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Computational modelling supports that dengue virus envelope antibodies can bind to SARS-CoV-2 receptor binding sites: Is pre-exposure to dengue virus protective against COVID-19 severity?

Himadri Nath [a,1], Abinash Mallick [a,1], Subrata Roy [a], Soumi Sukla [b], Subhajit Biswas [a,*]

[a] Infectious Diseases & Immunology Division, CSIR-Indian Institute of Chemical Biology (CSIR-IICB), 4, Raja S.C. Mullick Rd, Jadavpur, Kolkata 700032, West Bengal, India
[b] National Institute of Pharmaceutical Education and Research, 168, Maniktala Main Road, Kolkata 700054, West Bengal, India

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ABSTRACT

The world is going through the scourge of the COVID-19 pandemic since January 2020. However, the pandemic appears to be less severe in highly dengue endemic countries. In this connection, several studies reported that sero-diagnostic tests for dengue virus (DV) yielded considerable false-positive results for SARS-CoV-2 and *vice versa* in dengue endemic regions, thereby indicating towards potential cross-reactivity between these two viruses. We anticipated that SARS-CoV-2 and DV might share antigenic similarity and performed computational docking studies to test this hypothesis. Our results predicted with high confidence that human DV antibodies can indeed, bind to RBD of SARS-CoV-2 Spike protein. Some of these interactions can also potentially intercept human ACE2 receptor binding to RBM. Dengue serum samples predating the COVID-19, had been found to cross-react with SARS-CoV-2 Spike and this provides direct experimental validation of our predictions. Our analysis also showed that m396 and 80R antibodies (against SARS-CoV-1) did not dock with RBM of SARS-CoV-2, a fact already proven experimentally. This confirmed reliability and robustness of our approach. So, it is highly probable that immunological memory/antibodies to DV in endemic countries may reduce the severity and spread of COVID-19. It is not known whether SARS-CoV-2 antibodies will hinder DV infections by binding to DV particles and reduce dengue incidences in the future or, augment DV infection and severity by deploying antibody-dependent enhancement.

1. Introduction

Since the beginning of 2020, people around the world are confronting the COVID-19 pandemic, caused by SARS-CoV-2, a beta coronavirus. As of 26th August 2020, 23,697,273 confirmed cases with 814,438 deaths have been reported worldwide [1]. This infection is believed to originate from Wuhan city, Hubei province, China in December 2019. The virus is highly contagious and easily transmissible from human to human. The virus caused numerous outbreaks across the globe and WHO declared a public health emergency of international concern (PHEIC) on January 30, 2020.

Initially studying the global map of the COVID-19 pandemic, it occurred to us that SARS-CoV-2 is showing less transmission, severity and overall mortality per million population in highly dengue endemic countries [2], i.e. the COVID-19 and dengue global severity maps do not tend to overlap [3]. Despite having large population size, high population density, less public health awareness, relatively poor health and hygiene conditions and inadequate healthcare facilities, the highly dengue endemic countries in Southeast Asia, the Indian subcontinent, Latin America and Africa have experienced comparatively lower degree of COVID-19 severity so far.

On the other hand, developed countries in Europe, North America and Asia (China, Iran) with insignificant or sporadic dengue virus infection history, have been worst affected by SARS-CoV-2. The COVID-19 mortality in highly DV endemic countries was estimated at 24 per million population compared to 118 in the DV non-endemic regions as of 3rd June 2020 [3]. The epidemiological weekly update (17th to 23rd August 2020) reported cumulative deaths per million population for the Americas and Europe at 65

* Corresponding author.
E-mail address: subhajit.biswas@iicb.res.in (S. Biswas).
[1] These authors contributed equally to this work.

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and 32 respectively. During the same period cumulative deaths per million population in Southeast Asia was only 12 [4].

As an exception to our proposition, Brazil, a DV-endemic country recorded 3,622,861 infections and 115,309 deaths as on 26th August 2020 [1]. It is universally accepted and recommended that preventive measures are crucial to contain the spread of COVID-19 like social distancing, quarantine and lockdown in the early phases of the pandemic. In support of our hypothesis, a recent study from Brazil revealed that states reporting higher incidences of dengue during 2019–20 recorded lower COVID-19 cases and deaths. The exponential community transmission was also delayed due to slower SARS-CoV-2 growth rates [5]. The same study also described four major factors that contributed to the COVID-19 epidemic in Brazil including "super-spreader" events [5].

Even in the face of COVID-19 pandemic, dengue remains the most important arboviral disease of global concern. In last few years incidence of dengue cases has increased rapidly although a vast majority of the cases (~80%) are mild, asymptomatic and self-limiting. One report estimated 390 million (95% CI: 284–528) infections per year globally of which 96 million (CI: 67–136) manifested clinically. About 4 billion people across 129 countries are currently at the risk of DV infection, with 70% of global burden from Asia, namely the Indian subcontinent and Southeast Asia [6]. Consequently, COVID-19 pandemic overlapped with high dengue endemicity in many tropical and sub-tropical regions of the world as mentioned above.

So far, many theories have been put forward to explain why COVID-19 is less severe in many countries and we have discussed this elsewhere [3]. One such hypothesis was that COVID-19 spread was hindered by warmer climate. This could have been an alternative theory to explain why highly dengue endemic countries, falling in hot and humid regions of the world, were less affected by COVID-19. But several published reports on impact of weather conditions on virus spread suggest COVID-19 to be equally infectious under hot and humid conditions [7]. From the above observations, it appeared that pre-exposure to DV may render partial protection against COVID-19 as may be the case in highly dengue endemic regions of the world. This epidemiological observation has now been supported by biological evidences. One report from Singapore stated that an elderly man and a woman were actually SARS-CoV-2 positive but misdiagnosed for dengue due to similarities in disease presentation and more importantly, false-positive results in DV IgM and IgG serological tests. Both the patients were confirmed qRT-PCR negative for DV-, ZIKA- and Chikungunya-RNA [8]. Another study reported from our laboratory showed that the reverse scenario is also possible. We reported that five of thirteen DV NS1-positive serum samples from 2017 (predating the COVID-19 outbreak), gave COVID-19 IgG and IgM false-positive results [9]. Subsequently, another group from Israel confirmed both the scenarios i.e. approximately 22% cross-reactivity between dengue antibodies (Abs) and SARS CoV-2 antigen(s) and *vice versa* via lateral flow-based rapid tests and ELISA tests targeting antibodies to Spike protein in a larger number of patient samples [10].

Both the aforesaid scenarios unequivocally indicate towards some degree of antigenic similarities between SARS-CoV-2 and DV. This led us to investigate the effects of human DV Abs on SARS-CoV-2 Spike protein using molecular docking studies with two FFT algorithm-based docking servers i.e. ClusPro and ZDOCK 3.0.2. We chose four DV serotype 2 envelope Abs X-ray crystallography PDB structures (4UTA, 4UTB, 4UT6 and 4UT9) and SARS CoV-2 Spike protein trimer X-ray crystallography PDB structure (6VSB) for the docking studies.

2. Results

2.1. Dengue virus antibodies are predicted to bind to RBD of SARS-CoV-2 Spike protein

Four monoclonal antibodies namely EDE2 A11, EDE2 B7, EDE1 C8 and EDE1 C10 that are elicited in response to natural DV infection in humans [11,12], have been used in this study. Each Ab has been docked with the crystal structure of SARS-CoV-2 Spike protein (PDB ID: 6VSB) [13], using two docking servers ZDOCK and ClusPro. From the output of each algorithm, top 10 predictions were considered. All the selected interactions were within a distance cut-off of 3.5 Å [14]. Available PDB files of aforesaid Abs and SARS-CoV-2 Spike proteins were processed for docking as stated in "Methods and Models" section. In ZDOCK [15,16], protein–protein interaction and in ClusPro [17,18], protein–protein interaction with antibody mode were used. Only those common interactions that are predicted by both the algorithms were considered for interpretation. During analysis of docking results, interactions predicted to involve amino acid positions 333 to 527 of SARS-CoV-2 Spike protein (RBD), were only considered, as the immunogenic epitopes of the virus fall in this region [19].

For EDE1 C8 Ab docking with SARS-CoV-2 Spike protein, there were 48 occasions in total 20 predicted models (10 predicted model from each server), when EDE1 C8 Ab was found to bind to different amino acid residues in the SARS-CoV-2 Receptor Binding Domain (RBD, 333–527 amino acid positions on Spike protein[20]) (Fig. 1A). These 48 events include repetitions of different amino acids coming once in a particular prediction. Among these 48 interactions, 13 involved Ab binding to different residues of the Receptor Binding Motif (RBM, 438–506 amino acid positions on Spike protein [20]) and 35 involved Ab binding to RBD regions outside RBM.

Of twenty predictions for EDE1 C10 Ab binding (Fig. 1B), 38 events of interactions were observed in RBD which included 19 in RBM and 19 in RBD outside RBM. Similarly, EDE2 B7 Ab was found to bind to different amino acids in RBD for 30 times in 20 predictions. There were 20 incidences when EDE2 B7 Ab interacted with RBM of S protein and 10 interactions with RBD region outside RBM (Fig. 1C). In case of EDE2 A11 Ab, only five interactions were detected and all occurred involving the RBM (Fig. 1D). Representative images of docking have been presented (Fig. 2). Overall, there were 121 events in 80 predictions where DV MAbs interacted with SARS-CoV-2 RBD, including 57 events involving RBM (Fig. 3A).

2.2. DV antibodies are predicted to bind with Spike RBD amino acid residues which are crucial for interaction with ACE2 receptor

It is notable that DV antibodies were also found to bind to RBD amino acid residues that are crucial for interaction with the human ACE2 receptors, important for SARS-CoV-2 entry into the cells. SARS-CoV-2 Spike RBD interaction with ACE2 receptor has already been elucidated through crystal structure analysis with resolution of 2.45 Å [20]. A total of 17 residues (with a distance cut-off of 4 Å) of SARS-CoV-2 RBD interact with 20 residues of ACE2 receptor [20]. In our docking study, we discovered that DV-EDE antibodies bind with several of the above-mentioned S protein residues with a distance cut-off of 3.5 Å. EDE1 C10 Ab contacts with four amino acid residues with a total frequency of 13 among 20 predictions. Similarly, EDE1 C8 Ab interacts with four amino acid residues in RBM (with a total frequency of 9) that have been predicted to interact with ACE2 receptors. Likewise, EDE2 B7 Ab and EDE2 A11 Ab bind with different receptor-engaging amino acid residues in RBD on 14 and 5 occasions respectively (Table 1). Overall, the DV Abs used in

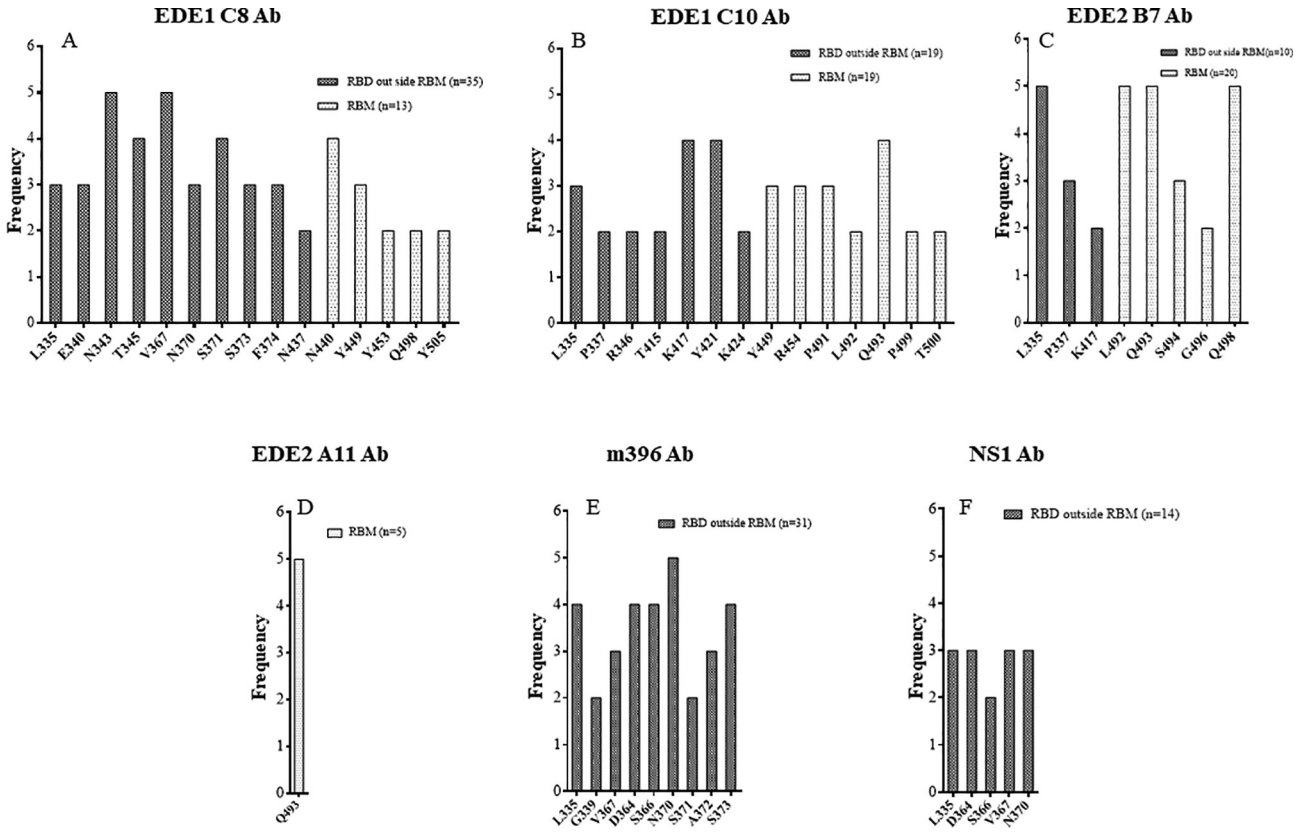

Fig. 1. Bar graphs representing frequency of each amino acid residue in the Spike protein predicted to bind to each antibody. Docking frequency of SARS-CoV-2 Spike protein amino acids with (A) EDE1 C8 Ab; (B) EDE1 C10 Ab; (C) EDE2 B7 Ab; (D) EDE2 A11 Ab; (E) m396 Ab and (F) NS1 Ab. Y axis represents frequency of specific SARS-CoV-2 Spike protein amino acid interacting with respective antibody in 20 predicted docking models. The X axis shows the positions of the Spike protein amino acids against the single letter amino acid codes. Common interactions between Abs and SARS-CoV-2 Spike protein predicted by both ZDOCK and ClusPro that fall within the distance cut-off of 3.5 Å, were considered only. "n" denotes the cumulative docking frequency for each type of interaction.

this study, docked with eight S protein amino acids that are crucial for binding to ACE2 receptor. These eight amino acid residues appeared on 41 occasions with repetitions in total 80 predictions (Table 1).

2.3. Reproducibility of this docking study with experimental data

Several neutralizing Abs against SARS-CoV-1 (like m396, 80R) are known to interact with RBD of Spike protein and compete with ACE2 receptor for binding [21]. But these antibodies do not bind with SARS-CoV-2 RBD as determined experimentally [13]. We "docked" m396 crystal structure (PDB ID: 2G75) with SARS-CoV-2 Spike in the same procedure as done before (Fig. 1E). Analysis of 10 ZDOCK and 10 ClusPro predictions revealed 31 interactions within amino acid positions L335 to S373 of SARS-CoV-2 Spike RBD. However, not a single common interaction was found to occur involving the RBM, from both algorithms. Similarly, docking of 80R (PDB ID: 2GHW) with SARS-CoV-2 Spike protein did not result in any common interaction between 10 ZDOCK and 10 ClusPro predictions. Furthermore the above 31 interaction points for m396 were far away from the ACE2 receptor interacting residues, which fall in the region spanning from K417-Y505 [20].

NS1 is an abundant viral protein in DV infected patients' serum [22] and elicits detectable antibodies [23]. So, we modeled another docking experiment to check, other than DV envelope antibodies (DV-EDE), whether or not NS1 antibodies have the potential to cross-react with SARS-CoV-2. There was no PDB structure available for DV NS1 antibody. We, therefore, used the three-dimensional structure available for NS1 of West Nile Virus (WNV), another flavivirus, in complex with the WNV NS1 Ab, known as 22NS1 (PDB ID: 4OII) for docking [24]. It has been already reported that the epitope for the 22NS1 Ab (i.e. WNV NS1 protein region 172–352) is similar but not identical to DV [24]. Thus, we chose 22NS1 as a representative flavivirus NS1 antibody to check cross-reactivity with SARS-CoV-2 Spike protein. The docking study revealed 14 interactions within RBD (333–527) but not a single interaction within RBM (438–506) (Fig. 1F). All these observations confirmed the reliability and robustness of our approach.

3. Discussion

Our computational modelling studies predicted, with high confidence, that DV Abs can interact with SARS-CoV-2 RBD (Fig. 1, Fig. 3A) and are also capable of intercepting eight key RBD interactions that are crucial for binding to ACE2 receptors (Fig. 3B, Table 1). From these findings we propose that DV Abs have the potential to compete with ACE2 receptors for access to RBD of SARS-CoV-2. So, theoretically, they can "mask" SARS-CoV-2 RBD and block its interaction with host cell receptors and thereby prevent virus entry. Our prediction is supported by the biological evidences of DV and SARS-CoV-2 cross-reactivity data as presented before [8–10] and provides a logical explanation to our previous observation that SARS-CoV-2 infections are causing less severity and mortality in the highly dengue endemic countries, where more than 80% of the population can be sero-positive for dengue [3]. First DV false-positivity report from Singapore [8]confirmed the absence of dengue infection in COVID-19 patients through DV-specific RT-PCR negativity. However, after 10 days of infection,

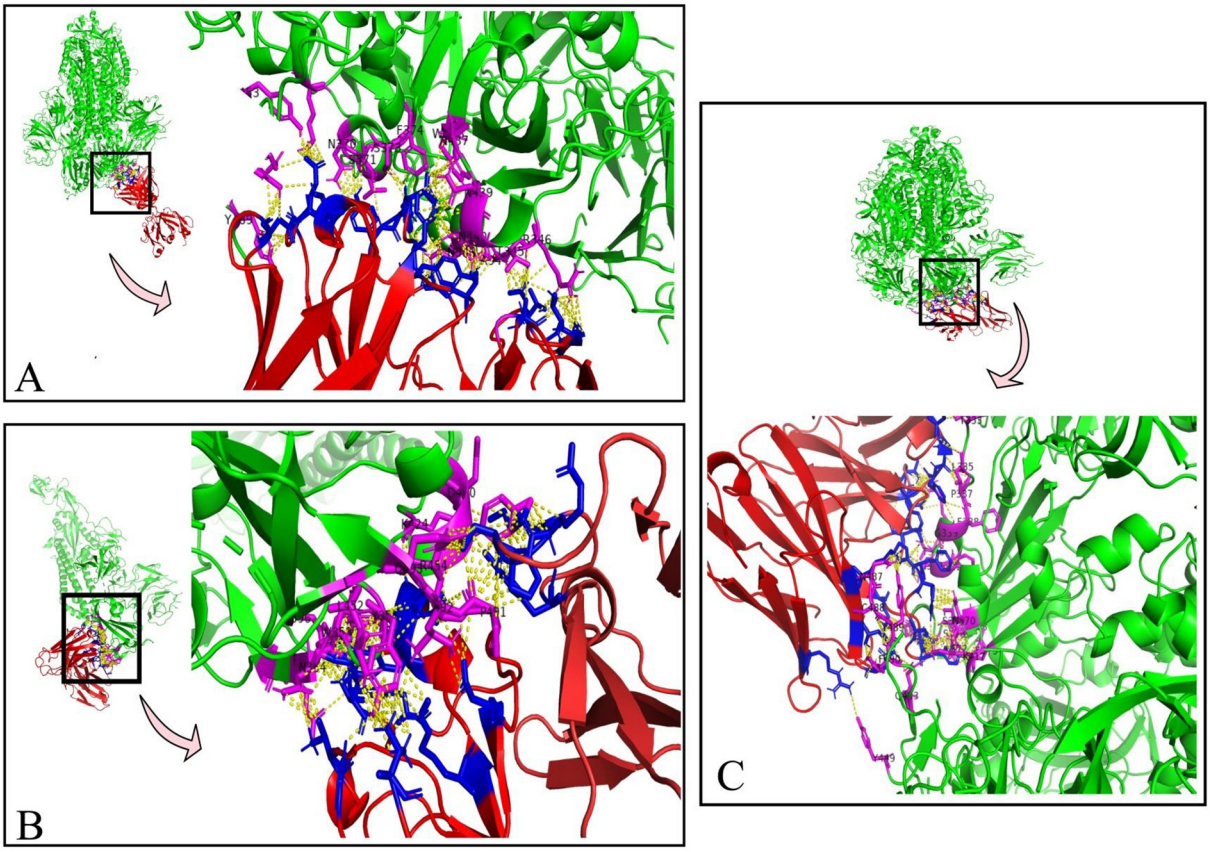

Fig. 2. Representative images of DV antibodies "docking" with SARS-CoV-2 Spike protein. (A) EDE1 C8 Ab (Red) is docked with Spike protein (green) of SARS-CoV-2 through ClusPro, Model 0. (B) EDE2 B7 Ab (Red) is docked with Spike protein (green) of SARS-CoV-2 through ZDOCK, Complex 5. (C) EDE1 C10 Ab (Red) is docked with Spike protein (green) of SARS-CoV-2 through ClusPro, Model 3. Spike protein amino acids involved in the interactions are depicted in violet. Interacting residues of respective antibodies are marked in blue. Hydrogen bonds within distance cut-off of 3.5 Å are marked as yellow dotted lines.

DV- RNA may not be detected. So, the possibility of previous infection could not be ruled out. This was taken care of in a subsequent study from Israel. The possibility of pre-existing DV antibodies (from previous infection) in COVID-19 serum samples was ruled out by anti-NS1 IgG ELISA [10] and such serum samples showed about 22% cross-reactivity in DV lateral flow-based antibody strip tests.

The reliability and robustness of our computational predictions were highlighted by the facts that m396 and 80R antibodies (against SARS-CoV-1) did not dock with RBM of SARS-CoV-2, a fact already confirmed experimentally by others [13] and that WNV NS1 antibody also did not bind satisfactorily with SARS-CoV-2 Spike protein RBD with no interaction in the RBM region. Although incapable of neutralizing SARS-CoV-2, a recent in-silico analysis suggested that suitable substitution of amino acids in the RBD region of m396 and 80R antibodies may increase computational docking efficiency [25]. The WNV NS1 Ab binds to WNV NS1 at an epitope which is similar but not identical to DV NS1 epitope. DV NS1 antibody could not be used in our docking studies as no X-ray crystallography structure for the same is available in the databases. The NS1 Ab and Spike protein docking results further support that it is the DV envelope Abs (and not NS1 Abs) in the DV diagnosed human serum samples that actually cross-reacted with the Spike antigens, immobilized in the SARS-CoV-2 rapid antibody tests and Spike Abs-detecting ELISA tests [9,10]. It has been predicted by others that some structural similarity may exist between DV envelope and the HR2 domain of the SARS-CoV-2 Spike protein [10]. However, the RBD domain is located far away from HR2 domain [20]. So, the results of our study do not correlate directly with the antigenic similarities proposed in the other study [10].

The four DV Abs used in this study, are known to neutralize DV and were identified from serum of dengue fever convalescent patients [11]. This ensures that both asymptomatic and symptomatic patients recovering from DV infection will possess immunological memory to these Abs. In highly Dengue endemic countries, where infections occur regularly, majority of the population has pre-exposure to DV and has turned DV sero-positive. In our present study only four Abs have been considered but immunological response against any pathogen comprises of a repertoire of Abs; so, it is likely there will a larger repertoire of DV antibodies which can bind to and block RBD in humans (Fig. 4). One limitation of our study is that we used only four DV antibodies to test binding to Spike protein, although there can be much higher number of antigen–antibody interactions in reality. Here, we were limited by available crystal structures for DV envelope antibodies. Nevertheless, we still believe these four antibodies were relevant and closer to real life scenario as they were originally isolated from dengue convalescent patients. As new variants of SARS-CoV-2 Spike protein emerge, it would be interesting to see how such amino acid substitution(s) impact on the interactions with DV antibodies.

Interestingly, some studies predicted pre-exposure of humans to animal coronaviruses from syananthropic animals such as bovines and dogs and it was speculated that antibodies elicited against animal coronaviruses could confer partial protection against SARS-CoV-2 [26–28]. In this One-Health approach, epitope mapping by homology modeling revealed high degree of similarity in nucleo-

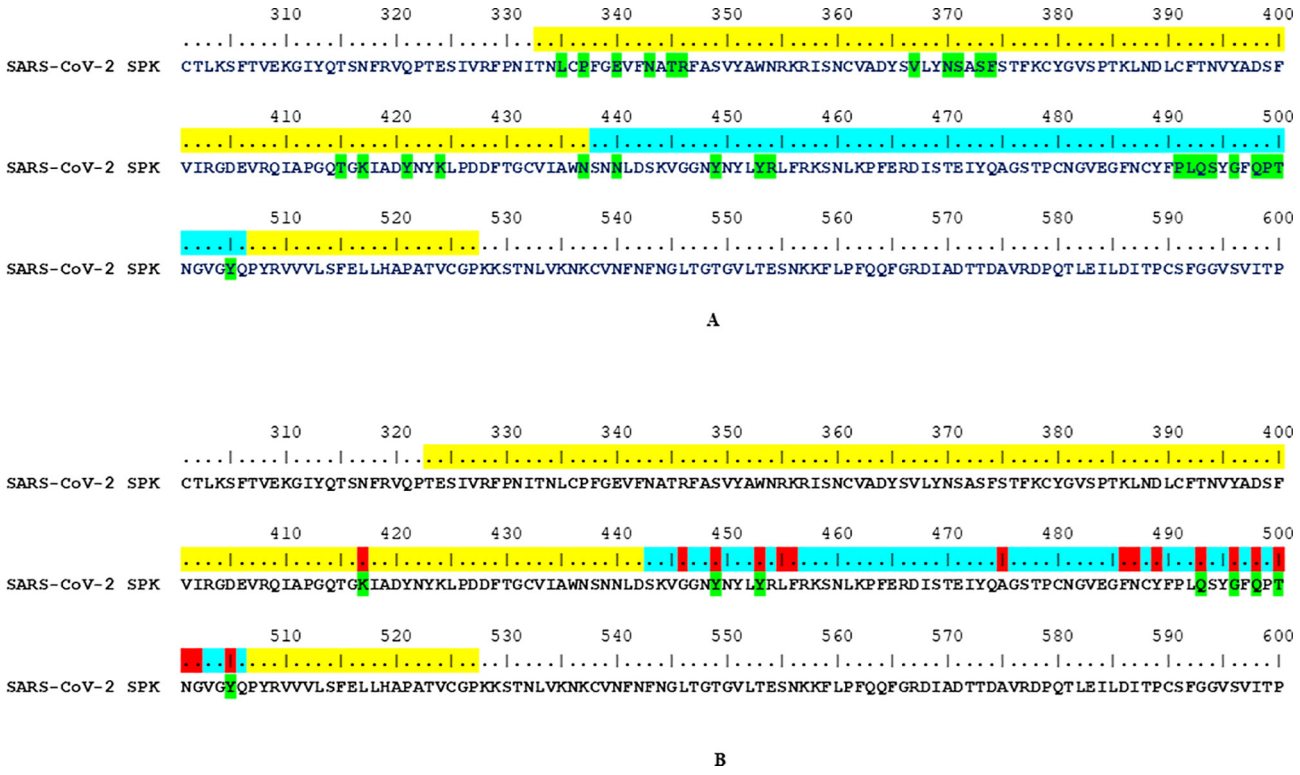

Fig. 3. Predicted interaction sites of DV antibodies with SARS-CoV-2 Spike protein. Yellow-marked region denotes RBD, spanning 333–527 amino acids. In RBD, sky blue-marked regions represent RBM, spanning 438–506 amino acids of the Spike protein. (A) All the amino acid residues in RBD, identified in overall 80 predictions to interact with DV antibodies, have been marked green. (B) Amino acids marked red are crucial for interactions between RBD and ACE2 receptor. Among these receptor binding residues of SARS-CoV-2 Spike, the green highlighted positions denote the residues of the RBD that were predicted to interact also with DV antibodies.

Table 1
Amino acid residues of SARS-CoV-2 RBD that interact with ACE2 receptor including the ones that were predicted to interact also with DV antibodies. Columns to the right show frequencies of interactions of DV Abs with some of the ACE2-engaging amino acid residues (bold, left column). These frequencies were obtained from the ZDOCK and ClusPro predictions.

	Frequency of interaction with DV antibody in ZDOCK and ClusPro models			
RBD residues that interact with ACE2 receptor [20]	EDE1 C8 Ab	EDE1 C10 Ab	EDE2 B7 Ab	EDE2 A11 Ab
K417	–	**4**	**2**	–
G446	–	–	–	–
Y449	**3**	**3**	–	–
Y453	**2**	–	–	–
L455	–	–	–	–
F456	–	–	–	–
A475	–	–	–	–
F486	–	–	–	–
N487	–	–	–	–
Y489	–	–	–	–
Q493	–	**4**	**5**	**5**
G496	–	–	**2**	–
Q498	**2**	–	**5**	–
T500	–	**2**	–	–
N501	–	–	–	–
G502	–	–	–	–
Y505	**2**	–	–	–
Total frequency	**9**	**13**	**14**	**5**

Total interaction events in 80 predictions: **41**.

capsid and envelope proteins between SARS-CoV-2 and taxonomically related coronaviruses [27,28]. In this context, it is noteworthy that human to animal transmission has been reported to be more common but the reverse is rare so far [29]. This proposition is thought-provoking and awaits experimental validation. Further research is warranted to confirm protective cross-reactivity between human and other animal coronaviruses.

One future challenge in case of COVID-19 is the yet undetermined impact of possible "Antibody Dependent Enhancement (ADE)" in already exposed populations [30,31]. ADE results from recurrent exposure to the immune stimulant within a defined time frame. This happens when antibodies to one SARS-CoV-2 strain fail to effectively neutralize another strain (s) (as often observed in case of dengue serotypes) and at the same time, enable the virus

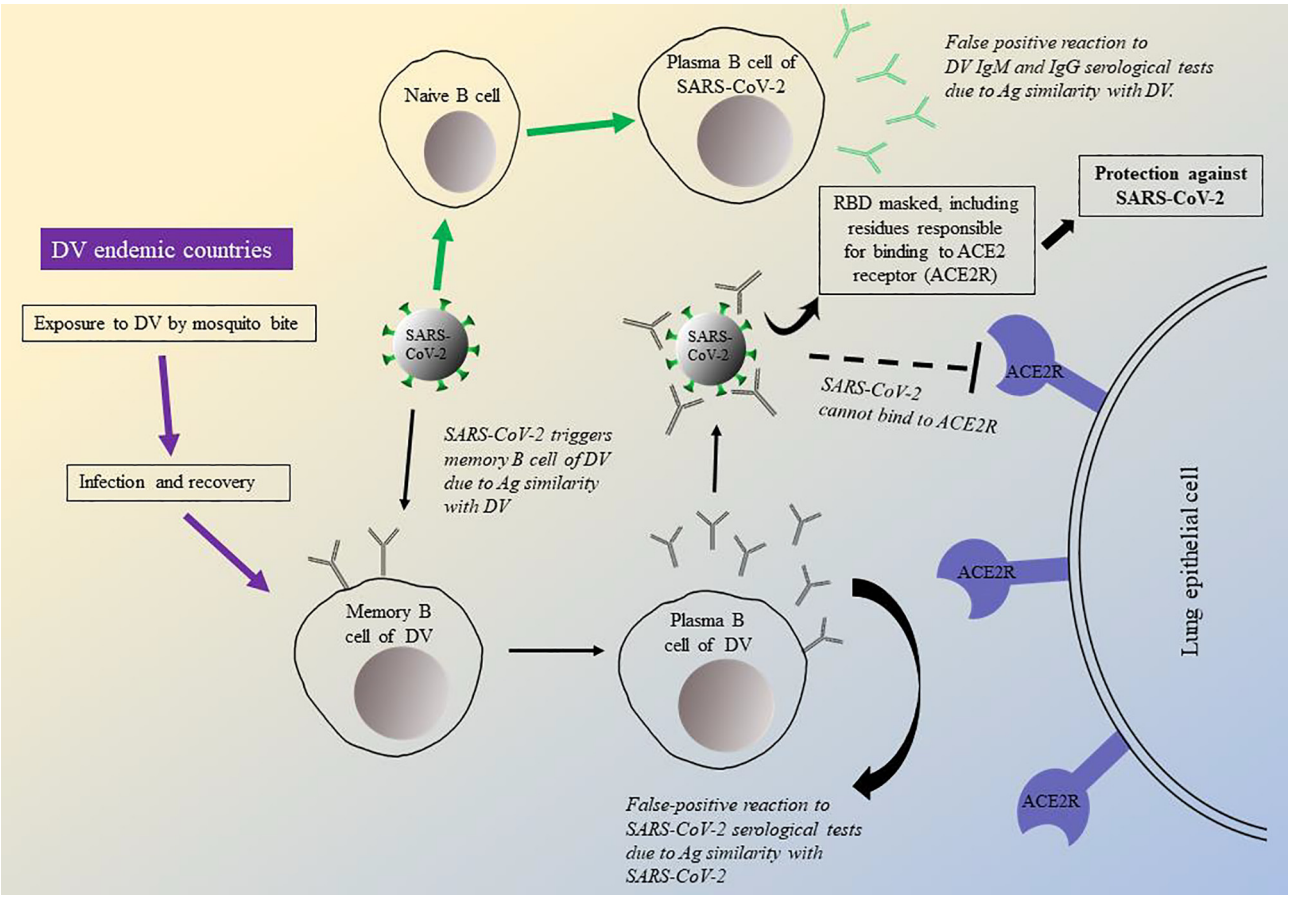

Fig. 4. Schematic diagram depicting why COVID-19 may be less severe in highly DV-endemic countries. In highly dengue-prone areas, SARS-CoV-2 infection may stimulate the immunological memory to DV in people with previous DV exposure (s), which could be asymptomatic. Due to antigenic similarity, the resultant dengue antibodies (grey "Y"-shaped) may bind to SARS-CoV-2 virus particles. DV Abs can even block Spike protein attachment to ACE2R by binding to Spike protein RBD and RBM. These are possible ways by which pre-exposure to DV infections can potentially reduce COVID-19 severity. SARS-CoV-2 Abs (green "Y-shaped") may cross-react in DV serological tests for detecting DV-specific IgM and/or IgG (top right) and *vice versa* (bottom).

of the other strain (s) to infect more cells by binding to the virus and bringing them closer to susceptible cells to which the antibodies are often attached by their Fc region.

From the results of our study, one can argue that DV Abs may also bind to SARS-CoV-2 and cause ADE for SARS-CoV-2 infections. But this does not appear to be the case, otherwise all highly dengue endemic countries would have been more hit by the COVID-19 pandemic than the dengue non-endemic countries due to pre-existing DV antibodies in the population. On the contrary, we are observing just the opposite scenario globally and therefore, DV antibodies are not involved in ADE of SARS-CoV-2 as per circumstantial evidence. Perhaps they are preventing SARS-CoV-2 severity as explained above [3]. But ADE can still represent a challenge for those individuals experiencing the first exposure to DV during this period of high SARS-CoV-2 presence/ transmission. This can happen due to the presence of SARS-CoV-2 Abs (potentially cross-reacting with DV) already in the COVID-19 affected individuals.

Our predicted computational models as well as growing experimental reports [9,10] of cross-reactivity between DV antibodies with SARS-CoV-2 and *vice versa* can affect sero-surveillance of COVID-19 in dengue endemic countries like India [32] and Brazil. Sero-diagnosis may come up with false-positive results in areas where both the viruses now co-exist. In such regions, due to antigenic similarity, SARS-CoV-2 Abs may cross-react in DV serological tests for detecting DV-specific IgM and/or IgG. Alternatively, SARS-CoV-2 infection may trigger the immunological memory to DV in people with previous DV exposure (s), which could be asymptomatic (Fig. 4). This will result in production of DV Abs, also resulting in false-positive results for COVID-19 patients in DV serological tests. It is now evident from the Spike protein ELISA results that DV Abs can, indeed, bind to SARS-CoV-2 Spike protein [10]. Further biological data to confirm the potential of DV EDE Abs to cross-react with SARS-CoV-2 are warranted. It would be also interesting to investigate whether, conversely, SARS-CoV-2 Abs can protect against DV.

4. Methods and models

4.1. Preparation of antibody for docking

The PDB files of all the antibodies were retrieved from RCSB PDB; PDB ID: 4UTA (EDE1 C8 Ab), 4UTB (EDE2 A11 Ab), 4UT6 (EDE2 B7 Ab), 4UT9 (EDE1 C10), 2G75 (m396 Ab), 2GHW (80R) and 4OII (WNV NS1-Ab). Most of the antibody structures in PDB were in neutralizing condition with their antigenic ligand molecule. From an original antibody PDB file, a separate PDB file consisting of only the antigen binding fragment, Fab (devoid of its natural antigenic counterpart) was created using PyMOL Molecular Graphics System, Version 2.3.3, Schrödinger, LLC software. These newly created PDB files were then processed in Chimera software using the Dock Prep plugin [33]. Subsequently solvent deletion, deletion of alternate positions (retaining only the highest-

occupancy positions), hydrogen addition, partial charge assignment, and output in Mol2 format were modulated through graphical interface. Standard residues (receptor amino acids) were assigned AMBER ff14sb partial charges [34,35]. AM1-BCC charges were computed for the receptor cofactors with ANTECHAMBER, which is included in Chimera [36,37]. The modified molecular structures were then used as receptor inputs in docking servers i.e. ZDOCK 3.0.2 [15,16] and ClusPro [17,18,38].

4.2. Refined protein data input in ZDOCK 3.0.2 and ClusPro web server for protein–protein docking predictions

For each antibody-antigen interaction, two FFT algorithm-based docking servers were used and the predictive results for each antibody were analyzed side by side to forage any similarity or pattern in the predictive interaction in accordance with our hypothesis. The ClusPro Server included FFT based protein–protein docking program PIPER. The simple user interface of the webserver allowed inputting PDB ID or PDB files for respective docking predictions. We used special antibody mode plugin in the server for the docking [39], where each dock- prepared antibody was uploaded as receptor input and SARS-CoV-2 trimeric spike protein (PDB ID: 6VSB) was uploaded as ligand input. Additional specification, such as automatic non-complement determining region masking of the antibody, was also enabled for the docking. The top 1000 results from the docking were then clustered using the optimal clustering algorithm in the server. The top 10 docking predictions were then downloaded as PDB files from the server for analysis.

ZDOCK 3.0.2 is also a FFT algorithm-based server for initial stage protein docking predictions. The user interface of the webpage enables uploading of PDB files or specifying PDB IDs. We put the dock-prepared antibody as Input protein 1. Due to PDB file size uploading restrain, we were unable to upload the entire trimeric Spike protein, 6VSB in the server as Input protein 2. Instead of the whole complex, we uploaded only one monomer of the Spike protein complex i.e. chain A as Input protein 2.

In the residue selection module of ZDOCK server, we blocked the SARS-CoV-2 Spike protein amino acid region 910–1146 of the monomeric chain A, as this region is unlikely to have any interaction with antibody [19] and stays mostly buried inside the trimeric Spike protein and envelope portion of the virus. After docking was done, the top 10 predictive structures were downloaded and analyzed in PyMOL. The unique interaction sites i.e. each amino acid residue and its position in the Spike protein, interacting with an antibody, and predicted by both the algorithms, have been tabulated (Supplementary data 1).

4.3. Analysis of predictions and image refinement using PyMOL

We used the PyMOL Molecular Graphics System, Version 2.3.3, Schrödinger, LLC for analyzing the predicted PDB structures obtained from both the servers. For each predicted docked complex, the interaction surface between antigen and antibody was determined through the "find any interaction within 3.5 Å cutoff" plugin. Amino acid residues of the Spike antigen within RBD region that were involved in an interaction with the target antibody, were identified and marked. All pictures were also refined and modified using the software.

4.4. Representative two-dimensional frequency bar graph generation

Two-dimensional frequency bar graph for each Spike protein amino acid interaction event with each antibody within RBD region from predicted docked complex, were created using the GraphPad Prism 6 software.

4.5. Data availability

The structures of docking models both in raw and analyzed format are available at Mendeley Data (https://data.mendeley.com/datasets/hpjyhjvrvv/1). Further information and requests for resources should be directed to and will be fulfilled by the corresponding author, Dr Subhajit Biswas (subhajit.biswas@iicb.res.in).

Author contributions

S.B., S.S., H.N. and A.M. conceived and designed the study. A.M. performed the docking experiments. H.N. and A.M. contributed equally and are joint first authors. All authors analysed and discussed the data. All authors wrote the manuscript to its final version. S.B. supervised the work and critically reviewed and edited the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2020.12.037.

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