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European Journal of Internal Medicine 104 (2022) 33–36

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Clinical Insights

Structural evolution of severe acute respiratory syndrome coronavirus 2: Implications for adhesivity to angiotensin-converting enzyme 2 receptors and vaccines

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ARTICLE INFO

Keywords:
SARS-CoV-2
Omicron BA.5
COVID-19
Vaccines
ACE2
Renin angiotensin system
Spike protein

The Omicron BA.5 variant currently accounts for the majority of cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (https://covid.cdc.gov/covid-data-tracker/#variant-proportions). This subvariant evolved from the Omicron lineage and became more contagious. Furthermore, data suggest that Omicron sublineages may evade polyclonal neutralizing antibody responses elicited by primary vaccine series [1,2]. Of note, development of antibodies to SARS-CoV-2 largely revolves around the viral spike (S) glycoprotein [3], which mediates host cell entry by binding to a cell surface receptor, angiotensin-converting enzyme 2 (ACE_2, Fig. 1) [4]. ACE_2 receptors are composed by 805 amino acids and use a single extracellular catalytic domain to cleave an amino acid from angiotensin (Ang) I to form $Ang_{1,9}$ and to remove an amino acid from Ang II to form $Ang_{1–7}$[4]. The process of internalization of ACE_2 receptors which accompanies the link with the viral S protein, eliminates the enzymatic activity of ACE_2 receptors on the outer cell surface. This phenomenon could dysregulate the renin-angiotensin system (RAS), with reduced final generation of $Ang_{1,7}$ from Ang II [5,6]. Since $Ang_{1,7}$ exert anti-inflammatory, antithrombotic and vasodilating effects, the ascendency of Ang II over $Ang_{1–7}$ might contribute to trigger inflammation, thrombosis and vasoconstriction [7,8]. Several investigations demonstrated the development of an “Ang II storm” or “Ang II intoxication” during the acute phase of infection[9]. Wu and co-workers demonstrated a significant increase in Ang II levels among coronavirus disease 2019 (COVID-19) patients [10]. More specifically, they found increased Ang II levels in 90.2% of COVID-19 patients, and a direct association between plasma Ang II levels and COVID-19 severity [10]. Similar results were obtained in a clinical study investigating disease severity in SARS-CoV-2 infected patients: Liu and co-workers found that Ang II levels in the plasma samples were significantly increased and linearly associated with viral load and lung damage [11].

We evaluated the effect of mutations of BA.5 subvariant on the

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S, spike; ACE_2, angiotensin-converting enzyme 2; Ang, sngiotensin; RAS, renin-angiotensin system; COVID-19, coronavirus disease 2019; RBD, receptor binding domain; ORF, open reading frame; Kd, dissociation constant.

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https://doi.org/10.1016/j.ejim.2022.08.012
Received 30 July 2022; Accepted 10 August 2022
Available online 12 August 2022

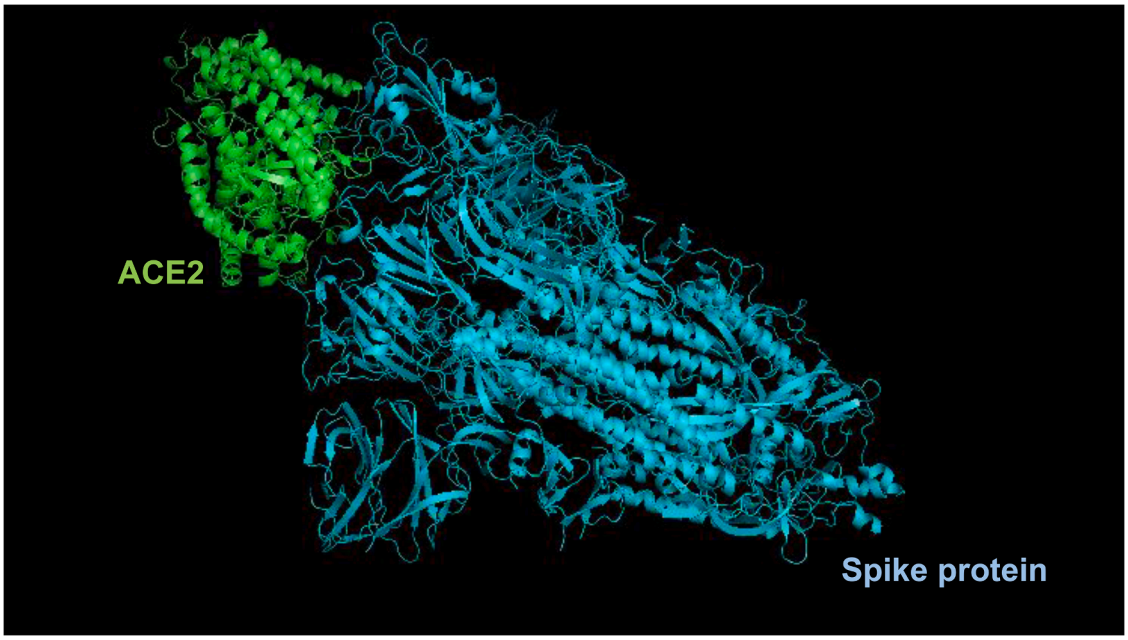


Fig. 1. Structure and interaction of SARS-CoV-2 spike glycoprotein with ACE_2 receptor (see text for details).

receptor binding domain (RBD) of the S protein, the overall conformational dynamics of the S protein and its adhesivity to ACE_2 receptors. We downloaded the proteins sequence and structure for SARS-CoV-2 S protein and ACE_2 receptor from the Protein Data Bank [12]. We analyzed the nucleotide sequence of BNT162b2 vaccine, accession MQ287666 (Patent WO2021214204). We annotated the open reading frame (ORF) coding for vaccine S and we translated into protein sequence with Translate tool (https://web.expasy.org/translate). The Pymol mutagenesis wizard (https://pymol.org) was used to introduce the specific mutations at the appropriate residues in wild type SARS-CoV-2 S in order to create Alpha B.1.1.7 and Omicron BA.5 variants. The online server EMBOSS Pepstats (https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/) was used to calculate the amino acid composition, molecular weight, distribution of charged residues, hydropathicity, aliphatic index and instability index (for both Alpha B.1.1.7 and Omicron BA.5 subvariants). In order to verify Pepstats results, we conducted a second analysis with ProtParam [13] and AA-prop (http://www.biogem.org/tool/aa-prop/). In addition, in order to compare and visualize residue-level physicochemical properties and to assess the impact of mutations on function we adopted PROVEAN (http://provean.jcvi.org/index.php) and VOLPES (http://volpes.univie.ac.at/) software, respectively. To evaluate binding affinity (ΔG) and Kd prediction we used the PRODIGY webserver (https://wenmr.science.uu.nl/prodigy/). After preparing the protein, Cluspro (https://cluspro.bu.edu/login.php) was used to dock the reference Alpha and Omicron to the ACE_2 receptor. Finally, we generate the 3D figure with Pymol graphical software (https://pymol.org/2/) and we did a pairwise alignment of the RBD's sequence of BNT162b2 vaccine, alpha and omicron variants with Clustal omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) to evaluate the possible sequence of new vaccine and their possible effects,

We assessed the impact of mutations on the function of S protein and computed the binding affinity (ΔG) and dissociation constant (Kd) (Fig. 2, upper panel). We found a 63-fold increase in binding affinity with ACE_2 of Omicron BA.5 S protein, when compared with Alpha B.1.1.7 variant (Fig. 2, middle panel). Fig. 2 shows an example of a new bond with the ACE_2 receptor resulting from the Q498R mutation in Omicron BA.5. Moreover, mutations in the Omicron BA.5 S protein have made it more positively charged than the Alpha variant's spike. This change in charge may attracts Omicron BA.5 toward the negatively charged ACE_2 protein even across relatively large distances (Figure, middle panel).

The pairwise alignment of the RBD's sequence of BNT162b2 vaccine revealed an identical sequence with Alpha B.1.1.7 variant (Fig. 2, lower panel). For effect of mutations, the aminoacidic sequences of the RBD markedly differed between Alpha B.1.1.7 and Omicron BA.5 with significant functional changes (Fig. 2, lower panel).

In conclusion, the mutated S protein of Omicron BA.5 showed a 63-fold higher adhesivity to ACE_2 receptors, with expected enhanced activity of Ang II and concomitant $Ang_{1–7}$ deficiency. Finally, the higher adhesivity to ACE_2 receptors by the S protein generated by new vaccines eventually built on the aminoacidic sequence of Omicron BA.5 might enhance the imbalance between Ang II overactivity and of $Ang_{1–7}$ deficiency.

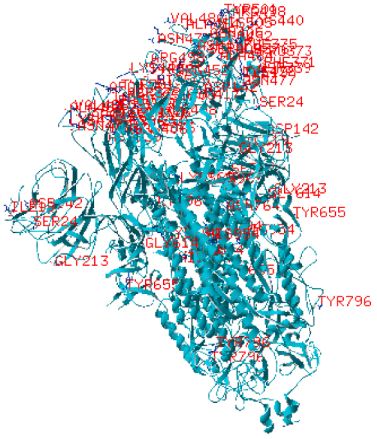
Basic and clinical research is urgently needed to investigate the clinical impact of dysregulated RAS axis on SARS-CoV-2 disease and vaccination. Moreover, the potentially detrimental impact of the interactions between S proteins (viral or vaccine-induced) and ACE_2 and other angiotensinases (involved in the processing of Ang II to $Ang_{1,7}$) remains to be determined [9]. New experimental and clinical data exploring the relationships between different mechanisms of Ang II cleavage and accumulation will be valuable in guiding the development of vaccines and other therapeutic strategies against SARS-CoV-2 pandemic [14,15].

Mutations from the wild type of SARS-CoV-2

Alpha B.1.1.7	Omicron BA.5		
del69/70	T19I	D405N (*)	Y505H (*)
del144/144	L24S	R408S (*)	D614G
N501Y (*)	del25/27	K417N (*)	H655Y
A570D	del69/70	N440K (*)	N679K
D614G	G142D	L452R (*)	P681H
P681H	V213G	S477N (*)	N764K
T716I	G339D (*)	T478K (*)	D796Y
S982A	S371F (*)	E484A (*)	Q954H
D1118H	S373P (*)	F486V (*)	N969K
	S375F (*)	Q498R (*)	
	T376A (*)	N501Y	

(*) refers to mutations involving the receptor binding domain (RBD) of the S protein

3D structure and site of mutations of Spike protein of Omicron BA.5

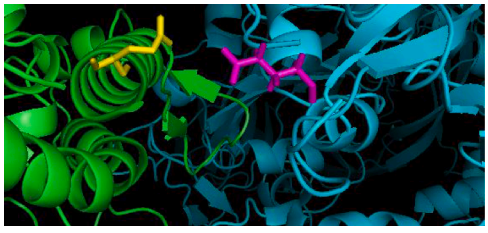


Variant	Molecular weight	Polarity		GRAVY	Charged Residues		Net Charge	Isoelectric Point	Extinction coefficient (1mg/ml)	Instability index	Aliphatic index
		Polar	Non-polar		Acidic	Basic					
Alpha B.1.1.7	118062.95	45.489	54.511	-0,101	7.989	9,211	6	6,94	0,968	32,84	82,99
Omicron BA.5	118079.04	45.386	54.614	-0,107	7.910	9.510	10	7,60	0,978	33.95	82.50

Binding affinity (ΔG) and dissociation constant (Kd) predicted values

Alpha B.1.1.7

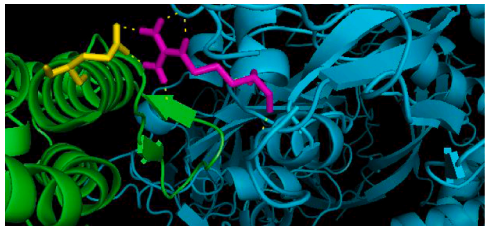
Protein-protein complex
ΔG (Kcal mol^{-1}): -58.7
Kd (M) at 25.0 °C: 8.3E-44



Q498R mutation in Omicron BA.5

Omicron BA.5

Protein-protein complex
ΔG (Kcal mol^{-1}): -61.2
Kd (M) at 25.0 °C: 1.3E-45



RBD sequence

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Alpha B.1.1.7   TNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF  60
BNT162b2        TNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCF  60
Omicron BA.5    TNLCPFDEVFNATRFASVYAWNRKRISNCVADYSVLYN FAPFFAFKCYGVSPTKLNDLCF 60
                ******.*********************************.*.*.:****************

Alpha B.1.1.7   TNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYL  120
BNT162b2        TNVYADSFVIRGDEVRQ IAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYL 120
Omicron BA.5    TNVYADSFVIRG NEVSQIAPGQTGNIADYNYKLPDDFTGCVIAWNSN KLDSKVGGNYNYR 120
                ************:**.********:**********************:***********.

Alpha B.1.1.7   YRLFRKSNLKPFERDISTEIYQAGSTPCNGVEG FNCYFPLQSYGFQPTNGVGYQPYRVVV 180
BNT162b2        YRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVV  180
Omicron BA.5    YRLFRKSNLKPFERDISTEIYQAG NKPCNGVAGVNCYFPLQSYGFRPTNGVGHQPYRVVV 180
                ************************..******..*.***********:******:*******

Alpha B.1.1.7   LSFELLHAP 189
BNT162b2        LSFELLHAP 189
Omicron BA.5    LSFELLHAP 189
                *********
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"." denotes a significant functional changes when compared with Alpha B.1.1.7.

Fig. 2. Mutations observed in Omicron BA.5 variant (and comparison with Alpha B1.1.7) with 3D structure of S protein (upper Panel). Affinity of both Alpha B.1.1.7 and Omicron BA.5 to ACE_2 (middle Panel) and comparison alignment of the receptor binding domain's sequence of BNT162b2 vaccine and the two variants (lower Panel) are also reported.

Declaration of Competing Interest

None.

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