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A Review

Computer-Aided vaccine design for selected positive-sense single stranded RNA viruses

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Spontaneous mutations and lack of replication fidelity in positive-sense single stranded RNA viruses (+ssRNA virus) result in emergence of genetic variants with diverse viral morphogenesis and surface proteins that affect its antigenicity. This high mutability in +ssRNA viruses has induced antiviral drug resistance and ability to overcome vaccines that subsequently resulted in rapid viral evolution and high mortality rate in human and livestock. Computer aided vaccine design and immunoinformatics play a crucial role in expediting the vaccine production protocols, antibody production and identifying suitable immunogenic regions or epitopes from the genome sequences of the pathogens. T cell and B cell epitopes can be identified in pathogens by immunoinformatics algorithms and methods that enhance the analysis of protective immunity, vaccine safety, immunity modelling and vaccine efficacy. This rapid and cost-effective computational vaccine design promotes development of potential vaccine that could induce immune response in host against rapidly mutating pathogens like +ssRNA viruses. Epitope-based vaccine is a striking concept that has been widely employed in recent years to construct vaccines targeting rapidly mutating +ssRNA viruses. Therefore, the present review provides an overview about the current progress and methodology in computer-aided vaccine design for the most notable +ssRNA viruses namely Hepatitis C virus, Dengue virus, Chikungunya virus and Coronaviruses. This review also highlights the applications of various immunoinformatics tools for vaccine design and for modelling immune response against +ssRNA viruses.

Keywords: Epitope prediction, Immunoinformatics, Hepatitis C virus, Dengue virus, Chikungunya virus, Coronaviruses

Introduction

Viruses are diverse and are classified into seven classes based on the genome replication and encapsidation. This includes i) single stranded (ss) DNA virus, ii) double stranded (ds) DNA virus, iii) mRNA sense ssRNA virus, iv) antisense ssRNA virus, v) antisense dsRNA virus, vi) reverse transcribing RNA virus and vii) reverse transcribing DNA virus[1]. Low replication fidelity due to lack of proof-reading mechanism by RNA dependent polymerases makes the RNA viruses to exhibit high mutation rate. This spontaneous mutation in the RNA viruses results in variety of mutants called 'quasispecies' that affects viral antigenicity by varying viral morphogenesis, altered surface glycoproteins, rapid viral evolution and antiviral resistance when compared to DNA viruses[2]. Another factor that influences the frequency of mutation in RNA viruses includes polarity of RNA namely positive-sense (5′ to 3′) ssRNA and negative- sense (3′ to 5′) ssRNA[3]. Positive-sense single stranded RNA (+ssRNA) viruses form one-third of known viral genera including various virulent pathogens that are also listed in potent bioterrorism agents. Positive strand RNA viruses use host machinery for its entry and replicate by modulating the host gene expression and also evades host innate immune system by co-opting the host factors[4]. +ssRNA adopts either of two strategies to evade host immune response namely covalent attachment of peptide and formation of 7-methulguanosine cap at the 5′ terminal of viral RNA. +ssRNA viral genome replication occurs in host cell cytoplasm tandemly along with nucleocapsid assembly which shows that there is a close association between species specific viral replication and nucleocapsid formation for genome packing. +ssRNA viral genome work as messenger RNA (mRNA) and act as template for viral replication by fostering interactions of host replication factors during various

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stages of viral RNA replication. Apolipoprotein B mRNA editing enzymes and endogenous deaminases in host can edit cytosines and inosines in viral genome into inosines and uracils, respectively, which could directly edit viral RNA or indirectly alter host cell response by editing host transcripts. Moreover, +ssRNA viruses employ various strategies to evade from this protective mechanism of host. +ssRNA viral families include Picornaviridae, Coronaviridae, Caliciviridae, Astroviridae and Togaviridae. Notable examples of +ssRNA viruses includes Hepatitis C virus and Dengue virus in Flaviviridae family, Chikungunya virus in Togaviridae family and Coronaviruses (Severe Acute Respiratory Syndrome - SARS, Middle-East Respiratory Syndrome-MERS, SARS-Coronavirus-2 -SARS-CoV-2) in Coronaviridae family. Coronaviruses attach a cap through triphosphate bridge and encode capping enzyme that result in formation of m7Gpp-Am-cap mediated by canonical capping pathway. Chikungunya viruses also produces a viral cap similar to eukaryotic cap by unconventional synthetic pathway. To date there are no effective vaccines developed for infectious diseases in human caused by aforesaid +ssRNA viruses. Therefore, it is crucial to identify the RNA mutations, mutation rate and genetic drift in +ssRNA viruses to design effective vaccines. Traditional vaccine development process takes more than 15 years right from discovery phase to clinical phase and its subsequent availability in the market. However, Bioinformatics and Immunoinformatics methods enable to predict and hasten the identification of potent vaccine candidates against several infectious diseases. The information obtained from biological experiments are converged, organized and stored using bioinformatics tools which enhance prediction, annotation and characterization of novel drug targets as well as potent antigens for vaccine design. Advance sequencing experiments have provided several information about clinical, epidemiological and functional data pertaining to immunology research. Therefore, the fast-growing resources in immunology have led to the emergence of new field called Immunoinformatics that deals with the conversion or organization of large amounts of immunological data into meaningful interpretations including retrieval of immunological domain, data mining or management, sequence analysis, target identification, prediction of antigenicity, allergenicity, cross-reactivity, molecular interactions and epitope classification using different computational tools and statistical methods. New hypothesis developed by immunoinformatics about virulence, pathogenesis and host immune response data has given rise to the progress in second-generation vaccine overtaking traditional time-consuming vaccine development methods. Therefore, integration of immunoinformatics tools and recombinant DNA data will further enhance in the development of third generation vaccines called reverse vaccinology. This approach will facilitate in identification of appropriate immunogenic regions in viral genomes and enhance development of potent vaccines that induce protective immune response in host. Recently multiepitope-based vaccine design strategies have been found effective for the design and development of vaccine against highly mutating viral pathogens. This review highlights the significant role of recent computer-aided immunoinformatics approach in developing safe and potent vaccines against notable +ssRNA viruses. The advanced open access bioinformatics tools or databases recently used for designing +ssRNA vaccine candidates are alone discussed throughout this review while the other earlier approaches and computational tools used are provided in the table.

Immunoinformatics and reverse vaccinology

Several public online immunoinformatics databases and bioinformatics tools have been developed for storage and investigation of huge immunology-associated data that facilitate researchers to retrieve the necessary information. Characteristics of the viruses selected for this study are summarized in the (Table 1). Three major repositories associated with immunoinformatics includes i) Immune epitope and analysis resource (IEDB), ii) Nucleic Acids Research (NAR) Databases Annual Issue and iii) Canadian Bioinformatics Link Directory. These open access repositories provide access to updated high-quality annotations of immunoinformatics resources. IEDB contains data on epitopes involved in adaptive immune responses especially in human and other mammals. IEDB has curated thousands of published articles with special focus on priority pathogens (Category A, B, C) specified by National Institute of Allergy and Infectious Diseases (*NIAID*) besides the experimental data of emerging and recurring infectious diseases. Information associated with epitopes from pathogens, autoantigens and allergens are available in open access web-based interface. NAR database maintains the

Table 1 — Characteristics of three selected +ssRNA viral families

Viral Characteristics	Virus Family		
	Flaviviridae	Togaviridae	Coronaviridae
Size (Diameter)	50 nm	65–70 nm	60–220 nm
Shape	Spherical	Spherical	Helical
Envelope	Non-enveloped	Enveloped	Enveloped
Capsid Symmetry	Icosahedral-like	Icosahedral or cubic	Spherical, pleomorphic (Tubular/ bacilliform)
Structural proteins	prM/M and E	Capsid, Envelope (E1, E2) and 3 Glycoproteins	Nucleocapsid and Membrane proteins (many copies)
Genome length	11 kb	10–12 kb	20–30 kb
Nº Capsomeres	32	80 trimers	-
Polarity	p-sense	p-sense	p-sense
Segmentation	n-seg	n-seg	n-seg
Replication	Cytoplasm	Cytoplasmic vesicles	Cytoplasm
Host	Vertebrates	Human, primates, amphibians, birds, reptiles, mosquitoes and other arthropods	Mammals, fish & birds
Entry receptor	Endocytosis,Cell surface	Endocytosis	Endocytosis, Cell surface
Triangulation	T = 3	T=4	-
Disease caused	Several diseases from mild symptoms to severe disease.	Fever, arthritis & encephalitis	Severe acute respiratory syndrome, Respiratory-track infections & gastroenteritis
Notable example	Hepatitis C & Dengue virus	Chikungunya virus	SARS, MERS & SARS-CoV-2

Molecular Biology Database Collection (MBDC) that describes all the available databases. Canadian Bioinformatics Link Directory includes the information extracted from NAR with conceptually annotated links containing tags to describe resources based on the terms of Medical Subject Headings (MeSH). Annotated contents in this repository is characteristic to the Link directory features and it excludes the dead contents which are currently unavailable. All the specified databases facilitate researchers to choose appropriate database and tools that could facilitate in the development of novel diagnostics and potent therapeutics.

Reverse vaccinology involves identification of new antigenic peptides or immunogenic entities in pathogens that can elicit immune response based on the genetic make-up of the pathogen analysed using bioinformatics tools. Therefore, reverse vaccinology employs bioinformatics tools to screen genomic information of pathogens and to identify its genes that could lead to identification of potent epitopes and surface proteins. It was pioneered by Rino Rappuoli[5] against serogroup B meningococcus. The genome sequence of pathogens provides complete information about the antigenic proteins that can be expressed. Therefore, reverse vaccinology approach also includes retrieval of genome sequence, computational analysis of genome sequences, prediction of antigen or epitopes and design of suitable vaccine. Whole genome sequencing data and computational tools also facilitate identification of effective antigenic proteins in pathogens. In the year 2000, Rino Rappuoli[5] applied reverse vaccinology for development of vaccine for *Neisseria meningitidis* that led to the successful development of vaccine against *Streptococcus pneumonia* and *Staphylococcus aureus*. In 2008, Vaxign program was developed by He and colleagues[6] that further improved reverse vaccinology by identifying probable antibody targets based on genomic information and grouping of protein data of specific pathogen. The features predicted by Vaxign program includes transmembrane domain of protein, subcellular location, proteome sequence similarity to host and epitope binding to class I or/and class II MHC molecules. Therefore, Vaxign has been widely used for prediction of vaccine targets against various pathogens. Reverse vaccinology also includes different pattern of antigenic function and high throughput screening necessary for developing suitable vaccine. However, the reverse vaccinology method cannot be applied for designing vaccines using lipid or polysaccharide antigens. Therefore, the two modified reverse vaccinology techniques include i) Pan genomic reverse vaccinology that involves genome comparison between different isolates of same pathogen, and ii) Comparative reverse vaccinology that compares genome of non-pathogenic strain with pathogenic strains of same species using computational tools[7]. Therefore, these two modified techniques involve differences in protein structure

within the same or different organisms. The basic steps involved in vaccine design using reverse vaccinology approach are shown in the (Fig. 1).

Retrieval of viral sequences and phylogenetic analysis

Hepatitis C virus (HCV) belonging to Flaviviridae comprises of 9,600 nucleotides of +ssRNA lacking 3′ polyA tract that causes chronic liver disease and liver cancer in several countries. The HCV genome has a single open reading frame that can be translated into a polyprotein containing 3000 amino acids whose sequence is found to be highly conserved among varied HCV isolates. The post translation of HCV proteins derived from a polyprotein includes signal sequence at C-terminal, envelope proteins E1/S (gp 35) and surface glycoprotein E2/Nsp1 (gp 70) which are found to contain immunogenic epitopes considered as potential targets for vaccine design. There are 6 genotypes, several subtypes in HCV with highly conserved Capsid protein and non-structural proteins (Nsp) especially Nsp3 and Nsp4 domains. The N-terminal of HCV genome particularly E2/Nsp1 regions was reported to have significant sequence heterogeneity and thereby provide strong immune selection[8].

This sequence heterogeneity and diversity amid isolates in N-terminal of E2/Nsp1 hinder the development of effective vaccine against HCV.

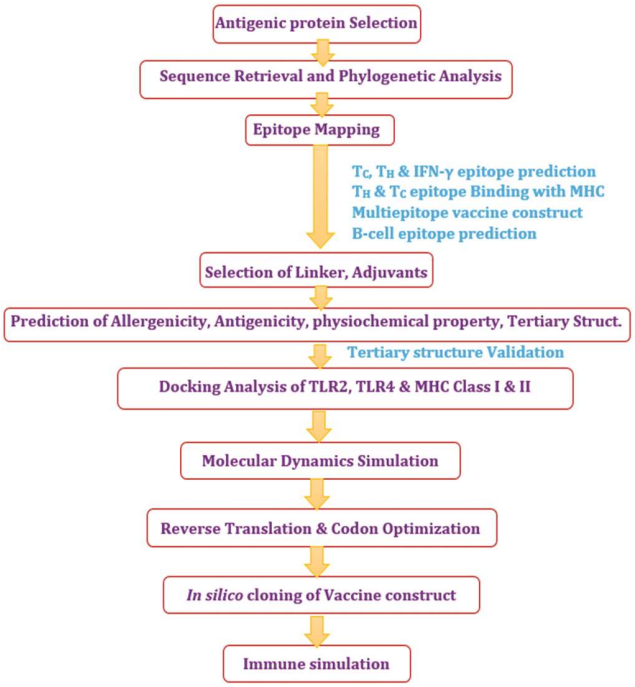

Fig. 1 — Basic Steps in Reverse Vaccinology

Therefore, vaccines developed against *Pestiviruses* and *Flaviviruses* can be used to develop a rational approach for designing an effective vaccine against HCV.

Dengue (DENV) virus is a +ssRNA virus belonging to family Flaviviridae and causing mosquito borne diseases in human ranging from self-limited dengue fever to most severe dengue shock syndrome or dengue hemorrhagic fever. There are five dengue viral serotypes namely DENV-1 to 5 based on their surface antigens. It is a 50 nm virus with genome of 11,000 bases coding for single polypeptide that is cleaved to several structural and non-structural proteins. The polyproteins are cleaved to form short coding regions in 3′ and 5′ terminal, seven non-structural proteins (Nsp) (Nsp1, Nsp2a, Nsp2b, Nsp3, Nsp4a, Nsp5) and three structural proteins (capsid, envelope (E) and membrane (M) proteins) originating from a single precursor called partial premembrane precursor (PrM). DENV-1, DENV-2 showed mutations in PrM, immunodominant regions in E protein, respectively while DENV-3 and DENV-4 did not show any mutations[9]. Phylogenetic analysis of DENV of sequences of PrM and E evidenced that genotype surveillance is essential for designing effective vaccine for DENV virus and the mutation in immunodominant E region in DENV-2 can facilitate the virus to evade the host immune response.

Chikungunya virus (CHIKV) is also a mosquito-borne virus belonging to family Togaviridae that causes acute febrile chinkungunya fever characterized by debilitating joint pain, arthritis, skin rash, headache and fever. The 11,800 nucleotides genome of CHIKV has two open reading frames (ORF) that code for three structural proteins (capsid ‘C’ and two envelope protein E1 and E2) and four non-structural proteins (Nsp1, Nsp2, Nsp3 and Ns4). The Nsp act as replication complex mediating replication of genomic RNA and transcription of sub genomic RNA which express structural proteins. Phylogenetic analysis of CHIKV have revealed that two nucleotide substitutions especially 6K L20M and E2 V368A amino acid substitutions occur at a rate of 5×10^{-4} substitutions/site/year. Vaccine candidate under experimental trial for CHIKV includes 1) inactivated CHIKV vaccine using silica microparticles, ii) E1 or E2 mediated CHIKV subunit vaccines, iii) Live attenuated CHIKV using vaccine developed by site-directed mutagenesis of Nuclear localization sequence (NoLs) in N terminal of capsid protein, iv) Viral-like

protein (VLP) vaccine for CHIKV with and without QuilA adjuvants, v) Chimeric CHIKV vaccine using Eilat (EILV) virus and vi) Nucleic acid CHIKV vaccine using CHIV mRNA designed to deliver monoclonal antibodies[10]. However, currently there is no licensed CHIKV vaccine available.

Human Coronavirus (hCoV) are +ssRNA viruses with 4 diverse genera such as α, β, γ and *δ* coronaviruses among which α and β coronaviruses are of recent interest due to their capacity to cause life-threatening infections by crossing animal-human barriers. Till now five human β-CoV namely SARS, MERS, SARS-CoV-2, hCoV-OC43 and hCoV-HKU1 whereas two α-CoV like hCoV-229E and hCoV-NL63 have been documented to cause infectious diseases in human. Moreover, hCoV-OC43, hCoV-HKU1, hCoV-229E and hCoV-NL63 accounts for 5-30% of common cold that normally cause wide range of symptoms from asymptomatic or mild symptoms to severe respiratory or gastrointestinal infections. However, the devastating outcomes of lethal β-hCoV namely SARS, MERS and SARS-CoV-2 have documented dreadful impacts on human causing global pandemic with severe complications like lower respiratory tract infections, acute lung injury, acute respiratory distress syndrome, multi-organ failure and septic-shock resulting in high fatality ratio. CoV are spherical viruses with spike proteins on its surface giving them a solar crown-like appearance called Corona. Among other RNA viruses, CoV has the small genome size of about 41 kb with 26-32 kb. The genome size of SARS, MERS and SARS-CoV-2 are 29.75 kb, 30.11 kb and 29.9 kb, respectively. Phylogenetic analysis of these three CoV suggested that SARS-COV-2 shares about 50% genome similarity with MERS and 79.5% with SARS. The genome of β-hCoV have 3′ polyadenylated tails ($1/3^{rd}$ of genome) that codes for structural proteins like nucleocapsid (N), envelope (E), Membrane (M) and spike (S) proteins essential for viral life cycle. However, 5′ methylated caps in CoV genome encodes for several non-structural proteins crucial for viral replication. Understanding the origin, virulence, host immune response, mode of transmission and viral genome information of CoV are essential for developing effective vaccine candidates. The morphology and genome organization of the selected +ssRNA viruses are shown in the (Figs 2 and 3).

The VIPR database (https://www.viprb rc.org/ brc/home.spg?decorator=vipr) can be used for retrieval of selected both viral sequences and the target protein sequences of different strains of viruses isolated from different countries across the world. The phylogeny tree for the viruses can be constructed using MUSCLE tool which align the protein sequences based on Neighbour Joining algorithm. The viral variants can be clustered together based on common ancestry so that the vaccine developed against one strain can be used for all other related strains. The close relationship between the strains isolated from different countries will also support in use of single vaccine that will be effective for all other viral strains.

Prediction of epitopes

Immunoinformatics includes designing of algorithm for mapping of T-cell and B-cell epitopes for development of potential vaccine candidates against viral pathogens. T and B lymphocytes play a vital role in acquired immunity by binding with the epitopes in the antigens. Human Leukocyte Antigens (HLA) or Major histocompatibility complex (MHC) molecules expressed on surface of Antigen Presenting Cells (APCs) present the peptide epitope to the T-lymphocytes. These linear peptide epitopes for T-cells can bind to Class I or Class II MHC cleft and can present the intracellular or extracellular antigens to Cytotoxic T-cells (T_C) or Helper T cells (T_H) through $CD8^+$ or $CD4^+$ receptors, respectively. In human, HLA system on chromosome 6 is coded with highly polymorphic genes (about 12 genes). MHC class I have three loci namely HLA-A, -B and -C that could detect antigenic peptides of 8-12 amino acids long, but MHC class II contains HLA-DR, DP and DQ bind to sequences of 15-24 amino acids. Identifying varied HLA alleles play a significant role in predicting T-cell epitopes. Therefore, B cells induce humoral-mediated immune response through identification of epitopes in antigen by the paratope of specific antibody while T cells mediate cell-mediated immune response when the processed antigens are presented to them by APCs. Hence identification of T and B cell epitope is crucial in designing of epitope-based vaccine. Frequently used computational tools for prediction of T-cell and B-cell epitopes are listed in (Tables 2 and 3).

Prediction of T-cell epitope in +ssRNA viruses

A good vaccine candidate should stimulate long term adaptive immunity especially cell-mediated immunity mediated by both T_C cells and T_H cells

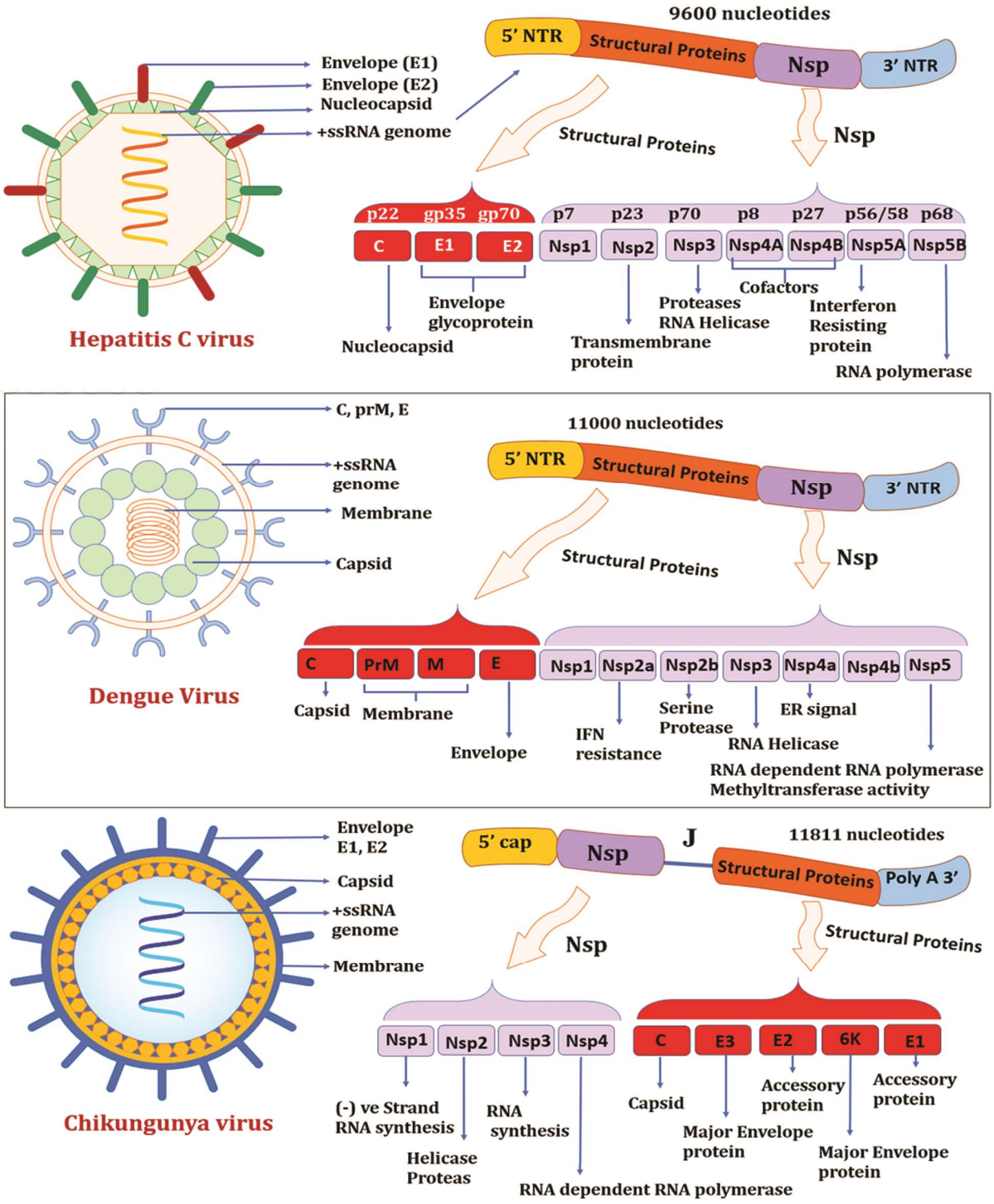

Fig. 2 — Morphology and Genome Organization of HCV, Dengue and CHIKV

epitopes. T_C cell epitopes can induce long term cellular immunity that has the capacity to kill viral infected cells and remove viruses circulating in blood. Whereas T_H epitopes induce both cell-mediated and humoral-mediated immune response by eliciting $CD4^+$ response that leads to stimulation of protective T_C cell memory and also activates B-lymphocytes for antibody production, respectively. Therefore, significant T_C cell and T_H cell epitopes should be included for effective vaccine design. Recent methods for prediction of Tc cell epitopes in viruses are IEDB consensus and NetCTL1.2 approaches. However,

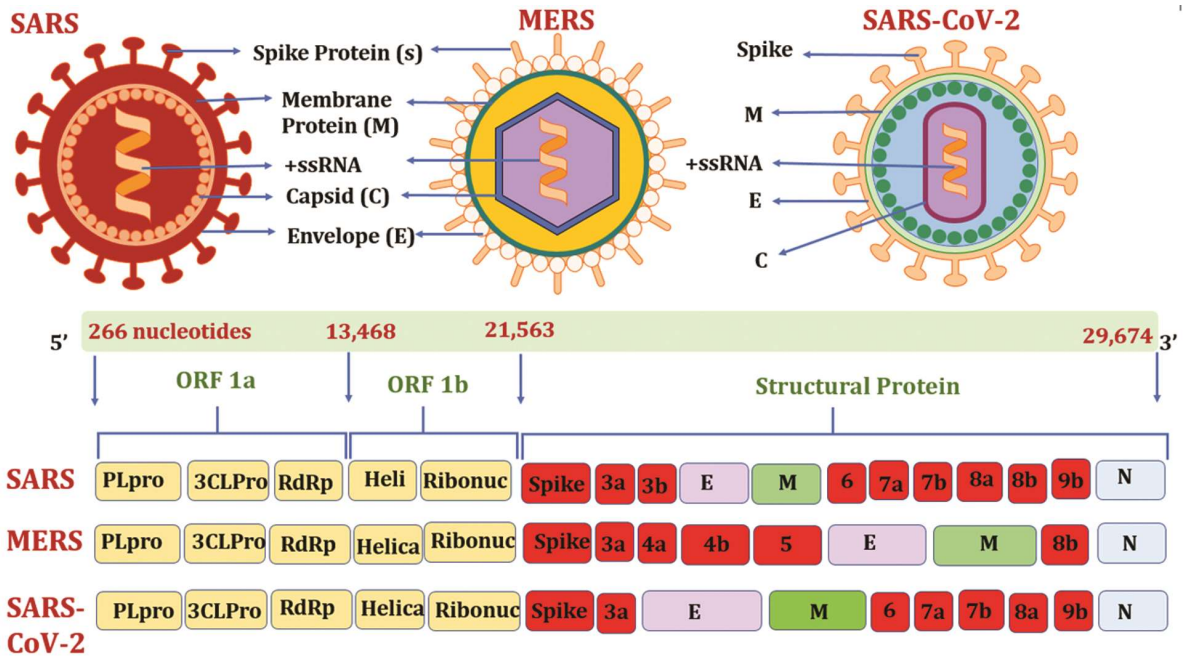

Fig. 3 — Morphology and Genome Organization of SARS, MERS and SARS-CoV-2

Table 2 — Tools and databases used for T-cell epitope prediction

Tool	Application in prediction of:	Web Link
TEPITOPE	Class II MHC epitope	https://www.vaccinome.com
Allele frequencies	HLA polymorphism and frequencies	http://www.allelefrequencies.net
NetCTL	Tc cell & MHC I, II epitope	http://www.cbs.dtu.dk/services/NetCTL
ElliPro	Antibody epitope	http://www.tools.immuneepitope.org/tools/ElliPro
IMGT/HLA	Database for MHC class I & II alleles	http://www.ebi.ac.uk/imgt/hla/allele.html
JenPep	Qualitative information of epitopes & peptide-MHC complexes	http://www.jenner.ac.uk/JenPep
MHCPred	Peptide-MHC binding	http://www.ddg-pharmfac.net/mhcpred/MHCPred/
NetMHC	MHC binding with peptides	http://www.cbs.dtu.dk/services/NetMHC
Pcleavage	Immuno-proteosome cleavage	http://www.imtech.res.in/raghava/pcleavage/
EpiVax	MHC class I and II conserved region	http://www.epivax.com Prediction of classes I/II conserved and promiscuous epitopes
EpiJen v 1.0	Algorithm for T-cell epitopes prediction	http://www.ddg-harmfac.net/epijen/ EpiJen/EpiJen.htm
MAPPP	Cleavage sites in proteasome	www.mpiib-berlin.mpg.de/MAPPP/cleavage.html
SYFPEITHI	MHC anchor motifs database	http://www.syfpeithi.de
PDB	Combination of HLA-peptide and T-cell receptor	https://www.rcsb.org/pdb/
CTLpred	Identify T_C cell epitope by artificial neural network	http://www.imtech.res.in/raghava/ctlpred
MMBPred	MHC class I binders and high affinity mutation	http://www.imtech.res.in/raghava/mmbpred/
EpiToolKit	Ligands for MHC class I and II	http://www.epitoolkit.org
BIMAS	HLA dissociation half-life	http://www.thr.cit.nih.gov/molbio/hla_bind
PREDEPP	Peptide-MHC binding Structure	http://margalit.huji.ac.il/Teppred/mhc-bind/index.html
IEDB	Database with peptide epitopes	http://www.immuneepitope.org/
TAPPred	TAP protein binding affinity	http://www.imtech.res.in/raghava/tappred/

NetMHC II pan 3.2 server is widely used for predicting T_H cell epitopes in viruses. Immune filters are used to screen the best T cell epitopes having high binding affinity with class I and II MHC molecules. The criteria for screening viral epitopes are: i) T cell epitope should be promiscuous and ii) Epitope should have both immunogenic and antigenic properties.

The immunogenicity and antigenicity of the viral T cell epitopes can be predicted by IEBD class I immunogenicity server while VaxiJen v2.0 is widely

Table 3 — Tools and databases used for B-cell epitope prediction

Tool	Application in B-cell Epitope prediction	Web Link
CED	Database for epitope conformation	http://immunet.cn/ced
BCIPEP	Database for B-cell epitope	http://www.imtech.res.in/raghava/bcipep
COBEpro	Linear B-cell epitope prediction	http://scratch.proteomics.ics.uci.edu
IMGT VR	Antibody, T-cell receptor and MHC	http://www.imgt.org
AntiJen	Quantified B-cell epitope Binding data	http://www.jenner.ac.uk/antijen/
Pepitope	Affinity based epitope mapping	http://www.pepitope.tau.ac.il/
IEDB	Database for epitope prediction	http://www.immuneepitope.org
Bepipred	B-cell epitope prediction via HMM	http://www.cbs.dtu.dk/services/BepiPred
CEP	B-cell epitope prediction	http://bioinfo.ernet.in/cep.htm
CED	Database for Epitope conformation	http://immunet.cn/ced
3DEX	Epitope mapping in 3D structure of protein	http://www.schreiber-abc.com/3dex/
MIMOX	Phage display tool for epitope mapping	http://www.immunet.cn/mimox/
AgAbDb	Database for interaction of antigen-antibody	http://www.115.111.37.206:8080/agabdb2/home.jsp
DiscoTope	Epitope conformation prediction	http://www.cbs.dtu.dk/services/DiscoTope/
MIMOP	3D epitope of mimotope peptide	franck.molina@cpbs.univ-montp1.fr

Table 4 — Illustration of Epitopes predicted for most notable +ssRNA viruses

+ssRNA virus	Protein	Epitope sequence	Amino acid position
Hepatitis C virus	Envelope Glycoprotein-2	LPCSFTPMPALST	675-687
		QLVNTNGSWHIN	409-420
Dengue virus	D2 Envelope	QLKLDWFKKGSS	386–397
Chikungunya virus	Nsp1	RAVPQQKPRGPGPGGANEGA	396
	Nsp2	IKLIEQGPGPGAPARRMMSD	231
	Nsp3	MAEIYTMGPGPGDRRRALAD	355
	Nsp4	ALPPLQAGPGPGRKFRSSRA	271
	Envelope protein (E1, E2)		
SARS	Spike protein	CYGVSATKL	366-374
MERS	Nucleocapsid protein	PAAVRAVSF	4-12
SARS-CoV-2	Spike protein	FSYTESLAGKREMAII	26

used for identification of T cell epitope antigenicity. The three-dimensional structure of the viral antigenic proteins and selected epitopes can be modelled and visualized by I-TASSER. For prediction of T_C cell epitope, NetCTL12 server can be used for recognition of MHC class I subtypes that frequently occur in human population. The threshold of the server can be set appropriately for epitope recognition using parameters like cleavage of C-terminal and Transporter associated with antigen processing (TAP). Moreover, the epitopes identified by other Class I MHC alleles can be detected by IEDB tool. Similarly, NetMHCII pan 3.2 server can be used to identify Tc cell epitopes and has to be classified as non-binders, intermediate binders and strong binders based on the percentile rank provided by the server.

The essential criteria for designing linear vaccine construct includes i) overlapping T_H and T_C cell epitopes, ii) epitope should be antigenic or immunogenic and not allergenic and iii) should have high affinity to MHC alleles. Based on the aforesaid criteria, linear vaccine can be constructed for +ssRNA viruses using T cell (T_H and T_C cells) epitopes and interferon-γ (IFN- γ) while GPGPG linkers (junctional epitopes can be avoided) are used to enhance immune processing of immunogen. The immune response of the vaccine can be boosted longer by attaching the adjuvants like cholera toxin B through EAAAK linker to N- terminal of vaccine construct[11]. Then the molecular weight and number of the amino acids in the designed vaccine should be determined. The trRosetta server can be used to generate three-dimensional structure of the designed vaccine and the quality of predicted vaccine model can be validated by ERRAT, Z-score analysis and Ramachandran plot. Normally, the ERRAT score higher than 50 evidences good quality of the designed vaccine model. The epitopes predicted for the selected +ssRNA viruses in earlier reports are provided in the (Table 4).

Selection of adjuvants

Optimization of adjuvants is essential for maximizing the prophylactic efficiency of vaccine. Adjuvants are molecular complexes that increase the immune response once included in the designed

vaccine. Though T and B cell epitopes are optimized, the designed peptide vaccines can act as a week immunogen intrinsically and therefore inclusion of appropriate adjuvants will enhance the strength as well as the duration of immune response. The advantages of using adjuvants in multiepitope-based vaccine construct includes: i) long term immunological memory to vaccine, ii) increase in antibody repertoire, iii) limited vaccine dosage and iv) stimulating the immune response even in older patients[12].

Selection of linkers

Linkers or spacers are generally used in the vaccine construct to enhance the interdomain interactions, vaccine function and structure stability. The linkers include three main components namely T cell epitope, B cell epitope and intramolecular adjuvants. But use of inappropriate linkers can cause undesirable effects like misfolding of protein, reduced production and feeble activity of vaccine construct. Therefore, the length, amino acid sequence, secondary structure, protease sensitivity, interactions with components in vaccine construct and hydrophobicity are the important properties to be considered while choosing appropriate linkers[13]. SynLinker server facilitates selection of linkers and fusion protein modelling. This sever contains about 2150 and 110 natural or peptide linkers and artificial or empirical linkers, respectively. Suitable linker can be chosen from this server based on the user-specified criteria like peptide length, amino acid composition, solvent accessibility and other properties that affect function and flexibility of the linker.

Immunogenic, allergenic and physicochemical evaluation of vaccine construct in +ssRNA viruses

Antigenicity is the capacity to be recognized as an antigen by the antibody produced due to immune response. While immunogenicity refers to stimulation of cell-mediated or humoral immune response to a particular immunogen. Therefore, the designed vaccine should have both immunogenic and antigenic characteristics. The allergenic or non-allergenic nature of the viral epitopes can be identified by webservers namely AllergenFP and AllerTOP. Vaxijen v2.0109 and viral databases can be used to check the antigenicity and to obtain whole-protein antigenicity identification models, respectively [14]. The produced models can be analysed using the data sets made up of identified and non-identified antigens employing external as well as leave-one-out cross validations. AllerTOP server groups protein sequences into equal length vectors by using autocross-covariance (ACC) that can be utilized for quantitative structure-activity relationships. The server employs K-nearest neighbour algorithm to predict allergens and non-allergens in different species. Efficacy and safety of vaccine candidate can be evaluated by physiochemical properties using ExPASy. Theoretical pI and aliphatic index of vaccine can be used to detect thermostability. Higher the value of protein aliphatic index, higher is the thermostability of the developed vaccine. The half-life of vaccine can be identified by ExPASy and the hydrophilic nature of the vaccine can be predicted by Grand average hydropathicity (GRAVY) score. Stability of the protein can be predicted by instability index whose value less than 40 indicates good stability. The designed vaccines can then be verified for transmembrane helices and signal peptides.

Prediction of B cell epitope

B-cell epitopes are responsible for binding of antigenic determinants in antibody to the antigen. B cell receptors or secretory antibodies bind to B cell epitopes on antigens to induce humoral mediated immune response. Therefore, presence of B-cell epitopes in the vaccine candidate is crucial for triggering immune response. B-cell epitopes are specific surface protein that can be of two types namely linear and conformational epitopes. The conformational epitopes are three-dimensional folded proteins while the linear epitopes are short peptides. Levitt hydrophobicity scale to evaluate the amino acids propensity value which is the prime factor to evaluate its location in B cell epitopes. ElliPro server can be used to identify the continuous or linear and discontinuous or conformational B-cell epitopes. PyMOL software can be used to visualize B-cell epitopes in the constructed vaccine. Continuous and discontinuous B cell epitopes can be identified using ElliPro tool in IEDB server using default parameters. The most commonly used prediction tools for antigenicity and allergenicity are provided in the (Table 5).

Prediction of Interferon-γ epitope

Interferon-γ (IFN- γ) plays an active role in innate immunity and in mediating humoral immune response by inducing antiviral activities. IFN- γ epitope prediction is essential to design multi-epitope-based

Table 5 — Prediction tools for antigenicity, allergenicity and epitope optimization

Tool	Antigenicity & Allergenicity Prediction	Web Link
Allergome	Information about allergens	www.allergome.org
Allermatch	Protein allergenicity prediction via slide window	http://www.allermatch.org
AllerTOP 2.0	Allergens prediction	http://www.ddg-pharmfac.net/AllerTOP
VaxiJen	Identification of subunit vaccines, tumor and protective antigens	www.ddg-pharmfac.net/vaxijen/
AllergenPro	Database for analysis of allergenicity	http://nabic.rda.go.kr/allergen/
ANTIGENpro	Protein antigenicity prediction	http://scratch.proteomics.ics.uci.edu/
SDAP	Database with structural information about allergenic proteins.	http://fermi.utmb.edu/SDAP/
APPEL	Allergenic proteins prediction	http://jing.cz3.nus.edu.sg/cgi-bin/APPEL
International Union of Immunological Societies	Allergens and isoallergens database	http://www.allergen.org
AlgPred	Allergenic protein prediction and IgE epitope mapping	http://www.imtech.res.in/raghava/algpred/
Tool	Application in Codon optimization	Web Link
Optimizer	Protein sequence optimization	http://genomes.urv.es/OPTIMIZER/
COOL	Synthetic gene design optimization	http://bioinfo.bti.a-star.edu.sg/COOL/
Codon usage database	Codon optimization of nucleotide sequence obtained from GenBank	http://www.kazusa.or.jp/codon/
GenScript	Gene expression parameter optimization	https://www.genscript.com/codon-opt.html
Jcat	Codon optimization of prokaryotic sequences	www.jcat.de/
Integrated DNA Technologies, Inc	Codon optimization	https://eu.idtdna.com/CodonOpt#

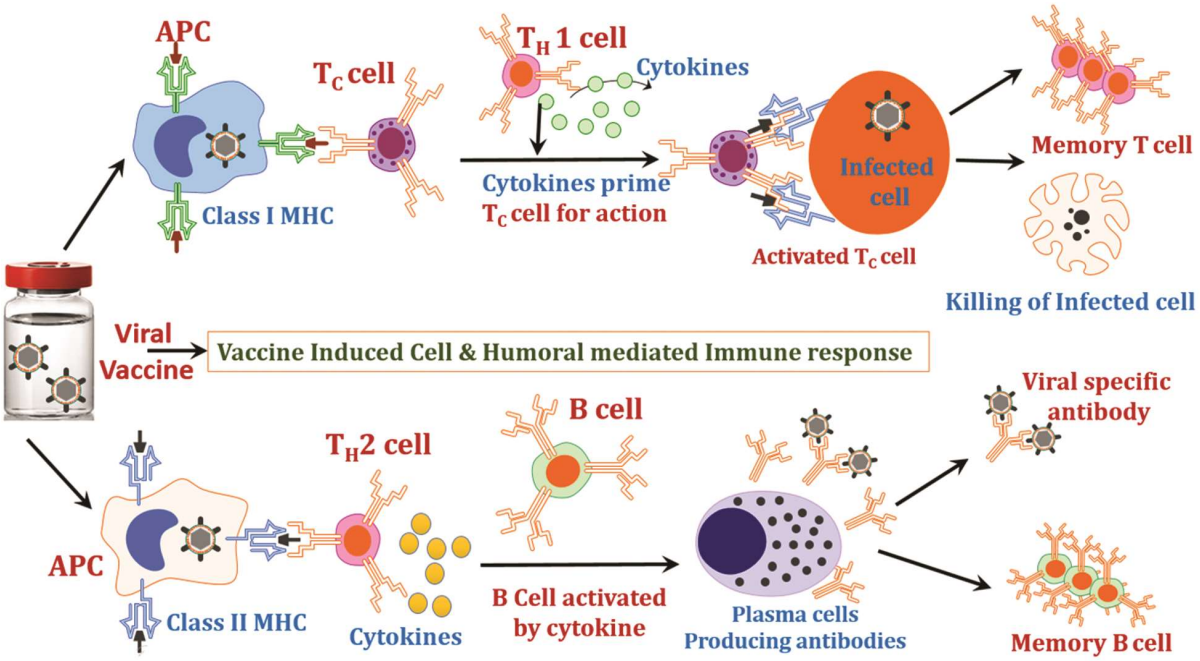

Fig. 4 — Schematic illustration of multi-epitope vaccine inducing both humoral and cell-mediated immune response

vaccine. IFN epitope server has an accuracy of 82% and can be used to predict the IFN- γ epitope in viral antigens[15]. Other approaches used for epitope prediction includes motive-based study, hybrid and machine learning approaches. The mechanism of eliciting humoral and cell mediated immune response by multi-epitope-based vaccine is shown in the (Fig. 4).

Population coverage

Distribution as well as expression of MHC alleles could differ with people across the globe based on the geographical regions and ethnicity. Therefore, development of successful vaccine demands evaluation of distribution of MHC alleles around global population. Since most of the global pandemic are caused by +ssRNA viruses, the designed epitopes for potent vaccine candidate should have a good total world population coverage. IEDB population coverage tool can be used to analyse if the epitope of the vaccine construct covers the total world population[16]. The default parameters in the IEDB server can be used to check the population coverage against MHC class I and II alleles.

Molecular docking analysis

Molecular docking of designed vaccine with Toll-like receptor (TLR)

An effective vaccine should have interactions with the receptors in target immune cells for production of prolonged immune response. Therefore, Molecular docking analysis can be performed to evaluate the interaction of vaccine candidate with the Toll-like receptors. TLRs identify the conserved pathogen-associated molecular patterns (PAMPs) on several viruses which lead to activation of innate immunity and subsequent stimulation of adaptive immunity[17]. The TLR's involved in recognition of structural proteins of viruses results in the production of inflammatory cytokine especially TLR4 and TLR2 since they are found on the surface of the cells and can elicit an immune response on administration of vaccine. Moreover, the vaccines should also be docked with the MHC class I and II receptors. Structure of TLR2, hetero-tetrameric structure of TLR4, structures of both MHC class I and II receptors can be obtained from Protein Data Bank. The passive residues and active sites involved in the interaction of TLR's and vaccine can be predicted using CPORT122[18]. The best docked clusters obtained by docking of vaccine candidate with the TLR and MHC receptors using HADDOCK 2.4 can be refined from the docked complex. Lower HADDOCK score in arbitrary units indicates strong protein interaction. The binding affinity of each docked complex can be analysed by PRODIGY web server and the interacting residues between TLR and vaccine can be mapped using PDBsum. Root-mean-square deviation of atomic positions (RMSD) values are significant in estimating the efficient docking studies since it facilitates identification of complex with lowest structural deviation and energy[19]. Lower RMSD score of the docked complex evidence good quality of the developed model. The structure validation of the docked complex can be validated by Ramachandran plot.

Molecular docking of designed vaccine with MHC class I and II receptors

The multi-epitope based viral vaccine candidate consisting of T_H and T_C cell epitopes can interact with MHC class I and II receptors leading to formation of epitope-MHC complex that activate T_H and T_C cells necessary for inducing cell-mediated immune response. These interactions can be evaluated by docking analysis of the designed vaccine candidate with the MHC receptors using water refined HADDOCK models[20] and the docked complex can be further validated by Ramachandran plot. The structure with lowest HADDOCK score is considered as the top cluster which can then be subjected to refinement using HADDOCK refinement server. The Gibbs free energy and binding affinity of the complex can be used to analyse the interactions occurring within the cell at specific conditions. The binding affinity can be determined using PRODIGY web server. The negative value of Gibbs free energy will indicate docking is energetically feasible.

Molecular dynamics simulation for predicted vaccine structure

Molecular dynamics simulation (MDS) can be used to evaluate the protein stability at varied thermobaric conditions as well as to validate the behaviour of vaccine construct in i*n vivo* biological system. The protein stability as well as energy minimization of the viral vaccine construct can be evaluated using GROningen MAchine for Chemical Simulations (GROMACS) algorithm (steepest descent)[21]. The topology file necessary for energy minimization can be generated using Optimized Potential for Liquid Simulation-All Atom (OPLS-AA) force field constrain. The spc16 is an equilibrated 3-point water model which act as solvent to simulate the vaccine through boundary conditions. The total charge of designed vaccine can be analysed and ions can be added or removed to neutralize the system. The root mean square fluctuation (RMSF) and RMSD of side chain and backbone, respectively, can be evaluated using simulation run performed for energy minimised structure. Xmgrace plotting tool can be used to visualize the graph while Trajectory analysis depicts the stability or flexibility of vaccine construct. The plots for RMSD, RMSF and radius of gyration provides the information regarding fluctuations, degree of flexibility and compactness of protein round the axes, respectively. Fig. 5 represents step-wise construction of epitope-based vaccine and tools to predict the properties of selected antigens.

In silico cloning of the vaccine

The constructed vaccine can then be expressed into *Escherichia coli* expression system by *in silico* cloning. Therefore, as a first step, the codon has to be optimized according to the usage of selected expression system for enhanced protein expression. Java Codon Adaptation Tool (JCat) tool can be

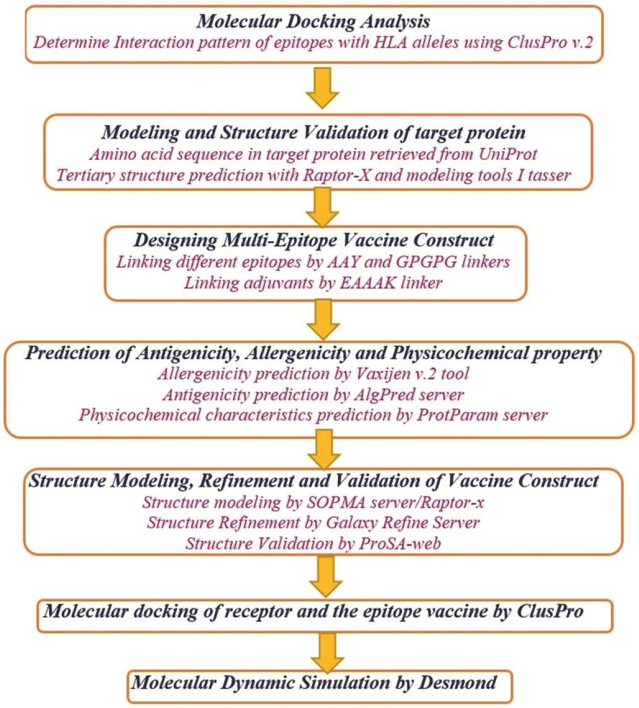

Fig. 5 — Steps to construct multi-epitope-based vaccine

employed for codon optimization in *E. coli* K-12 expression system[22]. The other databases or tools used in codon optimization are provided in the Table 5. Generally, cDNA sequence and codon adaptation (CAI) are performed after optimization of codon. The CAI value greater than 0.8 and GC content in the range of 30-70% indicates enhanced expression of protein in the host. SnapGene software is the widely applied tool for designing the recombinant plasmid pET-28a (+) vector in order to insert adapted codon sequence.

Immune simulation

Immunogenic profile of multi-epitope vaccine construct can be generated using C-IMMSIM immune server. Tertiary and secondary response produced by MDS are compared with primary response to evidence the decrease in concentration of antigen accompanied with higher levels of immunoglobulin activity. Moreover, presence of multiple B cells that last longer indicate memory formation and isotype switching. Increase in population of T_H and T_C cells along with enhanced activities of natural killer cells, macrophage and dendritic cells indicate good immune response. This can be evidenced by elevated levels of interferon-γ and Interleukin-2. Live replicating virus can be stimulated to check the efficiency of vaccine construct. The virtual absence of antigenic surge specifies the enhanced immune response primarily due to higher antibody concentration. Immune response and immunogenicity of the vaccine construct can be characterized by immune simulation using C-IMMSIM server which uses position-specific scoring matrices for prediction of peptides[23]. Therefore, computational tools could be used as a promising tool in construction of structurally stable multi-epitope vaccines that could induce specific immune response against +ssRNA viruses.

Success paradigms in development of vaccines against +ssRNA virus

Developing vaccines for +ssRNA viruses is no easy task as only one specific mutant predominates in a particular season. Therefore, it is very tricky to develop +ssRNA viral vaccines that require equal levels of protection against most of the mutants/genotypes/serotypes by eliciting an immune response. Computational approaches have been extensively used as primary tool to hasten and develop highly precise vaccines for +ssRNA viruses[24]. Though, the computationally designed vaccines need to be subjected for extensive experimental studies, the success rate of vaccines developed by this multi epitope approach is high since it is based on genomic information of the viruses when compared to random trial and error method.

Prophylactic vaccine candidate for HCV that have entered Phase 1b and Phase 2 clinical trial includes recombinant proteins made from envelope glycoproteins (gpE1/gpE2) in oil/water and a recombinant vector vaccine produced using modified vaccinia Ankara virus that *expresses* non-structural proteins (*NS3*, *NS4 and NS5B*) of HCV. Recently the membrane-associated oligomeric protein (p7) in HCV that is involved in ion channel activity was found to be the potential target in producing cytokines in inducing both $CD4^+$ and $CD8^+$ T-cells[25]. Currently there is no vaccine available in the market for hepatitis C.

Among four serotypes of Dengue virus (DENV-1 to 4), only one serotype predominates in a season and there for all the four serotype epitopes should be given equal attention to develop an effective vaccine. Sanofi Pasteur has developed the first licensed chimeric vaccine for dengue consisting of genes for structural proteins namely pre-membrane and envelope protein of all the four serotypes of Dengue virus recombined with genes encoding non-structural proteins of yellow fever viral strain as in 17D vaccine.

Other dengue vaccine candidates in phase III efficacy trials includes DENVax vaccine (Attenuated DENV-2 with recombined viruses expressing pre-membrane-(prM) and envelope (E) proteins of DENV 1, 3 and 4 serotypes), TV0003 (tetravalent vaccine developed by removing 30 nucleotides from 3′ untranslated region of all four Dengue serotypes), TDENV-LA (tetravalent vaccine with live attenuated dengue virus), TDENV-PIV (tetravalent vaccine made up of purified inactivated dengue virus), TDV (tetravalent prM/E-expressing plasmid DNA vaccine) and V180 (truncated recombinant DENV with 80% of N-terminal envelope (E) glycoproteins from all 4 DENV serotypes)[26]. World Health Organization (WHO) recommends use of live recombinant tetravalent Dengue vaccine (CYD-TDV) developed by Sanofi Pasteur (Mexico).

Several vaccine candidates for Chikungunya virus that are in pre-clinical studies were developed using chimeric-alphavirus, viral DNA vaccines, recombinant vectors, inactivated Chikungunya virus, live attenuated virus and virus-like particles. However, only two vaccines have successfully entered phase II clinical trials namely MV-CHIK (recombinant measles virus which expresses surface proteins of La Reunion East/Central/South African genotype of Chikungunya virus) and VRC-CHKVLP059-00-VP (is a viral-like particle vaccine that consists of plasmid encoding structural proteins like capsid (C) proteins and envelope (E1, E2) proteins[27]. No prophylactic *vaccines* for CHIKV infections are *currently* licensed.

The licenced prophylactic vaccines candidates for SARS-CoV-2 includes protein subunit, non-replicating viral vector, mRNA and inactivated virus. COVID-19 vaccine platforms and their ways to elicit immune response in cells is represented in (Fig. 6).

NVX-CoV2373 COVID-19 vaccine is a recombinant protein-based vaccine candidate developed by Novavax using genetically engineered baculovirus expressing SARS-CoV-2 spike protein on its surface and adjuvant with synthetic lipid nanoparticles (50 nm) (Matrix-M) displaying about 14 pike proteins that enhance immune responses and stimulate production of high levels of neutralizing antibodies. NVX-CoV2373 also showed cross-reactive immune responses against Omicron (B.1.1.529) and other variants[28]. India's indigenous approved COVID-19 vaccines includes COVAXIN made from inactivated whole NIV-2020-770 strain of SARS-CoV-2 antigen developed by Bharat Biotech and Covishield vaccine made from genetically engineered, non-replicating chimpanzee adenovirus to produce coronavirus proteins developed by Serum Institute of India. Several other COVID-19 vaccines approved by Drugs Controller General of India (DCGI) includes ZyCoV-D and Sputnik V.

Non-replicating viral vector vaccines signify is novel approach and no vaccine of this kind was permitted before this SARS-CoV-2 pandemic[29,30].

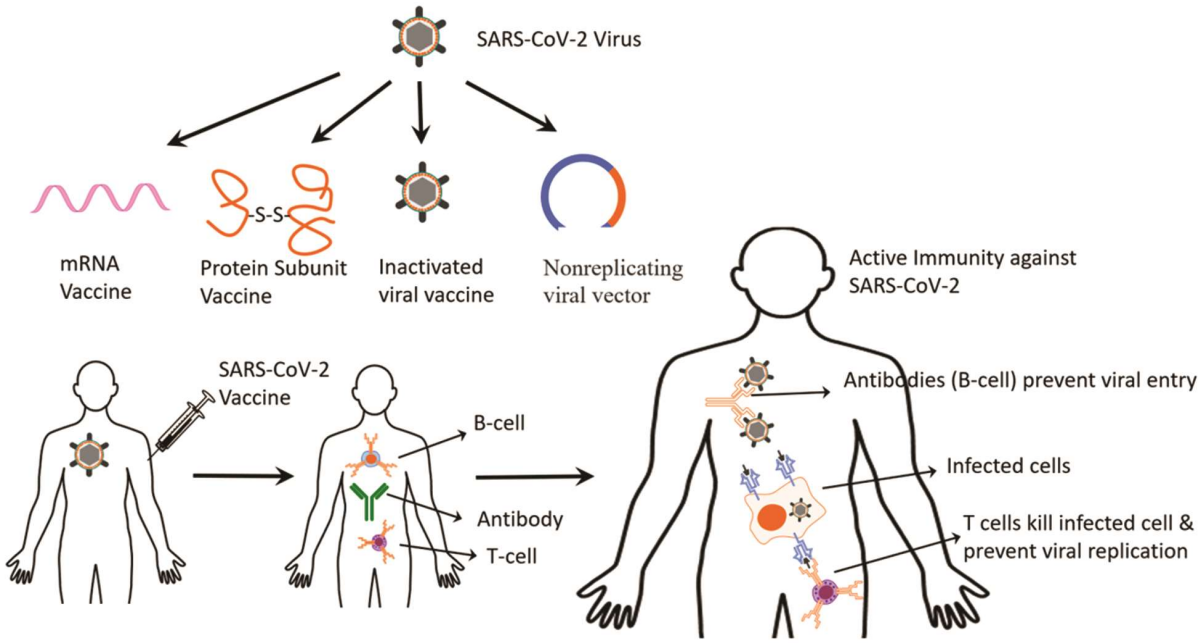

Fig. 6 —SARS-CoV-2 Vaccine platforms and their mechanism of stimulating Immune response

Most of the vaccine candidate developed by this method uses genetically modified adenoviral vectors by deleting the viral structural gene responsible for its replication in the host using helper virus thereby preventing assembly of virion in human cell. ChAdOx1 or AZD1222 is a non-replicating chimpanzee adenovirus vector vaccine that encodes spike protein of SARS-CoV-2 in genetically altered human embryonic kidney-293 cells. JNJ-78436735 is a non-replicating viral vector monovalent vaccines vaccine composed of genetically altered adenovirus vector (serotype 26) engineered to express SARS-CoV-2 spike protein. Convidecia is also a non-replicating vaccine developed using specific cell line for production of replication incompetent adenovirus type 5 vector that encodes spike protein of SARS-CoV-2 to elicit adaptive immune response. Sputnik V is another non-replicating viral vector vaccine with two Components I and II that contains serotype 26 and serotype 5 particles, respectively of recombinant adenovirus that comprises of genes expressing spike protein of SARS-CoV-2. Inactivated or killed vaccines are developed by allowing the virus to grow in an unfavourable culture medium and then deactivating it with chemicals or radiation or heat treatment so that the viruses are unable to replicate and cause infection in immunocompromised patients. For example, CoronaVac vaccine was produced by treating coronavirus with inactivating agent and adjuvant namely β-propiolactone and aluminium hydroxide, respectively to enhance immune response. BBIBP-CorV or Sinopharm vaccine is another inactivated vaccine that contains coronavirus inactivated in Vero Cells. Covaxin (BBV152) is also an inactivated vaccine that uses beta-propiolactone and TLR 7/8 agonist (imidazoquinolinone) to inactivate SARS-CoV-2 to stimulate Helper-T cell and lymphocyte responses against SARS-CoV-2.

Though mRNA vaccines for SARS-CoV-2 were the first of its kind to be approved conditionally, their stringent storage condition (−70°C to −20°C) restricts the usage of these vaccines in underdeveloped countries. mRNA-1273 (Moderna vaccine) is a mRNA vaccine that encodes synthesis of stabilized pre-fusion form of spike protein of SARS-CoV-2 encapsulated in lipid nanoparticle that acts as vector by expressing viral machinery in host cell inducing adaptive immune response against spike protein. mRNA-BNT162b2 or Comirnaty is a mRNA vaccine that encodes receptor binding domain of SARS-CoV-2 along with trimerization domain derived from T4 fibritin to elicit immune response (IgG levels). It is worth mentioning that recent researchers employ computational reverse vaccinology technology to identify multiepitope (B- cell epitopes, T-cell epitope, Interferon-γ Epitope) in structural proteins of +ssRNA viruses as presented in (Table 6).

Table 6 — Notable Epitopes predicted using Immunoinformatics and reverse vaccinology approaches for HCV, Dengue, Chikungunya virus and SARS-CoV-2

Virus	Protein	Epitope	Epitopes with High antigenicity Score (≥0.7)
Hepatitis C virus	T-cell epitope overlapping with epitopes of B-cell and IFN-γ		
	NS2	MHC II	YYHLPWGLL
	NS3/4A	MHCI	CHLGIGTVL
	NS5A	MHC II	ICDECHLGI
		MHC I	ASSASQLSL
	NS5B core	MHCI	GNTTCYKAA
		MHC II	LPILSNRNV
		MHC II	YRRCRAGVT
	E1 protein	MHC II	WPRDASYGC
Dengue virus	Top T-cell epitopes		
	DENV-1	MHC II	LKRARNRVS
	DENV-2	MHC II	RGFRKEIGR
	DENV-3	MHC II	KNGAIKVLR
	DENV-4	MHC I	KAINVLRGF
Chikungunya virus	CD8+ and CD4+ T-cell epitopes MHC binding		
	Frameshifted Structural polyproteins (S27 strain - African prototype)	MHC I	KPGDSGRPI
		MHC I	TGTMGHFIL
		MHC I	ALSVVTWNK
		MHC I	KPGRRERMC
		MHC I	GRRERMCMK
		MHC II	MCMKIENDCIFEVKH
		MHC II	DRTLLSQQSGNVKIT
SARS-CoV-2	B-cell and T-cell (CD8+ and CD4+) epitopes		
	Spike S protein	B-cell	VYDPLQPE
		MHC I	FTISVTTEI
		MHC I	FVFLVLLPL
		MHC I	VVFLHVTYV
		MHC I	VRFPNITNL
		MHC I	FAMQMAYRF
		MHC II	LLQYGSFCT
	Open reading frame that code for polyprotein pp1a and pp1ab (orf1ab)	B-cell	SLDTYPSL
		B-cell	KSVYYTSNP
		B-cell	DASGKPVPY
		B-cell	VKGLQPSVGPKQ
		MHC I	MMISAGFSL
		MHC I	SLENVAFNV
		MHC II	FFYVLGLAA
		MHC II	LRGTAVMSL
		MHC II	LVQMAPISA
		MHC II	LVQSTQWSL

Conclusion

Computer aided design of multiepitope-based peptide vaccine is the keystone in the development of vaccine against highly contagious and pathogenic +ssRNA viruses. The significance of immunoinformatics in viral infections mediated by +ssRNA virus is diverse with regard to computational methods used, however remain united in predicting common qualities associated with host–pathogen relationship. Bioinformatics tools can also assign functions to uncharacterized genes or hypothetical proteins that can be targeted as potent vaccine candidates. The essence of the present review is to highlight on the role of unique computational driven approaches to analyse host–pathogen interactions for facilitating vaccine development. This cost effective and rapid method enhances identification of suitable antigen composition, prediction of epitope, selection of intramolecular adjuvants and linkers to design epitope-based peptide vaccine with maximum prophylactic activity and minimum side effects. Therefore, this review has provided insights about the procedures for synthesizing *in silico* multi-epitope based peptide vaccine that can be applied for development of highly effective next-generation vaccines to combat epidemic potential of +ssRNA viruses. The predicted epitopes can also be computationally validated for their MHC binding affinity, C-Terminal Cleavage Affinity, TAP transport efficiency, Proteasome score, immune response and toxicity which can enhance rapid progression in production of low risk and high efficacy vaccine ensuring no corners are cut regarding safety evaluation.

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Conflict of interest

All authors declare no conflict of interest.

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