Distinct evolution of infection-enhancing and neutralizing epitopes in the spike protein of SARS-CoV-2 variants (alpha, beta, gamma, delta, lambda and mu) : a structural and molecular epidemiology study

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Research Article

Distinct evolution of infection-enhancing and neutralizing epitopes in the spike protein of SARS-CoV-2 variants (α, β, γ, δ, λ and μ) : a structural and molecular epidemiology study

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Abstract

Objectives. The efficiency of Covid-19 vaccination is determined by cellular and humoral immune responses, and for the latter, by the balance between neutralizing and infection-enhancing antibodies. Here we analyzed the evolution of neutralizing and facilitating epitopes in the spike protein among SARS-CoV-2 variants. **Methods.** Amino acid alignments were performed on 929,203 spike sequences over the 4 last months. Molecular modeling studies of the N-terminal domain (NTD) and rod-like regions of the spike protein were performed on a representative panel of SARS-CoV-2 variants that were structurally compared with the original Wuhan strain. **Results.** D614, which belongs to an antibody-dependent-enhancement (ADE) epitope common to SARS-CoV-1 and SARS-CoV-2, has rapidly mutated to D614G in the first months of 2020, explaining why ADE has not been detected following mass vaccination. We show that this epitope is conformationally linked to the main ADE epitope of the SARS-CoV-2 NTD which is highly conserved among most variants. In contrast, the neutralizing epitope of the NTD showed extensive variations in SARS-CoV-2 variants. **Conclusions.** This molecular epidemiology study coupled with structural analysis of the spike protein indicates that the balance between facilitating and neutralizing antibodies in vaccinated people is in favor of neutralization for the Wuhan strain, α and β variants, but not for γ, δ, λ and μ. The evolution of SARS-CoV-2 has dramatically affected the ADE/neutralization balance which is nowadays in favor of ADE. Future vaccines should consider these data to design new formulations adapted to SARS-CoV-2 variants and lacking ADE epitopes in the spike protein.

Key words: SARS-CoV-2 variants, vaccine, facilitating antibodies, neutralizing antibodies, molecular epidemiology.

Introduction

Cytotoxic T-cells and neutralizing antibodies play a key role in the control of viral infections, especially in the case of respiratory viruses [1, 2]. However, virus-specific antibodies can also promote pathology, a phenomenon referred to as antibody-dependent enhancement (ADE) [3]. ADE of virus infection is generally due to virus-specific antibodies that enhance the entry of virus into host cells, and in some cases, virus replication in monocytes, dendritic cells and macrophages through antibody binding to Fcγ receptors [4]. In addition, alternative mechanisms of ADE involving the complement component C1q have been reported [5]. ADE has been observed in two typical situations: i) reinfection with a virus variant after primary infection with a different strain [6] or a cross-reactive virus [7], and ii) as the result of viral infection in vaccinated people [8]. The ADE phenomenon was initially discovered in flaviviruses in the late 1960's [9] and experimentally demonstrated in the early 1970's [10]. It concerns a broad range of viruses including dengue [11], Ebola [12], Zika [13], HIV [14], influenza [15], and various animal and human coronaviruses [16].

As early as in June 2020, at a time when Covid-19 vaccines had just entered clinical evaluation, Akiko Iwasaki and Yexin Yang from Yale University School of Medicine alerted that "*ADE should be given full consideration in the safety evaluation of emerging candidate vaccines for SARS- CoV-2*" [17]. A similar warning on vaccine safety due to potential risks of ADE was independently published by Shibo Jiang [18]. In contrast, several authors considered the risk to be null or minimal in the case of SARS-CoV-2 [19] [20] [21] [22].

However, several pieces of evidence strongly argue in favor of an ADE issue for SARS-CoV-2. i) ADE has been reported for animal coronaviruses such as feline infectious peritonitis virus [23]. In the most dramatic cases, kittens previously vaccinated with a recombinant virus containing the spike protein gene succumbed of early death after a coronavirus challenge [24]. ii) ADE epitopes were characterized in the spike protein of this feline coronavirus [25]. iii) ADE epitopes have also been found in human coronaviruses related to SARS-CoV-2, i.e. SARS-CoV-1 [26] and MERS-CoV [27] [28]. The case of SARS-CoV-1 is particularly interesting since its spike protein displays a linear ADE epitope, 597-LYQDVNC-603 (recognized by the monoclonal antibody 43-3-14) [26] that is fully conserved in the SARS-CoV-2 spike protein sequence used for mRNA Covid-19 vaccines. iv) ADE antibodies directed against the N-terminal domain (NTD) of the spike protein have been detected and characterized in convalescent Covid-19 patients [29] [30]. v) ADE antibodies are suspected to

be particularly efficient in vaccinated Covid-19 patients infected with the δ variant [31] [32].

In this context, we recently reported that facilitating anti-spike antibodies targeting the NTD have a higher affinity for the δ variant than for the initial Wuhan strain. We also reported that the main neutralizing epitope of the NTD is almost lost in δ variants [31]. This finding is of critical importance since ADE infection of coronaviruses is known to be induced by the presence of sub-neutralizing levels of anti-spike antibodies [33].

Overall, our data suggested that the balance between neutralizing and facilitating antibodies may greatly differ according to the virus strain.

In the present study, we analyzed a panel of representative SARS-CoV-2 variants including α, β, γ, δ, λ and μ as well as the most recent South-Africa strain C.1.2 (with no attributed Greek letter at the time of submission). We used multiple amino acid sequence alignment methods combined with structural and molecular modeling approaches to determine the variability of ADE and neutralizing epitopes and the impact of this variability on antibody-spike protein interactions. Our main objectives were i) to decipher the evolution of neutralizing and facilitating epitopes since the beginning of the Covid-19 pandemic, and ii) to predict for each SARS-CoV-2 variant which way the balance between neutralization and facilitation is tipping.

Methods

Molecular modeling studies were performed with Hyperchem (http://www.hyper.com) and Deep View/Swiss-Pdb viewer (https://spdbv.vital-it.ch) programs, as described in previous studies [34] [35] [36] [37]. The energy of interaction (ΔG) of each antibody-spike protein complex was calculated with Molegro Molecular Viewer (http://molexus.io/molegro-molecular-viewer). The cluster of gangliosides GM1 in a typical lipid raft organization was generated as described previously from CHARMM-GUI Glycolipid Modeler [38] and submitted to several minimization steps with the Polak-Ribière algorithm [39].

Results

Description of two distinct ADE epitopes in SARS-CoV-2 spike protein

The mutational patterns and geographic origins of the SARS-CoV-2 variants analyzed in this study are summarized in **Table 1**. All variants have a dual nomenclature (lineage and Greek letter) except for C.1.2 which, at the date of submission of this article, had no Greek letter

attributed. Our analysis is focused on the NTD and on the rod-like domains of the spike protein. Other ADE and neutralization epitopes do exist in the RBD, but during the complex process of viral adhesion to target cells, this domain is involved at later step [36] [37]. Clearly, the NTD is key to understand how SARS-CoV-2 initially interacts with the plasma membrane of host cells.

The first ADE epitope studied is the 611-617 motif with the original amino acid sequence LYQDVNC recognized by the 43-3-14 antibody [26]. This ADE epitope is common to human coronaviruses SARS-CoV-1 and SARS CoV-2. Interestingly, this epitope is centered on position 614 which is an aspartic acid residue in the original Wuhan strain but has rapidly evolved to the ultra-dominant D614G during the first months of 2020 [40]. The localization of this epitope on the spike protein (Wuhan strain) is shown in **Figure 1A** (epitope colored in yellow, except for D614 highlighted in red). It is well exposed on the protein surface so that it can be recognized by facilitating antibodies generated during previous coronavirus infections in humans, especially in geographic areas previously exposed to SARS-CoV-1. The second ADE epitope targeted by facilitating antibodies is divided in two parts (both colored in blue in **Figure 1A**): one in the NTD (27-32, 64-69 and 211-218 segments) and the other one in the rod-like domain (600-607, 674-677 and 689-691 segments) of the spike protein. Antibodies directed against this epitope have been detected in sera from convalescent Covid-19 patients[30]. Although the two parts of this ADE epitope seem to be spatially distant, both are close to a flexible 20-amino acid residue loop (621-640) that is unresolved in PDB files but was added by molecular modeling in the structures shown in **Figure 1**. It is interesting to note that this loop (highlighted in green) is ideally located to connect the NTD and the RBD, but also to provide a conformational link between both ADE epitopes (**Figure 1B**).

Once the NTD is bound to the cell membrane of the host cell, a conformational change unmasks the RBD which becomes available for a functional interaction with a viral receptor, chiefly ACE2 [37]. This spatial reorganization leads to the open, fusion-compatible conformation of the trimeric spike protein [41]. In the Wuhan strain, the closed conformation of the trimer [42] is stabilized by a hydrogen bond between D614 of one subunit and T859 of its neighbor (respectively chains B and C in **Figure 2A**). The global spreading of the pandemic during the first months of 2020 has been associated with the breakthrough of the first SARS-CoV-2 variant with a unique mutation in spike protein, D614G. As shown in **Figure 2B**, this mutation induces the loss of the hydrogen bond that stabilized the closed conformation. Thus, we analyzed the status of this hydrogen bond in the complex between the

facilitating 1052 antibody and the spike protein trimer. As shown in **Figure 2C**, the antibody has a long range conformational effect on both D614 and T859, which renders impossible the formation of this hydrogen bond. It is likely that the 621-640 loop, which conformationally connects the 1052 and the 611-617 epitopes, mediates this distal effect. In this respect, it is interesting to note that this facilitation can be induced by two distinct mechanisms: i) the replacement of aspartic acid by a glycine at position 614 (D614G mutation), or ii) binding of the ADE antibody 1052 to the original Wuhan spike protein displaying an aspartic acid (D614) at this position.

Analysis of amino acid sequence variations in ADE and neutralizing epitopes during the global spreading of the Covid-19 pandemic

Then, we analyzed the evolution of the amino acid sequence of ADE epitopes among SARS-CoV-2 variants (**Figure 3**). The 611-617 epitope (lower left panel), which is common to SARS-CoV-1 and SARS-CoV-2, has a unique signature in all variants, i.e. the D614G mutation. As position 614 is central to the epitope, this epitope is probably no longer recognized by ADE antibodies generated by previous coronavirus infections in humans. The second ADE epitope is formed by several distinct areas in the NTD and in the rod-like regions of the spike protein (**Figure 3**, upper panel). In the NTD, the epitope is divided in three linear segments that represent ca. 80% of the total energy of interaction of the 1052 antibody-NTD complex (as calculated from PDB: 7LAB): 27-32, 64-69 and 211-219 (accounting respectively for 12, 19 and 51 % of the energy of interaction). The complex is further stabilized by auxiliary contacts with the rod-like region of the spike protein (chiefly 600-607, 674-677 and 689-691). Overall, the whole epitope appeared to be extremely well conserved, except at two amino acid residues positions: H69 and D215. Indeed, a deletion (ΔH69) is found in the α variant, and D215 is mutated in D215G in the β and the more recent C1.2 variants (**Figure 3**, upper panel).

In marked contrast with the conservation of the 1052 ADE epitope, the main neutralizing epitope of the NTD showed extensive amino acid sequence variations (**Figure 3**, lower right panel). The changes included deletions, insertions and single point mutations that are distributed among two key regions, 144-158 (N3 loop) and 242-249 (N5 loop) that constitute the three-dimensional site recognized by the neutralizing 4A8 antibody [43]. The localization of the neutralization epitope of the NTD at the virus/host cell interface is consistent with this

high variability as it is submitted to a strong pressure of selection for SARS-CoV-2 variants. Conversely, the ADE epitope, which is on the lateral side of the NTD, is not facing the plasma membrane of the host cell and for this reason is not subjected to such a high selective pressure.

The frequency of amino acid sequence variations of the ADE and neutralizing epitopes was analyzed by specific queries of the Los Alamos database over the last four-month period (2021-06-01 to 2021-09-26) (**Table 2**). All the epitopes listed in **Figure 3** were analyzed in 929,203 genomes. The ADE epitope of the NTD is highly conserved (>99% for all segments) except for the 64-69 motif at position H69 (variation of 8.95% with 1 mutation), mostly reflecting the α variant [44]. The ADE epitope 611-617 displays 1 mutation in 99.14% of cases, consistent with the worldwide dominance of the D614G mutant [45]. The situation of the neutralization epitope of the NTD is by far more complex, in particular for the 144-158 segment which shows high amino acid sequence variability (frequency of the Wuhan sequence <0.05%). Remarkably, 92.01% of the sequences have 2 mutations and some viruses with 3, 4 and even 5 mutations are currently detected. The second linear segment (242-249) is more conserved (99.11% of sequences are identical to the Wuhan strain), but viruses with up to 4 mutations have been characterized. Interestingly, the amino acid variations of the neutralization epitope are concentrated on positions that are associated with the variants analyzed in the present study: Y144, E156, F157 and R158, in the N3 loop, R246 in the N5 loop (**Figure 3**).

Estimating the risks of the facilitation phenomenon depending on the variant concerned: a molecular modeling approach

Finally, we used molecular modeling approaches to determine how mutations in ADE epitopes could impair the binding of facilitating antibodies. In our analysis of ADE epitopes in SARS-CoV-2 variants (**Figure 3**), we detected only two mutations that can potentially suppress the facilitation phenomenon: ΔH69 and D215G. Thus, we studied the localization of H69 and D215 in the molecular complex between ADE antibody 1052 and the spike protein (**Figure 4A**, left panel). Both H69 and D615 appeared critical for the 1052 antibody binding site on the NTD of the spike protein. These positions are fully conserved in the γ, δ, μ and λ SARS-CoV-2 variants, which are still recognized by the ADE antibody 1052. An illustration of the efficiency of this antibody to facilitate the infection by the δ variant is shown in **Figure**

4A (right panel). The plasma membrane of the host cell is represented by a cluster of gangliosides GM1 to figure the lipid raft that acts as a landing platform for the NTD [36]. In line with previous data from our group [31], once the 1052 antibody is bound to the NTD of the δ spike protein, a global conformation change involving both the NTD and the antibody allows the formation of a highly energetic trimolecular complex (antibody-NTD-lipid raft) with an obvious geometric complementarity of all partners. Then, we compared the structure of the δ variant NTD with the μ, λ, and C.1.2 variants (**Figure 4B**). Except for C.1.2 which displays a D215G mutation, all other variants have both H69 and D215 accessible on the NTD surface.

In line with these data, the energy of interaction of the C.1.2 variant spike protein with the 1052 antibody was less than half the value calculated for the Wuhan strain (-229 kJ.mol^{-1}), whereas it reached -246 kJ.mol^{-1} for the δ variant [31], -236 kJ.mol^{-1} for μ and -228 kJ.mol^{-1} for λ variants. Thus, the conservation of H69 and D215 (in γ, δ, μ and λ variants) is critical for virus infectivity as it favors the ADE phenomenon by allowing an optimal binding of the 1052 antibody to the spike protein. In contrast, the ADE epitope is affected as soon as at least one of these positions is mutated (as it the case for α, β and C.1.2 variants).

Discussion

Vaccine strategies against viral diseases are confronted to the risk of antibody facilitation (ADE), especially when the strain used for the immunization protocol is distinct from circulating viruses [46]. In the past, ADE has been evidenced for a broad range of human RNA viruses including HIV, influenza, filoviruses, and coronaviruses [4]. Although ADE antibodies have been consistently characterized in the serum from Covid-19 convalescent patients [29] [30], the risk of ADE linked to vaccination with spike protein-based vectors (either mRNA or adenovirus) has not been considered as critical. As a matter of fact, it has been generally assumed that ADE antibodies exhibited SARS-CoV-2 infection enhancement *in vitro* but not *in vivo* [30]. However, a potential caveat of these studies is that SARS-CoV-2 variants have not been specifically assessed. Moreover, surprising higher incidence rates in vaccinated vs. unvaccinated individuals in the 0-14 days after the first dose were recently reported in long-term care facility residents and health-care workers, which resulted in significant negative vaccine efficiency estimates of -37% and -113%, respectively [47]. To which extent this apparent enhancement of SARS-CoV-2 infection is due (or not due) to an

imbalance between vaccine-induced (and/or pre-existing) neutralizing and facilitating antibodies warrants further investigation. Moreover, a recent report revealed that there is no clear relationship between the percentage of fully vaccinated individuals and new Covid-19 cases in 68 countries including Israel, a pioneer in mass vaccination against SARS-CoV-2 [48]. Taken together, these observations suggested that ADE, or more specifically the ADE/neutralization balance, could pose a problem for Covid-19 vaccine strategies, especially during the outbreak of SARS-CoV-2 variants. Finally, it is worth noting that ADE has been suspected to increase the severity of Covid-19 symptoms in selected geographic areas [49].

The objective of the present study was thus to assess the potential risk of ADE in vaccinated individuals challenged with SARS-CoV-2 variants. To this end, we studied the amino acid sequence variability of ADE and neutralizing epitopes in the NTD and rod-like regions of the spike protein. Then we used our target-based molecular modeling strategy to interpret these data at the level of the three-dimensional structure of the spike proteins.

We focused our attention on two distinct ADE epitopes: one linear epitope common to SARS-CoV-1 and SARS-CoV-2 (611-617 in the rod-like region of the spike protein, recognized by the 43-3-14 antibody) [26] and a complex three-dimensional NTD epitope (recognized by the 1052 antibody) [30].

Both epitopes are present on the spike protein generated by mRNA vaccines as the original formulas are based on the Wuhan strain [50]. Therefore, it is of high importance to determine whether these epitopes are still expressed and accessible on SARS-CoV-2 variants. The 611-617 epitope has probably escaped facilitating antibodies because the D614G variant has rapidly replaced the original strain [45]. Although in the initial study of the D614G mutation the authors mentioned the presence of D614 in a conserved ADE epitope, they did not comment further this important issue [40]. Our modeling approaches revealed a common molecular mechanism leading to enhanced infectivity for the D614G variant and for ADE antibodies with the Wuhan strain (**Figure 2**). In both cases, the loss of a stabilizing hydrogen bond between amino acid residues 614 and 859 of two vicinal spike protein chains relaxes the trimer and facilitates the conformational change that unmasks the RBD. A major outcome of our study is the identification of the 621-640 loop, which is missing in PDB files, as the conformational transmitter that allows the 1052 antibody to induce distant effects on amino acid residue 614. In this respect, the enhancement of infection provided by this ADE antibody involves two distinct Fc□-independent mechanisms: a long range conformational effect and a stabilization of the NTD bound to a lipid raft [31].

From an epidemiologic point of view, we can propose a scenario according to which the first cases of SARS-CoV-2 infections in China had been facilitated by ADE antibodies directed against the 611-617 epitope in individuals previously infected by SARS-CoV-1 or similar coronaviruses. Then the global extension of SARS-CoV-2 has probably levied this constraint by selecting the D614G variant in SARS-CoV-1 free populations. This scenario is consistent with the rapid raise and long-term maintenance of D614G worldwide. It is also consistent with the discrepancy between the severity of Covid-19 cases observed in the Hubei province of China and those occurring elsewhere in the world at the beginning of the pandemic [49]. Moreover, it explains why ADE has not been detected during the first months following mass vaccination, since ADE antibodies directed against the 611-617 epitope are no longer active on D614G variants. The observation that anti-SARS-CoV-1 antibodies isolated from a convalescent patient could enhance virus infection mediated by civet virus spike proteins [51] also supports this notion. Retrospectively, it is important to note the statement of Helen Pearson in a Nature editorial commenting these data in 2005: "*a jab against one strain might even aggravate an infection with SARS virus from civets or another species*" [52].

After the first wave of D614G, several other SARS-CoV-2 variants have emerged until the rise of the δ variant which is now by far the most common strain worldwide. Indeed, key variations in the ADE epitope at positions 69 and 215 have probably protected patients infected with α or β strains from the ADE risk (**Figure 3**). Nevertheless, these variants also showed significant variability of the neutralizing epitope, which could have decreased vaccine efficiency [53]. The situation is more dramatic for the δ variant. Indeed, several studies converged to alert on the potential risk of ADE when a δ SARS-CoV-2 variant infects a vaccinated individual [31] [32]. Our study confirms this possibility and further extends it to other circulating variants, including λ and μ, for which the neutralization/facilitation balance is unfavorable. A useful approach to anticipate such ADE risk in face of any variant is to analyze both the ADE and neutralizing epitopes of the NTD, as developed in **Figure 3**. At first glance, one can determine the balance between neutralization and facilitation and assess the risks of virus escape, ADE and/or both. Our molecular modeling approaches confirmed that hot mutational spots in ADE and neutralizing epitopes of the NTD give reliable information on antibody recognition of the spike protein, allowing us to determine which way the balance between neutralization and facilitation is tipping.

We recently hypothesized that the δ variant is dominating because its electrostatic surface potential of the NTD region that faces the host cell membrane has evolved to a large electropositive flat area that is complementary to the electronegative surface of lipid raft gangliosides [37]. The electrostatic potential surface value, which reflects the kinetics of virus infection, is a key parameter of a mathematic formula giving the transmissibility score (T-index) of any SARS-CoV-2 strain. This original and straightforward approach allowed us to correctly predict the rapid emergence of the δ variant (T-index >10) over α (T-index <4) even though both variants display a similar affinity for ACE-2 [37]. At present, the T-index of the δ variant is still higher than all other circulating variants so that it is likely that it will remain predominant in the coming months. Proposing a third and potentially a fourth jab to improve vaccine efficiency to face the threat of the δ variant may not be a good idea as it may further increase the amount of ADE antibodies without significant gain in neutralizing activity. Instead, we believe that it is critical to design new vaccine formulations able to induce neutralizing antibodies against this strain and, most importantly, lacking ADE epitopes in the NTD. Molecular epidemiology surveillance of SARS-CoV-2 coupled with structural analysis of variant spike proteins will certainly help to reach this goal.

References

1. Schmidt ME, Varga SM: **The CD8 T Cell Response to Respiratory Virus Infections.** *Frontiers in Immunology* 2018, **9**.
2. Du L, Yang Y, Zhang X: **Neutralizing antibodies for the prevention and treatment of COVID-19.** *Cellular & Molecular Immunology* 2021.
3. Morens DM: **Antibody-Dependent Enhancement of Infection and the Pathogenesis of Viral Disease.** *Clinical Infectious Diseases* 1994, **19:**500-512.
4. Tirado SM, Yoon KJ: **Antibody-dependent enhancement of virus infection and disease.** *Viral Immunol* 2003, **16:**69-86.
5. Takada A, Feldmann H, Ksiazek TG, Kawaoka Y: **Antibody-dependent enhancement of Ebola virus infection.** *J Virol* 2003, **77:**7539-7544.
6. Narayan R, Tripathi S: **Intrinsic ADE: The Dark Side of Antibody Dependent Enhancement During Dengue Infection.** *Front Cell Infect Microbiol* 2020, **10:**580096.
7. Dejnirattisai W, Supasa P, Wongwiwat W, Rouvinski A, Barba-Spaeth G, Duangchinda T, Sakuntabhai A, Cao-Lormeau VM, Malasit P, Rey FA, et al: **Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus.** *Nat Immunol* 2016, **17:**1102-1108.
8. Shukla R, Ramasamy V, Shanmugam RK, Ahuja R, Khanna N: **Antibody-Dependent Enhancement: A Challenge for Developing a Safe Dengue Vaccine.** *Frontiers in Cellular and Infection Microbiology* 2020, **10**.
9. Halstead SB, Nimmannitya S, Yamarat C, Russell PK: **Hemorrhagic fever in Thailand; recent knowledge regarding etiology.** *Jpn J Med Sci Biol* 1967, **20 Suppl:**96-103.
10. Halstead SB, Shotwell H, Casals J: **Studies on the pathogenesis of dengue infection in monkeys. II. Clinical laboratory responses to heterologous infection.** *J Infect Dis* 1973, **128:**15-22.
11. Wang TT, Sewatanon J, Memoli MJ, Wrammert J, Bournazos S, Bhaumik SK, Pinsky BA, Chokephaibulkit K, Onlamoon N, Pattanapanyasat K, et al: **IgG antibodies to dengue enhanced for FcγRIIIA binding determine disease severity.** *Science* 2017, **355:**395-398.
12. Takada A, Watanabe S, Okazaki K, Kida H, Kawaoka Y: **Infectivity-enhancing antibodies to Ebola virus glycoprotein.** *J Virol* 2001, **75:**2324-2330.
13. Bardina SV, Bunduc P, Tripathi S, Duehr J, Frere JJ, Brown JA, Nachbagauer R, Foster GA, Krysztof D, Tortorella D, et al: **Enhancement of Zika virus pathogenesis by preexisting antiflavivirus immunity.** *Science* 2017, **356:**175-180.
14. Marasini B, Vyas HK, Lakhashe SK, Hariraju D, Akhtar A, Ratcliffe SJ, Ruprecht RM: **Mucosal AIDS virus transmission is enhanced by antiviral IgG isolated early in infection.** *Aids* 2021.
15. Winarski KL, Tang J, Klenow L, Lee J, Coyle EM, Manischewitz J, Turner HL, Takeda K, Ward AB, Golding H, Khurana S: **Antibody-dependent enhancement of influenza disease promoted by increase in hemagglutinin stem flexibility and virus fusion kinetics.** *Proc Natl Acad Sci U S A* 2019, **116:**15194-15199.
16. Wen J, Cheng Y, Ling R, Dai Y, Huang B, Huang W, Zhang S, Jiang Y: **Antibody-dependent enhancement of coronavirus.** *Int J Infect Dis* 2020, **100:**483-489.
17. Iwasaki A, Yang Y: **The potential danger of suboptimal antibody responses in COVID-19.** *Nature Reviews Immunology* 2020, **20:**339-341.

18.   Jiang S: **Don't rush to deploy COVID-19 vaccines and drugs without sufficient safety guarantees.** *Nature* 2020, **579:**321.
19.   Yager EJ: **Antibody-dependent enhancement and COVID-19: Moving toward acquittal.** *Clin Immunol* 2020, **217:**108496.
20.   van der Lubbe JEM, Rosendahl Huber SK, Vijayan A, Dekking L, van Huizen E, Vreugdenhil J, Choi Y, Baert MRM, Feddes-de Boer K, Izquierdo Gil A, et al: **Ad26.COV2.S protects Syrian hamsters against G614 spike variant SARS-CoV-2 and does not enhance respiratory disease.** *npj Vaccines* 2021, **6:**39.
21.   Clark NM, Janaka SK, Hartman W, Stramer S, Goodhue E, Weiss J, Evans DT, Connor JP: **Anti-SARS-CoV-2 IgG and IgA antibodies in COVID-19 convalescent plasma do not facilitate antibody-dependent enhance of viral infection.** *bioRxiv* 2021.
22.   de Alwis R, Chen S, Gan ES, Ooi EE: **Impact of immune enhancement on Covid-19 polyclonal hyperimmune globulin therapy and vaccine development.** *EBioMedicine* 2020, **55:**102768.
23.   Takano T, Kawakami C, Yamada S, Satoh R, Hohdatsu T: **Antibody-dependent enhancement occurs upon re-infection with the identical serotype virus in feline infectious peritonitis virus infection.** *The Journal of veterinary medical science* 2008, **70:**1315-1321.
24.   Vennema H, de Groot RJ, Harbour DA, Dalderup M, Gruffydd-Jones T, Horzinek MC, Spaan WJ: **Early death after feline infectious peritonitis virus challenge due to recombinant vaccinia virus immunization.** *J Virol* 1990, **64:**1407-1409.
25.   Takano T, Tomiyama Y, Katoh Y, Nakamura M, Satoh R, Hohdatsu T: **Mutation of neutralizing/antibody-dependent enhancing epitope on spike protein and 7b gene of feline infectious peritonitis virus: influences of viral replication in monocytes/macrophages and virulence in cats.** *Virus Res* 2011, **156:**72-80.
26.   Wang Q, Zhang L, Kuwahara K, Li L, Liu Z, Li T, Zhu H, Liu J, Xu Y, Xie J, et al: **Immunodominant SARS Coronavirus Epitopes in Humans Elicited both Enhancing and Neutralizing Effects on Infection in Non-human Primates.** *ACS Infect Dis* 2016, **2:**361-376.
27.   Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, He L, Chen Y, Wu J, Shi Z, et al: **Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry.** *J Virol* 2020, **94**.
28.   Agrawal AS, Tao X, Algaissi A, Garron T, Narayanan K, Peng BH, Couch RB, Tseng CT: **Immunization with inactivated Middle East Respiratory Syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus.** *Hum Vaccin Immunother* 2016, **12:**2351-2356.
29.   Liu Y, Soh WT, Kishikawa JI, Hirose M, Nakayama EE, Li S, Sasai M, Suzuki T, Tada A, Arakawa A, et al: **An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies.** *Cell* 2021, **184:**3452-3466.e3418.
30.   Li D, Edwards RJ, Manne K, Martinez DR, Schäfer A, Alam SM, Wiehe K, Lu X, Parks R, Sutherland LL, et al: **In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies.** *Cell* 2021, **184:**4203-4219.e4232.
31.   Yahi N, Chahinian H, Fantini J: **Infection-enhancing anti-SARS-CoV-2 antibodies recognize both the original Wuhan/D614G strain and Delta variants. A potential risk for mass vaccination?** *J Infect* 2021.
32.   Liu Y, Arase N, Kishikawa J-i, Hirose M, Li S, Tada A, Matsuoka S, Arakawa A, Akamatsu K, Ono C, et al: **The SARS-CoV-2 Delta variant is poised to acquire complete resistance to wild-type spike vaccines.** *bioRxiv* 2021**:**2021.2008.2022.457114.

33. Takano T, Yamada S, Doki T, Hohdatsu T: **Pathogenesis of oral type I feline infectious peritonitis virus (FIPV) infection: Antibody-dependent enhancement infection of cats with type I FIPV via the oral route.** *J Vet Med Sci* 2019, **81:**911-915.

34. Fantini J, Di Scala C, Chahinian H, Yahi N: **Structural and molecular modelling studies reveal a new mechanism of action of chloroquine and hydroxychloroquine against SARS-CoV-2 infection.** *Int J Antimicrob Agents* 2020, **55:**105960.

35. Fantini J, Chahinian H, Yahi N: **Synergistic antiviral effect of hydroxychloroquine and azithromycin in combination against SARS-CoV-2: What molecular dynamics studies of virus-host interactions reveal.** *Int J Antimicrob Agents* 2020, **56:**106020.

36. Fantini J, Chahinian H, Yahi N: **Leveraging coronavirus binding to gangliosides for innovative vaccine and therapeutic strategies against COVID-19.** *Biochem Biophys Res Commun* 2021, **538:**132-136.

37. Fantini J, Yahi N, Azzaz F, Chahinian H: **Structural dynamics of SARS-CoV-2 variants: A health monitoring strategy for anticipating Covid-19 outbreaks.** *J Infect* 2021, **83:**197-206.

38. Jo S, Kim T, Iyer VG, Im W: **CHARMM-GUI: a web-based graphical user interface for CHARMM.** *J Comput Chem* 2008, **29:**1859-1865.

39. Di Scala C, Fantini J: **Hybrid In Silico/In Vitro Approaches for the Identification of Functional Cholesterol-Binding Domains in Membrane Proteins.** *Methods Mol Biol* 2017, **1583:**7-19.

40. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, Hengartner N, Giorgi EE, Bhattacharya T, Foley B, et al: **Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus.** *Cell* 2020, **182:**812-827.e819.

41. Benton DJ, Wrobel AG, Xu P, Roustan C, Martin SR, Rosenthal PB, Skehel JJ, Gamblin SJ: **Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion.** *Nature* 2020, **588:**327-330.

42. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, Graham BS, McLellan JS: **Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.** *Science* 2020, **367:**1260-1263.

43. Chi X, Yan R, Zhang J, Zhang G, Zhang Y, Hao M, Zhang Z, Fan P, Dong Y, Yang Y, et al: **A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2.** *Science* 2020, **369:**650-655.

44. Meng B, Kemp SA, Papa G, Datir R, Ferreira IATM, Marelli S, Harvey WT, Lytras S, Mohamed A, Gallo G, et al: **Recurrent emergence of SARS-CoV-2 spike deletion H69/V70 and its role in the Alpha variant B.1.1.7.** *Cell Reports* 2021, **35:**109292.

45. Groves DC, Rowland-Jones SL, Angyal A: **The D614G mutations in the SARS-CoV-2 spike protein: Implications for viral infectivity, disease severity and vaccine design.** *Biochemical and Biophysical Research Communications* 2021, **538:**104-107.

46. Kulkarni R: **Antibody-Dependent Enhancement of Viral Infections.** *Dynamics of Immune Activation in Viral Diseases* 2019:9-41.

47. Emborg H-D, Valentiner-Branth P, Schelde AB, Nielsen KF, Gram MA, Moustsen-Helms IR, Chaine M, Seidelin UH, Nielsen J: **Vaccine effectiveness of the BNT162b2 mRNA COVID-19 vaccine against RT-PCR confirmed SARS-CoV-2 infections, hospitalisations and mortality in prioritised risk groups.** *medRxiv* 2021:2021.2005.2027.21257583.

48.   Subramanian SV, Kumar A: **Increases in COVID-19 are unrelated to levels of vaccination across 68 countries and 2947 counties in the United States.** *European Journal of Epidemiology* 2021.
49.   Tetro JA: **Is COVID-19 receiving ADE from other coronaviruses?** *Microbes Infect* 2020, **22:**72-73.
50.   Xia X: **Detailed Dissection and Critical Evaluation of the Pfizer/BioNTech and Moderna mRNA Vaccines.** *Vaccines* 2021, **9:**734.
51.   Yang ZY, Werner HC, Kong WP, Leung K, Traggiai E, Lanzavecchia A, Nabel GJ: **Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses.** *proceedings of the national academy of sciences of the united states of america* 2005, **102:**797-801.
52.   Pearson H: **Caution raised over SARS vaccine.** *Nature* 2005.
53.   Cevik M, Grubaugh ND, Iwasaki A, Openshaw P: **COVID-19 vaccines: Keeping pace with SARS-CoV-2 variants.** *Cell* 2021.

Table 1. Mutations in SARS-CoV-2 variants.

Virus strain	NTD	Rod
Alpha α **B.1.1.7 (UK)**	ΔH69 ΔV70 ΔY144	D614G P681H T716I S982A D1118H
Beta β **B.1.351 (S_Afr)**	L18F D80A D215G ΔL242 ΔA243 ΔL244	D614G A701V
n/a **C.1.2 (S_Afr)**	P9L C136F ΔY144 R190S D215G ΔA243 ΔL244	D614G H655Y N679K T716I T859N
Gamma γ **P.1 (Brazil)**	L18F T20N P26S D138Y R190S	D614G H655Y T1027I V1176F
Delta δ **B.1.617.2 (India)**	T19R T95I G142D ΔE156 ΔF157 R158G	D614G P681R D950N
Mu μ **B.621 (Columbia)**	T95I +143T Y144S Y145N	D614G P681H D950N
Lambda λ **C.37 (Peru)**	G75V T76I R246N ΔS247 ΔY248 ΔL249 Δ250 ΔP251 ΔG252 ΔD253	D614G T859N

Table 2. Frequency of ADE and neutralizing epitope sequences.

Number of mutations	1052 mAb (NTD) ADE 27-32 AYTNSF	1052 mAb (NTD) ADE 64-69 WFHAIH	1052 mAb (NTD) ADE 211-218 NLVRDLPQ	1052 mAb (Rod) ADE 600-607 PGTNTSNQ	1052 mAb (Rod) ADE 674---691 YQTQ---SQS	43-3-14 mAb (Rod) ADE 611-617 LYQDVNC	4A8 mAb (NTD) Neutralization 144-158 YYHKNNKSWMESEFR	4A8 mAb (NTD) Neutralization 242-249 LALHRSYL
0	99.08	90.75	99.19	99.93	99.47	< 0.05	< 0.05	99.11
1	0.88	8.95	0.78	0.07	0.05	99.14	4.36	0.34
2	< 0.05	0.3	< 0.05	-	< 0.05	0.85	92.01	0.06
3	-	-	-	-	-	-	3.39	0.11
4	-	-	-	-	-	-	0.10	0.31
5	-	-	-	-	-	-	< 0.05	-

The frequency of mutations of each epitope sequence is calculated as the percentage of identity with the reference amino acid sequence of the SARS-CoV-2 spike protein (Wuhan strain). The most variable amino acid residues of each epitope are underlined.
929,203 sequences were analyzed from 2021-06-01 to 2021-09-26.
The raw data were obtained from the Los Alamos website
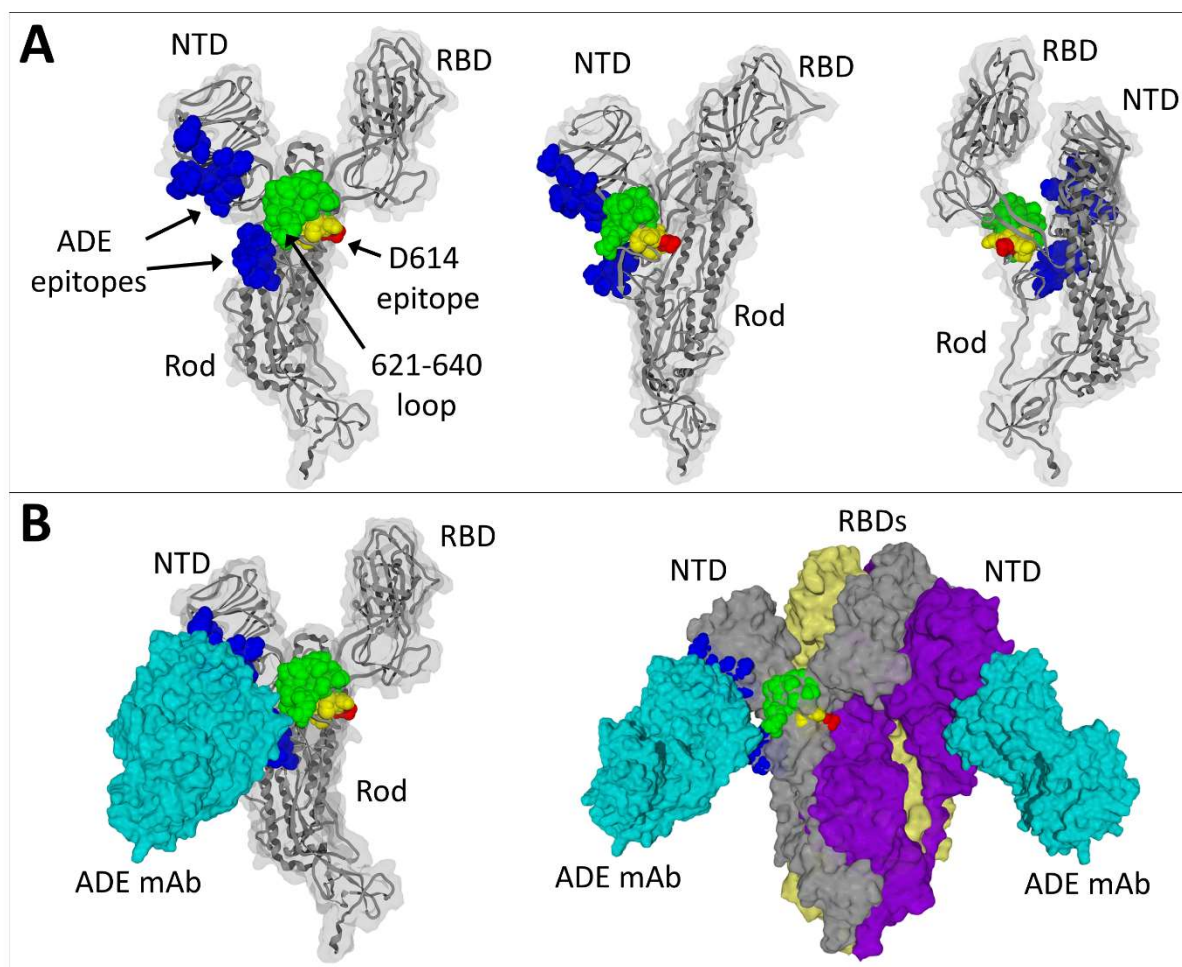(https://cov.lanl.gov/content/sequence/ANALYZEALIGN/analyze_align.html).

Figures

Figure 1. Localization of ADE epitopes on the spike protein.
A. Three distinct views of the SARS-CoV-2 spike protein (Wuhan strain). The ADE epitopes recognized by the 1052 antibody are colored in blue. The common coronavirus ADE epitope is colored in yellow, with amino acid residue D614 in yellow. The 621-640 loop that is missing in PDB: 7LAB is in green. **B.** ADE antibody 1052 (in cyan) bound to the monomeric spike (left panel) or to the trimeric spike protein (right panel). The N-terminal domain (NTD) and receptor-binding domain (RBD) are indicated in all models.
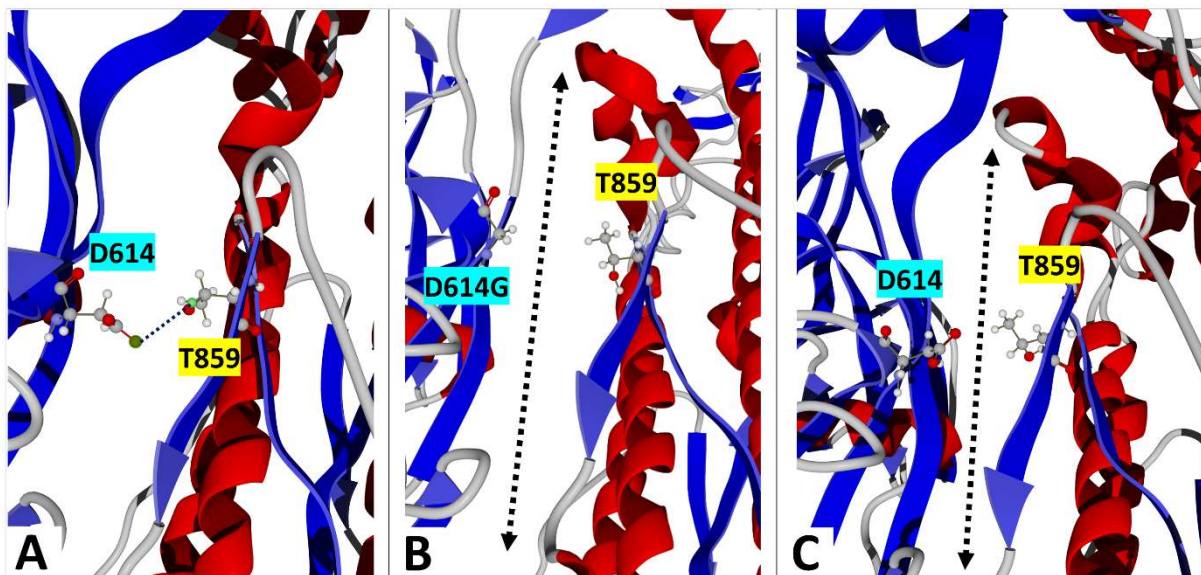
Figure 2. How the D614G mutation and the ADE antibody 1052 enhance SARS-CoV-2 infectivity.
A. Hydrogen bond between D614 (chain B) and T859 (chain C) stabilizing the trimeric spike protein (PDB: 6VSB). **B.** The D614G mutation renders impossible the formation of the hydrogen bond and facilitate the conformational change inducing the demasking of the RBD (PDB: 7BNM). **C.** Binding ADE of ADE antibody 1052 breaks the hydrogen bond between D614 and T859 (PDB: 7LAB). The arrow in panels B and C illustrates the lack of contact between vicinal spike protein monomers in the context of the trimeric association.

```
                                            ADE mAb 1052
                    27-32      64-69      211-218    600-607    674-677  689-691
Wuhan Origin        AYTNSF     WFHAIH     NLVRDLPQ   PGTNTSNQ   YQTQ     SQS
α B.1.1.7 UK        ------     -----Δ     --------   --------   ----     ---
β B.1.351 S_Afr     ------     ------     ----G---   --------   ----     ---
n/a C.1.2 S_Afr     ------     ------     ----G---   --------   ----     ---
γ P.1 Brazil        ------     ------     --------   --------   ----     ---
δ B.1.617.2 India   ------     ------     --------   --------   ----     ---
μ B.621 Columbia    ------     ------     --------   --------   ----     ---
λ C.37 Peru         ------     ------     --------   --------   ----     ---


                    ADE mAb 43-3-14        NEUTRALIZING mAb 4A8
                    611-617                144-158          242-249
Wuhan Origin        LYQDVNC                YYHKNNKSWMESEFR  LALHRSYL
α B.1.1.7 UK        ---G---                Δ--------------  --------
β B.1.351 S_Afr     ---G---                ---------------  ΔΔΔ-----
n/a C.1.2 S_Afr     ---G---                Δ--------------  -ΔΔ-----
γ P.1 Brazil        ---G---                ---------------  --------
δ B.1.617.2 India   ---G---                -------------ΔΔG --------
μ B.621 Columbia    ---G---                YTSNKNNKSWMESEFR --------
λ C.37 Peru         ---G---                ---------------  ----NΔΔΔ
```

Figure 3. Amino acid sequence alignments of ADE and neutralizing epitopes in SARS-CoV-2 variants.
Amino acid residue variations are highlighted in yellow. -, identity; Δ, deletion. Note that the 144-158 neutralizing epitope of the μ variant displays a threonine residue (T, in red) inserted after Y144, then two mutations after this insertion (colored in blue). The insertion induces a shift of the amino acid sequence (highlighted in grey).
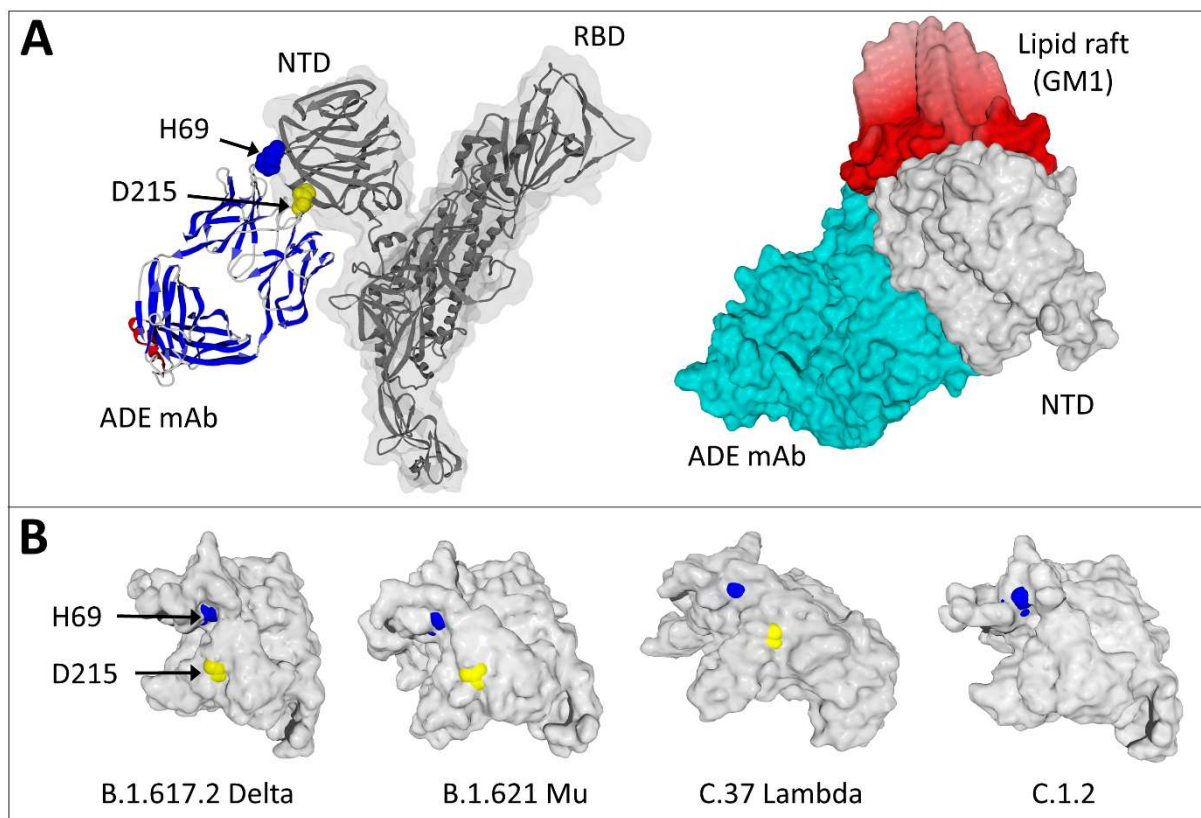
Figure 4. Critical amino acid residues control the binding of the ADE antibody 1052 to variant spike proteins.

A. Binding of the 1052 antibody (ADE mAb) to the Wuhan spike protein (PDB: 7LAB) with the NTD and RBD indicated. In the left panel, the light and heavy chains of the antibody are represented in standard secondary structures (red, α-helix, blue, β-strand). H69 (in blue) and D215 (in yellow) are highlighted. In the right panel, a surface representation illustrates the geometric complementarity of the δ spike protein-antibody complex bound to a cluster of gangliosides GM1 figuring a lipid raft on the plasma membrane of a host cell. Note that the 1052 antibody binds simultaneously to the NTD of the spike protein and to the edge of the lipid raft. **B.** Molecular modeling of the NTD of several SARS-CoV-2 variants showing different levels of surface exposure of H69 (in blue) and D215 (in yellow) amino acid residues.