SEQUENCE | MW (Da) | SCORE | % OPT. |
|---------------|------|---|----------|---------|--------|--------|
| IaAb          | 152  | GPQ | DYGPNIPAS CRG | 914.98 | 14.911 | 41.85% |
| IaAb          | 51   | HKV | WQAAPIAIV VG  | 941.18 | 13.831 | 38.82% |
| IaAb          | 50   | LHK | VWGAAPIAI IV  | 927.15 | 12.589 | 35.33% |
| IaAb          | 142  | GSS | FHCCGAKQGQ DOY | 901.07 | 10.703 | 30.04% |
| IaAb          | 97   | LLI | AELAAAI AA VY | 809.97 | 10.117 | 28.39% |
| IaAd          | 100  | AEL | AAAAVVYV KD R | 858.05 | 13.112 | 24.67% |
| IaAd          | 71   | VSF | LSVGCAIGKE NVC | 875.07 | 9.244 | 17.39% |
| IaAd          | 192  | CVA | FVCGFQOLL SIV | 1055.31 | 8.643 | 16.26% |
| IaAd          | 186  | NLY | IVACVAFGV CFF | 860.08 | 7.5 | 14.11% |
| IaAg7         | 42   | KFS | QYGDNLHKV WQA | 1055.15 | 11.605 | 28.39% |
| IaAg7         | 101  | ELA | AAIAVVYK DRI | 915.14 | 11.112 | 27.19% |
| IaAg7         | 28   | CSL | VLGAGAYV EVK | 844.02 | 8.935 | 22.96% |
| IaAg7         | 103  | AAA | IVAVVYKR IDS | 1044.26 | 8.49 | 20.77% |
| IaAg7         | 203  | LSI | VIACCLQRG I KE | 944.10 | 8.458 | 20.69% |

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

Discussion and Conclusion

Gomase method (2007), BepiPred Server, Hopf 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 regions of this peptide and shows high antigenicity [Fig-1, [Fig-2], [Fig-3], [Fig-4], [Fig-5], [Fig-6]. In this essay we predicted the binding affinity of S. haematobium 23-kDa transmembrane protein having 218 amino acids, which shows 210 nonamers. We predicted MHC-I binding peptides for 9mer_H2_D allele (optimal score is 14.128), 9mer_H2_Db allele (optimal score is 20.065), 10mer_H2_Db allele (optimal score is 13.776), 11mer_H2_Db allele (optimal score is 31.213) [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 S. haematobium 23-kDa protein, analysis shows epitopes present in the S. haematobium 23-kDa protein are adequate to induce desired immune response. The region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because C-terminal regions of S. haematobium 23-kDa transmembrane protein are solvent accessible and unstructured; antibodies against those regions are also likely to recognize the native protein. During prediction of antigenic determinant site of S. haematobium 23-kDa protein, we found Six antigenic determinant sites in the sequence. The highest pick is recorded between sequence of amino acid in the region are 7-GMRLKSCVFVLNIICLCSLVLIGAGAY VEYKFS-41 (35AA) and 47-LHKKWQAAPIAIVGVIILVFSPLGC GAIKVENCMLYMyAF- FLILLILAEAAIAIVAVYKID-110 (64AA) [Table-1]. The average propensity for the S. haematobium 23-kDa protein found is 1.094 [Fig-2]. All residues having above 1.0 propensity are always potentially antigenic [Table-1]. The predicted MHC-Peptide binding is a log-transformed value related to the IC50 values in nM units. These MHC binding peptides can decently elicited the desired immune response. Predicted MHC binding regions in an antigen sequence and these are directly associated with immune reactions, in analysis we found the MHC-I and MHC-II binding region.

Future Perspectives

This method will be applicable in cellular immunology, Vaccine design, immunodiagnostics, immunotherapeutics and molecular understanding of autoimmune susceptibility. S. haematobium 23-kDa transmembrane protein sequence contains multiple antigenic components to direct and empower the immune system to protect the host against lymphatic filariasis disease. 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 the parent antigen. So a small fragment of antigen can induce immune response against complete antigen. 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.

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