

SARS-CoV-2 neutralizing antibody epitopes are overlapping and highly mutated which raises the chances of escape variants and requires development of broadly reactive vaccines

V. Stalin Raj¹, Jeswin Joseph¹, Sukhada Darpe¹, and Grishma Kulkarni¹

¹Indian Institute of Science Education and Research Thiruvananthapuram

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Abstract

The rapid adaptation of SARS-CoV-2 within the host species and the increased viral transmission triggered the evolution of different SARS-CoV-2 variants. Though numerous monoclonal antibodies (mAbs) have been identified as prophylactic therapy for SARS-CoV-2, the ongoing surge in the number of SARS-CoV-2 infections shows the importance of understanding the mutations in the spike and developing novel vaccine strategies to target all variants. Here, we report the map of experimentally validated 74 SARS-CoV-2 neutralizing mAb binding epitopes of all variants. The majority (87.84%) of the potent neutralizing epitopes are localized to the receptor-binding domain (RBD) and overlap with each other, whereas limited (12.16%) epitopes are found in the N-terminal domain (NTD). Notably, 69 out of 74 mAb targets have at least one mutation at the epitope sites. The potent epitopes found in the RBD show higher mutations (4-10aa) compared to lower or modest neutralizing antibodies, suggesting that these epitopes might co-evolve with the immune pressure. The current study shows the importance of determining the critical mutations at the antibody recognition epitopes, leading to the development of broadly reactive immunogens targeting multiple SARS-CoV-2 variants. Further, vaccines inducing both humoral and cell-mediated immune responses might prevent the escape of SARS-CoV-2 variants from neutralizing antibodies.

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Jeswin Joseph ¹, Sukhada Darpe ¹, Grishma Kulkarni ¹, V. Stalin Raj ¹, *

¹Virology Scientific Research (VSR) Laboratory, School of Biology, Indian Institute of Science Education and Research Thiruvananthapuram, India.

*Corresponding author: *stalin@iisertvm.ac.in*

Abstract

The rapid adaptation of SARS-CoV-2 within the host species and the increased viral transmission triggered the evolution of different SARS-CoV-2 variants. Though numerous monoclonal antibodies (mAbs) have been identified as prophylactic therapy for SARS-CoV-2, the ongoing surge in the number of SARS-CoV-2 infections shows the importance of understanding the mutations in the spike and developing novel vaccine strategies to target all variants. Here, we report the map of experimentally validated 74 SARS-CoV-2 neutralizing mAb binding epitopes of all variants. The majority (87.84%) of the potent neutralizing epitopes are localized to the receptor-binding domain (RBD) and overlap with each other, whereas limited (12.16%) epitopes are found in the N-terminal domain (NTD). Notably, 69 out of 74 mAb targets have at least one mutation at the epitope sites. The potent epitopes found in the RBD show higher mutations (4-10aa) compared to lower or modest neutralizing antibodies, suggesting that these epitopes might co-evolve with

the immune pressure. The current study shows the importance of determining the critical mutations at the antibody recognition epitopes, leading to the development of broadly reactive immunogens targeting multiple SARS-CoV-2 variants. Further, vaccines inducing both humoral and cell-mediated immune responses might prevent the escape of SARS-CoV-2 variants from neutralizing antibodies.

Keywords: SARS-CoV-2, Epitopes, Omicron variant, Delta variant, Monoclonal antibodies

Introduction

Severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2) emerged in Wuhan, China in 2019 [1] and rapidly spread across the globe with more than 220 countries being affected [2]. SARS-CoV-2 is highly contagious and causes mild to severe respiratory illness in humans and more than 600 million confirmed cases with 6 million deaths have been reported worldwide [2]. Coronavirus infection begins by the attachment of its trimeric spike glycoprotein to the host cell membrane receptor to enter into the cells. The spike protein consists of S1 and S2 domains, S1 contains the receptor-binding domain (RBD) which interacts with the cellular receptor, whereas S2 is involved in cell fusion [3]. Intervening the interaction of spike and its receptor would aid in the early prevention of virus infections. Therefore, spike glycoprotein is a key target for developing vaccines or therapeutics. The spike protein of SARS-CoV-2 uses angiotensin-converting enzyme-2 (ACE2) [3] an ectopeptidase as an entry receptor to initiate the infection cycle. Several vaccine candidates have been developed soon after the SARS-CoV-2 outbreak using the spike glycoprotein, which induces robust humoral re-sponses against SARS-CoV-2 and has been approved and licensed by WHO for human administration [4,5]. However, during the pandemic, the sudden increase in the human-human transmission and severity of the disease mainly caused by the spike variants such as the Delta promoted the usage of convalescent serum/plasma therapy as an immediate treatment option for SARS-CoV-2[6,7]. The modest efficacy of the plasma therapy [8] due to the diverse non-specific neutralizing antibodies led several research groups to develop highly specific potent monoclonal antibodies (mAbs) targeting spike protein. The recent advancements in molecular biology led to the rapid identification, engineering and production of potent mAbs against infectious diseases in a short time frame [9,10]. This has been exemplified from the recent SARS-CoV-2 outbreak wherein several potent neutralizing mAbs have been developed either directly from the B-cells or by phage display library of lymphocytes from the patients recovered from SARS-CoV-2 infection [5,11]. Due to the rapid clearance of the virus from severely infected patients, several mAbs with high sensitivity or specificity have been approved by FDA for immediate human administration [5].

The increasing trend of human-human transmission and accumulation of diverse favorable mutations in the spike protein of SARS-CoV-2 leads to the generation of several naturally occurring variants including the Delta and Omicron [7]. Recent reports demonstrates that selective pressures mediated by mass vaccinations as well as adaptation of SARS-CoV-2 to mAb treatment might facilitate the emergence of novel variants which are resistant to antibodies and shows higher transmission [12,13]. Proper understanding of the conservation of amino acid mutations within the Alpha, Beta, Gamma, Delta, and Omicron variants and changes associated at critical epitope residues of neutralizing monoclonal antibodies delineates the importance of developing novel vaccine candidates or therapeutics targeting current or future SARS-CoV-2 variants. A combined comparison between the neutralizing antibody binding epitopes and their mutations in the spike protein of all major variants of SARS-CoV-2 remain unclear which is important to understand the chances of immune escape by SARS-CoV-2 variants. In this study, we report the map of experimentally evaluated 74 SARS-CoV-2 potent neutralizing monoclonal antibody epitopes and compared them with naturally occurring escape variants of SARS-CoV-2.

Materials and Methods

2.1. Global map of SARS-CoV-2 variants of concern (VOC) and its timeline of emergence

Data reporting the emergence of different VOCs of SARS-CoV-2 was collected on February 17, 2022 from the World Health Organization (WHO) and was depicted manually in the world map generated in Adobe Illustrator. Independent color-coded circles were assigned for each variant (Beta- dark blue, Alpha-green, Delta- yellow, Gamma- red and Omicron- light blue) and were annotated based on the country of its first

occurrence. To represent the global timeline of SARS-CoV-2 variants, the number of SARS-CoV-2 confirmed cases were retrieved from the Global Initiative on Sharing All Influenza Data (GISAI) database on February 17, 2022. The submission count per week for each variant was summed for all countries and plotted using Microsoft Excel as an area plot. The X-axis denotes the timeline and the Y-axis represents the weekly total number of cases worldwide in thousands. Each variant was depicted with different colors (Beta- dark blue, Alpha-green, Delta- yellow, Gamma- red and Omicron- light blue).

2.2. Annotation of mutations in SARS-CoV-2 spike variants of concern

SARS-CoV-2 spike mutation details corresponding to the five variants of concern were collected from WHO on 17th February 2022. Independent mutations in each variant were manually represented on the schematic of SARS-CoV-2 spike glycoprotein. Substitutions were represented as yellow circles, deletions with red triangles and insertions with green triangles. Common mutations to multiple VOC have been connected through a standard line at that residue position. The spike subdomains (N-terminal domain -dark blue, receptor binding domain- green, fusion peptide- dark yellow, heptad repeat sequence 1- light yellow, heptad repeat sequence 2- light blue, transmembrane domain- brown) were represented with unique colors.

The Protein Data Bank (PDB) structure of SARS-CoV-2 S-ACE2 complex (7DF4) was used to annotate the mutations in five variants of concern (VOC) based on the WHO classification. All the mutations in the VOCs were manually highlighted in the spike glycoprotein using PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC). The receptor binding domain (RBD) of spike glycoprotein in open conformation was highlighted in orange color and N-terminal domain (NTD) in green, while the regions excluding NTD and RBD of spike protein was colored in grey. All the mutated residues were shown in sphere representation highlighted in red color and spike monomer was shown in cartoon representation on chain B of the structure.

2.3. Acquisition of SARS-CoV-2 spike epitopes of neutralizing monoclonal antibodies Experimentally validated 74 SARS-CoV-2 neutralizing mAbs with varying ranges of efficacy (modest to highly neutralizing) were selected from previous published articles for generating a map of individual antibody binding sites. Data on epitope residues was collected from the pre-analyzed crystal structure of monoclonal neutralizing antibodies in complex with SARS-CoV-2 spike glycoprotein and were retrieved manually for epitope annotation. Independent mAb binding residues were tabulated in an excel sheet which was further used for generating the map of SARS-CoV-2 spike glycoprotein mAb binding residues.

2.4. Generation of monoclonal antibody epitope heat map The python library Seaborn was used to visualize the heatmap displaying epitopes of the antibodies. Briefly, the number of antibodies containing a given epitope residue were divided by the total number of antibodies to generate the strength of the given epitope. These strengths were then represented in the form of a heatmap, with the colours with highest intensity representing highly represented epitopes.

2.5. Generation of antibody-epitope scatter plot

All the mAb binding residues in the SARS-CoV-2 spike glycoprotein were represented as a scatter plot with spike residue positions on X-axis and each individual mAbs on Y-axis. The scatter plot and the variant schemes were generated using the pyplot module in the python graphical plotting library Matplotlib [14]. Briefly, the excel file containing information about mAbs and their epitopes were processed using the pandas library [15]. Subsequently, the data was plotted using the scatter function in pyplot. The Matplotlib patches module was used to generate the background colour schemes in the plots corresponding to spike glycoprotein subdomains. Variants of Concern (VOC) and wild type spike glycoprotein schemes were generated using the pyplot subplots function while text annotations and final image export were done using Adobe Illustrator. The variant mutations were referred from VOC profiles of spike protein made available by WHO [16]. The mutated epitope residues with respect to the VOCs were represented as red filled circles while non-mutated residues were shown as green filled circles.

2.6. Frequency analysis of the neutralizing mAb epitope residues

Frequency of 74 experimentally validated mAb epitope residues occurring in SARS-CoV-2 spike protein was computed using python script. Briefly, the excel file with the details of epitope residues was processed using the pandas library [15]. Frequency of independent epitope residues was calculated and normalized by the total number of antibodies (n=74) and converted into percentage. The percentage frequency for those epitope residues that have been observed to be mutated in the SARS-CoV-2 spike variants of concern was plotted as a bar plot using the pyplot module in the python graphical plotting library Matplotlib [14]. To generate the colorbar for frequency, percentage frequency values were normalized by the maximum value among the computed frequency and ‘jet’ colormap was used to plot the colorbar using Scalar Mappable class.

2.7. Frequency analysis of mutations in SARS-CoV-2 spike glycoprotein corresponding to the mutated mAb epitope residues

Data from the GISAID hCoV-19 spike glycoprotein mutation surveillance dashboard was obtained for all spike protein variations in SARS-CoV-2. A total of 81,79,987 SARS-CoV-2 spike sequences (updated on February 19, 2022, by Raphael Tze Chuen Lee, GISAID) were compared to the reference sequence EPI_ISL_402124 for the annotation of individual mutations (mutation data obtained from GISAID). Among the spike glycoprotein mutations, the residues that are mAb binding sites as well as mutated in VOCs were analysed for naturally occurring mutations. The occurrence reported for each such mutation was divided by the total number of sequences (n=8179987) and converted into percentage. The mutations occurring at these particular residues with a frequency greater than 0.01% were selected for plotting. A stacked plot was generated with series in ascending order of frequency percentage values. Y-axis represents the frequency in logarithmic scale while X-axis represents the mutated mAb binding sites in the spike glycoprotein.

2.8. Structural annotation of mAb epitopes in SARS-CoV-2 spike glycoprotein

The Protein Data Bank (PDB) structure of SARS-CoV-2 S-ACE2 complex (7DF4) was used to annotate the epitope residues of SARS-CoV-2 neutralizing mAbs. Independent mAb binding residues tabulated in an excel sheet were used for highlighting the mAb binding residues. All the non-mutated mAb binding residues on SARS-CoV-2 spike were depicted in yellow surface representation. The mAb binding residues that are mutated in the SARS-CoV-2 VOCs were marked in sphere representation in red color while the regions excluding all the epitope residues were colored in grey.

2.9. Analysis of Kd and IC50 value of monoclonal antibodies against SARS-CoV-2 spike variants

The Kd values of the 74 monoclonal antibodies which are available publicly in research articles were collected and was tabulated against the wildtype as well spike variants. Similarly, the IC₅₀ values were also tabulated from the available in vitro studies from different research articles and was compared with that of wild type or other SARS-CoV-2 spike variants. The variations in the Kd value as well as IC₅₀ was denoted with red color which denotes the reduction in binding affinity as well as the efficacy of antibody neutralization.

2.10. Statistical analysis

A multiple variable two tailed correlation analysis was performed using GraphPad Prism 5. Pearson correlation coefficient was analysed and statistical significance was annotated for p value less than 0.05. The fold change in susceptibility of SARS-CoV-2 to mAbs were based on the coronavirus antiviral & resistance database (<https://covdb.stanford.edu/susceptibility-data/table-mab-susc/>) [17]. The presence of a mutation is marked as 1 and 0 represents no mutation.

Results and Discussion

3.1. Emergence of SARS-CoV-2 variants and Spike mutations

Since the first emergence of SARS-CoV-2 in 2019 [1], the virus spread globally with minimal mutations in the spike protein until the end of 2020. From late 2020, Alpha, Beta and Gamma variants dominated over the wild type SARS-CoV-2. By the mid and late 2021, Delta and Omicron subtypes dominated Alpha, Beta and Gamma variants as shown in figure 1A and B. Until November 2021, the Delta variant was considered

highly transmissible and pathogenic. In contrast, by the end of 2021, a new variant (Omicron) with high mutations emerged in South Africa [18] with increased transmissibility and modest pathogenicity (Figure 1A and B). The number of SARS-CoV-2 cases rapidly increased after the emergence of the Omicron variant which gradually transmitted across different countries within a short time span.

The random accumulation of mutations gradually increased in different lineages of SARS-CoV-2 variants due to the rapid transmission and disease severity (Figure 2 A and B). The variants reported early (Alpha, Beta, Gamma and Delta) had minimal spike mutations (8-12 aa). However, the recent Omicron variant shows the highest (>30 aa) mutations across the spike protein (Figure 2A and B). These variants might have co-evolved with host adaptation and selective immune pressure, raising the chances of having antibody escape variants [7,12,13].

3.2. Epitope map of SARS-CoV-2 neutralizing monoclonal antibodies

In order to understand the chances of antibody escape phenotype which is mediated by the naturally occurring mutations at the antibody binding epitopes, we selected 74 monoclonal antibodies capable of neutralizing SARS-CoV-2 and its variants. These mAbs have varying levels of neutralization efficacy ranging from modest to highly potent and their binding sites were characterized by structural studies. The antibody binding epitopes of these mAbs span the S1 domain of the spike and the details of the epitopes used in this study are shown in Figure 3 and Supplementary Table 1. We found 65 out of 74 mAbs having epitopes localized to the receptor-binding domain (RBD), whereas few (9 out of 74) are targeting the N-terminal domain (NTD) (Figure 4A). Moreover, most of the potent neutralizing antibodies were mapped to receptor binding motif (RBM), while the NTD targeting antibodies had less neutralizing efficacy than RBD [11].

Next, we analyzed the distribution of epitopes within the S1 domain and found that NTD or RBD neutralizing epitopes overlap with each other (Figure 4A). The NTD directed mAbs target the different loops within S1 (annotated as N1, N2, N3 and N4) and share the epitope sites at N1, N3 and N4. In contrast, one of the selected mAb 2-17 has an additional recognition site at N2 (Figure 4B). Similarly, majority of the RBD binding mAbs recognise spike RBM within the RBD (Figure 4B). These data suggest that both NTD and RBD binding mAbs share conserved recognition region/s independently within spike protein similar to other viruses including HIV, Influenza virus and Middle East respiratory syndrome (MERS) coronavirus, wherein the mAbs targets common conserved recognition sites [19-21].

3.4. Spike mutations and its role in emergence of antibody escape phenotypes

The mutations at the antibody binding sites and their conservation within the NTD or RBD raises the chances of escape from neutralizing antibodies through naturally occurring spike mutations. Here, we found several mutations in the antibody binding sites in either NTD or RBD with at least one variation in either of the analyzed lineages (Alpha, Beta, Gamma, Delta or Omicron). Notably, the Omicron variant (BA.1 and BA.2) showed the highest mutations (20 aa and 18 aa respectively) at different mAb binding epitopes (Figure 4A). These data are consistent with the recent experimental evidence which suggests the decreased neutralization efficacy of few monoclonal antibodies against the Omicron variant [22]. Similarly, majority of the antibody binding epitopes are highly mutated in different SARS-CoV-2 variants. The highest mutations were found in the epitopes of highly neutralizing mAbs (4-10 aa changes) whereas, less neutralizing antibodies were not affected by mutations in either of the variants. Interestingly, 5 out of 74 mAb binding sites had no mutations in the SARS-CoV-2 variants, but these mAbs have less neutralization potential, which predicts the chances of a natural selection based on antibody neutralization of SARS-CoV-2 (Figure 4A). In addition, analysis of the K_d (dissociation constant) and IC₅₀ values of the mAbs suggest a strong binding affinity to wildtype spike protein. However, the mAb binding affinity has decreased with respect to the spike variants of SARS-CoV-2 and the IC₅₀ values have increased which shows the potential chances of escape from neutralizing antibodies (Supplementary Table 2). The decrease in affinity of mAbs towards the spike variants is clearly demonstrated by the increased K_d and IC₅₀ values of mAbs including the well characterized REGN10933, LY-CoV555, LY-CoV016, and REGN10987. The data suggest that consequences in host adaptation along with other factors including immune pressure might trigger the chances of emergence of antibody escape

variants with increased tropism and transmissibility.

3.5. Frequency of SARS-CoV-2 spike neutralizing epitopes with respect to variants

Next, we calculated the frequency of mAb binding sites and observed that residues E484 and Q493 has more than 40% frequency (with respect to the 74 mAbs) compared to other antibody binding sites (Figure 5A). The occurrence of E484 and Q493 in majority of the mAb binding sites may drastically affect the neutralization potential of diverse mAbs. Surprisingly, Beta, Gamma, and Omicron (BA.1 and BA.2) variants showed mutations at E484 residue. In addition, the Omicron variants showed mutations at Q493 residue and other epitope residues (Figure 5A) which might help the variant to escape antibody neutralization. In order to test the correlation of the spike RBD residue mutation with respect to mAbs efficacy, we performed a correlation analysis with fold change values of mAbs retrieved from the coronavirus antiviral & resistance database (<https://covdb.stanford.edu/susceptibility-data/table-mab-susc/>). We found that RBD residues including G339, S371, S373, S375, T376, R408, K417, N440, G446, S447, E484, Q493, G496, Q498, Y505, T547 were statistically more significant ($p < 0.0001$) with respect to multiple mAbs (Supplementary table 3). These RBD residues might play the key role in the fold change values of the mAbs with respect to the SARS-CoV-2 variants. The mutational profile associated with each mAbs vary with similar mutations as well as diverse mutations contributing to the fold change. The fold change of mAb LyCoV555 was correlated with mutations at G339, S373, S375, K417, L452, S477, E484, Q493, Q498, Y505. Similarly, residues G339, S373, S375, T376, R408, K417, S477, E484, Q493, Q498, N501, Y505 were related to the fold change of LyCoV016. The combination of LyCoV555 and LyCoV016 also was correlated with the similar mutations with major significance contributed by G339, S373, S375, K417, S477, E484, Q493, Q498, N501, Y505. Amino acid residues G339, S373, S375, S477, Q493, Q498, Y505 were found to be highly significant with respect to mAbs including COV2-2196, CT-P59, P2C-1F11, C135 and combination of REGN10987 and REGN10933. Residue R408 was significant in the case of mAbs CoV2-2196 and S309. Similarly, N440 and G446 were highly significant with respect to mAbs C135 and REGN10987 respectively. Moreover, G496 and T547 were both significant for mAbs REGN10933 and REGN1097. Altogether a mutational hotspot spanning the RBD region was identified to be statistically significant which correlated with our bioinformatic analysis as well. The analysis was performed based on the presence or absence of a mutation in a variant with respect to the mAbs which resulted in few residues showing significance outside the RBD region. These residues within the spike outside the RBD may or may not be involved in increasing the fold change of mAbs. Point mutation of the non RBD regions are necessary to conclude their role in the fold changes of mAbs and experimental evidence is necessary to validate the findings made from the study which can pinpoint the exact mutational hotspot.

Further, we represented the frequency of different amino acid changes at the epitope sites (Figure 5B) by using the SARS-CoV-2 spike protein mutation data from the GISAID database. The amino acid changes at the epitope sites are critical as they can lead to conformational changes in the epitope sites, leading to an antibody escape phenotype. The changes in amino acid residues with similar properties may not affect the spike recognition by mAbs thereby providing a potential neutralization against SARS-CoV-2 variants. However, with the emergence of new variants, notably Delta and Omicron, there is a gradual increase in the mutations at the epitope residues, affecting the mAb efficacy. There is a high probability of outbreaks associated with new variants of SARS-CoV-2 in the near future with more complicated mutations in the spike protein as seen in the Omicron variant. The conserved epitopes which are non-mutated in either of the lineages may serve as a checkpoint for designing novel vaccines, antibodies or therapeutics against current or future variants.

4. Conclusions

In summary, the mapping of mutations in the mAb binding sites in SARS-CoV-2 spike protein shows the conserved non-mutated epitope sites and the mutated epitope regions in different variants. The study will help better understand the chances of antibody evasion and will aid in designing novel vaccine candidates which target broad range of variants. The analysis is fundamental as new variants of concern with a combination of mutations from different lineages may evolve in the future, thereby affecting the efficacy of available

vaccines. Generation of a broadly reactive vaccine candidate which can elicit both humoral and cellular immune responses might help in targeting multiple current or future SARS-CoV-2 variants.

Author Contributions: V.S.R. designed and coordinated the study. J.J., S.D., and G.K conducted the analysis. S.D and G.K wrote the custom python scripts and J.J, S.D, and G.K performed the epitope mapping and variant annotations. All authors contributed to the interpretations and conclusions presented. J.J, and V.S.R. wrote the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interests.

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Figure Legends

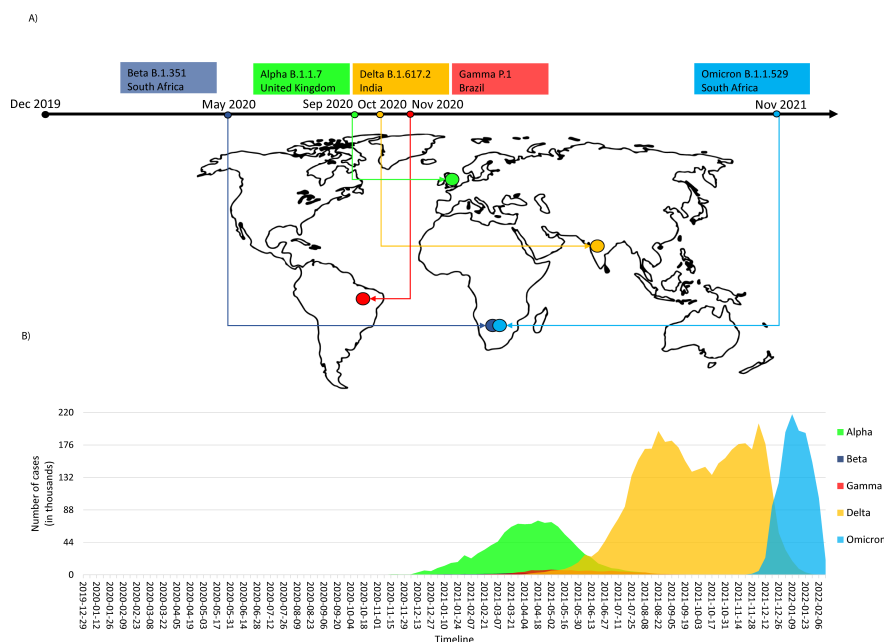
FIGURE 1 : Geographic mapping of SARS-CoV-2 outbreak and its timeline of infection. A) SARS-CoV-2 variants of concern (VOC) as classified by the World Health Organization (WHO) and their first reported case represented on a world map. B) Global timeline of SARS-CoV-2 cases in thousands wherein weekly cases of SARS-CoV-2 VOC as obtained from the GISAID database from December 2019 to February 2022

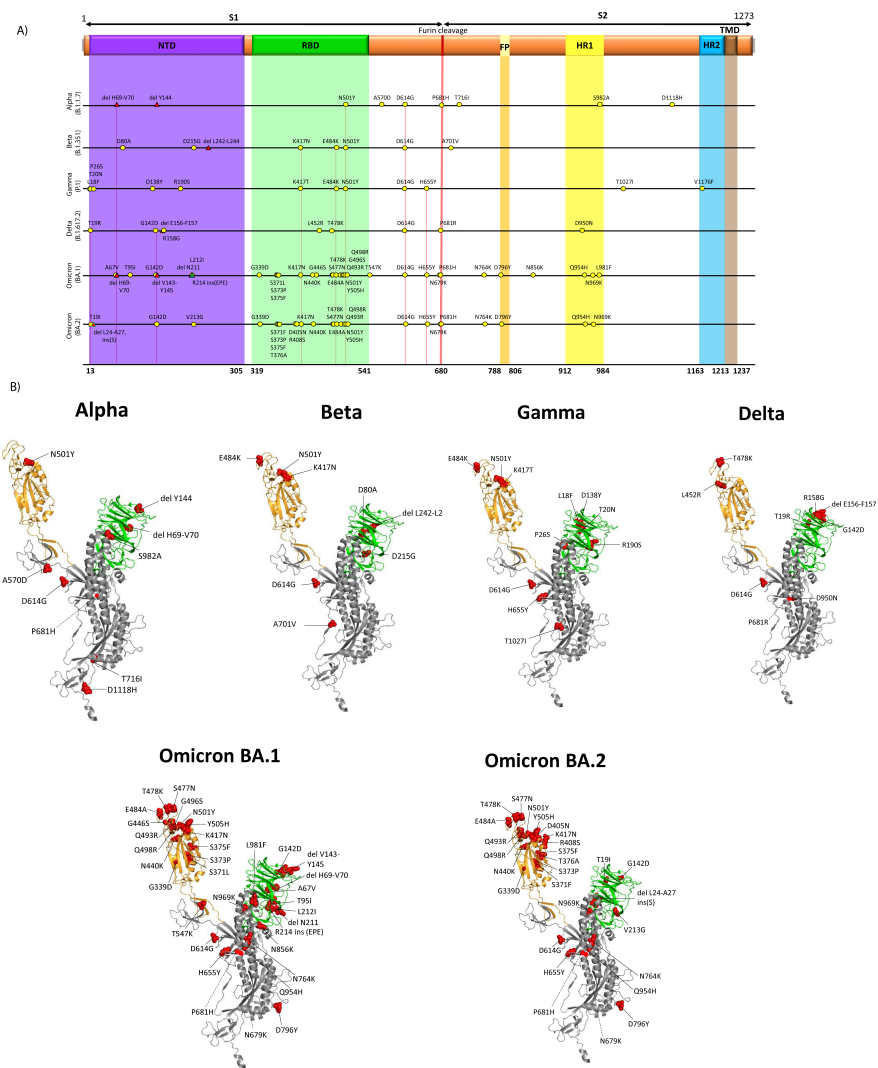
FIGURE 2 : Schematic and structural annotation of SARS-CoV-2 spike glycoprotein variants. A) Mutations in each of the VOC- Alpha, Beta, Gamma, Delta and Omicron mapped on the SARS-CoV-2 spike gene. Substitutions are represented with yellow circles, deletions with red triangles and insertions with green triangles. Common mutations to multiple VOC have been connected through a standard line at that residue position. NTD: N-terminal domain, RBD: receptor binding domain, FP: fusion peptide, HR1: heptad repeat sequence 1, HR2: heptad repeat sequence 2, TMD: transmembrane domain. B) Mutations involved in each of the VOC of SARS-CoV-2 mapped on spike monomer. Mutated residues are depicted in red spherical representation and spike monomer shown in cartoon representation (PDB: 7df4). RBD in open conformation is depicted in orange and NTD in green. Epitopes that could not be marked in the PDB structure are marked with a dashed line and red spot at the location of the neighboring residues. V1176F mutation in the Gamma variant has not been shown since it belongs to the stalk region of spike which was not available in the spike PDB structure of 7df4.

FIGURE 3 : Heat map of epitope residues of monoclonal antibodies. The epitope residues of the analysed mAbs are plotted as a heat map wherein the highest intensity colour is denoting the most frequent epitope site followed by the decreasing the colour gradient which depicts the less frequent epitope sites.

FIGURE 4 : Epitope mapping of monoclonal antibodies targeting SARS-CoV-2 spike. Mapping of epitopes of 74 experimentally validated mAbs against SARS-CoV-2 variants. The mutated epitope sites with respect to the Alpha, Beta, Gamma, Delta and Omicron variants are annotated as red, whereas the non-mutated sites are shown in green. The antibodies selected for the analysis are depicted on the y axis. The mutated epitope regions within the spike N-terminal domain are subdivided into N1, N2, N3 and N4 and the receptor-binding domain (RBD) is sub annotated as receptor binding motif (RBM) within RBD.

FIGURE 5 : Frequency of mutated epitopes and the amino acid changes at the epitope sites. A). The frequency of mutated epitopes among the 74 mAbs was analyzed based on the region of occurrence and is shown as colormap with different frequencies. B) The percentage of amino acid variations at the epitope site among all reported SARS-CoV-2 variants were mapped and shown as a bar graph with different colors depicting each amino acid change in a series. In addition, the mutated epitope regions within the spike protein are denoted structurally and are shown as residues labeled in red. In contrast, the complete epitope recognition sites are marked as yellow. Some of the epitope recognition sites (Amino acid residues- 12, 13,14,15,74,75,76,248,249,250,251,252,253,254) which are non-mutated couldn't be marked as yellow as these were not depicted in the structure.





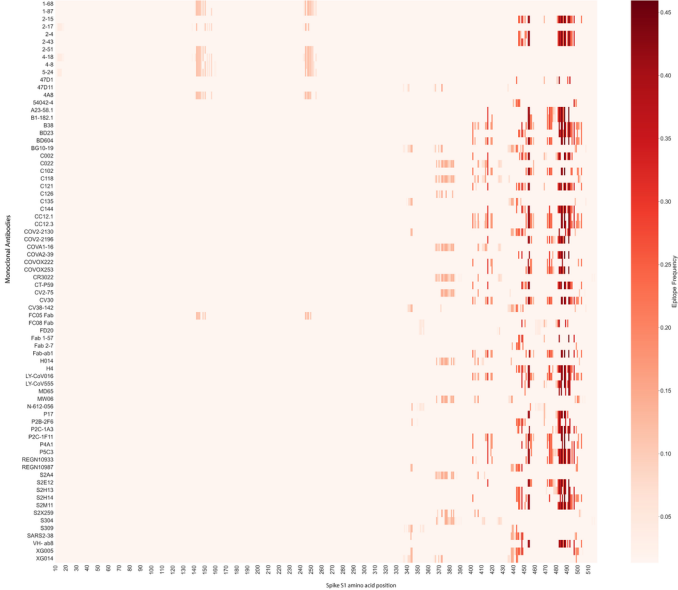


Figure 4

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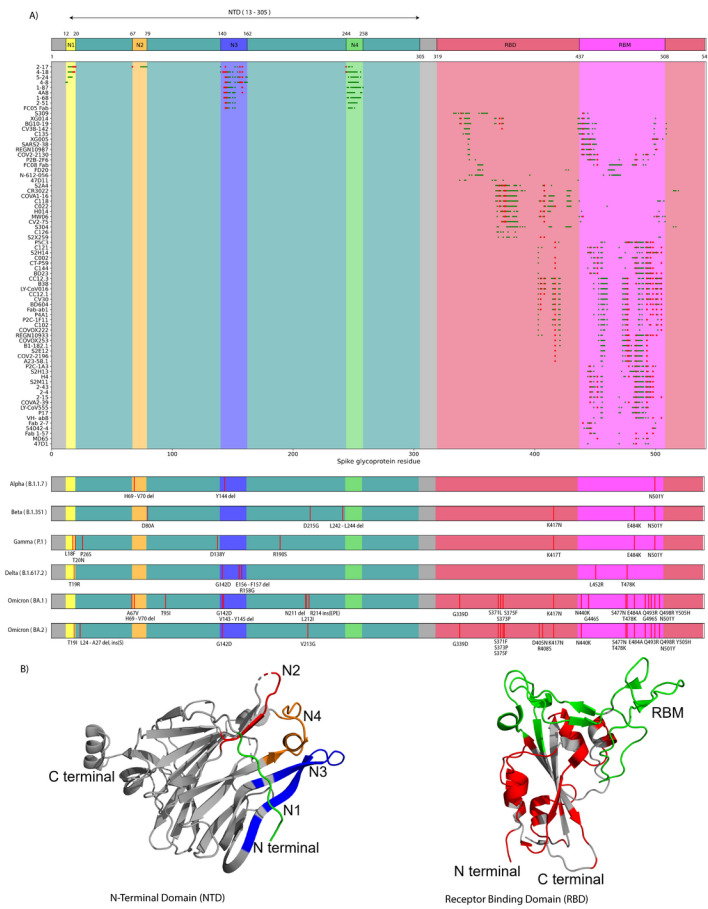


Figure 4

