Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Peculiar Variations of the Electrostatic Potential of Spike Protein N-terminal Domain Associated with the Emergence of Successive SARS-CoV-2 Omicron Lineages

Dear Editor

Zeng and collaborators (1) have recently discussed the potential of the porcine tyrosine-protein kinase receptor UFO (AXL) to interact with the N-terminal domain (NTD) of the spike (S) protein of some SARS-CoV-2 Variants of Concern (VOC). Omicron (BA.1.1.529) is the last VOC that, after its first detection in South Africa in the late 2021, has spread worldwide and has generated several subvariants of which those belonging to the BA.2 lineage (BA.2.12.1, BA.4 and BA.5) are now the most prevalent in several countries (www.who.int). Omicron subvariants markedly differ in resistance to antibody neutralization, that has been largely attributed to changes in the mutational landscape of RBD region of the Spike (S) protein (2) Little comparative attention is currently reserved to the mutational landscape of the S protein NTD although this domain also carries a distinctive set of mutations which markedly distinguish BA.1 (and BA.3) from the subvariants of the BA.2 lineage (BA.2.12.1,and BA4/5). In addition, BA.4 and BA.5 carry a HV69-70 deletion that is absent in the BA.2 and BA.2.12.1 subvariants.

We have recently shown that the mutational landscape of both RBD and NTD largely determines their net surface charge, i.e. an indirect estimate of the dominant charge of the surface electrostatic potential (EP) (3,4) . Changes in these potentials can modify the kinetics/strength of receptors recognition, or other suggested NTD functions, hence influencing the biological properties of SARS-CoV-2. in particular its transmissibility and infectivity(4-7). In all the pre-Omicron VOC, the EP of both RBD and NTD is dominantly positive, a finding that has been interpreted to favour their binding to negatively charged surfaces of the ACE2 (RBD) or the less characterized receptor(s) of NTD (4-7: see also below). Interestingly, the first emerged Omicron VOC (BA.1.1.529), while maintaining the usual positive net charge of the RBD region, showed a negative net charge of the NTD region, differently from all other previous VOC(4).

We have therefore considered to be of interest reporting here the net-charge values of all Omicron subvariants. Surprisingly, these EP-NTD values differed in the different subvariants. As shown in Table 1, only the first appeared Omicron strain had a dominantly negative EP. All others had a neutral (BA.3) or slightly positive (BA.2 and BA.2.12.1) or frankly positive (BA.4/5) value. Interestingly, the EP value of these last two subvariants falls in the range of all pre-Omicron VOC, being equal to that of the Delta variant. In contrast, no appreciable changes were observed in the high positive value of the RBD-EP of all Omicron subvariants (Table 1) demonstrating that variations in the electrostatic potentials of the NTD regions occur independently on those of the RBD region.

Table 1
Predicted net charge (electrostatic potential) of the Spike RBD and NTD (folded state) of SARS-CoV-2 VOC, compared with previous main VOC[1]

SARS-CoV-2 VOC	Pango Lineage	EP-RBD	EP-NTD
Wuhan	B.1	2.15	1.30
Alpha	B.1.1.7	3.18	1.69
Delta	B.1.617.2	4.15	1.28
Omicron	BA.1.1.529	5.22	-1.10
Omicron	BA.3	5.22	0.02
Omicron	BA.2	5.18	0.80
Omicron	BA.2.12.1	5.18	0.80
Omicron	BA.4	5.19	1.39
Omicron	BA.5	5.19	1.39

[1] Calculated as described in Ref.4.

We notice that the negativity of the BA.1 Omicron variant is probably contributed to or just determined by its unique EPE insertion at the position 214 of NTD sequence, meaning the double acquisition of the negatively charged (at physiologic pH) glutamic acid. Thus, the trend toward positivity of all other Omicron subvariants could be mostly due to the loss of the EPE insertion. In-silico mutagenesis of the Glu residues of the EPE insertions with Ala moves the net charge toward neutrality. The same effect can be seen by replacing Asp142 with Ala. Interestingly, Asp142 is shared by all the BA subvariants and by Delta. Also in this case, replacement with Ala increases the positivity of the domain net charge.

We previously (4) suggested that the negative EP value of BA.1 NTD might have hindered the NTD recognition by known or postulated,NTD- receptors, including gangliosides and, particularly, the AXL receptor which is mostly expressed in lung cells (5-7). In fact, the net charge of the AXL domain that is putatively involved in the interaction with NTD (as reported in the PDB structure 2C5D) is negative at around -5.5 according to our calculations. The electrostatic potential of AXL has been displayed and the most negative portion of its surface appears to coincide with the predicted interface with NTD (1). If so, the EP-NTD reversion to positivity of the BA.2 subvariants, in particular BA.4/5 could actually imply the rescue of NTD receptor recognition function that was lost or decreased in the progenitor Omicron BA.1n this line, it is of some interest that these EP-NTD variations appear to parallel the increased resistance of the BA.2 lineage subvariants to neutralization by antibodies as well as their increase in the experimental pathogenicity reported by Kimura and collaborators, as compared to BA1 lineage (8). In particular, the gradient of fusogenicity, a marker of SARS-CoV-2 pathogenicity, of these subvariants (BA.1 toBA.2 to BA-4/5 in increasing order) coarsely parallel the gradient 0f EP-NTD trend to positivity from BA.1 to BA4/5. . In addition,

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evasion of innate immunity appears to be markedly higher in BA.5 than in BA.1 and BA.2 (9,10).

We are aware of the rather speculative nature of our data interpretation above. Nonetheless, the here reported, peculiar variations of the electrostatic potential of the S-protein NTD region of the Omicron lineages may be virologically relevant, thus worthy being carefully investigated.

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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