Review Article

Structural Insights into the Dynamics of the SARS-CoV-2 Receptor Binding Domain

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Abstract |The spike protein of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is crucial for host cell entrance and host specificity, making it a significant target for vaccine and treatment development. In reaction to immunological pressure and adaptive selection, the S protein has undergone significant evolution throughout the COVID-19 pandemic. The majority of these alterations were attributed to the receptor binding domain (RBD) of the S protein, resulting in modifications to viral characteristics, including transmission dynamics and immune evasion from vaccinations and antiviral treatments. The SARS-CoV-2 virus is always evolving in reaction to extraordinary difficulties, allowing it to flourish and altering its core structure. This short review seeks to delineate the evolutionary and structural challenges that have transformed the characteristics of SARS-CoV-2, in relation to the high-resolution data concerning the functional and structural specifics of the receptor-binding domain of the spike proteins. The findings illuminate the adaptability and evolution of the RBD, providing essential insights for the development of innovative vaccines and next-generation antivirals aimed at persistent and developing forms of SARS-CoV-2.

Received | February 09, 2025; **Accepted** | March 23, 2025;**Published** | April 25, 2025
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Citation | Israr, M., R. Naseem, L. Rasheed, S. Abbas, A. Taqveem, A. Fatima, A. John and A. Haider. 2025. Structural Insights into the Dynamics of the SARS-CoV-2 Receptor Binding Domain. Hosts and Viruses. 12: 111-122.
DOI | https://dx.doi.org/10.17582/journal.hv/2025/12.111.122
Keywords| SARS-CoV-2, Structural proteins, Spike protein

Introduction

The pathogen responsible for COVID-19, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), possesses a single-stranded, positive-sense, non-segmented RNA genome. The genomic structure of SARS-CoV-2 comprises around 30,000 nucleotides and contains at least twelve open reading frames (ORFs) [1]. The longest open reading frame (ORF) is ORF1ab, which encompasses approximately two-thirds of the SARS-CoV-2 genome, and following its production, it is cleaved into 16 polyproteins referred to as nonstructural proteins. Essential structural viral proteins comprise the spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N). These proteins serve essential roles as viral structural components and are also involved in the replication and pathogenesis of SARS-CoV-2. The S protein is omnipresent across all variants and is crucial for the development of vaccines and treatments [2].

The full-length S protein (1273 aa) consists of subunits of N-terminal S1 (1-685 aa) and C-terminal S2 (686-1273 aa) (**Figure 1A**). By coordinating S1 and S2, S protein executes an important restructuring step as it transitions between pre and postfusion configurations and allows for the merging of viral and host cell membranes [3]. Interacting with a host cell receptor is the responsibility of S1, and S2 aids the fusion of viral and cellular membranes [4]. The S1 subdomain displays a motion analogous to a hinge which permits it to achieve the two states of "down" and "up." While the receptor's "down" posture is not accessible prior to fusion, the "up" posture is accessible for binding to host ACE2 and has available antibody epitopes [5]. The S1 subunit contains the N-terminal domain (NTD), receptor-binding domain (RBD), subdomain 1 (SD1), and subdomain 2 (SD2). The S1 portion from SARS-CoV-2 and SARS-CoV has a considerable degree of resemblance in the architecture of domains. The major difference is in direction of RBDs tilt downwards. When the SARS-CoV RBDs are in its downward conformation, they are situated at a compact angle to the central cavity of the trimer and are closely compressed against the NTD of the neighboring protomer [6]. These strands are designated as β2 (residues 376-379), β1 (residues 354-358), β6 (residues 492-494), β4 (residues 432-437), β3 (residues 394-403), β5 (residues 452-454) and β7 (residues 507-516). The five strands, namely β2, β1, β4, β3, and β7, are arranged in an antiparallel orientation relative to each other.

The structural analysis of different components of the S protein, especially RBD, has highlighted rhythmical and sequential arrangements to facilitate viral entry into the host which are further attributed to host proteases including transmembrane serine protease 2 (TMPRSS2) among others [7]. Since the emergence of COVID-19, SARS-COV-2 has acquired numerous mutations throughout the length of the S protein, impacting viral pathobiology, virulence, and transmission. The purpose of this review is to critically evaluate contemporary mutations in the receptor binding domains of the S protein over the evolutionary journey of SARS-COV-2. Due to the decisive roles of the RBD of the S protein in the development of novel vaccines and the design of effective therapeutics, understanding the molecular evolutionary dynamics will enhance our potential to formulate more effective control strategies for COVID-19.

Critical Roles of RBD in the Entry Mechanisms of SARS-CoV-2

To initiate an effective replication cycle, SARS-COV-2 uses homotrimeric S glycoprotein homotrimeric RBD and enters cells expressing ACE2. Binding between RBD and ACE2 initiates a sequence of activities that ultimately result in the fusion of the cell membrane with the viral membrane, thus facilitating the entry of the virus into the cell. This RBD:ACE2 interaction determines the host range, cell tropism, and contributes to the transmission potential of SARS-COV-2 [8]. Structural analysis of the S protein of SARS-CoV-2 revealed that RBD was not adequately represented: notably, the receptor-binding motif (RBM) with which ACE2 interacts directly [9]. Computer-based simulations of the interaction between ACE2 and the SARS-CoV-2 RBD have identified many residues that may contribute to contact (Figure 1B). However, the exact residues responsible for mediating this interaction remain unidentified. The findings indicated that the SARS-CoV-2 and SARS-CoV RBDs exhibit distinct variations in both their genetic makeup and physical arrangement. Previous cryo-electron microscopy experiments have shown that the SARS-CoV spike protein interacts with the ACE2 cell receptor, leading to the dissociation of S1 from ACE2 [7]. This separation initiates the change of S2 from a metastable prefusion state to a more stable postfusion state, which is

crucial for membrane fusion. Recent findings indicate that ACE2 is crucial for facilitating the entrance of SARS-CoV-2. Hela cells that express ACE2 exhibit increased sensitivity to SARS-CoV-2 infection relative to ACE2-negative Hela cells. The interaction between ACE2 and SARS-CoV-2 RBD has been measured in a controlled environment, demonstrating a significant affinity in the low nanomolar range. This discovery further substantiates that RBD is essential for the interaction between SARS-CoV-2 and ACE2 [10]. The effective entry of SARS-CoV into target cells relies on the initial binding of the receptor-binding domain (RBD) to the ACE2 receptor.

Structural Characteristics and Evolutionary Triggers for Mutations in the RBD of the S Protein

Throughout the SARS-COV-2 pandemics, a range of mutations were observed in multiple proteins; however, the dynamic evolution in S proteins remained the primary concern for vaccine escape and increased transmission and pathogenicity. The primary inherent factor that influenced the evolution was the genetic nature of SARS-COV-2. As an RNA virus, SARS-COV-2 showed a high vulnerability to replication-induced mutations with a rate of approximately $1x10^{-3}$ nucleotides per site per year [11]. This error-prone replication, even under the proofreading capability of the replication complex, improved the transmissibility of the virus and promoted its successful adaptation to the host, which was further compounded by positive selection-driven host selection [2]. Due to the high transmissibility and infection of the population on an international scale, a high level of immune pressure induced by infection or vaccination triggered mutations that evaded the immune system further empower the evolutionary capabilities of SARS-COV-2. Collectively, rapid mutation rates, immune pressure, and widespread infection resulted in high evolution within the S protein, especially the evolution observed within the short RBD, which has been extraordinary and highlights the plasticity and dynamic of viral evolution in suspensible hosts.

Analysis of the S protein has highlighted strong positive selection, especially around the S1 region. This substantiative positive selection was not only restricted to SARS-COV-2 but has been observed across all classes of coronaviruses identified [12]. Although mutations across the length of the S protein have an adaptive advantage, mutations in the RBD can enhance molecular interaction with receptors through epistatic changes. Evolutions observed within the S protein, even with the exception of RBD, have been shown to impact the RBD interaction in the ACE-accessible state [13]. A range of mutations with meaningful impact on viral phenotypes include residues 439, 483 and 493 within the RBD domain of the S protein. Furthermore, the residues under positive selection were expanded to positions 483, 484, 490, 493, and 494 with the RBD region of the S protein [14]. Of more than 100 mutations observed within the S protein, at least 20 amino acids (e.g. T372A, L452R, S477N, E484K, F486P, N501Y and D614G) have shown increased transmission, while 19 of these amino acids (EG K417N, G446S, L455F, L455S, F456L, E484A, F486V, G496S, Q498R and Y505H) contributed to the reduction in SARS-COV-2 transmission (Figure 1C).

Three strains had been rapidly spreading after their identification: one originating from the United Kingdom with a spike mutation of N501Y; another from South Africa with three simultaneous mutations: E484K, N501Y and K417N; and finally, a strain from Brazil with mutations N501Y, E484K and K417T [15]. Asparagine and tyrosine have the potential to establish hydrogen bonds with Tyr-41 of ACE2. Consequently, from a structural perspective, it is improbable that the N501Y mutation would have any effect on enhancing the stability of the complex. A potential π-π stacking interaction involving Tyr-41 could arise due to the replacement of tyrosine [16]. The *in silico* binding energy study revealed that the N501Y mutant and the wild-type spike exhibit identical overall binding energies, considering the differential interaction of ACE2, the N501Y mutation may have use for this particular strain [17]. However, recent computational investigations have shown that the binding energy interactions between ACE2 and spike RBD strains from Brazil and Africa, which have triple mutations, are much greater than those seen in the wild type (WT) spike [18]. The South African variant of the virus has hydrogen bonds between Lys-484, Tyr-501, and Asn-417, and the ACE2 residues Glu-75, Glu-35, and Glu-30. Lys-484 generates an extra salt bridge when it is linked to Glu-35 of ACE2. Furthermore, Glu-35 and Lys-353 of ACE2 in the Brazilian variant formed hydrogen bonds with Lys-484 and Tyr-501, respectively. Both variations exhibited a higher concentration of electrostatic contacts compared to the wild-type spike protein when exposed to ACE2. Despite the presence of the E484K mutant spike, the outcome remained unchanged.

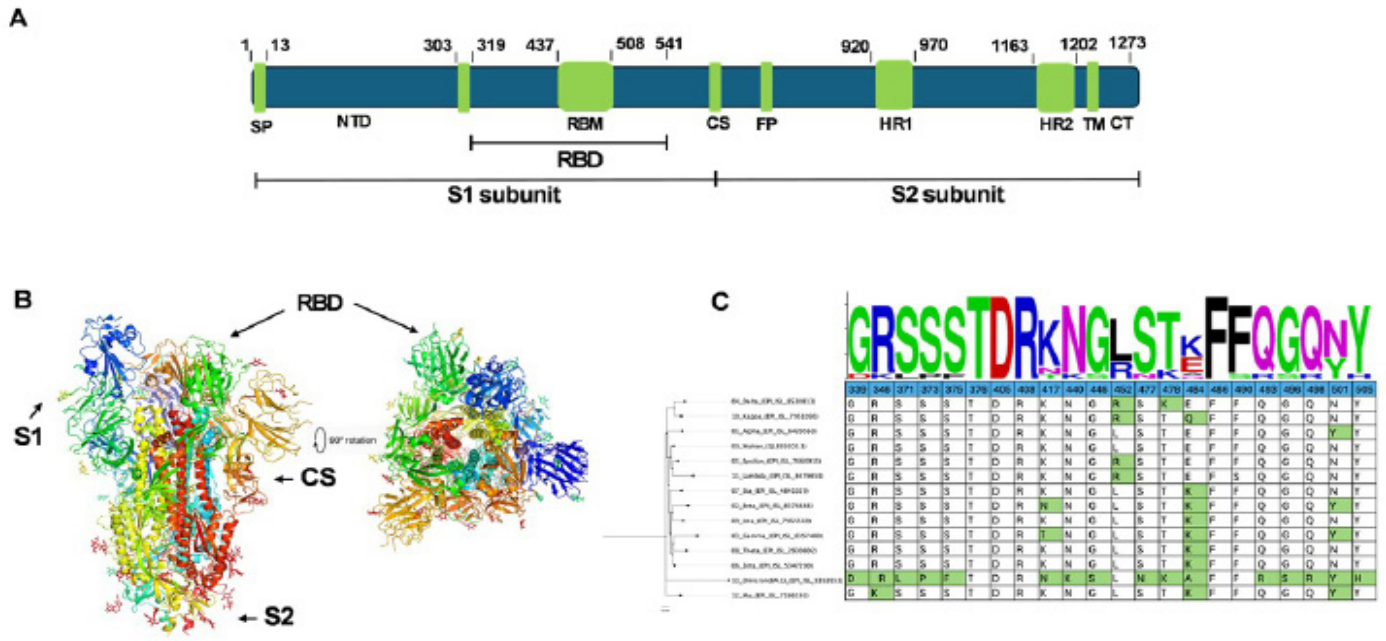

Figure 1: *Structural architecture of the S protein of SARS-COV-2 and RBD conservation among main variants of concerns across the COVID-19 pandemic. (***A***) The S protein is divided into two subunits called S1 and S2 at the cleavage site (CS). The signal peptide (SP), N-terminal domain (NTD) and the receptor biding domain (RBD) are located within the C-terminus of the S1 subunit. The active receptor binding motif within the RBD is highlighted. The fusion peptide (FP), heptad repeats (HR) 1 and 2, and transmembrane (TM) and cytoplasmic tail (CT) are located within the S2 subunit. Amino-acid position relative to Wuhan S protein is marked at the top of the schematic illustration. (***B***) The structure of the S protein (PDB ID: 6XR8) is shown where S1, S2 and CS are labelled both in the vertical and top view confirmation. (***C***) Conservation and evolutionary changes in the RBD residues that interact with ACE2 in major variants of concern since the inception of the pandemic. The phylogenetic tree was constructed based on the full S protein in the Genious Prime and visualized in Figure Tree. The conservation of residues in all variants was drawn using Web Logo.*

S477N and N439K are prevalent mutations seen in the spike protein of SARS-CoV-2. The absence of Asn-439 in the wild-type spike does not affect the interaction with ACE2, since it forms hydrogen bonds within the spike protein with Spike Ser-443 and Pro-499. However, a mutation in the lysine residue would lead to complex stabilization due to electrostatic contact with Glu-329 of ACE2. The S447N mutation, found in a spike surface loop, is also expected to have a similar impact. In its natural form, Ser-447 does not have any interaction with ACE2. However, when asparagine is present, an additional hydrogen bond may be created between Ser-447 and ACE2 Ser-19. The stability of the interaction with ACE2 was facilitated by the presence of both mutations [19].

In October 2021, the Spike Q493R escape mutation was detected in a COVID patient who had previously received antibody treatment [20]. This specific mutation has caused the virus to become resistant to the monoclonal antibody cocktail medications Bamlanivimab and Etesevimab. The ACE2 receptor Glu-35 establishes a hydrogen bond with the interfacial residue Gln-493 [17]. To accommodate the positively charged arginine side chain in a pocket on the surface of ACE2 formed by Asp-30, His-34 and Glu-35, the Q493R mutation would be required. This mutation would greatly improve the stability of the bond. *In silico* molecular dynamics simulations indicate that the Q493R mutation facilitates the creation of two additional salt bridges between ACE2 and Glu-35 (which enhances stability) and Asp-38. These salt bridges are not seen in the wild-type spike. However, the model also showed a detrimental interaction between Lys-353 and a disruption of contact with ACE2 Lys-31. Similarly, Q498R enhances the binding affinity to ACE2. Here, an extra salt bridge was formed using Asp-38. ACE2-Tyr-41 and Gln-42 exhibited a higher level of interaction compared to the WT spike [21] (Figure 1C).

Receptor ACE2 exhibits high binding affinity to mu-

tant spike variations located in solvent exposed loop regions, specifically in N440K and G476S. Both of the aforementioned mutations cause significant structural changes, with the RBM loop relocating to a position closer to the binding surface which results in a larger number of contacts being formed than what was possible with the spike in its original position. G446S and Y505H are examples of harmful mutations that possess high affinity for binding to ACE2 while at the same time causing RBD to become destabilized [22]. Yajima et al. (2024) offer cryo-EM data demonstrating an ACE2-bound, down-RBD conformation which occurs prior to the RBD-up posture. This structural observation suggests that during the binding of ACE2, an RBD has a considerable amount of mobility, transitioning from an all-down to one or more RBD-up regions. This intermediate conformation is critical in switching to the fully active RBD-up structure that is bound to ACE2 and emphasizes how astonishing the spike protein is. The analysis is focused on the spike protein K356T mutation of the BA.2.86 variant. This particular mutation has a remarkable impact on both the degree of infectivity and the degree of immune evasion. Yajima et al. (2024) suggest that the K356T change, together with some other changes, enables the BA.2.86 variant to more efficiently neutralize antibodies that are supposed to block it, thus improving its fitness and resistance to immune response. This is especially pertinent for the viral SARS-CoV-2 evolution. The data of Yajima et al. (2024) demonstrates the structural studies of the SARS-CoV-2 virus are indeed pivotal. The substitution of K356T with other changes and finding of the intermediate confromation of RBD are remarkable in understanding how the virus deals with immune selection pressure and furthers its evolution. These studies have a profound impact on how to deal with the virus and its emerging mutants [23].

Critical Mutations in RBD since the Inception of Pandemic

To investigate the structural effects of these amino acid alterations, the RCSB protein data bank (https://www.rcsb.org) was used as a source for the S trimer or RBD-hACE2 complexes. Using publicly available X-ray crystallography and cryo-electron microscopy (cryo-EM) structures as templates, we conducted homology modelling via the SWISS-MODEL service (https://swissmodel.expasy.org). With the help of Py MOL software, we were able to examine and visualise the molecular interactions of mutants [24].

Impact of RBD mutations at 371-373 on SARS-COV-2

Proteins featuring the N-X-T/S pattern commence with asparagine, followed by an amino acid distinct from proline, and subsequently threonine or serine. Upon recognition of an N-X-T/S motif, the cellular machinery may conjugate a carbohydrate moiety to the N residue (**Figure 1C**). The process is referred to as nested glycosylation. Three amino acid residues, N370, S371, and T372, in the S protein exhibit a pattern designated as N-X-T/S in many bat-associated coronaviruses closely related to SARS-CoV-2. In accordance with this pattern, a sugar molecule is affixed to the N370 residue of the S protein, a process referred to as glycosylation. The closed conformation of the RBD is stabilized by this glycosylation [25]. Moreover, bat coronaviruses exhibit resistance to trypsin digestion at pH 5.5 owing to the N-linked glycosylation of N370 in the spike protein. This provides selective benefits in preserving viral stability during fecal-oral transmission [26]. A specific amino acid modification transpired in the S protein of SARS-CoV-2 during the initial animal-to-human transmission. A mutation converting the ACU/ACC codon to GCA is detected in nearly all analyzed SARS-CoV-2 genomes [27]. The glycan binding cleft on the RBD is liberated when the T372A mutation in SARS-CoV-2 disrupts the N-X-T/S motif and abolishes the N-linked glycosylation at N370. This modification enables SARS-CoV-2 to bind more efficiently to hACE2, enhancing its interaction with carbohydrate molecules on the host cell surface. Consequently, SARS-CoV-2 exhibits significantly enhanced infectivity towards human cells when possessing the T372A mutation. In general, the study indicates that bat coronaviruses have a T372 residue that contributes to the stability of the virus during fecal oral transmission, whereas SARS-CoV-2 contains an A372 residue, enhancing transmission via aerosols.

Impact of the mutation 452 mutation on vaccine escape

Both Delta variation and Omicron BA.5 lineages include the S protein mutation L452R, which is associated with increased infectiousness and reduced ability to be neutralized by antibodies [28] [29] (Figure 1C). The L452R mutation interacts with a negatively charged area on hACE2, including E35, E37, and D38, through electrostatic interactions. Variants with this mutation148 exhibit increased infectiousness due

in part to this interaction. Furthermore, the L452R mutation causes an electrically charged region on the surface of the virus, which influences the strength of the link with monoclonal antibodies and allows the virus to evade detection by the immune system [30]. The L452R mutation improves the interaction with hACE2 and aids immune system evasion. Experts contend that the L452Q mutation observed in the Lambda variants has the same biological effects as the L452R mutation [31].

Mutations that Impact RBD: ACE2 Interaction

S477N mutations

The S477N mutation present in the S protein of every Omicron sublineage enhances the hACE2 binding of RBD to hACE2170 by introducing two more hydrogen bonds with S19 of hACE2. Moreover, the S477N mutation exhibits resistance to a number of monoclonal antibodies of different types [32]**.** In particular, the Omicron variant exhibits immune evasion and weakened hACE2 binding due to the alterations K417N, G446S, E484A, G496S, and Y505H [29] [33]. One S477N substitution might account for the lowered binding affinity from these previously discussed mutations (Figure 1C).

Mutations E484K and E484A

A fewer percentage of Alpha strains and E484K mutations are present in Beta and Gama lineages. For hACE2, it amplifies the RBD's allure [34]. The fact that it becomes much more resistant to antibody neutralization when coupled with the N501Y mutation further suggests that it contributes to immunological evasion [35] [36]. The local rearrangement caused by the E484K mutation enhances the hydrogen connection between the S protein's Q493 and H34 of hACE2. As a result, K31 of hACE2 and Y489 of the S protein form a cation-π connection as a result. Furthermore, the E484K mutation positions and aligns the positively charged amino acid K484 with the similarly charged amino acid K31 of hACE2. With favourable results, this can mitigate electrostatic repulsion between K31148 and K484. An increased binding affinity of RBD for hACE2 has been associated with the E484K mutation (Figure 1C).

Every lineage derived from the Omicron variety possesses the E484A mutation. Unlike E484K, E484A diminishes the binding affinity of the receptor binding domain to human angiotensin-converting enzyme [37]. The E484A mutation diminishes the cation-π interaction between Y489 of the S protein and K31 of hACE2, and disrupts the salt bridge between E484 and hACE2 K31. This results in a reduction of the binding affinity of hACE2. The E484A mutation significantly enhances the capacity to evade neutralizing antibodies. Omicron variants possessing the E484A mutation may have been favored due to a compromise between increased transmissibility and evasion of the immune system. The E484D mutation, absent in currently available variant strains, may augment the binding of the S RBD to TMEM106B. In light of this reality, it is essential to recognize that SARS-CoV-2 infection may transpire via a mechanism independent of hACE2-mediated viral entry [38].

L455F and F456L mutations

The "Flip" mutations, which consist of codons 455F and 456L, are found in a number of XBB sub lineages (Figure 1C). These include XBB.1.5.70, HK.3, JG.3, and JD.1.1. The capacity to propagate and evade the immune system is enhanced by these alterations. That is equal to 176/177 A smaller leucine side chain is responsible for the little impact of the F456L mutation on the RBD-hACE2 interaction. In general, the interaction has not altered much as expected. Furthermore, the affinity for RBD-hACE2 is independently and significantly decreased by the L455F mutation. On the other hand, H34 in hACE2 was repositioned due to the presence of both F456L and L455F, leading to the creation of two additional hydrogen bonds with RBD. As shown, this resulted in an increase in the binding affinity via an epistatic effect [39]. This SARS-CoV-2 RBD may have more evolutionary potential as a result of the interaction between the L455F and F456L mutations. This could allow the virus to change in a way that evades the immune system while still attaching strongly to receptors, like the A475V mutation in "Flip" variant strains such as JD.1.1.

The current strain JN.1, which is genetically distinct from the XBB lineages and descends from BA.2.86, is characterised by the L455S mutation, which is generally acknowledged as the distinguishing mutation in this strain. While decreasing the binding affinity to hACE2, the L455S mutation greatly improves immune evasion [40] [41]`. Further exploration is needed to understand the molecular ramifications of L455S in relation to hACE2 affinity and immune evasion that led to the establishment of the JN.1 dominant strain.

Mechanisms Of Immune Escape in Sars-co V-2 Variants Structural Modifications in the Spike Protein

The spike protein of SARS-Cov-2 is literally one of the main components of concern for the human immune system which tries to preemptively block the virus from getting into the human body. While the spike protein connects with the ACE2 receptor utilized by human cells, the RBD region is referred as antibody binding necleus for the neutralizing antibodies. Any change in the DNA sequence of the spike protein, particularly those which exist within the RBD, strongly diminishes the likelihood of the virus being detected and neutralized by the immune system. The Alpha, Beta, Delta, and Omicron variants of concern (VOCs) have different mutations in the spike protein that aid in immune system evasion. An excellent example is the RBD L452R mutation which augments the binding of the virus to ACE2, while simultaneously diminishing the binding of the neutralizing antibody to the virus. Other RBD mutations like E484K and N501Y were also shown to help the virus evade neutralizing antibodies, augment immune evasion [42, 43].

Additionally, immune evasion also seems to arise from the other immune evasion regions of the N-terminal domain (NTD) of the spike protein. Dramatic deletions in the NTD, such as in the ΔN135 variant, normally change the protein's antigenic contour leaving the antibodies with the NTD, unable to engage, in a more challenging predicament. The structural examination of the spike proteins of these variants indicate that such deletions have the ability to change the entire NTD supersite architecture which is extremely negative for the binding of antibodies [44, 45].

The Omicron strain has displayed considerable alterations in the RBD and NTD domains, which have furthered its ability to evade immune response. Research suggests that Omicron has numerous mutations throughout the spike protein, especially in areas important for antibody interaction, resulting in substantial loss of vaccine and monoclonal antibody treatment effectiveness. The spike protein of Omicron's greater mutation load permits it to at least partially circumvent humoral as well as cellular immunity, thus causing breakthrough infection in vaccinated persons [46].

Moreover, alterations not located within the RBD, like the D614G change, have been associated with improving stability of the spike protein, permitting greater viral penetration and facilitating immune evasion. Multiple studies have shown the D614G substitution increases the spike protein's conformational rigidity, which promotes infection and immune evasion. It has been established by structural studies that this mutation could potentially aid in maintaining the spike protein in a position that is optimal for ACE2 binding while also being suboptimal for neutralizing antibody action. [47, 48].

Impact on Immune Response and Vaccine Efficacy

The immune system's capacity to counteract SARS-CoV-2 infection is predominantly dependent on antibody production and T-cell function. Mutations in the virus's spike protein remain a significant problem for vaccine-induced immunity. Vaccine-escape variants with mutations in the spike protein, particularly in the RBD and NTD, are significantly more challenging for the immune system to identify, rendering vaccinations derived from earlier viral strains less efficacious.

The discovery of the Delta and Omicron forms represented the newest advancements in vaccine evasion, rendering vaccinations considerably less effective. For instance, mRNA vaccines, such as the Pfizer-BioNTech BNT162b2 and Moderna mRNA-1273, have demonstrated reduced efficacy against the Delta and Omicron variants compared to earlier forms. Despite these vaccines maintaining strong protection against severe sickness and hospitalization, their effectiveness in preventing infection has diminished due to changes in the spike protein [49].

A major discovery in vaccine efficiency research is the change of the RBD mutations which reduce the neutralizing antibodies binding affinity to the spike protein. This phenomenon has been particularly pronounced during the Omicron variant that has disfiguring mutations that cap the binding of the majority of monoclonal antibodies. As a result, there is an increasing necessity to modify the vaccines and design booster vaccines to these variants. The research data suggests that booster vaccines including the vaccine for the Omicron specific spike protein appear to enhance immune response and help in protection from infections and adverse outcomes [1].

Furthermore, vaccines targeting specific conserved regions of the spike protein, like the RBD, may be use-

ful against more recent variants. Some multivariant vaccines targeting Omicron and some of its sub-variants are focused on particular epitopes of the spike protein and have shown greater efficacy against many VOCs, including Omicron. These multivariant vaccines aim to provide greater protective efficacy against many variants of SARS-CoV-2 to mitigate immune escape [50].

Ongoing Challenges and Future Directions

It will be critical to keep track on the rise of new variants of the SARS-CoV-2 virus as it evolves, especially in assessing potential immune escape mechanisms. The emergence of Delta, Omicron, and its subvariants has also emphasised the importance of improving surveillance and modifying existing vaccines. In addition, nuanced approaches to vaccination may be necessary in the future if the evolving virus is to be effectively neutralised by broad immune responses, including neutralizing antibodies and T-cells.

Recent evidence suggests that T-cell centric vaccines might offer stronger and more effective protection against emerging variants of viruses. In addition, as T-cell responses are vital on viral replication, they are able to be more resistant to the surrounding mutations of the spike protein compared to the response mediated by antibodies. This opens doors for newer vaccine platforms such as T-cell active based vaccines, which might produce longer-lasting immunity for future variants of SARS-CoV-2 [51, 52].

Focusing on the development of universal vaccines due to the conserved regions of the SARS-CoV-2 spike protiens, which have a low chance for mutation, could serve as a T-cell based approach which furthermore aids in fide fense against emerging variants. Their goal is to provoke an immune response strong enough to identify and incapacitate the virus irrespective of the mutations it has. Other scientists are exploring the possibility of nanoparticle vaccines capable of displaying several viral antigens to increase the prospects of inducing a cross-reactive immunity to several different virus strains [52].

In summary, understanding the immune evasive strategies adopted by different variants of SARS-CoV-2 is crucial for developing effective vaccines and treatment. With the ongoing improvements to the virus, ensuring that the research on changes to the spike protein's structure and its functional outcomes remains pertinent will be necessary in assisting vaccine deployment strategies along with guarantees for extensive coverage of new variants.

Future Therapeutic Strategies for SARS-CoV-2 Infections

This part will cover three main strategies: the development of next-generation vaccines centered around conserved receptor-binding domain (RBD) epitopes, broad-spectrum neutralizing monoclonal antibodies (mA bs) for new variant mAbs, and the antiviral medication aimed at the spike-ACE2 binding level interaction.

Next-Generation Vaccines Targeting Conserved RBD Epitopes

The advancement of new vaccines for SARS-CoV-2 should contend with the problem of immune escape due to evolving variants, especially those with spike protein mutations. One of the possibilities is focusing on conserved parts of the receptor-binding domain (RBD) of the spike protein, which binds the ACE2 receptor and enables viral entry. These conserved epitopes are less prone to mutation than other parts of the virus and therefore, make for good targets for vaccine development.

There is considerable research literature documenting attempts to design vaccines concentrating on the conserved RBD regions. For example, one recent study reported developing a pan-sarbecovirus vaccine using the RBD from the original SARS-CoV-2 strain. This vaccine was shown to produce strong neutralizing antibody responses against Omicron subvariants as well as the original virus, also in rhesus macaques [53]. Moreover, the extraction of human monoclonal antibodies aimed at a conserved linear neutralizing epitope within the RBD domains has been recognized as a critical approach for formulating broadly effective vaccines [54]. These studies imply that concentrating on the most conserved regions of RBD can offer enduring defense from new variants.

Broad-Spectrum Monoclonal Antibodies (m Abs) for Neutralization of Emerging Variants

The fast development of SARS-CoV-2 has resulted in the emergence of new variants capable of escaping neutralization by both vaccine and infection-derived antibodies. Thus, broad-spectrum monoclonal antibodies are valuable as countermeasures to neutralize a wide range of variants, including those resistant to existing therapies.

Cross-neutralization of a broad spectrum of SARS-CoV-2 variants with monoclonal antibodies was recently documented. One of the studies showed that equine hyperimmune antibodies were effective by demonstrating marked neutralization for Delta and Omicron variants among others [55]. In this same regard, human monoclonal antibodies like iC1 are capable of neutralizing variants such as BA.5 and XBB.1.5, thus proving their potential as therapeutic agents [56].

The S2 sub-unit of the spike protein is also a major target for designing broadly neutralizing antibodies. For instance, the monoclonal antibody 2-36 described in the study was noted to neutralized not only SARS-CoV-2 but also other sarbecoviruses like SARS-CoV because it targets an epitope integral to the RBD [57]. The mAbs discussed provide a potential solution for both existing and emerging viral variants, thereby expanding the range of therapeutic choices available.

Antiviral Drugs Targeting Spike-ACE2 Interactions

The spike-ACE2 interaction facilitates the infection of host cells by SARS-CoV-2. This particular protein-protein interaction (PPI) offers an opportunity to design an effective antiviral drug surfacing a great deal of interest in PPI- centred drug design. Inhibiting spike-ACE2 interactions allows the blockage mechanisms of antiviral strategies, preventing the virus from entering cells and stopping infection.

The spike-ACE2 binding has been targeted by small-molecule inhibitors and natural products in some studies. For example, a study by Bojadzic et al. (2020) demonstrated the potential of oral or inhaled antiviral therapies, as some small molecules, such as Congo red and DRI-C23041, were found to inhibit the spike-ACE2 interaction. Evidence already exists that some other compounds, like methylene blue, which blocks spike-ACE2 interaction, have antiviral activity and can be used for the treatment of COVID-19 [58].

Furthermore, a study on the plant species Acacia farnesiana found that some of its phytochemicals can inhibit the formation of the spike-ACE2 complex, thus blocking viral cell infection [59]. This research indicates that natural compounds, unlike synthetic drugs, may have a lesser side effect profile in preventing viral entry.

As discussed, the next lines of intervention for SARS-CoV-2 and its new variants should explore formulating next-generation vaccines aimed at the conserved RBD epitopes, broad-spectrum monoclonal antibodies targeting multiple variants, and peptide drugs that prevent spike-ACE2 interaction. Backed by current evidence, these methods stand as reliable options to combat the problems arising from mutations of SARS-CoV-2.

Conclusions and Recommendations

All in all, the biology of the assemblies of some S proteins of SARS-CoV-2 has made considerable progress and now enables atomic level study of the complex molecular events associated with the life cycle of the virus, its transmissibility, and vaccine evasion. Structural analysis clearly articulates that adaptive evolution at critical RBD determines the architecture of the protein and influences the receptor interaction. The ongoing evolutionary adaptation of SARS-COV-2, especially in RBD, overtakes the vaccine effectiveness and thus warrants continued genetic, functional and structural analysis of the S protein to guide novel vaccines and therapeutic developments in the future.

Remaining Questions

The complex intricacies of RBD interactions with receptors are further contributed by mutations in the S proteins outside the RBD, and roles of cofactors and co-receptors. Our knowledge is limited to these axillary and essential factors.

Multiple mutations in and around RBD may have synergistic and/or antagonistic impact on the receptor interaction, thus warranting studying them in conjunction in contrast to their impact individually.

Additional and silent mutations could influence protein metabolism in infecting hosts and require ongoing investigations.

Perspectives

Evolution in RBD significantly impacts replication, infectivity, transmission, and evasion of both innate and adaptive immunity of SARS-COV-2

The RBD continues to mutate, and the viral biology of influenza indicates the dynamic plasticity and af-

fordability of additional mutations in a short stretch of the RBD.

Novel therapeutics (antiviral or monoclonal antibodies) and an advanced vaccine targeting RBD need to be revisited during the evolutionary trajectory of SARS-COV-2.

Funding

Not applicable.

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