

Importance of the SARS-CoV-2 genome and spike protein in the immunopathogenesis of COVID-19 and in the efficacy of vaccines

Importancia del genoma y de la proteína espiga del SARS-CoV-2 en la inmunopatogénesis de la COVID-19 y en la eficacia de las vacunas

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Abstract

Coronavirus (CoV) infections cause respiratory and enteric diseases with clinical manifestations ranging from faint to severe, even lead to death of patients. High connectivity between nations and infectivity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), represent a global health problem as the coronavirus disease 19 (COVID-19). This CoV-2 that cause SARS, which appeared in Wuhan, China, in December 2019 originated COVID-19 and declared as pandemic a few months posterior its appearance. In this review, the genomic and spike protein characteristics of SARS-CoV-2, the role of SARS-CoV-2 in the COVID-19 pathogenesis, cytokine storm, the role of cytotoxic T and B cells against SARS-CoV-2, as well as the vaccines efficacy (taking into account mutations in the spike protein) are described.

Keywords: SARS-CoV-2. ACE2. COVID-19. Mutations. Immune response. Vaccines

Resumen

Los coronavirus (CoV) causan enfermedades respiratorias y entéricas leves, graves o críticas, pudiendo ocasionar la muerte del paciente. Debido a la alta conectividad entre naciones y a la transmisión, actualmente la COVID-19 representa un verdadero problema de salud pública en todo el mundo. El CoV-2 causante del síndrome respiratorio agudo grave (SARS-CoV-2) apareció a finales de diciembre de 2019 en Wuhan, China, y en marzo de 2020 la COVID-19 fue declarada pandemia. En esta revisión se describen las características del genoma y de la proteína espiga del SARS-CoV-2, su papel en la inmunopatogénesis de la COVID-19, la tormenta de citocinas, la actividad citotóxica inducida por células T y la producción de anticuerpos contra el SARS-CoV-2 mediada por células B, así como la eficacia de algunas vacunas, tomando en cuenta las mutaciones presentes en la proteína espiga.

Palabras clave: SARS-CoV-2. ACE2. COVID-19. Mutaciones. Respuesta inmunitaria. Vacunas.

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Introduction

The 2019 coronavirus disease (COVID-19), caused by the coronavirus (CoV) 2 of severe acute respiratory syndrome (SARS-CoV-2), was first described back in December 2019 in Wuhan, capital of Hubei, China¹, and declared a pandemic in March 2020. As of November 17, 2022, a total of 632,953,782 infections and 6,593,715 deaths have been reported worldwide². The first human CoV, identified in 1965 and captured in an electron microscopy image in 1967, was associated with mild symptoms like the common cold³. Since then, 7 of them have proven capable of infecting humans, being the SARS-CoV reported in China in 2002, the Middle East Respiratory Syndrome coronavirus (MERS-CoV) identified in 2012 in Saudi Arabia, and SARS-CoV-2 the most pathogenic of them all⁴. Recent studies have helped understand the virus and the role it plays in COVID-19; however, new mutations have generated variants of concern (VOC) that show increased transmission capability and immune system evasion⁵. The aim of this review is to describe the role of the genome and spike protein (S protein) of SARS-CoV-2 in COVID-19, cytokine storms, T and B cell activity, immune system evasion, and the efficacy of some vaccines, while taking into account mutations in the S protein.

Origin of SARS-CoV-2

Some CoVs (*Coronaviridae* family, *Orthocoronavirinae* subfamily, and *Betacoronavirus* genus) have acquired the ability to infect humans over time¹. HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 cause common colds or mild respiratory diseases, compared to SARS-CoV, MERS-CoV, and SARS-CoV-2, which can lead to lung infections, pneumonia, respiratory distress, and potentially the patient's death^{1,3-5}. MERS-CoV and SARS-CoV-2 share a nucleotide sequence, which is identical to the bat CoV *Taphozous perforatus*^{1,5}, which, along with the pangolin, have been proposed as intermediaries for transmission and infection in humans¹. Their origin could have 3 different scenarios: 1) the viral ancestor incubated in bats for decades, accumulating mutations that prompted the transmission, 2) a less virulent ancestor remained latent until acquiring new mutations, thus increasing its virulence, or 3) SARS-CoV-2 remained circulating in an intermediate host until it was transmitted to humans^{1,4,5}.

Genome

The genome of SARS-CoV-2 consists of a single-stranded positive-sense RNA with 29 881 base pairs arranged into 14 open reading frames (ORFs), encoding 4 structural proteins, 16 non-structural proteins (NSPs), and 9 accessory proteins involved in infection and viral release^{6,7}. Its ORFs are flanked by untranslated regions at the 5' and 3' ends; the former is adjacent to a leader sequence, while the latter contains a polyadenylated tail^{6,7}. ORF1a and ORF1b constitute nearly two thirds of the genome and code for 16 NSPs. ORF1a (NSPs 1-11) is involved in viral genome expression, encoding 2 proteases. ORF1b (NSPs 12-16) produces the RNA-dependent RNA polymerase (RdRp), a helicase (NSP13), an exonuclease (NSP14), an endoribonuclease (NSP15), and a methyltransferase (NSP16). The remaining ORFs produce the S, E, M, and N proteins and 9 accessory proteins (3a, 3b, 6, 7a, 7b, 8a, 8b, 9b, and 9c) (Fig. 1)^{6,7}. During genome replication, mutations occur at an estimated rate of 2.9 to 3.7×10^{-6} per nucleotide per cycle. The S gene mutates 4 to 5 times more than the remaining genome⁵⁻⁷. RdRp appears to have no effect on mutation correction or removal^{5,6}.

Structural proteins

The N protein, the most abundant in SARS-CoV-2, has an N-terminal domain (NTD) and a c-terminal domain (CTD). It binds to viral RNA and aids in entry and cellular interactions after viral fusion⁶. The E protein has an NTD, a hydrophobic domain, a CTD, with a weight of 8-12 kDa, and is involved in virus assembly and release: the viroporins⁶. The M protein provides the virion's morphology and participates in scaffolding and assembly⁸. The S protein, responsible for recognizing and binding to the human ACE2 receptor (angiotensin-converting enzyme 2), consists of 1273 amino acids (aa) (with a weight of 180-200 kDa) divided into subunits 1 (S1) and 2 (S2), and in the NTD, it consists of a transmembrane domain, and an NTD. Its active form is a trimer^{6,7}. S1 (1-685 aa) contains the signal peptide (1-13 aa), the NTD (14-305 aa), the receptor-binding domain (RBD) (319-541 aa), and subdomains SD1 and SD2. The RBD is the target of neutralizing antibodies that block the interaction with ACE2. S2 (686-1273 aa) contains the fusion peptide (788-806), HR1 (heptapeptide repeat sequence 1) (912-984 aa), HR2 (1163-1213 aa), the transmembrane domain (1213-1237 aa), and the cytoplasmic tail domain (1237-1273 aa) and possesses anchor and fusion amino acids with the host cell membrane⁷.

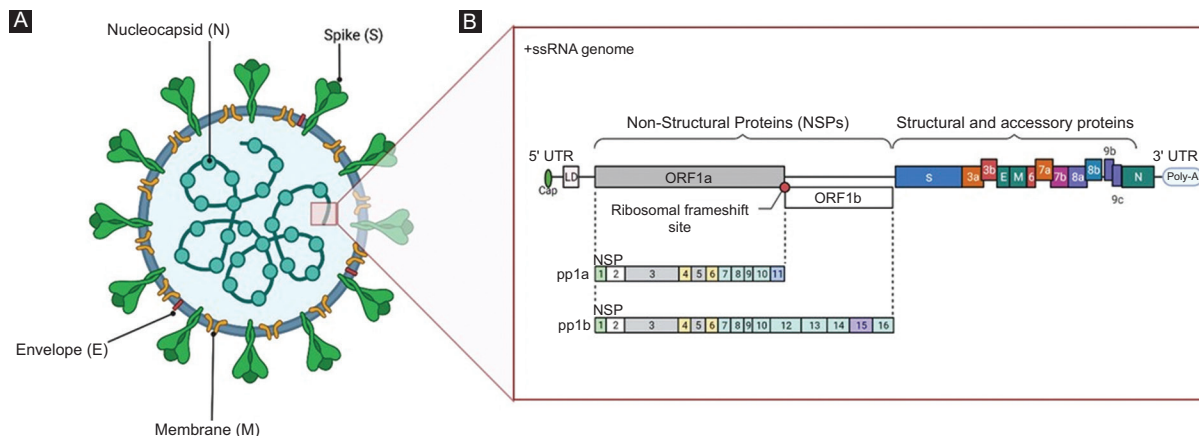


Figure 1. Structure and genomic organization of SARS-CoV-2. **A:** the structural proteins S, M, E, and N that make up SARS-CoV-2 are found schematized here. **B:** the structure of the viral genome is depicted. The single-stranded positive-sense RNA (+ssRNA) is divided into open reading frames (ORF) encoding non-structural proteins (NSP), structural proteins, and accessory proteins. The NSPs are encoded by ORF1a and ORF1b through a ribosomal frameshift mechanism in gene 1b, resulting in polyproteins pp1a and pp1ab, which are subsequently processed by viral proteases to produce 16 proteins. LD: leader sequence; PP1: polyprotein 1; UTR: untranslated region.

S Protein, ACE2, and proteases

In SARS-CoV-2 infection, various proteases cleave the S protein at 2 different sites: the furin site located between S1 and S2 and the S2' site located in S2, recognized by the transmembrane protease serine 2 (TMPRSS2) (Fig. 2A), cathepsin L (CTSL) (Fig. 2A), and neutrophil elastase^{9,10}. Furin cleavage promotes the entry of SARS-CoV-2 by inducing fusion with the host membrane, TMPRSS2 cleaves the S protein on the cell surface while inducing binding to human cells (Fig. 2), while CTSL cleaves it in the late endosome/lysosome (Fig. 2)⁹. The interaction of the S/ACE2 proteins induces changes in S2, exposing the S2' cleavage site. Therefore, endocytosis releases the fusion peptide, initiating pore formation and infection in human cells¹⁰⁻¹². ACE2, expressed in the lower airways, upper bronchial and nasal epithelium, small intestine, cardiac muscle, etc., facilitates SARS-CoV-2 penetration into the cells, thus potentially affecting these organs^{6,7,11}. The viral genome in the cell must be translated into ribosomes to produce viral particles, and thus, after replication of its genome and translation of its proteins, they will translocate to the rough endoplasmic reticulum and the Golgi complex, where assembly occurs.

Innate immunity and cytokine storm

SARS-CoV-2 and its variants produce different signs and symptoms within 3 to 14 days, causing headache, chest and body pain, fever, difficulty breathing, fatigue,

dry cough, loss of taste, etc. Nearly 20% of patients develop severe or critical COVID-19, including respiratory lesions, septic shock, and death¹¹. The immune response begins when SARS-CoV-2 enters the cell; depending on different circumstances, it can evade, attenuate, or induce innate immune attacks, thus potentially activating inflammatory signaling pathways, including nuclear factor kappa B (involved in the production of proinflammatory cytokines) and JAK/STAT (involved in the transcription of interferon-stimulated genes), among others¹³. The epithelial cells of the airways, when infected, release the virus and infect other cells. At this stage, macrophages derived from monocytes or dendritic cells activate a viral clearance defense mechanism, producing a cytokine and chemokine-mediated immune response, among other factors^{13,14}. However, some patients release proinflammatory cytokines, chemokines, and growth factors uncontrollably, which triggers a cytokine storm associated with fever and myalgia in mild cases or in the presence of hyperinflammation, pneumonia, acute respiratory distress, cardiovascular abnormalities, and multiple organ failure in critical cases^{13,14}. Tumor necrosis factor, interferon, granulocyte colony-stimulating factor, transforming growth factor-beta, and hepatocyte growth factor are key players in the "cytokine release syndrome" during COVID-19. High levels of interferon are associated with headaches, chills, dizziness, and fever¹⁴. Neutrophils, macrophages, lymphocytes, endothelial and epithelial cells, fibroblasts, etc., through different cytokines, can contribute to lung scarring and the death of the patient with COVID-19¹⁴.

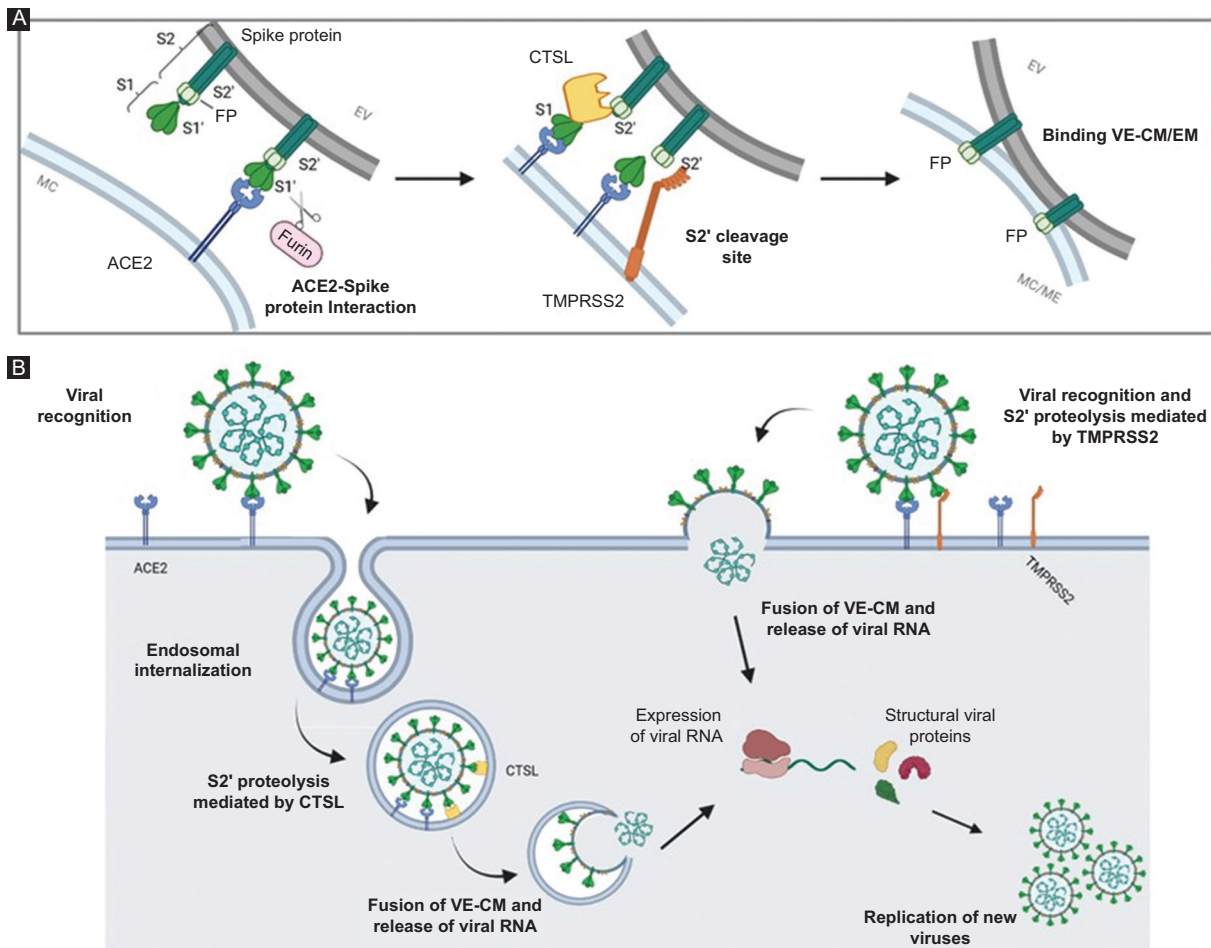


Figure 2. Mechanisms of recognition and cellular internalization of SARS-CoV-2. **A:** viral recognition is mediated by the interaction of the S1 subunit of the viral protein S and its human receptor, angiotensin-converting enzyme 2 (ACE2), in the host cell. The ACE2-S protein binding induces proteolytic cleavage at S1' mediated by furin while exposing the S2' cleavage site, thus facilitating recognition by proteases (TMPRSS2, CTSL). Cleavage exposes the fusion peptide (FP) of protein S, which binds to the cell membrane (CM), or endosomal membrane (EM) promoting membrane fusion. **B:** SARS-CoV-2 internalization can occur through the S1-ACE2 recognition pathway, thus leading to endosome formation (left), where cathepsin L (CTSL) cleaves the S2' site, resulting in viral envelope fusion with the endosomal membrane. If the transmembrane serine protease 2 (TMPRSS2) is expressed on the cell surface, the viral envelope, and the cell membrane fuse, thus releasing viral RNA into the cytoplasm (right). Viral RNA is replicated and expressed in the cytoplasm, where eventually the assembly of new viruses occurs. CM: cell membrane; EM: endosomal membrane; FP: fusion peptide; NE: nuclear envelope; VE: viral envelope.

Adaptive immunity, T and B cells

Cytotoxic T lymphocytes (CTL) eliminate virus-infected cells. Patients with COVID-19 who had CTL and responded to viral peptides had better clinical outcomes¹⁵, while those with a significant decrease in these cells had a more severe illness¹⁶. CTL recognize antigenic peptides from S, M, or N proteins, suggesting that this type of immunity is equally or more relevant than antibodies¹⁵. Robust activation of CTL has been observed 10 to 12 days after the first vaccination¹⁵. Changes to CTL, such as low perforin protein expression, are associated with severe COVID-19¹⁷.

B lymphocytes represent the most important adaptive immune response for protection vs SARS-CoV-2 since they produce specific antibodies against an amino acid sequence of the RBD induced by vaccines or natural infection. However, variations in amino acids following mutations make antibodies less accurate in identifying different SARS-CoV-2 variants, thus reducing their neutralizing effect¹⁸. B cells generate memory, and thus, a second infection could go unnoticed as antibodies will protect the individual for just a few months. Still, if the RBD has differences in amino acid sequence compared to the strain or variant that previously infected it, or if the vaccine was designed

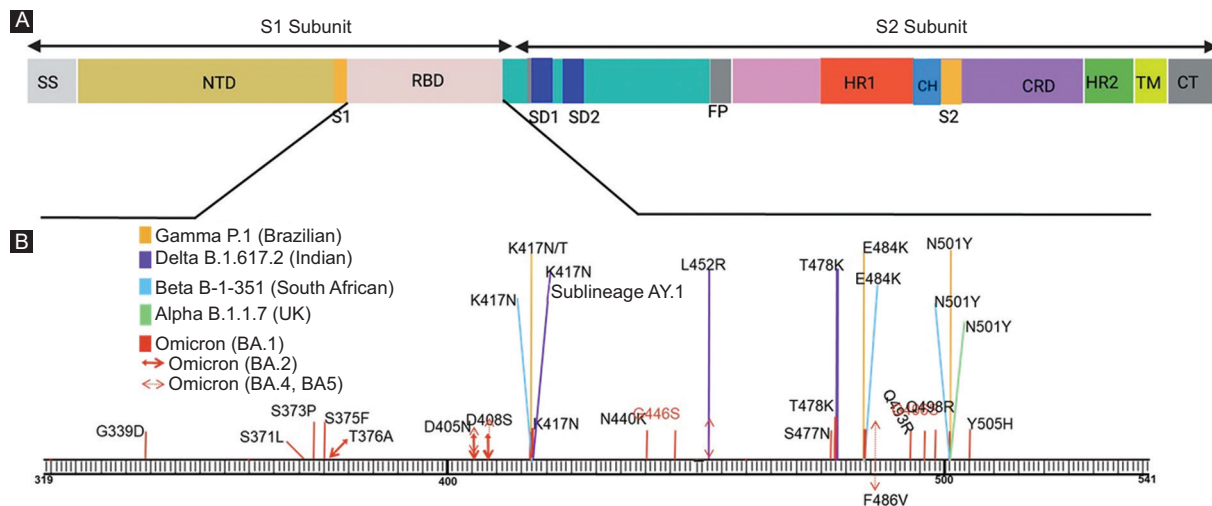


Figure 3. Mutations identified in the variants of concern (VOC). **A:** structure of the spike protein (S protein). **B:** mutations located in the receptor-binding domain (RBD) of alpha, beta, gamma, delta, and omicron VOCs. The amino acid changes in each mutation and their positions in the protein are indicated. Red lettering indicates mutations in omicron, mustard lettering shows mutations in gamma, violet color lettering shows mutations in delta, blue lettering shows mutations in beta, and green lettering indicates mutations in alpha. CH: central helix; CRD: connector domain; CT: cytoplasmic tail; FP: fusion peptide; HR1: heptad repeat region 1; HR2: heptad repeat region 2; NTD: N-terminal domain; S1: subunit 1; S2: subunit 2; SD1: subdomain 1; SD2: subdomain 2; SS: signal sequence; TM: transmembrane domain.

for a specific strain or variant, then protection will decrease. Antibodies IgM, IgG, and IgA vs SARS-CoV-2 will neutralize and eradicate the virus^{19,20}. High IgG titers are associated with delayed clearance of SARS-CoV-2 and severe COVID-19. The duration of antibody-mediated protection in asymptomatic individuals and those with moderate COVID-19 ranges from 3 to 6 months, with a gradual decline²¹.

Mutations in VOCs and biological effects

The S gene, the primary target of mutations, showed the D614G mutation (aspartic acid to glycine) in the early months of the pandemic in the United Kingdom (March 2020) (Fig. 3), and 2 months later, it became common in Europe and across the world. D614G increased infectivity and viral load^{22,23}. In September 2020, the alpha variant (lineage B.1.1.7) was first identified in the United Kingdom (first VOC), with 9 mutations in the S protein compared to the original strain (Fig. 3). The N501Y mutation in the RBD (asparagine to tyrosine) enhanced viral transmission and ACE2 affinity, and reduced binding to neutralizing antibodies (Table 1)²³. One month later, the beta or South African variant (lineage B.1.351) was reported, with 10 mutations in the S protein (Fig. 3) compared to the original strain, 3 of which were located in the RBD: N501Y, K417N (lysine to asparagine), and

E484K (glutamic acid to lysine) (Table 1). These mutations increased ACE2 binding, transmissibility, and evasion of neutralization by monoclonal antibodies²⁴. In December 2020, the gamma or Brazilian variant (lineage B.1.1.248) emerged, with 10 mutations in the S protein compared to the original strain. It included E484K, N501Y, and K417T (lysine to threonine) in the RBD, increasing ACE2 binding, transmission, and evasion of neutralization by monoclonal antibodies²⁵. The delta variant (lineage B.1.617), with 9 mutations in the S protein (Fig. 3), appeared in India back in October 2020²⁶. Its RBD showed the mutations T478K (threonine to lysine) and L452R (leucine to arginine), while P681R (proline to arginine) generated a furin cleavage site. The delta variant was exceptionally infectious and showed reduced neutralization by antibodies in vaccinated individuals. The L452R mutation increased the stability of the S protein, infection, and viral replication²⁷. The omicron variant (lineage B.1.1.529), with over 30 mutations in the S protein (Fig. 3), was identified in South Africa in November 2021²⁸. As of the second week of November 2022, it presented lineages BA.1, BA.2, BA.3, BA.4, BA.5, BQ.1, and BQ.1.1 (Table 1)²⁹. More than 15 mutations were reported in its RBD,^{30,31} thus contributing to lower antibody binding affinity and increased immune system evasion, with a higher risk of reinfection, etc^{30,31}. Lineages BA.2.12.1 and BA.4/

Table 1. Variants of concern, mutations of SARS-CoV-2 spike protein, and biological effect

Variant	Mutations of the S protein					Effects ^{ref.}
	NTD	RBD	Furin site	Other	Relevant mutations	
(B.1)				D614G		It increases the density of the spike protein, and the rate of infection ^{22,23}
Alfa (B.1.1.7)	Δ 69-70 Δ 144	N501Y	P681H	A570D, D614G, T716I S982A D1118H	N501Y	10 times more affinity to ACE2 than the original strain, increases transmissibility and viral load, and has a minimal effect on immune system evasion ^{23,30}
Beta (B.1.351)	L18F D80A Δ211 D215G Δ241-243	K417N E484K N501Y		D614G, A701V	E484K	Increases transmissibility, has a moderate effect on antibody escape, and immune system evasion ^{23,24,30}
Gamma (P.1)	L18F T20N P26S D138Y D164G R190S	K417T E484K N501Y		D614G H655Y T1027I	K417T	Increases transmissibility, has a moderate effect on antibody escape, and immune system evasion ^{23,25,30}
Delta (B.1.617.2)	T19R Δ156-157 R158G	L452R T478K	P681R	D614G D950N	L452R	Increases transmissibility, has a moderate effect on antibody escape, immune system evasion, and increased S1/S2 cleavage ^{26,27,30}
Omicron	A67V Δ69-70	G339D		T547K	N501Y	Increases binding affinity to ACE ²⁸⁻³¹
(B.1.1.529)	T95I G142D Δ143-145 L212I Δ211	S371L S373P S375F K417N N440K G446S S477N T478K E484A Q493R G496A Q498R N501Y Y505H		D614G H655Y N679K N764K D769Y N856K Q954H N969K	E484A	Reduces neutralization of the drug bamlanivimab ²⁸⁻³¹
Omicron BA.1/ B.1.1.529.1	A67V Δ69-70 T95I Δ143-145 Δ211 L212I +214EPE	G339D S371L S373P S375F K417K N440K G446S S477N T478K E484A Q493R G496S Q498R N501Y Y505H	P681H	T547K D614G H655Y N679K N764K D796Y N856K Q954H N969K L981F		Increases transmissibility, has a moderate effect on antibody escape, and immune system evasion ²⁸⁻³¹
Omicron BA.2/ B.1.1.529.2	T19I Δ24-26 A27S	G339D S371F S373P	P681H	D614G H655Y N679K		Increases transmissibility by 30% compared to BA.1, exhibits better antibody escape, and higher immune system evasion ²⁸⁻³¹

(Continues)

Table 1. Variants of concern, mutations of SARS-CoV-2 spike protein, and biological effect (continued)

Variant	Mutations of the S protein					Effects ^{ref.}
	NTD	RBD	Furin site	Other	Relevant mutations	
	G142D V213G	S375F T376A D405N R408S K417N N440K S477N T478K E484A Q493R Q498R N501Y Y505H		N764K D796Y Q954H N969K		
Omicron BA.3/ B.1.1.529.3	A67V Δ69-70 T95I G142D Δ143-145 Δ211 L212I +214EPE	G339D S371L/F S373P S375F D405N K417N N440K G446S S477N T478K E484A Q493R Q498R N501Y Y505H	P681H	D614G H655Y N679K N764K D796Y Q954H N969K		Increases transmissibility, shows better antibody escape, and higher immune system evasion ²⁸⁻³¹
Omicron BA.4/ B.1.1.529.4	T19I Δ24-26 A27S Δ69-70 G142D V213G	G339D S371F S373P S375F T376A D405N R408S K417N N440K L452R S477N T478K E484A F486V Q498R N501Y Y505H	P681H	D614G H655Y N679K N764K D796Y Q954H N969K		Increases transmissibility, immune system evasion, and antibody escape compared to BA.2 in vaccinated individuals or those infected by BA ²⁸⁻³¹
Omicron BA.5/ B.1.1.529.5	T19I Δ24-26 A27S Δ69-70 G142D V213G	G339D S371F S373P S375F T376A D405N R408S K417N N440K L452R S477N T478K E484A F486V Q498R N501Y Y505H	P681H	D614G H655Y N679K N764K D796Y Q954H N969K		Increases transmissibility, immune system evasion, and antibody escape compared to BA.2 in vaccinated individuals or those infected by BA ²⁸⁻³¹

ACE2: angiotensin-converting enzyme 2; NTD: N-terminal domain; RBD: receptor binding domain.

BA.5 showed increased immune evasion vs neutralizing antibodies compared to BA.2³¹. The mutations reported in the VOCs are shown in table 1.

Mutations in the RBD and vaccine efficacy

Vaccines generate specific antibodies, and changes in amino acids contribute to immune evasion and a reduction in their affinity for binding to neutralizing antibodies vs SARS-CoV-2¹⁸⁻²⁰. Vaccines developed based on the amino acid sequence of the original strain do not generate specific antibodies vs the VOCs, leading to a decrease in their efficacy. The Pfizer-BioNTech vaccine showed a 95% efficacy profile vs the original strain and 89.5% and 75% efficacy rates vs the alpha and beta variants, respectively, 14 days after the second dose. Protection vs severe or critical hospital admission was 97.5%³². For the delta variant, the efficacy rate was 59% 14 days after the first dose, 66% on days 21-27 after the first dose, and 90% on days 7-21 after the second dose. Against COVID-19 symptoms, the efficacy was 57% on days 14-20 after the first dose, 82% on days 21-27 after the first dose, and 93% on days 7-21 after the second dose³³. The efficacy vs the omicron variant was significantly reduced at 41% with 2 doses. However, it went up to 85% for hospital admission after 3 months and then went down to 55% after that time period³³. The efficacy of the AstraZeneca vaccine (ChAdOx1-S) after 2 doses vs the alpha and delta variants was 74.5% and 67.0%, respectively³⁴, with reduced efficacy vs symptomatic disease due to omicron with 2 doses³⁴. Finally, recent research has shown an increased protection vs the different VOCs if individuals receive different vaccines or a combination of them³⁵.

Conclusions

The COVID-19 pandemic due to SARS-CoV-2 remains a significant global health challenge. To date, efforts to halt transmission have been hindered by the numerous mutations present in the RBD of the virus, giving the virus an increased transmissibility and the ability to evade the immune system and reduce the neutralization effectiveness of antibodies. Recent studies show that individuals previously infected with one variant or subvariant are at a high risk of reinfection with other variants, which stresses the importance of developing new vaccines and achieving complete vaccination coverage worldwide.

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Conflicts of interest

None declared.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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