

Epitope-based vaccine design against the membrane and nucleocapsid proteins of SARS-CoV-2

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Abstract

Background. The high prevalence and mortality rate of coronavirus disease 2019 (COVID-19) is a major global concern. Bioinformatics approaches have helped to develop new strategies to combat infectious agents, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Indeed, the structural proteins of microorganisms provide suitable epitopes for the development of vaccines to prevent infectious diseases.

Objectives. The present study aimed to use bioinformatics tools to find peptides from the membrane (M) and nucleocapsid (N) proteins with effective cellular and humoral immunogenicity.

Material and methods. Sequences of the M and N proteins were sourced from the National Center for Biotechnology Information (NCBI). The conserved regions of the proteins with the highest immunogenicity were identified and assessed using different servers, and the physicochemical and biochemical properties of the epitopes were evaluated. Finally, allergenicity, antigenicity and docking to human leukocyte antigen (HLA) were investigated.

Results. The data indicated that the best epitopes were LVIGFLFLT and LFLTWICLL (as membrane epitopes), and KLDDKDPNFKDQ (as a nucleocapsid epitope), with significant immunogenicity and no evidence of allergenicity. The 3 epitopes are stable peptides that can interact with HLA to induce strong immune responses.

Conclusions. The findings indicate that 3 common epitopes could effectively elicit an immune response against the disease. Hence, in vitro and in vivo studies are recommended to confirm the theoretical information.

Keywords: HLA, COVID-19, SARS-CoV-2, bioinformatics, multi-epitope vaccine

Introduction

Coronavirinae is a subfamily of *Coronaviridae*, and it includes 4 genera – alpha, beta, gamma, and delta coronaviruses. Human coronaviruses were first identified in 1965; they are responsible for respiratory tract infections in large populations in various countries around the world.^{1,2} A novel type of coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or 2019-nCoV), the causative agent of coronavirus disease 2019 (COVID-19), was first reported in Wuhan, Hubei Province, China, in December 2019.² During the COVID-19 pandemic, several viral lineages of various clinical and public health impact were identified, and divided into variants of interest (VOIs) and variants of concern (VOCs).^{3–6}

The whole genome of SARS-CoV-2 contains 30 kb single-stranded ribonucleic acid (RNA) that encodes 29 different proteins. The most prominent structural proteins are spike (S), envelope (E), membrane (M), and nucleocapsid (N) ones. These proteins play a vital role in the pathogenesis of the virus, and each has distinct functions.⁷

The S protein is the major surface protein that binds to the host cell receptors and facilitates entrance into human cells; in approx. 86%, it is similar to the SARS-CoV protein. Moreover, it seems that mutations in the gene encoding the S protein cause differences in the pathogenic potential of SARS-CoV-2 as compared to SARS-CoV.⁸

The E protein is one of the small structural proteins involved in the viral life cycle and pathogenesis; it participates in virus assembly, budding and envelope formation.⁹ The protein in SARS-CoV-2 is very similar to the SARS-CoV variant, with similarity estimated to be 94.74%.¹⁰

The M protein, as the most abundant glycoprotein, plays a critical role in virus size and shape maintenance, and assists in the assembly and budding stages of the virus. In addition, the M protein cooperates with the S protein to facilitate virus attachment and entry into host cells.¹⁰ According to studies, the M protein of SARS-CoV-2 resembles the M protein of SARS-CoV in 90%, which means there is a remarkable similarity between the 2 types of virus.¹¹

The N protein is a multi-purpose protein with several functions. It is involved in the viral life cycle, including virus core formation, assembly, budding, envelope formation, genomic messenger RNA (mRNA) replication, and genomic RNA synthesis. It is also vital for the cellular response, playing a role in chaperone activity, cell cycle regulation, cell stress responses, pathogenesis, and signal transduction.¹² In addition, the SARS-CoV-2 N protein has 90% similarity with the SARS-CoV N protein, which highlights its functional importance.¹³

Several vaccines were developed during the coronavirus pandemic and produced promising results. However, the high mutation rate of SARS-CoV-2 prompted the use of computational approaches to increase knowledge on

the prevention and treatment strategies.^{14,15} Although several SARS-CoV-2 epitopes have been reported in numerous vaccine development studies, the search for specific and unique epitopes has opened the door for future discoveries. Therefore, the present study aimed to identify and screen distinct epitopes of the M and N proteins of VOCs by using bioinformatics databases.

Material and methods

Sequences of membrane and nucleocapsid proteins, and the phylogenetic tree

Several sequences representing all the circulating VOCs of SARS-CoV-2, presented by the World Health Organization (WHO) (<https://www.who.int/activities/tracking-SARS-CoV-2-variants>), including GenBank accession numbers OX008586.1, OL790194.1, OW998408.1, OW996240.1, ON286809.1, ON286831.1, and OX014251.1, were acquired from the National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov>). The sequences were saved in the FASTA format, and a phylogenetic tree was designed using Molecular Evolutionary Genetics Analysis Version 11 (MEGA 11). The FASTA format of the SARS-CoV-2 wild-type (WT) (Wuhan-Hu-1) strain (accession number NC_045512.2) was included for comparison. The conserved regions of the proteins were identified and evaluated for further work.

Prediction of T-cell epitopes

In order to predict the cellular immunoreactivity of epitopes, several online servers, including NetMHC (<https://services.healthtech.dtu.dk/service.php?NetMHC-4.0>), Immune Epitope Database (IEDB) (<https://www.iedb.org>), NetCTL (<https://services.healthtech.dtu.dk/service.php?NetCTL-1.2>), MHC2Pred (<http://crdd.osdd.net/raghava/mhc2pred>), and SYFPEITHI (<http://www.syfpeithi.de>), were used. The epitopes with the highest scores for intensely stimulating the cellular immune system were selected for the study.

Validation of B-cell epitopes

The B-cell epitopes of the M and N proteins, containing at least 10 amino acids were predicted by IEDB and the artificial neural network-based B-cell epitope prediction server (ABCpred) (<https://webs.iitd.edu.in/raghava/abcpred>), with threshold values of 0.5. Next, the selected epitopes were assessed based on their hydrophilicity, flexibility, polarity, surface area, and three-dimensional (3D) structures.

Evaluation of allergenicity and antigenicity

The allergenicity and antigenicity of the sequences were evaluated using AllerTOP (<https://www.ddg-pharmfac.net/allertop>) and VaxiJen (<https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html>), respectively.

Structure and docking analysis

The physicochemical and biochemical properties of the epitopes, including molecular weight, stability and hydrophobicity, were characterized using the ProtParam tool (<https://web.expasy.org/protparam>), and the 3D structures of peptides were drawn using the Molegro Virtual Docker software, v. 6.0.1 (Molegro ApS, Aarhus, Denmark). To analyze the molecular docking interaction with the highly-replicated epitopes, the tertiary structures of human leukocyte antigen (HLA) were gained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (<https://www.rcsb.org>). Then, the best ligand with minimum energy was assessed through Molegro Virtual Docker.

Results

The phylogenetic analysis and multiple sequence alignment of the complete SARS-CoV-2 genome revealed a close relationship between different variants of the virus (Supplementary material available on request from the corresponding author). Protein Basic Local Alignment Search Tool (BLAST) findings in NCBI also showed that the M and N proteins were highly conserved among the SARS-CoV-2 subtypes.

Stimulation of the cellular and humoral immune system

Of all the M epitopes identified in this study, LVIGFLFLT, WLLWPVTLA, LFLTWICLL, and FLYIIKLIF were capable of evoking cellular immunity, and seemed to induce a stronger host response. Also, they could correctly bind to the major histocompatibility complex class I (MHC-I) located at positions 22–30, 55–63, 27–35, and 45–53, respectively (Table 1). After immunogenicity and allergenicity evaluation, the final epitopes, including LVIGFLFLT and LFLTWICLL, were predicted as candidates for vaccine development.

The sequences KLDDKDPNFKDQ, RGPEQTQGNFGD and HIGTRNPANNA, were identified as the frequented epitopes of the N protein that could interfere with cellular and humoral immune responses (Table 2). Based on the evaluation with the use of different servers, the best epitope of the N protein that could stimulate B-cells was KLDDKDPNFKDQ, located at position

338–349 of the sequence. Conversely, none of the M protein epitopes could elicit a robust humoral immune response (Table 3).

Table 1. List of the high-scored major histocompatibility complex class I (MHC-I) predicted epitopes of the membrane (M) protein

Epitopes	Alleles	Servers	AllerTOP results	VaxiJen results
LVIGFLFLT	HLA-B44:03, HLA-B44:02, HLA-B07:02, HLA-B08:01, HLA-A24:02, HLA-A01:01, HLA-B15:01, HLA-B35:01, HLA-B40:01, HLA-B53:01, HLA-A23:01, HLA-B51:01	NetMHC IEDB NetCTL MHC2Pred SYFPEITHI	probable non-allergen	probable antigen
WLLWPVTLA	HLA-B44:02, HLA-B44:03, HLA-B57:01, HLA-B58:01, HLA-A24:02, HLA-B53:01, HLA-A23:01, HLA-B35:01, HLA-B40:01, HLA-A68:01, HLA-A31:01	NetMHC IEDB NetCTL MHC2Pred SYFPEITHI	probable allergen	probable antigen
LFLTWICLL	HLA-A01:01, HLA-A68:01, HLA-B40:01, HLA-B44:03, HLA-B15:01, HLA-A11:01, HLA-A26:01, HLA-B44:02, HLA-A03:01, HLA-B35:01, HLA-B07:02, HLA-A68:02, HLA-B53:01	IEDB NetCTL MHC2Pred SYFPEITHI	probable non-allergen	probable antigen
FLYIIKLIF	HLA-A68:02, HLA-A30:01, HLA-A11:01, HLA-A68:01, HLA-B44:03, HLA-A31:01, HLA-B44:02, HLA-A33:01, HLA-B07:02, HLA-B40:01, HLA-A03:01, HLA-A02:06, HLA-A02:03, HLA-A01:01, HLA-B51:01	IEDB NetCTL MHC2Pred SYFPEITHI	probable non-allergen	probable antigen

IEDB – Immune Epitope Database.

Table 2. List of the high-scored major histocompatibility complex class I (MHC-I) predicted epitopes of the nucleoplastic (N) protein

Epitopes	Alleles	Servers	AllerTOP results	VaxiJen results
KLDDKDPNFKDQ	HLA-B4001, HLA-A0101, HLA-A0201, HLA-A0301, HLA-A2402, HLA-A2601, HLA-B2705, HLA-B3901, HLA-B5801, HLA-B1501	NetMHC		
	HLA-A32:01, HLA-B08:01, HLA-A02:03, HLA-A02:01, HLA-A68:02, HLA-A26:01, HLA-A02:06, HLA-A33:01, HLA-A23:01, HLA-A30:01, HLA-B57:01, HLA-A30:02, HLA-A03:01, HLA-A24:02, HLA-B51:01, HLA-A02:01, HLA-B35:01	IEDB	probable non-allergen	probable antigen
	HLA-A01, HLA-A02:01, HLA-A03, HLA-A11:01, HLA-A24:02, HLA-A26, HLA-A68:01, HLA-B07:02, HLA-B08, HLA-B13, HLA-B14:02, HLA-B15:01, HLA-B15:10, HLA-B15:16, HLA-B18, HLA-B27:05, HLA-B27:09, HLA-B35:01, HLA-B37, HLA-B38:01, HLA-B39:01, HLA-B39:02, HLA-B40:01, HLA-B41:01, HLA-B44:02, HLA-B45:01, HLA-B47:01, HLA-B49:01, HLA-B50:01, HLA-B51:01, HLA-B53:01, HLA-B57:01, HLA-B58:02	SYFPEITHI		
RGPEQTQGNFGD	HLA-B4001, HLA-A0101, HLA-A0201, HLA-A0301, HLA-A2402, HLA-A2601, HLA-B0702, HLA-B0801, HLA-B2705, HLA-B3901, HLA-B5801, HLA-B1501	NetMHC		
	HLA-B57:01, HLA-B08:01, HLA-A30:02, HLA-A30:01, HLA-A01:01, HLA-B53:01, HLA-B40:01, HLA-B35:01, HLA-B15:01, HLA-B07:02, HLA-A68:02, HLA-A68:01, HLA-A33:01, HLA-A32:01, HLA-A31:01, HLA-A26:01, HLA-A24:02, HLA-A11:01, HLA-A03:01, HLA-A02:06, HLA-A02:03, HLA-A02:01	IEDB	probable non-allergen	probable antigen
	HLA-A01, HLA-A02:01, HLA-A03, HLA-A11:01, HLA-A24:02, HLA-A26, HLA-A68:01, HLA-B07:02, HLA-B08, HLA-B13, HLA-B14:02, HLA-B15:01, HLA-B15:10, HLA-B15:16, HLA-B18, HLA-B27:05, HLA-B27:09, HLA-B35:01, HLA-B37, HLA-B38:01, HLA-B39:01, HLA-B39:02, HLA-B40:01, HLA-B41:01, HLA-B44:02, HLA-B45:01, HLA-B47:01, HLA-B49:01, HLA-B50:01, HLA-B51:01, HLA-B53:01, HLA-B57:01, HLA-B58:02	SYFPEITHI		
HIGTRNPANNA	HLA-B40:01, HLA-B44:03	IEDB		
	HLA-B40:01, HLA-B35:01, HLA-A24:02, HLA-B51:01, HLA-B07:02, HLA-A02:03, HLA-B15:01, HLA-A02:01, HLA-A33:01, HLA-B44:02, HLA-A68:02, HLA-A26:01, HLA-A02:06, HLA-A32:01	SYFPEITHI	probable allergen	probable antigen

Table 3. Most frequent B-cell epitopes of the nucleocapsid (N) protein

Epitopes	Servers	AllerTOP results	VaxiJen results
KKDKKKKADETQ	ABCpred	probable non-allergen	probable antigen
HIGTRNPANNA		probable allergen	probable antigen
PSDSTGSNQNGE		probable non-allergen	probable antigen
GTGPEAGLPYGA		probable allergen	probable antigen
QRRPQGLPNNTA		probable non-allergen	probable non-antigen
NSSRNSTPGSSR		probable non-allergen	probable antigen
ELIRQGTDYKHW		probable allergen	probable non-antigen
KLDDKDPNFKDQ	IEDB	probable non-allergen	probable antigen
RIRGGDGKMKDL		probable non-allergen	probable antigen
GTGPEAGLPYGANK		probable non-allergen	probable antigen
ALNTPKDHIGTRNPANN		probable allergen	probable antigen
AFGRRGPEQTQGNFG		probable allergen	probable antigen
EVTSGTWL		probable non-allergen	probable non-antigen
KLDDKDPNFK		probable non-allergen	probable antigen

ABCpred – artificial neural network-based B-cell epitope prediction server.

Determination of structural conformations and molecular docking

According to the ProtParam server, 3 epitopes were defined as stable peptides, and the molecular weight, iso-electric point and hydrophobicity were 1,462.58 g/mol, 4.58 and 2.308 for KLDDKDPNFKDQ, 1,022.30 g/mol, 5.52 and 2.733 for LVIGFLFLT, and 1,121.45 g/mol, 5.52 and 2.600 for LFLTWICLL, respectively.

The 3D structures of the best peptides are shown in Fig. 1. The predicted results obtained from RCSB PDB showed that the epitopes could interact with HLA and induce strong immune responses. Moreover, the findings showed that there was an interaction between MHC-I/II (HLA-A0201:KLDDKDPNFKDQ, HLA-B51:LFLTWICLL and HLA-DRB1:LVIGFLFLT) and the suggested epitopes (Fig. 2).

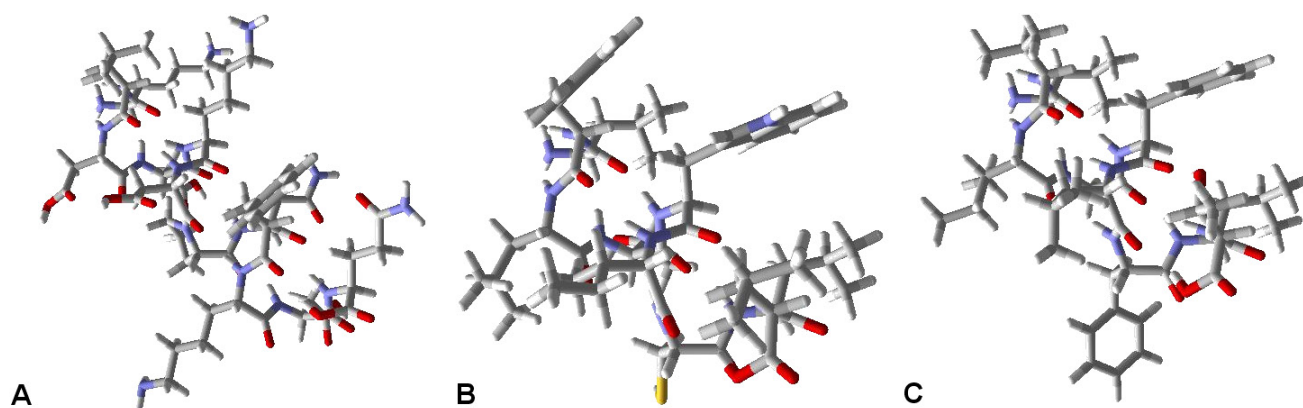


Fig. 1. Structures of the 3 epitopes, designed by means of the Molegro Virtual Docker software
A – KLDDKDPNFKDQ; B – LFLTWCILL; C – LVIGFLFLT.

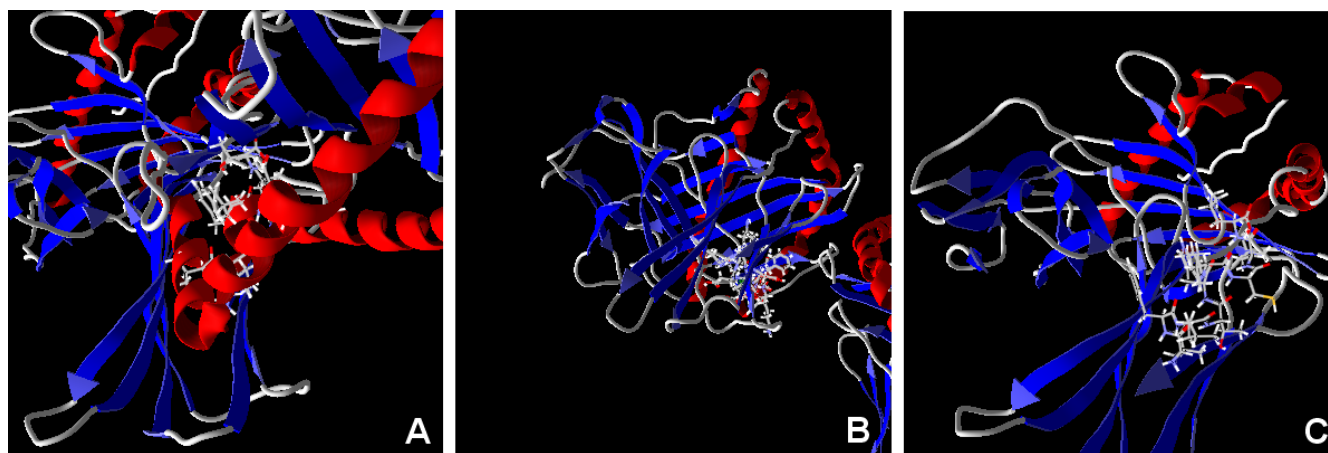


Fig. 2. Docking interaction and bond formation between the types of human leukocyte antigen (HLA) and the 3 epitopes, obtained using Molegro Virtual Docker
A – HLA-A0201 and KLDDKDPNFKDQ; B – HLA-B51 and LFLTWCILL; C – HLA-DRB1 and LVIGFLFLT.

Discussion

COVID-19 has been reported in many parts of the world as having a wide collection of clinical symptoms, ranging from fever and a dry cough to respiratory involvement, heart failure and kidney damage.^{16–18} The increasing mortality, especially in patients with underlying diseases, such as diabetes, cancer, cardiovascular complications, and bacterial co-infections, as well as the spread of viral mutations, emphasizes the importance of disease prevention and treatment.^{19–22}

Bioinformatics databases have proven to be useful sources of information on disease prevention, especially in the case of the diseases associated with life-threatening infections. Furthermore, bioinformatics methods have created a new avenue for designing effective vaccines at a low cost and with high efficiency. Indeed, the knowledge available in the bioinformatics field makes it possible to design effective vaccines against viruses and other infectious agents through using extensive information about the structural and immune features of microorganisms and humans.^{23,24}

An ideal vaccine should be able to activate both the cellular and humoral arms of the immune system.

Different platforms are used for vaccine development, and each of them presents several advantages and disadvantages. Typically, the subunit vaccines using recombinant peptides and proteins are among the most effective, inexpensive and safe vaccines that can be designed. Moreover, this type of vaccine provides effective immunogenicity by evoking host immune responses.^{25,26}

According to studies on the prevention of COVID-19, there are more than 60 vaccine candidates for SARS-CoV-2, most of which are aimed at inducing the release of neutralizing antibodies against the S protein.²⁷ Unfortunately, several reports have shown that the S protein is an antigen that can mutate rapidly. For example, the most common type of coronavirus, omicron, has more than 30 S protein mutations.^{28–31} Furthermore, a number of reports have confirmed that the M and N proteins are good targets for stimulating the antibody-producing B-cell and T-cell responses.^{32,33} Indeed, studies published by Enayatkhani et al.,³³ Rahman et al.³⁴ and Quayum et al.³⁵ emphasized the importance of the M and N proteins in the viral structure, confirming their potential role as suitable candidates for predicting multi-epitope vaccines. However, the current work focused on the M and N protein epitopes to predict a novel subunit vaccine.

In the present study, all epitopes were evaluated based on different immune responses, and the findings indicate that the M protein may be a useful target for eliciting a cellular immune response, while the N peptides could elicit a strong humoral immune response. Also, the results demonstrate that, among many epitopes, 2 highly antigenic M proteins, LVIGFLFLT and LFLTWICLL, and the KLDDKDPNFKDQ N protein could be used to construct an epitope-based vaccine. The LVIGFLFLT and LFLTWICLL M proteins were the best options among T-cell epitopes, while KLDDKDPNFKDQ was identified as a powerful B-cell epitope.

A number of publications reported on the LVIGFLFLT and LFLTWICLL M epitopes, including works by Behmard et al.³⁶ and Naveed et al.³⁷ In addition, Heffron et al. introduced KLDDKDPNFKDQ as part of the AIKLDDKDPNFKDQVI and KLDDKDPNFKDQVILLNKH peptides in a study on antibodies against the SARS-CoV-2 N protein.³⁸ In comparison with those studies, the findings of this study were more specific. Indeed, this work assessed specific epitopes to determine their distinct sites in the protein sequences, and introduced unique epitopes for vaccine and antibody research.

The present study included the SARS-CoV-2 WT (Wuhan-Hu-1) strain sequence and compared the results with other VOCs, especially lineage B.1.1.529, with respect to the worldwide omicron epidemic. All the suggested epitopes were matched with the new variants of COVID-19.

Conclusions

COVID-19 continues to be an alarming global disease, as observed in the reports of new cases and deaths, which increases the importance of developing a more effective vaccine. The immunoinformatics results obtained for the M and N proteins identified 3 top epitopes, including LVIGFLFLT, LFLTWICLL and KLDDKDPNFKDQ, that could effectively stimulate T-cells and B-cells with the lowest binding energy. Therefore, additional in vitro and in vivo studies are recommended to confirm this theoretical information. Moreover, bioinformatics tools are suggested to be used against future epidemics to design new vaccines for other infectious diseases, and researchers should pay more attention to this issue.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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