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Research Article

PREDICTION OF B-CELL EPITOPES FROM THE NUCLEOCAPSID PROTEIN OF SARS-COV2 COMBINING SEQUENCE BASED AND MOLECULAR DOCKING APPROACHES

Anjali Vasudevan[1]., Srinivas Rao D[2]., Dilip Mathai[3]., Mahendra Kumar Verma[4] and Raghavendra Rao M.V*[5]

[1]Union Christian College, Aluva. Affiliated to MG University, Kerala, India
[2]Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India
[3]American University School of Medicine Aruba, Caribbean Islands,
[4]Department of Medicine, Dean, Apollo Institute of Medical Sciences and Research, Jubilee Hills, Hyderabad, Telangana, India
[5]Department of Laboratory Medicine, Apollo Institute of Medical Sciences and Research, Hyderabad, TS, India

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A B S T R A C T

In December 2019, an outbreak of novel beta-coronavirus started in Wuhan, China, spread globally as COVID19 pandemic is still underway. The nSARS-CoV2 primarily targets the respiratory tract and results in severe acute respiratory distress (SARD), leading to respiratory tract collapse. The virus internalizes primarily via ACEII receptor, and many tissues reported a significant level of expression of ACEII receptor including lungs, hearts, kidney and gastrointestinal tract. The clinical manifestations of COVID19 are diverse, but growing evidence suggests that gut dysbiosis is one of them and poses a threat to native immunity. The viral Nucleocapsid protein could be an ideal target for therapeutic and vaccine development. In the present study a system biology approach was used to figure out a close affinity between B cell epitope and N protein of nSARS-CoV2.

INTRODUCTION

Corona Virus Disease 2019 (COVID-19), a respiratory illness, found to be originated from Wuhan city; Hubei province of China has spread worldwide recording 19,187,943 confirmed cases and 716,075 deaths across 216 countries as of 8th August 2020. Efforts to discover an effective vaccine or antiviral therapy are being undertaken worldwide. Several vaccines are already in their clinical trials. The disease is known to cause asymptomatic infection or a mild to severe pneumonia(1). The COVID-19 strains have been found to show similarity with Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome coronavirus (MERS-CoV) and epidemiologic similarity with SARS-CoV. The novel coronavirus (SARS-CoV2) belong to the genus Beta coronavirus with genome size of ~30kb coding for both structural and non-structural proteins. There is a total of 4 structural proteins; the Spike glycoprotein (S), Envelope (E) protein, membrane (M) protein and nucleocapsid (N) protein.

Corresponding author:* **Raghavendra Rao M.V
Department of Laboratory Medicine, Apollo Institute of Medical Sciences and Research, Hyderabad, TS, India

Studies show that SARS-CoV2 and SARS-CoV are quite similar in nature suggesting similar cell entry mechanism and receptor usage mechanisms. Protective immune responses against SARS-CoV may be used to aid vaccine development in SARS-CoV2(2). Previous studies point out that both humoral and cell-mediated immune response are known to have prominent roles and also shows that antibodies against the N protein were prevalent in SARS-CoV infected patients but was short-lived in convalescent SARS-CoV patients 3-5.

B cell epitopes are a collection of amino acid residues on an antigen that the antibody recognizes and specifically bind to eliciting an immune response. There are mainly two different classifications based on their orientation in space; linear and conformational. Linear epitopes comprise contiguous residues and conformational epitopes includes distant residues, brought into close proximity due to the folding of the protein. Identification of B-cell epitopes is a crucial step in designing effective vaccines. With the advent of bioinformatic approaches, recombinant vaccine containing single or multiple B-cell epitopes can be designed in a much cost, time-effective and safe way. Designing of protein subunits with structural and immunogenic properties for therapeutic and diagnostic tools

are also possible (6). Another key tool involved in computer aided drug design (CADD) is the molecular docking approaches. The ligand-protein docking aims to predict the predominant binding modes of a ligand with a protein of known three-dimensional structure (7). Two major pillars for a successful docking experiment are correct pose and affinity prediction (8). In the present study, we obtained the FASTA sequence of SARS-CoV2 from the NCBI database and B-cell epitope prediction was done for the Nucleocapsid protein (N). The obtained epitopes were compared with those of SARS-CoV having immune responses. The protein structure obtained from I-TASSER was then docked with the antibody S230 (known to trigger immune responses in SARS-CoV patients) and checked whether these known antibodies could be used to potentially bind to SARS-Cov2 N protein.

METHODOLOGY

B-cell epitope prediction

The linear epitopes of the Nucleocapsid protein of SARS-CoV2 was predicted using the following tools. The whole genome sequence in the fasta format of SARS-CoV2 was obtained from the NCBI Database.

Ellipro

Ellipro is a web tool that uses Thornton's method (identification of continuous epitopes protruding from the globular surface which are more accessible for antibody binding taking into account the correlation between antigenicity and solvent accessibility) along with a residue clustering algorithm, the MODELLER and the Jmol viewer (9). Ellipro is also available as a standalone version besides as an online server, which is a part of the Immune Epitope Database Analysis Resource (6). It is available at http://tools. immuneepitope.org/tools/Ellipro.

BepiPred 2.0

Bepipred 2.0 is a web-server for B-cell epitope prediction from antigen sequence. This tool is based on a random forest algorithm that has been trained on epitopes annotated from antibody-antigen protein structures. The results are displayed in a user-friendly and informative way (10). BepiPred is more of a machine learning approach and can be accessed at (11) http://www.cbs.dtu.dk/services/BepiPred/

ABCPred

ABCPred is an artificial neural network-based tool used to predict B-cell epitopes from an antigen sequence. They are predicted using standard feed forward (SFF) and recurring neural network (RNN) which are trained on data set containing 700 non-redundant B-cell epitopes obtained from Bcipep database and equal number of non-epitopes obtained randomly from Swissprot database. The highest overall accuracy obtained is 65.93% using RNN. Users can choose a window length of 10, 12, 14, 16 or 20 as predicted epitope length. The results are presented in tabular as well as graphical form (12-13). It can be accessed from http://www.imtech.res.in/ raghava/abcpred

Epitope cluster analysis from IEDB: Clustering algorithms help to facilitate analysis of epitope-related data. Peptide sequences are clustered on the basis of a specified level of identity listing out three possible algorithms (14). This novel clustering tool is available at http://tools.iedb.org/cluster2/.

Antibody-epitope docking

Cluspro: Available at https://cluspro.org, this particular web server present before the user a simple home page for basic use requiring only two files in the Protein Data Bank (PDB) format, the ligand and the receptor. This tool also offers a number of advanced options to modify the search, including removing of unstructured region of protein, application of attraction or repulsion and consideration of small angle X-ray scattering data (15).

RESULTS

Linear B-cell epitope prediction of Nucleocapsid protein of SARS-CoV2

Using Ellipro, a total of 5 epitopes were obtained having a score of above 0.7. Epitope prediction using ABCPred yielded a total of 10 epitopes when the threshold was kept above 0.8. Bepipred tool gave a total of 8 epitopes for the Nucleocapsid protein.

Table 1 Epitope analysis using Ellipro

S No	Residues	Score
1	LGTGPEAGLPYGANKDGIIWVATEGALNTPKD	0.83
2	LQLPQGTTLPKGFYAEG	0.793
3	LTQHGKEDLKFPRGQGVPINTNSSPDDQIGYYR RATRRIRGGDGKMKDLSPRW	0.744
4	ESKMSGKGQQQQGQTVTKKS	0.733
5	GSNQNGERSGARSKQRRPQ	0.721

Table 2 Epitope prediction using ABCPred

S. No.	Residues	Score
1.	TRRIRGGDGKMKDLSP	0.94
2.	KSAAEASKKPRQKRTA	0.93
3.	EGALNTPKDHIGTRNP	0.91
4.	TGSNQNGERSGARSKQ	0.91
5.	KDGIIWVATEGALNTP	0.91
6.	SGTWLTYTGAIKLDDK	0.88
7.	HGKEDLKFPRGQGVPI	0.87
8.	ASSRSSSRSRNSSRNS	0.87
9.	ADETQALPQRQKKQQT	0.86

Table 3 Epitope prediction using BepiPred

S. No.	Residues
1	NGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGL PNN
2	HGKEDLKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGK MKDLS
3	AGLPYGAN
4	GALNTPKDHIGTRNPANNAAI
5	TLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGTSPA RMAGNGGD
6	LNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATK
7	RRGPEQTQGNFGDQELIRQGTDYK
8	DAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLPAA DLDDFSKQLQQSMSSADS

Linear B-cell epitope clustering using Epitope Cluster Analysis from IEDB

The minimum length for the epitopes was set to 5 and the maximum to 25. The minimum sequence identity threshold was 70% ant he chosen clustering method was Cluster-break for clear representative sequence. These were aligned then with known B-cell epitopes from SARS-CoV using the Basic Local Alignemnt Search Tool and from the predicted epitopes; the 9 epitopes were known to show percentage identity above 50% with SARS-CoV epitopes.

Table 4 Predicted epitopes of SARS-CoV2 Nucleocapsid protein showing more than 50% percentage identity with already known SARS-CoV epitopes.

S. No	Residues	Percentage identity (%)	Query coverage (%)
1.	LQLPQGTTLPKGFYAEG	100	94
2.	GSNQNGERSGARSKQRRPQ	100	89
3.	EGALNTPKDHIGTRNP	55.56	43
4.	KDGIIWVATEGALNTP	100	12
5.	NKHIDAYKTFPPTEPK	100	100
6.	SGTWLTYTGAIKLDDK	100	25
7.	HGKEDLKFPRGQGVPI	100	12
8.	ASSRSSSRSRNSSRNS	65	87
9.	ADETQALPQRQKKQQT	100	50

These predicted epitopes were displayed on the Nucleocapsid protein. Since the complete structure of the protein was not available in the Protein Data Bank, here, a predicted model was used from I-TASSER.

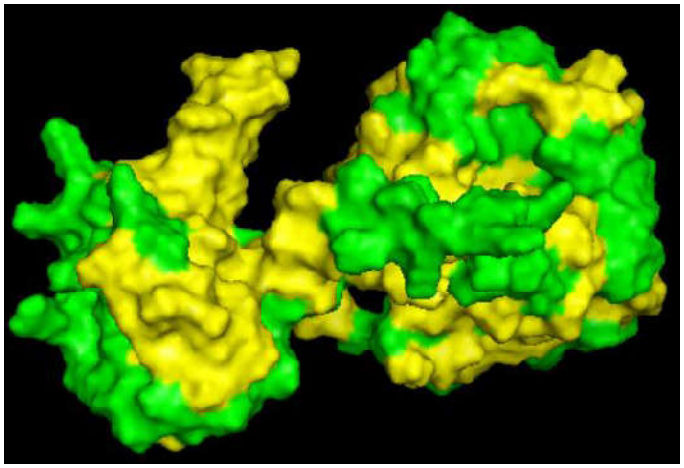

Figure 1 The Nucleocapsid protein is in yellow and the predicted epitopes in green colour. Both are in surface display style views in Pymol.

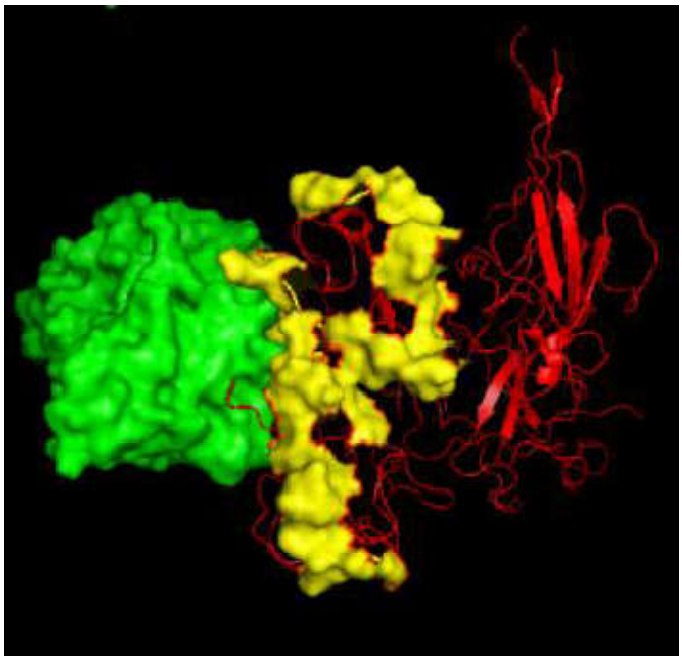

Figure 2 The ligand is shown in green (surface display style) and the nucleocapsid is shown in red colour (ribbon display style). They yellow part represents the epitope region that was predicted in this study.

Molecular docking of known antibodies against the Nucleocapsid protein of SARS-CoV2

Several SARS-CoV specific antibodies like S230, m396 and 80R have already been identified known to stimulate immune responses in patients2. The structure of the antibody S230 was obtained from AbDb Antibody data resources and using ClusPro tool, docking was carried out. Interactions of epitope binding domain of antibody with precited epitopes were analysed using Pymol. The cluster contains a total of 73 members with a weighted score in the centre -753.2 and with the lowest energy -758.5.

CONCLUSION

As time progresses, so does the effort to come up with an effective vaccine against the COVID-19 pandemic. Studies are being conducted to understand the complete genomic constituent, structural behavior, and immunological activity of the SARS-CoV2 virus. The present study can contribute effectively towards the first phases of vaccine development by providing epitopes that could be incorporated to develop a successful vaccine. Although proper immune responses of these epitopes could not be predicted accurately, since 9 of the predicted epitopes of SARS-CoV2 have shown similarity to already known SARS-CoV, they may elicit similar responses. From the docked models, it is clear that these predicted epitopes lie in the protein-antibody interface, suggesting that the antibody may be capable of eliciting immune responses against the novel coronavirus SARS-CoV2.

Future Perspective

Similar to sequence-based epitope prediction, structure-based epitope prediction can also contribute effectively to vaccine development. Docking with more known antibodies can help in the understanding of the underlying immunological aspects and behavior. It can give a clear picture regarding the immune responses that could be generated in the novel viruses. Multi epitopes vaccine candidates can be designed by stitching most immunogenic epitopes into a template using molecular modeling approaches.

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