Computational Identification of T-Cell Epitopes in Conserved Sequences Belonging to Genotype 6 of Hepatitis C Virus

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Abstract

Purpose: Hepatitis C Virus (HCV) is the leading cause of hepatocellular carcinoma and liver infections, which can be acute or chronic. For vaccine development, computational methods give us the best approaches for epitope-based vaccines against different types of viruses. In the early phase of acute hepatitis infection, only CD8+ and CD4+ T cells play a major role in fighting against HCV infection. In the current study, computational identification of multiple T-cell epitopes was done for the human genome with genotype 6.

Methods: Various immunoinformatic tools were used for the prediction of unique sequences of epitopes containing HCV glycoproteins, mainly E1 and E2 transmembrane proteins that show interaction with unique HLA allele sequences. The objective of the T-cell prediction is to find out the shortest sequence of antigen peptides that elicit the T cell immune response.

Results: Class I HLA alleles bound to the 8–11 amino acids, while Class II HLA bound to the 15–24 amino acids. The various tools used for epitope prediction of MHC class I CD8+ T cells were SYFPEITHI, NetCTL1.2, and IEDB. Similarly, the tools used for MHC class II CD4+ T cells were ProPred, MHC2 Pred, and IEDB for epitope prediction. Various unique HLA allele sequences were enlisted, for which various unique epitopes were predicted. A possible treatment for HCV is not known yet.

Conclusion: This study provides a future strategy for of HCV vaccine via in silico selection, which can be further validated by in vivo and in vitro methods.

Keywords: Hepatitis C virus, Epitopes, HLA alleles, T-cells.

Introduction

The genetic structure and characteristics of HCV have been described well in history.[1] The viral HCV could be a major blood-borne human infectious agent. There are currently 170 and 200 million cases of the virus worldwide, and 40,000 new cases are added each year. The HCV virus is an RNA-positive, positively polarized, 9500 nucleotide long virus.[2] It is considered as a significant public health issue since the virus is the etiological issue of chronic liver disease that often results in cirrhosis of the liver and malignant hepatoma (HCC). In developed countries, the foremost vital route of HCV transmission is blood vessel misuse, whereas in resource-poor countries, invasive procedures or injection-based therapies with contaminated instruments are the predominant sources of the latest infections.

Interferon-alpha and drugs like telaprevir and sofosbuvir can be used for the treatment of the HCV infection, but these therapies are costly.[3-4] Since people in developing countries can't afford such expensive treatments, there is a need to develop an affordable antiviral treatment. Thus, we need to gain knowledge about the epitopes of the virus for the advancement of the vaccine. At present, two vaccines are clinically used: one is developed by Chiron, which is recombinant protein E1 and E2, and the other, which makes CD8+ active, is made from the non-structural protein of the recombinant virus.[5]

Cytotoxic T cells (CTLs) are effective against the short sequences of peptide epitopes that are bonded with the human leucocyte antigen (HLA) and are expressed by the antigen-presenting cells. Through this, the evasion of HCV infection by immune responses could be avoided.[6]

Epitopes, also called antigen determinants, are the specific short peptide sequences in an antigen that stimulate the immune response when bound to the specific antibody. T-cell receptors (TCRs) are present on the surface of T cells and recognize the APC when bonded to the major

histocompatibility complex (MHC). T-cell epitopes consist of two classes: MHC Class I and MHC Class II. CD4 cells bond to the MHC Class II through the receptors present on their surface, while CD8 cells bond with the MHC Class I. The MHC is present on chromosome 6, which codes for 21 genes and shows polymorphism. The three loci present on MHC Class I (A, B, and C) are only able to detect peptide sequences of eight to twelve amino acids in length. While Class II has HLA (DP, DQ, and DR) and detects amino acids of length eighteen to twenty-four, if we want to predict the T-cell epitopes, we must first know about the various HLA alleles.[7]

Various bioinformatics tools are available for the prediction of epitopes. Among this Class I bound with 8 to 11 AA, the C-terminal region and the 2nd position are known as anchor sites because these are important positions for the binding. A very limited number of proteins are acceptable for binding to the anchor site and binding to an HLA allele. Matrix-based methods are not able to correlate the relationship between the amino acids and how the binding affinity at one position of AA depends on the other amino acid position. In this study, we use three types of tools for class I prediction: SYFPEITHI, NetCTL1.2, and IEDB.

For Class II, prediction became difficult because both ends are open and allow more peptides to be bound, which shows higher polymorphism. Also, their binding motifs have weaker relativity. For their prediction, it was assumed that the 9 AA core motifs determined the affinity of the protein, MHC. This idea was used by TEPITOPE.[8]

In the early days, this was only for the use of PCs, but after a long time, this was provided as a part of the web server for the class II predictor, namely ProPred.[9] In this study, we have used IEDB, ProPred, and MHC2pred.

Epitope-based vaccines are becoming increasingly popular for the development of vaccines against viruses with conserved sequences or pathogens that have mutated rapidly. Using these various tools, appropriate in silico selection of epitopes could be possible.

Methods

Sequence retrieval

ViPR

ViPR stores information for virus families that are classified as either human priority pathogens or potential public health threats. ViPR works to combine data from three different sorts of sources: (i) data transmitted to ViPR from public archives; (ii) unique data created by ViPR utilizing a variety of computational algorithms and bioinformatics methodologies; and (iii) data directly submitted to ViPR by independent researchers. In a relational database, these data are kept so that user-specified queries can quickly retrieve them.[10]

Whole genome sequences for E2 unique protein sequences were selected for downloading. Then, 121 protein sequences in FASTA format were retrieved for genotype VI.

Multiple sequence alignment

Multiple sequence alignment is a method for aligning more than two homologous nucleotide or amino acid sequences so that homologous residues from the respective sequences line up in columns as far as possible.

Clustal Omega

Clustal Omega is one of the bioinformatics tools used for aligning various multiple amino acid and nucleotide sequences precisely and rapidly. For decades, this has become one of the most used bioinformatics tools since it is a requirement for most phylogenetic or comparative studies of

homologous genes or proteins.[11] In this study, downloaded E2 protein sequences of Hepatitis C virus genotype 6 were used as input for Clustal Omega for multiple sequence alignment.

Conservancy analysis

AVANA

AVANA was further used to analyze the conserved region in the E2 protein after multiple sequence alignments through Clustal Omega. AVANA stands for Antigen Variability Analyzer Tool. This was used for the alignment of various subsets of the collected sequence that were entirely based on the viral subtype of the host. The tool provides useful information about the conserved region that is entirely based on information entropy analysis.[12] Information entropy is measured by the uncertainty of a random variable, or in the case of proteins, by protein mutation.[11] Highly conserved sites are shown due to lower entropy, which displays the degree of randomness.

To retrieve the conserved region of E2 protein, multiple sequence alignment of E2 protein in FASTA format was used by setting the parameter in AVANA as conservation region 70%, with a minimum length of 9 bp and a maximum length of 25 bp.

T-cell epitope prediction

Tools used for CLASS I MHC Epitope and HLA allele prediction

SYFPEITHI

The MHC databank is called SYFPEITHI. It regularly updates its database of MHC class I and class II ligands and peptide patterns from humans and other species, including apes, cattle, chicken, and mice, among others. There are distinct entries available for each motif that is currently available. It is possible to conduct searches for references, source proteins/organisms,

T-cell epitopes, natural ligands, MHC alleles, and MHC motifs. Links are provided to the EMBL and PubMed databases.[13]

In this study, MHC-I prediction was performed by SYFPEITHI. To perform this task, all HLA were selected from HLA-A * 01 through HLA-DRB1 * 1501 (DR2b). Other parameters include the selection of nanomers, and 70% of the conserved sequence obtained from the above step was used as input. As a result, the HLA, epitope, and score appeared. The score must be less than or equal to 20.

NetCTL1.2

The NetCTL1.2 service is used to predict CTL epitopes in protein sequences. Version 1.2 of the MHC class I binding prediction now includes 12 MHC supertypes, including supertypes A26 and B39. In the technique, the efficiency of TAP transport, proteasomal C-terminal cleavage, and peptide MHC class I binding are all predicted. The service can be used to anticipate CTL epitopes that are restricted to 12 MHC class I supertypes. In order to achieve MHC class I binding and proteasomal cleavage, artificial neural networks are used. To forecast TAP transit effectiveness, use the weight matrix.[14] Subtype A1 was used to choose input from 70% of conserved sequences, starting with C1. The ordering by score was then altered to "Combination Score."

The remaining C1-C7 sequences were processed using the same procedures. The threshold for epitope recognition, the weight of C-terminal cleavage, and the weight of TAP transport effectiveness are all unchanged.

IEDB

Researchers from the La Jolla Institute for Allergy and Immunology (LIAI) have developed the Immune Epitope Database and Analysis Resource (IEDB) in collaboration with the Department of Health and Human Services (HHS) and the National Institute of Allergy and Infectious Diseases (NIAID). In order to generate new research tools, diagnostic techniques, vaccines, and therapies, immunological epitope data must be disseminated. Data curation for the ongoing NIAID Emerging and Re-emerging Infectious Diseases and Category A, B, and C priority pathogens (which includes influenza) is given top priority. Epitopes from diverse allergens, autoantigens, and infectious pathogens are also being catalogued. The database also contains information on immune epitopes from the TopBank, FIMM, HLA Ligand, and MHC binding databases, as well as information on MHC binding from a number of antigenic sources.[15]

C1-C7 conserved sequences were used as input, much as the prior technique. The ANN.4.0.0 prediction format was chosen. When you type HLA-A * 01:01, the outcome is shown. Afterward, choose HLA and epitope based on a score of 500 or fewer.

Tools used for CLASS II MHC epitope and HLA allele prediction

PROPRED

MHC class II binding sites in antigenic protein sequences were predicted using ProPred, a graphical online application. The server uses an amino-acid/position coefficient database derived from literature to create a matrix-based prediction method. In the graphical interface, the predicted binders may be seen as peaks, whereas in the HTML interface, they can be seen as colored residues. This website might help researchers find promiscuous binding areas that can connect to several HLA-DR alleles.[9]

Conserved sequences obtained from AVANA were used as input sequences. Select HLA alleles and epitopes on the basis of red and blue color. At a time, only nine sequences can be selected from ProPred.

MHC2 Pred

An SVM-based method is employed to predict the peptides that bind to MHC class II. SVM-based techniques are 80% accurate for 42 alleles.

Because the dataset was smaller, the method's performance was worse for a few alleles. The method's performance was evaluated using 5-fold cross-validation. MHC2Pred input sequences were 70% AVANA conserved sequences for the human allele HLA-DR1-HLA-DRB5 * 0101, with a score of 0.5. The display mode would be tabular, and the tabular results would be 50. The results were then presented by HLA, epitope, and score. The score must be 0.5 or greater.

IEDB Analysis resource

MHC-2 prediction using IEDB was similar to one that we used for MHC-1 epitope prediction. By selecting human, HLA-DR, HLA-DP, and HLA-DQ species or loci one at a time. Then the results appear, with HLA, core sequence, peptide sequence, and score. Their selection was based on the score, which must be less than or equal to 500.

Results

Sequence retrieval

ViPR

In sequence retrieval, the unique sequence of 121 E2 proteins of the human genome had genotype 6, was retrieved in the FASTA format, and all had a unique sequence ID for each protein. The

results for the sequence retrieval are shown in the **Table *1*. A unique sequence of E2 protein in FASTA formatTable 1**.

Table 1. A unique sequence of E2 protein in FASTA format

S. No.	GenBank ID	Host	Country	Sequence ID
1	KC191671	Human	Malaysia	>gb:VIPR_ALG4_440234843_1468_2565
2	KJ567649	Human	Vietnam	>gb:VIPR_ALG4_685430775_1488_2594
3	KJ567648	Human	Vietnam	>gb:VIPR_ALG4_685430773_1489_2583
4	KJ567646	Human	Vietnam	>gb:VIPR_ALG4_685430769_1488_2588
5	KJ567651	Human	Vietnam	>gb:VIPR_ALG4_685430779_1488_2597
6	KJ567650	Human	Vietnam	>gb:VIPR_ALG4_685430777_1488_2594
7	KJ567652	Human	Vietnam	>gb:VIPR_ALG4_685430781_1488_2591
8	KJ567645	Human	Vietnam	>gb:VIPR_ALG4_685430767_1488_2582
9	KJ567644	Human	Vietnam	>gb:VIPR_ALG4_685430765_1473_2567
10	KJ567647	Human	Vietnam	>gb:VIPR_ALG4_685430771_1489_2580
11	KM504109	Human	-N/A	>gb:VIPR_ALG4_758739587_1489_2580
12	KJ678744	Human	China	>gb:VIPR_ALG4_758739124_1492_2595
13	KJ678745	Human	China	>gb:VIPR_ALG4_758739126_1492_2595
14	KM504110	Human	Thailand	>gb:VIPR_ALG4_758739589_1488_2576
15	KY014622	Human	China	>gb:VIPR_ALG4_AQT18930_1_1489_2583
16	JX183557	Human	China	>gb:VIPR_ALG4_407751085_1488_2585
17	KJ678746	Human	China	>gb:VIPR_ALG4_758739128_1449_2552
18	KJ678747	Human	China	>gb:VIPR_ALG4_758739130_1449_2552
19	KJ678748	Human	China	>gb:VIPR_ALG4_758739132_1429_2532
20	KJ678749	Human	China	>gb:VIPR_ALG4_758739134_1448_2551
21	KJ678750	Human	China	>gb:VIPR_ALG4_758739136_1449_2552
22	KJ678751	Human	China	>gb:VIPR_ALG4_758739138_1449_2552
23	KJ678752	Human	China	>gb:VIPR_ALG4_758739140_1425_2528
24	KJ678753	Human	China	>gb:VIPR_ALG4_758739142_1449_2549
25	KJ678754	Human	China	>gb:VIPR_ALG4_758739144_1492_2595
26	KJ678755	Human	China	>gb:VIPR_ALG4_758739146_1449_2552
27	KJ678756	Human	China	>gb:VIPR_ALG4_758739148_1491_2594
28	KJ678757	Human	China	>gb:VIPR_ALG4_758739150_1491_2594
29	KJ678758	Human	China	>gb:VIPR_ALG4_758739152_1491_2594
30	KJ678759	Human	China	>gb:VIPR_ALG4_758739154_1491_2594

S. No.	GenBank ID	Host	Country	Sequence ID
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32	KJ678761	Human	China	>gb:VIPR_ALG4_758739158_1491_2594
33	KJ678762	Human	China	>gb:VIPR_ALG4_758739160_1491_2594
35	KJ678764	Human	China	>gb:VIPR_ALG4_758739164_1491_2594
36	KJ678765	Human	China	>gb:VIPR_ALG4_758739166_1491_2594
37	KJ678766	Human	China	>gb:VIPR_ALG4_758739168_1449_2552
38	KJ678767	Human	China	>gb:VIPR_ALG4_758739170_1449_2552
39	KJ678768	Human	China	>gb:VIPR_ALG4_758739172_1446_2549
40	KJ678769	Human	China	>gb:VIPR_ALG4_758739174_1449_2552
41	KJ678770	Human	China	>gb:VIPR_ALG4_758739176_1449_2552
42	JX183549	Human	China	>gb:VIPR_ALG4_407751069_1488_2579
43	KM252792	Human	China	>gb:VIPR_ALG4_749396734_1488_2585
44	KM252793	Human	Laos	>gb:VIPR_ALG4_749396736_1488_2579
45	KM252794	Human	Laos	>gb:VIPR_ALG4_749396738_1488_2585
46	KM252789	Human	Laos	>gb:VIPR_ALG4_749396728_1491_2594
47	KM252795	Human	Laos	>gb:VIPR_ALG4_749396740_1488_2582
48	KM252796	Human	Laos	>gb:VIPR_ALG4_749396742_1488_2582
49	KM252797	Human	Laos	>gb:VIPR_ALG4_749396744_1474_2568
50	KM252790	Human	Laos	>gb:VIPR_ALG4_749396730_1491_2597
51	JX183554	Human	Laos	>gb:VIPR_ALG4_407751079_1488_2582
52	KM252798	Human	Laos	>gb:VIPR_ALG4_749396746_1491_2582
53	KM252799	Human	Laos	>gb:VIPR_ALG4_749396748_1491_2591
54	KM252800	Human	Laos	>gb:VIPR_ALG4_749396750_1488_2579
55	KM252791	Human	Laos	>gb:VIPR_ALG4_749396732_1491_2597
56	KM587629	Human	USA	>gb:VIPR_ALG4_751660982_1488_2585
57	KM587630	Human	USA	>gb:VIPR_ALG4_751660984_1488_2582
58	KM504112	Human	Canada	>gb:VIPR_ALG4_758739593_1488_2588
59	KM504113	Human	Canada	>gb:VIPR_ALG4_758739595_1489_2580
60	KM504114	Human	Canada	>gb:VIPR_ALG4_758739597_1488_2579
61	KJ678771	Human	Canada	>gb:VIPR_ALG4_758739178_1449_2552
62	KJ678772	Human	Canada	>gb:VIPR_ALG4_758739180_1449_2552
63	KJ678773	Human	Canada	>gb:VIPR_ALG4_758739182_1449_2552
64	KM504115	Human	Canada	>gb:VIPR_ALG4_758739599_1489_2580
65	KM504116	Human	Canada	>gb:VIPR_ALG4_758739601_1489_2580
66	KM504118	Human	Canada	>gb:VIPR_ALG4_758739603_1488_2579

S. No.	GenBank ID	Host	Country	Sequence ID
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68	KM504118	Human	Canada	>gb:VIPR_ALG4_758739605_1488_2579
69	KM504119	Human	Canada	>gb:VIPR_ALG4_758739607_1488_2570
70	KJ678775	Human	Canada	>gb:VIPR_ALG4_758739186_1449_2552
71	KM504120	Human	Canada	>gb:VIPR_ALG4_758739609_1488_2576
72	KM504121	Human	Canada	>gb:VIPR_ALG4_758739611_1489_2580
73	JX183558	Human	Canada	>gb:VIPR_ALG4_407751087_1488_2582
74	JX183550	Human	Canada	>gb:VIPR_ALG4_407751071_1488_2579
75	KM504122	Human	Canada	>gb:VIPR_ALG4_758739613_1488_2579
76	KJ678776	Human	Canada	>gb:VIPR_ALG4_758739188_1449_2552
77	KJ678777	Human	Canada	>gb:VIPR_ALG4_758739190_1449_2549
78	KM504124	Human	Canada	>gb:VIPR_ALG4_758739617_1489_2577
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82	KM504111	Human	Canada	>gb:VIPR_ALG4_758739591_1488_2579
83	KJ678781	Human	China	>gb:VIPR_ALG4_758739198_1492_2595
84	KJ678782	Human	China	>gb:VIPR_ALG4_758739200_1492_2595
85	KJ678784	Human	China	>gb:VIPR_ALG4_758739202_1492_2595
86	KJ678784	Human	China	>gb:VIPR_ALG4_758739204_1424_2527
87	KJ678785	Human	China	>gb:VIPR_ALG4_758739206_1449_2552
88	JX183551	Human	Vietnam	>gb:VIPR_ALG4_407751073_1488_2582
89	KM252779	Human	Vietnam	>gb:VIPR_ALG4_749396708_1488_2588
90	JX183555	Human	Vietnam	>gb:VIPR_ALG4_407751081_1488_2582
91	KM252780	Human	Vietnam	>gb:VIPR_ALG4_749396710_1488_2588
92	KM252787	Human	Vietnam	>gb:VIPR_ALG4_749396724_1488_2582
93	KM252786	Human	Vietnam	>gb:VIPR_ALG4_749396722_1488_2579
94	KM252782	Human	Vietnam	>gb:VIPR_ALG4_749396714_1489_2583
95	KM252783	Human	Vietnam	>gb:VIPR_ALG4_749396716_1474_2562
96	KM252785	Human	Vietnam	>gb:VIPR_ALG4_749396720_1489_2580
97	KJ678786	Human	Vietnam	>gb:VIPR_ALG4_758739208_1492_2595
98	KJ678787	Human	Vietnam	>gb:VIPR_ALG4_758739210_1449_2552
99	KJ678788	Human	Vietnam	>gb:VIPR_ALG4_758739212_1491_2594
100	JX183552	Human	Vietnam	>gb:VIPR_ALG4_407751075_1488_2582
101	KJ678789	Human	Vietnam	>gb:VIPR_ALG4_758739214_1491_2594

S. No.	GenBank ID	Host	Country	Sequence ID
102	KJ678790	Human	Vietnam	>gb:VIPR_ALG4_758739216_1449_2552
103	KJ678791	Human	-N/A-	>gb:VIPR_ALG4_758739218_1492_2595
104	KJ678792	Human	Vietnam	>gb:VIPR_ALG4_758739220_1492_2595
105	JX183556	Human	Vietnam	>gb:VIPR_ALG4_407751083_1488_2582
106	KM252782	Human	-N/A-	>gb:VIPR_ALG4_749396712_1488_2588
107	KJ678793	Human	Vietnam	>gb:VIPR_ALG4_758739222_1449_2552
108	KJ678794	Human	Vietnam	>gb:VIPR_ALG4_758739224_1492_2595
109	KJ678795	Human	Vietnam	>gb:VIPR_ALG4_758739226_1492_2595
110	KJ678796	Human	Vietnam	>gb:VIPR_ALG4_758739228_1449_2552
111	JX183553	Human	Vietnam	>gb:VIPR_ALG4_407751077_1488_2582
112	KM252788	Human	Vietnam	>gb:VIPR_ALG4_749396726_1489_2586
113	KJ678797	Human	Vietnam	>gb:VIPR_ALG4_758739230_1449_2552
114	KJ678798	Human	-N/A-	>gb:VIPR_ALG4_758739232_1449_2552
115	KM252784	Human	Vietnam	>gb:VIPR_ALG4_749396718_1487_2581
116	KC844040	Human	China	>gb:VIPR_ALG4_576294938_1488_2585
117	KC844039	Human	China	>gb:VIPR_ALG4_576294935_1488_2600
118	HQ912955	Human	China	>gb:VIPR_ALG4_324330491_1150_2253
119	HQ912954	Human	China	>gb:VIPR_ALG4_324330489_1150_2253
120	KC844037	Human	China	>gb:VIPR_ALG4_576294931_1448_2551
121	KC844038	Human	China	>gb:VIPR_ALG4_576294933_1448_2551

Multiple Sequence Alignment

Clustal Omega

The multiple sequence alignment shows the Consensus sequence of each unique 121 E2 protein sequence. The results for E2 protein sequences of HCV genotype 6 is shown below:

GENOTYPE VI

xxTTTVGxAVxRtTxGLxxLFSxGxKQNLQLINTNGSWHINRTALNCNDSLQTGFIASLFYxH

KFNSSGCPERMAACKPLxDFRQGWGxITYKxNISGPSDDRPYCWHYAPRPCxVVPARTV

CGPVYCFTPSPVVVGTTDRRGNPTYTWGENETDVFMLESLRPPxGGWFGCTWMNSTGF

TKTCGAPPCQIVPGDYNSxxxSxNELLCPTDCFRKHPEATYQRCGSGPWLTPRCLVDYPY

RLWHYPCTVNFTLHKVRMFVGGIEHRFDAACNWTRGERCELxDRDRIEMSPLLFSTTQL AILPCSFTTMPALSTGLIHLHQNIVDVQYLYGVSSSVTSWVVKWEYVVLMFLVLADARI CTCLWLMLLIxxVEA

Conservancy analysis

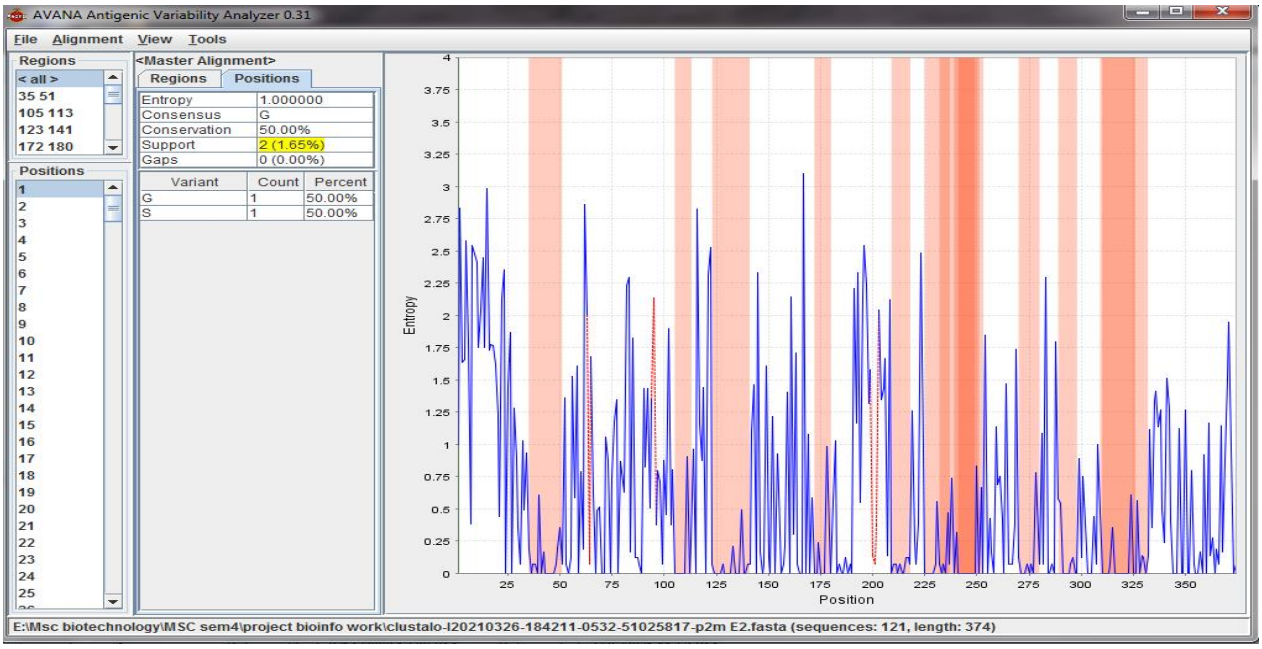
AVANA

The tool AVANA was used for the conservancy analysis. In this analysis, we took 70% of the conserved region as it shows the more unique sequence had a sequence length greater than 9. C1, C2, C3, C4, C5, C6, and C7 show the unique and non-repeated sequences with lengths greater than 9 (**Table 2** and **Figure 1**). This also represents the location of each sequence in the consensus sequence.

Table 2. 70% conserved region had unique sequence more than 9 length

Sr. No.	Sequence	Location
C1	NGSWHINRTALNCNDSL	35-51
C2	VCGPVYCFTPSPVVVGTTD	122-140
C3	CPTDCFRKHP	208-217
C4	CGSGPWLTPRCLVDYPYRLWHYPCTVNFT	224-252
C5	AACNWTRGERCE	269-280
C6	EMSPLLFSTTQ	288-298
C7	TMPALSTGLIHLHQNIVDVQYLYG	308-331

Figure 1. Orange color shows the 70% conserved region by using AVANA



Epitope prediction

It was done for both MHC class I and II with different tools. The results of these tools are mentioned below based on their category and tools.

MHC Class I

In class I epitope prediction, epitopes were predicted for conserved sequences C1, C2, C4, and C7 by using the tools SYFPEITHI and NetCTL1.2 (**Table 3**). In the case of the IEDB epitope, it was predicted for conserved sequences C2, C4, C6, and C7. The SYPEITHI tool had predicted 1 epitope for C1, 2 epitopes for C2, 10 for C4, and 7 for C7. Similarly, NetCTL1.2 predicted 1 epitope for C1, 2 epitopes for C2, 10 for C4, and 6 for C7, while 1 epitope for C2, 7 epitopes for C4, 1 epitope for C5, 1 epitope for C6, and 6 epitopes for C7 were predicted by IEDB (**Table 3**).

Table 3. Epitopes predicted by SYEPEITHI, NetCTL1.2, and IEDB tools for MHC I

Conserved Sequence	SYEPEITHI	NetCTL1.2	IEDB
C1	TALNCNDSL	SWHINRTAL	Not applicable
C2	TPSPVVVGT, CFTPSPVVV	VYCFTPSPV,GPVYCFTPS	VYCFTPSPV
C4	LTPRCLVDY,RLWHYPCTV,CLVDYPYRL,WLTPRCLVD,GSGPWLTPR, DYPYRLWHY,GPWLTPRCL,WHYPCTVNF,PRCLVDYPY LVDYPYRLW	LTPRCLVDY,RLWHYPCTV,CLVDYPYRL,DYPYRLWHY,HYPCTVNFT, DYPYURLWHY,GPWLTPRCL,YRLWHYPCT,PRCLVDYPY WHYPCTVNF	RLWHYPCTV,GSGPWLTPR,CLVDYPYRL,WHYPCTVNF, RCLVDYPYR,GPWLTPRCL,LVDYPYRLW
C5	Not applicable	Not applicable	ACNWTRGER
C6	Not applicable	Not applicable	EMSPLLFST
C7	ALSTGLIHL,HLHQNIVDV,GLIHLHQNI LIHLHQNIV,TMPALSTGL,HQNIVDVQY MPALSTGLI	NIVDVQYLY,HQNIVDVQY,ALSTGLTHL,TMPALSTGL ,HLHQNIVDV,MPALSTGLI	NIVDVQYLY,ALSTGLIHL,MPALSTGLI,TMPALSTGL, HQNIVDVQY,GLIHLHQNI

MHC Class II

In class II epitope prediction, epitopes were predicted for conserved sequences C1, C2, C4, and C7 by using ProPred, while MHC2Pred predicted epitopes for all conserved sequences C1, C2, C3, C4, C5, C6, and C7. Whereas in the case of IEDB, it was predicted for conserved sequences C1, C2, C4, C5, C6, and C7. ProPred predicted 1 epitope for C1, 3 for C2, 3 for C4, and 3 for C7 as well. MHC2Pred predicted 9 epitopes for C1, 11 for C2, 2 for C3, 21 for C4, 5 for C5, 3 for C6, and 14 for C7. IEDB predicted 4 epitopes for C1, 12 for C2, 16 for C4, 2 each for C5, and C6, and 16 for C7 **(Table 4**).

As compared to MHC class I tools, MHC class II tools have predicted epitopes for more conserved sequences.

Table 4. Epitopes predicted by ProPred, MHC2Pred, and IEDB tools for MHCII

Conserved Sequence	ProPred	MHC2Pred	IEDB
C1	WHINRTALN	NGSWHINRT,INRTALNCN,NRTALNCND,WHINRTALN, GSWHINRTA,TALNCNDSL,HINRTALNC , SWHINRTAL, RTALNCNDS	WHINRTALN,GSWHINRTA,INRTALNCN,SWHINRTAL
C2	VYCFTPSPV ,FTPSPVVVG, YCFTPSPVV	PSPVVVGTT,CGPVYCFTP,SPVVVGTTD,FTPSPVVVG, CFTPSPVVV,YCFTPSPVV,TPSPVVVGT,VCGPVYCFT,GPVYCFTPS PVYCFTPSP,VYCFTPSPV	YCFTPSPVV,VYCFTPSPV,CFTPSPVVV,FTPSPVVVG,VCGPVYCFT,PVYCFTPSP TPSPVVVGT,YCFTPSPVX,GPVYCFTPS,SPVVVGTTD,CGPVYCFTP,PSPVVVGTT
C3	Not applicable	CPTDCFRKH,PTDCFRKHP	Not applicable
C4	WHYPCTVNF ,YRLWHYPCT, YRLWHYPC	PWLTPRCLV,CGSGPWLTP,HYPCTVNFT,LWHYPCTVN,WLTPRCLVD, LVDYPYRLW,TPRCLVDYP,RLWHYPCTV,LTPRCLVDY,GPWLTPRCL, SGPWLTPRC,CLVDYPYRL,DYPYRLWHY,RCLVDYPYR,WHYPCTVNF GSGPWLTPR,PRCLVDYPY,YPYRLWHYP,PYRLWHYPC,VDYPYRLWH, YRLWHYPCT	YPYRLWHYP,CLVDYPYRL,PYRLWHYPC,LVDYPYRLW,VDYPYRLWH, LWHYPCTVN,RLWHYPCTV, YRLWHYPCT,WHYPCTVNF, PWLTPRCLV, RCLVDYPYR,CGSGPWLTP,LTPRCLVDY,WLTPRCLVD,PRCLVDYPY HYPCTVNFT
C5	Not applicable	NWTRGERCE,CNWTRGERC, NWTRGERC,EACNWTRGER, AACNWTRGE	ACNWTRGER,CNWTRGERC
C6	Not applicable	MSPLLFSTT,EMSPLLFST,SPLLFSTTQ	EMSPLLFST,MSPLLFSTT
C7	LIHLHQNIV, LHQNIVDVQ, IHLHQNIVD	STGLIHLHQ,LSTGLIHLH,HQNIVDVQY,QNIVDVQYL,LHQNIVDVQ,MPALSTGLI HLHQNIVDV,IVDVQYLYG,TMPALSTGL,NIVDVQYLY,TGLIHLHQN,PALSTGLIH LIHLHQNIV,IHLHQNIVD,ALSTGLIHL,GLIHLHQNI	LIHLHQNIV,LSTGLIHLH,IHLHQNIVD,ALSTGLIHL,HLHQNIVDV,GLIHLHQNI MPALSTGLI,LHQNIVDVQ,PALSTGLIH,NIVDVQYLY,TGLIHLHQN,TMPALSTGL IVDVQYLYG,HQNIVDVQY, QNIVDVQYL,STGLIHLHQ

HLA analysis

MHC Class I

In class I HLA allele prediction, HLA alleles were predicted for conserved sequences C1, C2, C4, and C7 by tools SYFPEITHI and NetCTL 1.2. But in the case of IEDB, HLA alleles are predicted for C2, C4, C5, C6, and C7. SYFPEITHI predicted 1 HLA allele for C1, 2 for C2, 15 for C4, 4 for C7, whereas NetCTL1.2 predicted 3 HLA alleles for C1, 2 for C2, 6 for C4, 5 alleles for C7. IEDB predicted 1 HLA allele for C2, 12 for C4, 1 for C5, 2 for C6, and 12 for C7 **(Table 5)**.

Table 5. HLA alleles predicted by SYFPEITHI, NetCTL1.2, and IEDB tools for MHC class I

Conserved Sequence	SYFPEITHI	NetCTL1.2	IEDB
C1	HLA-B*51:01	A24,B8,B39	Not applicable
C2	HLA-B*07:02 ,HLA-B*15:16	A24,B7	HLA-A*23:01
C4	HLA-A*01,HLA-A*02:01,HLA-A*03 ,HLA-A*11:01,HLA-A*26 , HLA-A*68:01 ,HLA-B*07:02,HLA-B*13 ,HLA-B*15:10 HLA-B*15:16 ,HLA-B*27:05 ,HLA-B*35:01 HLA-B*38:01 ,HLA-B*51:01 ,HLA-B*58:02	A1,A2,A24,A26,B27,B39	HLA-A*02:01,HLA-A*02:02,HLA-A*02:03 HLA-A*02:04,HLA-A*02:05,HLA-A*02:06 HLA-A*02:07,HLA-A*02:08,HLA-A*02:09 HLA-A*02:10,HLA-A*02:11,HLA-A*02:12
C5	Not applicable	Not applicable	HLA-A*31:01
C6	Not applicable	Not applicable	HLA-A*02:06,HLA-A*68:02
C7	HLA-A*02:01 ,HLA-B*13 ,HLA-B*15:01 (B62) ,HLA-B*51:01	A1,A2,A26,B7,B62	HLA-A*29:02,HLA-A*02:01,HLA-A*02:06 HLA-A*68:01,HLA-B*07:02,HLA-B*53:01 HLA-A*26:01,HLA-A*30:02,HLA-B*51:01

Conserved Sequence	SYFPEITHI	NetCTL1.2	IEDB
			HLA-B*15:01,HLA-B*35:01,HLA-A*68:02

MHC Class II

In the case of Class II HLA prediction, HLA alleles were predicted for conserved sequences C1, C2, C4, and C7 by using the tool ProPred. In MHC2Pred, it was predicted for conserved sequences C1, C2, C3, C4, C5, C6, and C7 and in the case of IEDB, the HLA allele was predicted for conserved sequences C1, C2, C4, C5, C6, and C7. ProPred predicted 2 HLA alleles for C1, 8 for C2, 6 for C4, and 14 for C7. MHC2Pred predicted HLA allele 17 for C1, 16 for C2, 5 for C3, 22 for C4, 5 for C5, 9 for C6, and 23 for C7. The IEDB tool predicted 17 HLA alleles for C1, 32 for C2, 38 for C4, 3 for C5, 4 for C6, and 35 for C7. The maximum HLA alleles were predicted by the IEDB tool for MHCII as compared to MHCI **(Table 6).**

Table 6. HLA alleles predicted by ProPred, MHC2Pred, and IEDB tools for MHC II

Conserved Sequence	ProPred	MHC2Pred	IEDB
C1	DRB1_0305,DRB1_0309	HLA-DR9,HLA-DR2,HLA-DR3,HLA-DR51 HLA-DQ4,HLA-DQ6,HLA-DQ7,HLA-DQ8 HLA-DQB1*03,HLA-DQB1*0301,HLA-DRB1*0101 HLA-DRB4*0101,HLA-DRB1*0301,HLA-DRB1*0405 HLA-DRB1*0901,HLA-DRB1*1101,HLA-DRB1*1501	HLA-DRB3*02:02,HLA-DQA1*02:01/DQB1*04:02 HLA-DQA1*03:03/DQB1*04:02 HLA-DQA1*06:01/DQB1*04:02 HLA-DRB1*04:05,HLA-DRB1*11:01 HLA-DRB1*08:01,HLA-DQA1*05:01/DQB1*04:02 HLA-DRB3*03:01,HLA-DQA1*01:02/DQB1*06:02 HLA-DQA1*01:02/DQB1*05:01 HLA-DRB1*13:02,HLA-DRB1*07:01 HLA-DRB1*01:01,HLA-DRB1*04:04 HLA-DRB1*13:01,HLA-DRB4*01:03

Conserved Sequence	ProPred	MHC2Pred	IEDB
			HLA-DRB1*07:01,HLA-DRB1*09:01
			HLA-DQA1*05:01/DQB1*03:03
			HLA-DQA1*06:01/DQB1*04:02
			HLA-DQA1*02:01/DQB1*04:02
			HLA-DQA1*05:01/DQB1*03:02,HLA-DRB3*02:02
			HLA-DQA1*05:01/DQB1*04:02
			HLA-DQA1*02:01/DQB1*03:03
			HLA-DQA1*02:01/DQB1*03:01,HLA-DRB3*03:01
		HLA-DR9,HLA-DR2,HLA-DR3,HLA-DR13	HLA-DQA1*01:03/DQB1*06:03,HLA-DRB1*04:01
	DRB1_0309,DRB1_0309,DRB1_0421	HLA-DR15,HLA-DR52,HLA-DQ4,HLA-DQ6	HLA-DRB1*04:04,HLA-DPA1*01:03/DPB1*02:01
C2	DRB1_0423,DRB1_0701,DRB1_0703	HLA-DQ7,HLA-DQ8,HLA-DQB1*0301	HLA-DRB1*13:02,HLA-DRB1*04:05
	DRB1_1501,DRB1_1506	HLA-DRB1*0101,HLA-DRB5*0101,HLA-DRB1*0401	HLA-DQA1*05:01/DQB1*03:01
		HLA-DRB1*0405,HLA-DRB1*0901	HLA-DRB1*16:02,HLA-DRB1*01:01
			HLA-DRB1*08:02,HLA-DPA1*01:03/DPB1*06:01
			HLA-DRB1*10:01,HLA-DQA1*03:01/DQB1*03:01
			HLA-DRB1*13:01,HLA-DQA1*01:02/DQB1*05:01
			HLA-DRB4*01:03,HLA-DRB1*08:01
			HLA-DRB1*15:01,HLA-DRB1*11:01
			HLA-DRB3*01:01,HLA-DPA1*02:01/DPB1*14:01
C3	Not applicable	HLA-DR9,HLA-DQ4,HLA-DQ7,HLA-DRB1*0405 HLA-DRB1*0901	Not applicable
C4	DRB1_0309,DRB1_0405,DRB1_0421	HLA-DR4,HLA-DR9,HLA-DR2,HLA-DR3	HLA-DQA1*05:01/DQB1*04:02,HLA-DRB3*01:01

Conserved Sequence	ProPred	MHC2Pred	IEDB
	DRB1_0703,DRB1_1502,DRB1_1506	HLA-DR8,HLA-DR13,HLA-DR15 HLA-DR52,HLA-DQ4,HLA-DQ6 HLA-DQ7,HLA-DQ8,HLA-DQB1*0301 HLA-DRB4*0101,HLA-DRB5*0101 HLA-DRB1*0301,HLA-DRB1*0401 HLA-DRB1*0405,HLA-DRB1*0802 HLA-DRB1*0901,HLA-DRB1*1501, HLA-DRB5*0101	HLA-DPA1*01:03/DPB1*06:01 HLA-DPA1*01:03/DPB1*02:01,HLA-DRB3*03:01 HLA-DRB1*15:01,HLA-DPA1*03:01/DPB1*04:02 HLA-DQA1*01:01/DQB1*05:01,HLA-DRB1*03:01 HLA-DRB4*01:03,HLA-DRB1*09:01 HLA-DRB1*04:04,HLA-DRB1*16:02 HLA-DPA1*01:03/DPB1*03:01,HLA-DRB1*13:01 HLA-DRB1*04:05,HLA-DQA1*02:01/DQB1*04:02 HLA-DRB1*10:01,HLA-DPA1*02:01/DPB1*01:01 HLA-DQA1*01:02/DQB1*05:01,HLA-DRB1*01:01 HLA-DPA1*01:03/DPB1*04:01 HLA-DQA1*02:01/DQB1*03:01,HLA-DRB1*04:02 HLA-DRB1*07:01,HLA-DRB1*13:02,HLA-DRB1*12:01,HLA-DPA1*02:01/DPB1*05:01 HLA-DQA1*03:01/DQB1*03:01 HLA-DQA1*03:03/DQB1*04:02,HLA-DRB5*01:01 HLA-DRB1*08:02,HLA-DQA1*01:02/DQB1*05:02 HLA-DQA1*06:01/DQB1*04:02,HLA-DRB1*08:01 HLA-DRB3*02:02,HLA-DQA1*05:01/DQB1*03:01 HLA-DQA1*05:01/DQB1*03:03

Conserved Sequence	ProPred	MHC2Pred	IEDB
C5	Not applicable	HLA-DR4,HLA-DR9,HLA-DRB1*0301 HLA-DRB1*0405,HLA-DRB1*0901	HLA-DRB1*13:01,HLA-DRB1*07:01 HLA-DRB4*01:03
C6	Not applicable	HLA-DR9,HLA-DQ4,HLA-DQ7,HLA-DQ8, HLA-DQB1*0301,HLA-DRB1*0401 HLA-DRB1*0405,HLA-DRB1*0901 HLA-DRB1*1501	HLA-DRB4*01:03,HLA-DQA1*01:02/DQB1*05:01 HLA-DQA1*05:01/DQB1*03:02 HLA-DPA1*01:03/DPB1*06:01
C7	DRB1_0102,DRB1_0306,DRB1_0307 DRB1_0308,DRB1_0311,DRB1_0401 DRB1_0402,DRB1_0405,DRB1_0410 DRB1_0421,DRB1_0426,DRB1_1107 DRB1_1304,DRB1_1506	HLA-DR4,HLA-DR9,HLA-DR2 HLA-DR7,HLA-DR13,HLA-DR15 HLA-DR52,HLA-DR53,HLA-DQ4 HLA-DQ6,HLA-DQ7,HLA-DQ8 HLA-DQB1*03,HLA-DQB1*0301 HLA-DRB1*0101,HLA-DRB4*0101 HLA-DRB1*0301,HLA-DRB1*0401 HLA-DRB1*0405,HLA-DRB1*0802 HLA-DRB1*0901,HLA-DRB1*1101 HLA-DRB1*1501	HLA-DRB3*03:01,HLA-DRB1*13:01 HLA-DRB1*01:01,HLA-DPA1*03:01/DPB1*04:02 HLA-DQA1*01:02/DQB1*05:01,HLA-DRB1*04:04 HLA-DRB4*01:01,HLA-DRB1*13:02 HLA-DQA1*05:01/DQB1*03:03 HLA-DQA1*02:01/DQB1*03:01 HLA-DRB4*01:03,HLA-DQA1*01:02/DQB1*05:02 HLA-DPA1*01:03/DPB1*06:01,HLA-DRB1*07:01 HLA-DQA1*03:01/DQB1*03:01 HLA-DRB1*10:01,HLA-DRB3*02:02 HLA-DQA1*01:02/DQB1*06:02 HLA-DPA1*02:01/DPB1*01:01,HLA-DRB1*04:05 HLA-DQA1*02:01/DQB1*03:03 HLA-DQA1*01:01/DQB1*05:01,HLA-DRB1*16:02 HLA-DRB1*12:01,HLA-DQA1*05:01/DQB1*03:01 HLA-DRB1*15:01,HLA-DQA1*05:01/DQB1*03:02

Conserved Sequence	ProPred	MHC2Pred	IEDB
			HLA-DRB1*09:01,HLA-DQA1*02:01/DQB1*04:02 HLA-DPA1*01:03/DPB1*02:01,HLA-DRB1*04:02 HLA-DRB1*04:01,HLA-DQA1*05:01/DQB1*04:02 HLA-DRB1*03:01,HLA-DQA1*05:01/DQB1*02:01

Discussion

There is identification of the requirement to stimulate each arm of the adaptive reaction for an efficient preventative HCV immunogen and proof to support the inclusion of each structural and non-structural protein.[16] Previous studies have shown proof of cross-reactive neutralizing antibodies (NAbs), significantly within the great ape model.[17-19] However, they have restricted knowledge on immunogen candidates that elicit neutralizing and HCV-specific T-lymphocyte responses.

Some positive results have been shown in a research on African monkeys and immunized mice by mixing the glycoproteins E1, E2, non-structural protein 3 (NS3), and the core protein in the alum (consisting of conserved T-cell epitopes) for the induction of sensitive neutralizing antibodies and wide cellular responses (CD4+ and CD8+).[20] Few vectors and DNA-based vaccines were also present, but results from the phase 2 trial showed that they increased productivity because they were likely to be in an "at-risk' population.[14]

In the relatively large and currently thirty-year field of HIV immunizing agent development, the dearth of an efficient immunizing agent points to the various remaining barriers to inducement, generally neutralizing antibodies or effective CD8+ T cells capable of acting and continuous at the positioning of tissue layer HIV entry. Several current immunizing agent methods going into

clinical studies look to handle a number of the evasion mechanisms mentioned here. For instance, there has been testing of assorted diversity-combating antigen-style approaches as well as mosaic vaccines, during which the inclusion of variant epitopes is optimized, as well as ways to preserve immunogen sequences. There are varied adjuvants, vectors, and delivery vehicles designed to boost the potency of the presentation of immunizing agent antigens so as to stimulate effective antiviral responses. A unique "tolerogenic" immunizing agent consisting of inactivated simian immunological disorder virus (SIV) mac239 particles with explicit microorganism adjuvants has been shown to elicit CD8+ T-regulatory cells in immunized macaques.

T lymphocytes were not lysed but were ready to suppress the activation of SIV-positive CD4+ T cells, making them less prone to SIV infection once challenged. Additionally, these CD8+ T cells were found to have unusual resistance provided by non-classical MHCIb/E molecules[20], such as HLA-E in humans. Curiously, recent knowledge shows HLA-E expression in liver biopsies correlates with HCV microorganism load in chronic HCV-infected subjects, and NK cells lacking the repressive receptor for HLA-E (NKG2A) are related to protection from HCV infection in bad-exposure subjects.[21] Thus far, there has been no examination of non-classical HLA-restricted CD8+ T cells in HCV infection. In distinction to the CD8+ "T-regulatory" sort cells represented above, an immunizing agent supported by a rhesus herpes virus vector has made sturdy protection or clearance of SIV challenge infections in unsusceptible macaques related to the induction of effector memory CD8+ T lymphocyte responses. However, these CD8+ T cells are found to focus on a variety of promiscuous or dominant epitopes restricted by HLA category II alleles instead of HLA category I.[22-23]

Conclusion

In this study, computational identification of multiple T cell epitopes was done for genotype 6 of the human genome with the help of various epitope prediction tools. The tools used for MHC class I epitope prediction were SYFPEITHI, NetCTL1.2, and IEDB for CD8+ T cells. Similarly, for MHC class II epitope prediction, the tools were ProPred, MHC2Pred, and IEDB for CD4+ T cells. These tools give unique epitope sequences. However, various unique HLA allele sequences were enlisted, for which the epitopes were predicted. This study gives the platform to identify future Hepatitis C vaccine candidates for genotype 6 for further in silico and experimental validation in vivo and in vitro cultures.

Conflict of interest

The author declares conflict of interest due to being the editor-in-chief at the Journal of Neoteric Life Sciences. To mitigate this conflict, the author will abstain from participating in any decision-making processes related to this manuscript and agree to comply with any approach outlined by the Journal of Neoteric Life Sciences to manage this conflict.

Data availability statement

The data can be made available upon request from the author.

Ethics statement

Not applicable.

Acknowledgement

Not applicable.

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