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MHC binding peptides for designing of vaccines against Japanese encephalitis virus: A computational approach

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Abstract Japanese encephalitis (JE), a viral disease has seen a drastic and fatal enlargement in the northern states of India in the current decade. The better and exact cure for the disease is still in waiting. For the cause an in silico strategy in the development of the peptide vaccine has been taken here for the study. A computational approach to find out the Major Histocompatibility Complex (MHC) binding peptide has been implemented. The prediction analysis identified MHC class I (using propred I) and MHC class II (using propred) binding peptides at an expectable percent predicted IC (50) threshold values. These predicted Human leukocyte antigen [HLA] allele binding peptides were further analyzed for potential conserved region using an Immune Epitope Database and Analysis Resource (IEDB). This analysis shows that HLA-DRB1*0101, HLA-DRB3*0101, HLA-DRB1*0401, HLA-DRB1*0102 and HLA-DRB1*07:01% of class II (in genotype 2) and HLA-A*0101, HLA-A*02, HLA-A*0301, HLA-A*2402, HLA-B*0702 and HLA-B*4402% of HLA I (in genotype 3) bound peptides are conserved. The predicted peptides MHC class I are IDLSNGDIIGLY, FVMDEAHFTDPA, KTRKILPQIIK, RLMSPNRVPNYLF, APTRVVAAEMAEAL,
1. Introduction

Japanese encephalitis (JE) a major viral disease among human beings of developing countries is caused by Japanese encephalitis virus (JEV) that belongs to the Flaviviridae family of dengue virus and yellow fever virus. JE virus causes membrane inflammation in the brain and leads to deleterious effects on central nervous system (CNS). It is a known fact that JEV is a single stranded RNA virus that mainly consist of three structural proteins: capsid protein, precursor membrane protein and envelope protein along with seven nonstructural (NS) proteins: NS1 (NP_775667.1) NS2A (NP_775668.1), NS2B (NP_775669.1), NS3 (NP_775670.1), NS4A (NP_775671.1), NS4B (NP_775673.1) and NS5 (NP_775674.1). In response to JEV infection, the host cell produces virus neutralizing antibodies and cytotoxic T cells (CTLs). It has been shown that defense against JEV infection is primarily antibody dependent, and virus-neutralizing antibodies lacking help are sufficient to convey protection (Mishra et al., 2009; Wu et al., 2003). As our knowledge of the immune responses to a protein antigen has progressed, epitope identification has become a challenging immune-informatics problem within vaccine design. To be an antigenic and a causative agent for pathogenicity, a protein, it must be located on the virus such that its surface is exposed externally towards the outer environment, it should be antigenically related to the HLA. Out of these the predicted peptide YENVFHTLW and MHC class II molecule are TTGVYRIMARGILGT, NYNLVFMEDEAHFTDP, AAAIFMTATPPGTD, GDTTTGVYRIMARG and FGEVGA found to be top ranking with potential super antigenic property by binding to all HLA. Out of these the predicted peptide FVMDEAHTFDP for allele HLA-A*02:01 in MHC class I and NYNLVFMEDEAHFTDP for allele HLA-DRB3*01:01 in MHC class II was observed to be most potent and can be further proposed as a significant vaccine in the process. The reported results revealed that the immune-informatics techniques implemented in the development of small size peptide is useful in the development of vaccines against the Japanese encephalitis virus (JEV).

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Table 1  MHC-1 best alleles HLA-A*01:01, HLA-A*02:01, HLAA*03:01, HLAA*24:02, HLAB*07:02, HLA-B*44:02.

| S.I. | Alleles | Seq num | Start | End | Length | Peptide | Method | Percentile rank | Receptor Energy |
|-----|---------|---------|-------|-----|--------|---------|--------|----------------|----------------|
| 1.  | HLA-A*01:01 | 1       | 139   | 150 | 12     | ILDSNGDIHGLY | ANN    | 0.2            | NS3: −597.74    |
| 2.  | HLA-A*02:01 | 1       | 282   | 293 | 12     | FVMDEAHTFDP | ANN    | 0.3            | NS3: −638.11    |
| 3.  | HLA-A*03:01 | 1       | 200   | 211 | 11     | KTRKILPQIKH | Consensus (ANN/ SMM) | 0.2 | NS3: −583.94    |
| 4.  | HLA-A*24:02 | 1       | 269   | 282 | 14     | RLMSPNRVPYNYL | ANN    | 0.2            | NS3: −519.28    |
| 5.  | HLA-B*07:02 | 1       | 223   | 236 | 14     | APTTRVVAEMEAEL | ANN | 0.3            | NS3: −509.21    |
| 6.  | HLA-B*44:02 | 1       | 42    | 50  | 9      | YENVFHTLW | Consensus (ANN/ SMM) | 0.15 | NS3: −578.85    |

The bold value indicate best binding affinity and energy score.
2.3. Epitope prediction

Initially, the amino acid sequence of the NS3 protein of JEV was obtained from the NCBI protein database. The sequence was obtained in FASTA format. For the prediction of MHC class-I epitope IEDB analysis tool (an online tool) was used (Saini and Vrati, 2003). The sequence was submitted here in FASTA format and with specific selected alleles. The epitopes were predicted for different alleles of MHC class-I. The predicted epitopes were in the form of small peptide sequences. For the prediction of MHC class-II epitopes same IEDB analysis tool was used but with different parameters. All the predicted epitopes were in the form of small peptide sequences.

2.4. Immune Epitope Database Analysis Resource

This server provides a collection of tools for the prediction and analysis of immune epitopes MHC class-I and MHC class-II epitope. The T-cell MHC class-I epitopes of NS3 was predicted for six alleles i.e., HLA-A*01:01 allele, HLA-A*02:01 allele, HLA-A*03:01 allele, HLA-A*24:02 allele, HLA-B*07:02 allele, and HLA-B*44:02 allele as well as for five alleles i.e., HLA-DRB1*01:01, HLA-DRB3*0101, HLA-DRB1*04:01, HLA-DRB1*01:02, and HLA-DRB1*07:01 of MHC class II epitopes of NS3. These small sequence epitopes were obtained from the IEDB analysis tool that uses and cross validated via SVMHC server which uses SYFPEITHI methods of epitope prediction while IEDB analysis tool uses artificial neural network [ANN] and stabilized matrix method SMM method of epitope prediction (Zhang et al., 2008).

2.5. NetMHC-3.0

This server predicts the binding affinity of either a list of peptides with a defined length (8-11 residues) or all possible

| S.I. | Alleles       | Start position | End position | Percentie rank | Predicted epitope                            | Length | Receptor | Energy   |
|------|---------------|----------------|--------------|----------------|----------------------------------------------|--------|----------|----------|
| 1.   | HLA-DRB1*01:01 19 | 33             | 3.95         | TTGVYRIMARGILGT | 14 NS3                                      |        |          | -556.82  |
| 2.   | HLA-DRB3*01:01 278 | 292            | 0.01         | NYNLFVMDEAHTDP  | 14 NS3                                      |        |          | -694.77  |
| 3.   | HLA-DRB1*04:01 310 | 324            | 1.31         | AAAIFMTATPPGTDT | 14 NS3                                      |        |          | -572.89  |
| 4.   | HLA-DRB1*01:02 16  | 30             | 0.22         | GDTTTGVYRIMARGI | 14 NS3                                      |        |          | -561.69  |
| 5.   | HLA-DRB1*07:01 114 | 128            | 0.66         | FGEVGAYSL       | 14 NS3                                      |        |          | -483.12  |

Table 3 | Interacting amino acid of allele with NS3.

| S. No. | Allele                  | Interacting amino acid residues |
|--------|-------------------------|---------------------------------|
| 1.     | HLA-A*01:01             | I1, L2, D3, S4, N5, G6, D7, I8, I9, G10, L11 and Y12 |
| 2.     | HLA-A*02:01             | F1, V2, M3, D4, G5, A6, H7, F8, T9, D10, P11 and A12 |
| 3.     | HLA-DRB3*01:01          | N1, Y2, N3, L4, F5, V6, M7, D8, E9, A10, H11, F12, T13, D14 and P15 |
| 4.     | HLA-DRB1*04:01          | A1, A2, A3, I4, M6, T7, A8, T9, P10, P11, G12, P13, T14 and D15 |

Figure 1 | Showing the interaction of HLA-A*01:01 (in purple color) with NS3. The green dotted lines are showing the hydrogen bond. Graphics developed by Discovery Studio Visualizer 4.1.
sub-peptides hosted within full-length proteins. NetMHC-3.0 is trained on a large number of quantitative peptide data using both affinity data from the IEDB and elution data from SYFPEITHI. The method generates high-accuracy predictions of MHC: peptide binding. The predictions are based on ANN trained on data from 55 MHC alleles (43 human and 12 non-human), and position-specific scoring matrices (PSSMs) for additional 67 HLA alleles. As only the MHC class I prediction server is available, predictions are possible for peptides of length 8–11 for all 122 alleles. Artificial ANN was given as actual inhibition constant IC50 values whereas position-specific scoring matrix PSSM predictions are given as a log-odds likelihood scores (Lundegaard et al., 2008).

2.6. Structure-based modeling of T-cell epitopes

The PEPstr server predicts the tertiary structure of small peptides with sequence length varying between 7 and 25 residues (Kaur et al., 2007). The prediction strategy is based on the realization that β-turn is an important and consistent feature of small peptides in addition to regular structures. Thus, the methods uses both the regular secondary structure information predicted from PSIPRED and β-turns information predicted from Beta Turns. The side-chain angles are placed using standard backbone-dependent rotamer library. The structure is further refined with energy minimization and molecular dynamic simulations using Amber (Case et al., 1999).

2.7. Peptide-NS interaction

The Peptide NS Interaction analysis was performed using HEX program (Ritchie and Kemp, 2000) owing to its promising results in the CAPRI (Critical Assessment of Predicted Interactions; http://capri.ebi.ac.uk/) competition with respect to proposing good docking solutions. HEX determines the steric shape, electrostatic potential, and charge density of each
protein as expansions of spherical polar Fourier basis functions. The protein surface shapes are calculated to determine the match potential of two proteins. Then, candidate-docking solutions are refined using a “soft” molecular mechanics energy minimization procedure, and the list of docking solutions is clustered to assist in identifying distinct orientations (Wang et al., 2012).

3. Results and discussion

The antigenic protein retrieved from Uniprot in FASTA format was used to predict the T-cell epitopes for MHC class-I and MHC class-II molecules of Homo sapiens using the IEBD analysis resource tool. These predictions were not easy to make because their respective alleles need to be specified. HLA-A*01:01, HLA-A*02:01, HLA*03:01, HLA*24:02, HLAB*07:02, HLA-B*44:02, HLADRBI*0101, HLA-DRB3*0101, HLA-DRB1*04:01, HLA-DRB1*01:02, and HLA-DRB1*07:01 alleles were considered for epitope prediction. Docked conformation of both proteins MHC class I and MHC class II with predicted epitopes were analyzed and graphical interpretation has been done using Discovery Studio 2.5 tool (Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 4.5, San Diego: Dassault Systèmes, 2015). Interacting interface residues have been identified between both biomolecules. Interface residue identification would be helpful for prediction of epitopes to generate a vaccine against JEV.

3.1. Results for MHC class-I binding epitope prediction

Table 1 shows the predicted T-cell MHC class-I epitopes for various HLA alleles. These small sequence epitopes were obtained from the IEDB analysis tool using ANN method of epitope prediction and cross validated via SVMHC server which uses SYFPEITHI methods for epitope prediction. The structure of docked complexes of predicted epitopes with MHC class I was visualized using Discovery Studio Visualization tool. There are six alleles i.e., HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLAB*07:02 and HLAB-B*44:02 of MHC class-I molecule for which epitopes are predicted. The complex with best binding affinity and energy score obtained is –638.11 for ‘FVMDEAHFTDPA’ epitope against HLA-A*02:01 allele docked with NS3 protein (Table 1). The important amino acids involved in interaction of epitope with NS3 protein are I1, L2, D3, S4, N5, G6, D7, I8, J9, G10, L11 and Y12 (Table 3).

The structure of ‘ILDSNGDIIGLY’ epitope docked with NS3 protein showed highest binding affinity and energy score of –597.74 kJ/mol while the structure of ‘FVMDEAHFTDPA’ epitope docked with NS3 protein showed best binding affinity and energy score of –638.11 kJ/mol (Table 1). The structure of ‘KTRKILPQIIK’ epitope docked with NS3 protein produced highest binding affinity and energy score of –583.94 kJ/mol while the structure of ‘RLMSPNVRYNLK’ epitope docked with NS3 protein produced highest binding affinity and energy score of –519.28 (Table 1). Similarly the structure of ‘APTRVVAEMAEL’ epitope docked with NS3 protein showed highest binding affinity and energy score of –509.21 kJ/mol and the structure of ‘YENVFHTLW’ epitope docked with NS3 protein showed highest binding affinity and energy score of –578.85 kJ/mol (Table 1). The predicted epitope of NS3 protein for six allele were arranged in accordance to their start and end positions along with their percentile rank.

3.2. Result for MHC class-II binding epitope prediction

Prediction of MHC-II binding peptides is difficult as compared to MHC class-I binding peptides due to their variable size. There are five alleles i.e., HLADRBI*0101, HLA-DRB3*0101, HLA-DRB1*04:01, HLA-DRB1*01:02, and HLA-DRB1*07:01 of MHC class-II molecule for which epitopes are predicted using IEDB analysis tool and cross
validating by SVMHC server. Only those epitopes having a peptide score above the threshold value and 50% cut off have been selected. The structure of predicted ‘NYYLFVMDEAHFTDP’ epitope against allele HLA-DRB3*01:01 docked with NS3 protein produced the best binding affinity and energy score of –694.77 kJ/mol (Table 2). The important amino acids involved in interaction of epitope ‘NYYLFVMDEAHFTDP’ with NS3 protein are N1, Y2, N3, L4, F5, V6, M7, D8, E9, A10, H11, F12, T13, D14 and P15 (Table 3).

The structure of ‘TTTGVYRIMARGILG’ epitope docked with NS3 protein showed binding affinity and energy score of –556.82 kJ/mol while the structure of ‘AAAIFMTATPPGTID’ epitope docked with NS3 protein showed binding affinity and energy score of –572.89 kJ/mol.

Similarly the structure of ‘GDTTTGYRIMARGIR’ epitope docked with NS3 protein produced binding affinity and energy score of –561.69 kJ/mol while the structure of ‘FGEVGAVSL’ epitope docked with NS3 protein produced the binding affinity and energy score of –483.12 kJ/mol

Table 2.

Design and development of short peptides as vaccine candidates for JEV is gaining momentum in recent years. Therefore, in the present study we have predicted epitope like region in the NS3 protein having RPN interaction. On the basis of energy score two best dock figure of each MHC-I and MHC-II were attached in this study for better understanding (Figs. 1–4). Hence, these data could be useful in designing candidates capable of producing antipeptide antibodies which are competent of recognizing JEV specific nucleocapsid protein.

4. Conclusions

The T-cell epitopes against MHC class I molecule are ILDSNGDIIGLY, FMDEAHFTDPA, KTRKILPQIK, RLMSPNRVPNYNLF, APTRVVAEMAEAL, YENVFHTLW respectively for each of the alleles. Among these FMDEAHFTDPA epitope has best binding affinity with docking score of –638.11 kJ/mol. The T-cell epitope for MHC class-II molecule are TTTGVYRIMARGILG, NYYLFVMDEAHFTDPA, AAAIFMTATPPGTID, GDTTTGYRIMARGIR and FGEVGAVSL out of which NYYLFVMDEAHFTDPA has the highest binding affinity with docking energy of –694.77 kJ/mol.

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