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REVIEW

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The variants of SARS-CoV-2 and the challenges of vaccines

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Abstract

Since the outbreak of coronavirus disease 2019 (COVID-19), countries all over the world have suffered severe losses. It affects not only human life and health but also the economy. In response to COVID-19, countries have made tremendous efforts to vaccine development. The newly discovered variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have brought major challenges to the effectiveness and research of vaccines. This article reviews the existing literature and summarizes the main variants of the SARS-CoV-2 and its impact on vaccines, and provides new ideas for the later development of vaccines. An excellent job in developing and applying vaccines will be an important measure for epidemic prevention and control.

KEYWORDS

COVID-19, SARS-CoV-2, spike protein, vaccines, variants

1 | INTRODUCTION

Coronavirus diease 2019 (COVID-19) is a respiratory-transmitted disease caused by a new type of coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). After being first discovered in Wuhan in 2019, it has now become a global trend. Coronaviruses are a group of enveloped viruses with a positive single-stranded RNA genome of about 30 000 bases, a 5′cap structure, and a 3′poly A tail, belonging to the Coronaviridaes family of the order Nidovirales.[1] Since then, many companies and research institutions have competed to develop SARS-CoV-2 vaccines, from traditional and protein-based vaccines to more cutting-edge vaccines, including vaccines based on DNA and messenger RNA (mRNA). Each vaccine exhibits different efficacy and duration, depending on the antigen design, adjuvant molecule, vaccine delivery platform, and immune method.[2]

The design of the COVID-19 vaccine must consider both humoral immunity and cellular immunity. In addition, COVID-19 is mainly spread through the respiratory tract and contact, so more attention should be paid to the role of mucosal immunity in preventing viral infections. The virus contains four structural proteins, spike S protein, envelope E protein, membrane/matrix protein, and nucleocapsid N protein. The S protein has two sections, S1 and S2. The S protein binds to a specific receptor, causing the virus to infect cells.[3–5] Neutralizing antibodies against S protein can block this process and prevent virus invasion.[6] S protein can effectively stimulate the T-cell immune response, so it is the most important target antigen for vaccine design. N and M proteins have also been shown to induce effective cellular immune responses in the body.[7–9]

The receptors of many viruses are expressed in many organs other than the airway. ACE2 is expressed in almost all organs but is expressed in higher levels, especially in the lung,[10] intestine,[11] and brain.[12] Therefore, SARS-CoV-2 has a wider biological distribution and may cause considerable damage outside the respiratory system. It has adverse effects on the genitourinary, digestive, circulatory, and central nervous systems. The universality of ACE2 receptor distribution leads to multiple changes in symptoms, such as dyspnea, headache, diarrhea, venous thromboembolism, hypertension, and so forth.[13]

The S protein binds to ACE2 on the cell to mediate infection. The S1 subunit contains the receptor-binding domain (RBD), which is responsible for the initial attachment of the host cell through the ACE2 receptor, while the S2 subunit promotes the fusion of the virus and the cell to initiate infection.[14] The S protein is a common target of vaccines,[14] but variants or deletions in the S protein region's genome may affect the vaccine's efficacy. Several of the mutations in the S protein were observed. At position E484 in RBD of the S protein, the mutation had already been predicted to escape neutralization by convalescent sera and affect the binding of monoclonal antibodies.[15] Near 700 nondegenerate mutations, contributing many mutations in emerging variants, that is, N501Y for alpha, K417N, E484K, and N501Y for beta, K417T, E484K, and

N501Y for gamma, L452R, and T478K for delta, L452Q, and F490S for lambda, and so forth, were observed in RBD.[16]

The World Health Organization (WHO) identified variants of concern (VOCs) and variants of interest (VOIs). VOCs have an increment in the virulence and transmissibility or adversely affect the effectiveness of vaccines, diagnostics with clear clinical correlation evidence, and therapeutics. VOIs carry genetic changes, which are predicted or known to reduce the neutralizing effect of antibodies produced against vaccination, affect virulence, transmissibility, immune escape, diagnostics, and the efficacy of treatments. VOIs also can cause significant community transmission. Currently, WHO listed four VOCs, that is, variants B.1.1.7 (alpha),[17–19] B.1.351 (beta),[18,20] P.1 (gamma),[18] and B.1.617.2 (delta)[21]), and some VOIs, that is, variants B.1.525 (eta),[22] B.1.526 (iota),[22–23] B.1.617.1 (kappa),[24] C.37 (lambda),[25] B.1.621 (Mu), and B.1.621.[26]

So, we focus on reviewing several variants and exploring their impact on several vaccines in the existing market and have expected to find new ideas for mutant response and vaccines development.

2 | MAIN VARIANTS OF SARS-COV-2 AND THE EFFECTIVENESS AND CHALLENGES OF VACCINES

Adaptive variants in the SARS-CoV-2 genome may change its pathogenic potential and increase drug and vaccine development difficulty (Figure 1). A study found that when the SARS-CoV-2 was incubated with highly neutralized plasma from COVID-19 convalescent patients, the plasma could wholly neutralize the virus for seven passages. However, after 45 days, the deletion of F140 in the N3 loop of the S protein N-terminal domain (NTD) caused a partial breakthrough. On the 73rd day, an E484K substitution occurred in RBD, and then on the 80th day, an NTD N5 loop containing the new glycan sequence was inserted, resulting in an entirely resistant variant to plasma neutralization. The computational model predicts that deletions and insertions in the N3 and N5 loops will prevent the binding of neutralizing antibodies.[27]

Viral variants may pose great challenges to current vaccines. If SARS-CoV-2 variants increase their transmissibility or virulence, the importance of public health measures and vaccination will increase. The global response strategy should also be adjusted in time. Tracking the variants of viruses requires global efforts. Many research groups isolated SARS-CoV-2 and sequenced it, and the sequencing results were shared in public databases to facilitate the tracking of virus evolution.[28] WHO also uses the shared database to develop and continuously improve the SARS-CoV-2 risk monitoring and evaluation framework to identify and evaluate worrisome variants.

2.1 | Variants of concern

Compared to the SARS-CoV-2 Wuhan reference sequence, all current VOCs have the D614G substitution. Experimental studies have confirmed that the variant containing the D614G substitution has enhanced the binding ability of ACE2 and the replication ability in the human ACE2 knock-in mouse model and in vitro culture (nasal airway

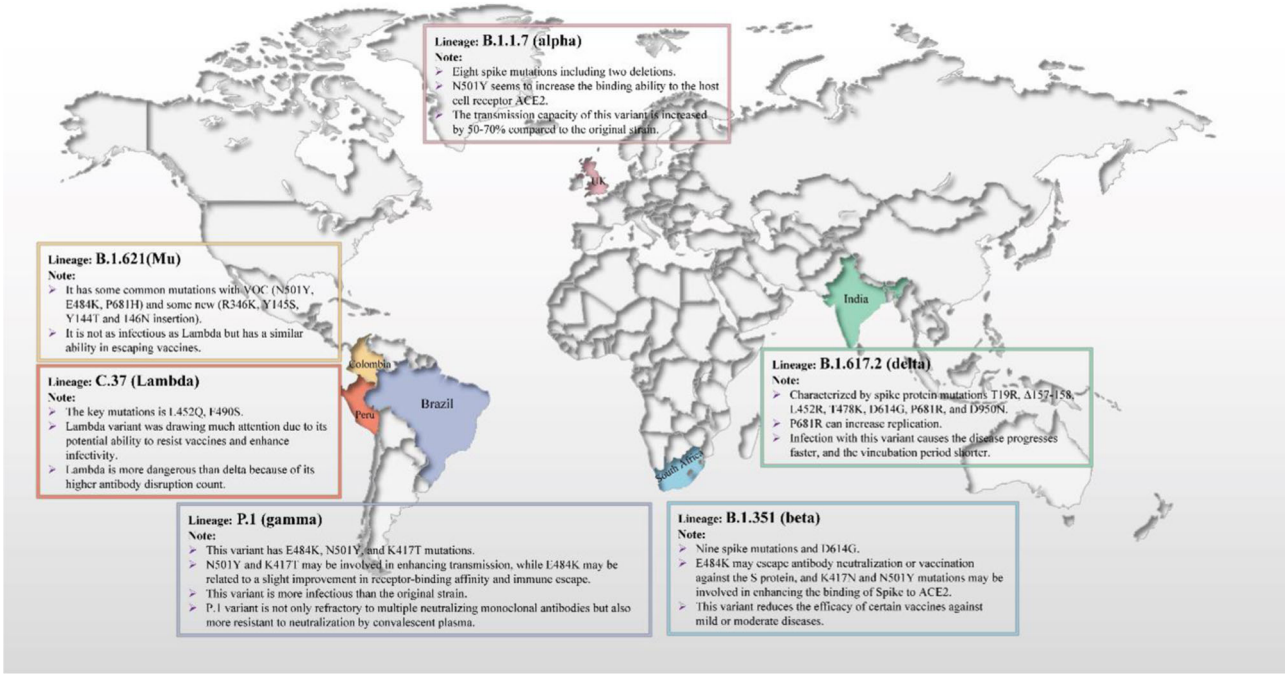

FIGURE 1 The place of the SARS-CoV-2 variants was the first reported (data were cited from WHO) and their brief characteristics. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization

epithelium and primary human bronchial culture).[29] The D614G substitution also enhanced the replication and transmissibility of SARS-CoV-2 in hamster and ferret models.[29]

The B.1.1.7 (or alpha) variant, reported by the United Kingdom to the WHO, has eight spike mutations which include two deletions, one is in an antibody supersite epitope (Y144), and the other increases infectivity but has little impact on immune escape.[30] The sole RBD mutation is N501Y, which seems to increase the binding ability to the host cell receptor ACE2. Epidemiological analyses quickly ascertained that B.1.1.7 was more transmissible.[31] So far, the B.1.1.7 variant has spread worldwide and has also been noted to acquire the E484K mutation. It is estimated that the sensitivity of this E484K mutant virus to the immune sera of individuals vaccinated with Pfizer/BioNTech mRNA vaccine is reduced by six times, and the sensitivity to serum during the recovery period is reduced by 11 times.[15,32] The transmission capacity of this variant is increased by 50%–70% compared to the original strain. There are many key site variants in the gene sequence of this variant. At present, this variant is becoming an increasingly common variant.[31] Chronic infection may have played a role in the origin of this mutation, but there is not enough evidence.

The P.1 (or gamma) variant was first discovered in Brazil, and it has E484K, N501Y, and K417T mutations, which are located at the RBD of the S protein. N501Y and K417T may be involved in enhancing transmission, while E484K may be related to a slight improvement in receptor-binding affinity and immune escape.[33–34] This variant is 1.4–2.2 times more infectious than the original strain. At the same time, it can cause serious illness in previously infected people, although this lacks clear evidence.[35]

The B.1.351 (or beta) variant was first discovered in South Africa, including D80A, L242del, A243del, L244del, R246I, K417N, E484K, N501Y, D614G, and A701V mutations. B.1.351 has nine spike mutations and D614G, including a cluster of mutations (e.g., R246I and 242–244del) in the NTD, three mutations (K417N, N501Y, and E484K) in the RBD, and one mutation (A701V) near the furin cleavage site.[36–37]

Mutations in S protein characterize this variant: E484K may be involved in the structural modification of the end of the spike, which may escape antibody neutralization or vaccination against the S protein, and K417N and N501Y mutations may be involved in enhancing the binding of the spike to ACE2.[34,38–39]

Compared with the prototype virus on which the vaccine antigen is based, the B.1.351 (or beta) variant is less likely to be neutralized by the convalescent plasma of patients infected with the previous variant and the serum of the vaccine.[40] Preliminary evidence shows this variation reduces the efficacy of certain vaccines against mild or moderate diseases.[41–43]

B.1.617.2 (or delta) variants appeared, causing more deaths. The delta variant is characterized by spike protein mutations T19R, Δ157-158, L452R, T478K, D614G, P681R, and D950N. Some of these mutations may affect the immune response to key antigen regions of the receptor-binding protein (452 and 478) and deletion of part of the NTD.[44] P681R is located at the cleavage site of S1–S2, and it seems that strains mutated at this site may have increased replication, resulting in a higher viral load and increased spread.[45–46]

After the patient is infected with this variant, the nucleic acid load of the new coronavirus is particularly high, and the course of the disease progresses faster, and the incubation period is shortened, which is only about 3.2 days.[47] Delta mutant strains can cause hearing impairment, gastrointestinal diseases, and thrombosis. Doctors in India found that patients infected with the delta variant would be more likely to have stomach pain, nausea, vomiting, loss of appetite, hearing impairment, and joint pain. There is little or no evidence that the variants beta and gamma can cause such abnormal clinical symptoms. At the same time, Ganesh Manudhane, a cardiologist from Mumbai, found that some patients infected with delta variant had micro thrombosis or small thrombosis, which could even become gangrene in severe cases.

2.2 | Variants of interest

In addition to the above four variants, we also need to pay attention to other variants types. On June 16, 2021, the WHO announced a new variant of SARS-CoV-2 called lambda (C.37) [L452Q, F490S], which has been discovered in 29 countries/regions, reportedly originated in South America and was first reported in Peru. Based on current data, this virus variant may be more infectious and resistant to vaccines. Lambda variant was drawing much attention due to its potential ability to resist vaccines and enhance infectivity. Lambda has already spread out in every country.[26] Another VOI variant is the Mu variant. It emerged in Colombia, which carries several spike mutations. Some mutations are common with VOC (N501Y, E484K, P681H), while others are new (R346K, Y145S, Y144T, and 146N insertion). Hence the Mu variant is not as infectious as lambda but has a similar ability in escaping vaccines.[26]

2.3 | Effect of variants on vaccines

At present, there is not enough evidence to show that these variants have entirely escaped from the vaccines. The fact that some of the key amino acids in the S protein have independently changed in variants identified around the world indicates that these are convergent changes and that vaccines containing these selected residues can cover multiple variants. The humoral and cellular immune responses of 121 medical workers (H. C. W.) vaccinated with the BNT162b2 mRNA vaccine against wild-type SARS-CoV-2 and B.1.1.7 and B.1.351 variants can reach a high level in all individuals after the second vaccination. The functional antibodies and the antibodies induced by the cellular immune response vaccine can cross-neutralize the B.1.1.7 and B.1.351 mutants, but the neutralizing ability is 2–4 times lower. No difference in the activation of CD4+ T cells in response to different antigens was observed, which indicates that B.1.1.7 and B.1.351S protein did not escape the T-cell-mediated immunity caused by wild-type S protein.[48] The recent surge in COVID-19 infections is

due to the occurrence of RBD comutations that combine two or more infectivity-strengthening mutations.

An experimental study has shown that RBD 2 comutation set [L452R, T478K] (delta variant) has the highest frequency (219 362) and the highest binding free energy (BFE) change (1.575 kcal/mol). The delta variant disrupts 40 antibody-RBD complexes, indicating that the delta enhances infectivity and is a breakthrough vaccine variant. [L452Q, F490S] (lambda variant) is another comutation set with high frequency, high BFE changes (1.421 kcal/mol), and high antibody disruption count (59). Lambda is considered to be more dangerous than delta because of its higher antibody disruption count. Furthermore, [R346K, E484K, N501Y] (Mu variant) also have a high antibody disruption count (60). The beta variant has the highest ability to breakthrough vaccines in all variants, but its infectivity is low (BFE change: 0.656 kcal/mol). The high-frequency two comutation sets [E484K, N501Y], [S494P, N501Y], and [F490S, N501Y] are considered to have the potential to escape vaccines.[26]

Neutralization experiments with real viruses have shown that the early antibodies that can neutralize the original SARS-CoV-2 have limited reactivity to B.1.351 (501Y.V2) and P.1 (501Y.V3) variants.[49] In the study of vaccination against AstraZeneca and Pfizer/BioTech in Scotland, the protective effect of vaccination was shown 28 days after the first dose. The study used S gene-positive polymerase chain reaction (PCR) to identify mutant and nonmutant infections. It was found that the protection of two doses of Pfizer/BioTech vaccine against nonmutant strains was 92%, and the protection against mutant strains was 79%. AstraZeneca vaccine had 73% protection against nonmutant strains and 60% protection against mutant strains.[50] Novavax announced that its recombinant protein-based nanoparticle vaccine candidate NVX-CoV2373 had reached the primary endpoint in a pivotal phase 3 clinical trial, with an overall protective effect of 90.4%. In addition, its protective efficacy against moderate and severe COVID-19 is 100%, and its effectiveness in high-risk groups is 91%. Moreover, NVX-CoV2373 also showed an excellent protective effect on VOCs/VOIs.

The emerging data showed protection from severe infection and death for all vaccines in all settings, although preventing asymptomatic transmission and mild-to-moderate disease is variable. The AstraZeneca ChAdOx1 vaccine showed only 10% protection against mild-to-moderate disease associated with the B.1.351 variant in a young population with a median age of 30 in South Africa. By contrast, in the United Kingdom, ChAdOx1 demonstrated 75% protection against B.1.1.7 (including asymptomatic infection). The Novavax vaccine, consisting of purified spike protein, showed approximately 50% protection against infection in South Africa (largely the B.1.351 variant) and 86% protection against infection in the United Kingdom (predominantly the B.1.1.7 variant). Pfizer/BioNTech BNT162b2 mRNA vaccine's estimated effectiveness against any documented infection with the B.1.1.7 variant was 87.0% (95% confidence interval [CI]: 81.8–90.7) at 14 or more days after the second dose in Qatar.[51]

Johnson & Johnson's human adenovirus-vectored vaccine showed 64% protection against moderate-to-severe disease in South Africa (dominated by the B.1.351 variant) and 66% protection against moderate-to-severe disease in the United States of America (mainly the Wuhan-1 variant with D614G), as assessed 29 days after vaccination.[52] The Pfizer/BioNTech BNT162b2 mRNA vaccine was less effective against B.1.351 than against non-B.1.351 variants based on a small analysis of breakthrough infections enriched for B.1.351 in Israel.[53] However, the Pfizer/BioNTech BNT162b2 mRNA vaccine's estimated effectiveness against any documented infection with the B.1.351 variant was 72.1% (95% CI: 66.4–76.8) at 14 or more days after the second dose in Qatar.[15]

The efficacy of the CoronaVac/Sinovac inactivated virus vaccine in Brazil, where 75% of infections were with the P.1 variant, was estimated at around 50% against symptomatic infection.[15] One study has shown that the P.1 variant is not only refractory to multiple neutralizing monoclonal antibodies but also more resistant to neutralization by convalescent plasma (3.4-fold) and vaccine sera (3.8–4.8 folds).[18]

The efficacy of the BNT162b2 vaccine was 88.0% (95% CI: 85.3–90.1) with the delta variant after the second dose, while the effectiveness with two doses of the ChAdOx1 nCoV-19 vaccine was lower than with the BNT162b2 vaccine, which was 67.0% (95% CI: 61.3–71.8).[46]

3 | THOUGHTS AND PREVENTIVE SOLUTIONS

We need more information regarding the protection from an infection that is afforded by the current generation of SARS-CoV-2 vaccines in light of the existing and potential emerging viral VOC.

3.1 | Extensive vaccination of current vaccines

Although the protection of current vaccines against variants has declined to various degrees, they are still higher or close to the level of protection defined by the WHO of at least 50%. The vaccine is very effective against severe, critical or fatal diseases caused by SARS-CoV-2 infection. In other words, the vaccine retains a certain degree of effectiveness against the variant. Therefore, urgently launching current vaccines to immunize most of the population is currently the best choice to deal with the threat of emerging mutations.

3.2 | Improving the immunogenicity of current vaccines

The efficacy of vaccines depends mainly on the immunogenicity of the vaccines. The vaccine-induced immune response level maybe helps to prevent further transmission of variants. Increasing the

number of doses and alternating vaccines should be considered to boost the immune response.

Moderna has considered adding a third dose after the first two doses of vaccine to increase the titers of neutralizing antibodies, thereby ensuring the protective efficacy against SARS-CoV-2 variants.[54] Public Health of the United Kingdom updated the SARS-CoV-2 vaccination guidelines and announced that alternative vaccines could be used in the case of a shortage of the first dose of the individual vaccine.[55] The US Food and Drug Administration has also noticed mixed and matched vaccines.[37,56]

3.3 | Discovery of mutations timely

The current diagnostic criteria for SARS-CoV-2 are based on molecular testing of reverse transcription-quantitative real-time PCR (qRT-PCR). The qRT-PCR oligonucleotides bind to a small area of approximately 20 bp. Mutations in these targets reduce effective amplification or probe binding, resulting in false-negative results. The application of high-throughput sequencing comparison helps us discover new mutations and variants timely. Strict control and other measures can reduce the spread of variants the first time and gain more precious time for clinical diagnosis and treatment.

3.4 | Effectiveness evaluation and development of new vaccines for variants

The universality of the variants of SARS-CoV-2 is a severe challenge not only for vaccine development but also for clinical testing. Although important information can be obtained from animal models and in vitro studies on the effectiveness of existing vaccines, clinical data is still needed to determine whether the existing vaccines are losing their effectiveness against variants. Clinical data can be obtained from carefully planned observational studies and controlled protocols (e.g., vaccine vs. placebo, different doses, number of doses, time intervals between doses, etc.). Nonrandom observational studies that estimate vaccine effectiveness are prone to certain biases. In areas where several variants coexist, observational studies of virus genotypes in vaccinated and unvaccinated cases can yield a relatively reliable estimate of the relative effectiveness of vaccination against various variants. Observational studies can also discover whether protection against previously infected variants is lost. When drawing relevant conclusions, relevant confounding factors must be eliminated. If the vaccination rate in the region is too low or the mutation rate is too high to be conducive to statistical stability, observational studies will lack accuracy. The confounding factors of the study can be limited by testing negative studies.[57–59]

In the research on the effectiveness of vaccines, complete sequencing of the isolates at the selected location can reduce the bias in the selection of samples. Samples obtained from unselected vaccine recipients with breakthrough infections and matched unvaccinated controls can be used to assess the impact of specific genomic or antigenic characteristics of interest on vaccine efficacy. Using these methods in trials or research after vaccine deployment can gather important insights about the correlation of specific virus characteristics, improving the selection of vaccine strains.[60]

The trial of new vaccines uses randomized evaluation to get reliable and interpretable results on immunology and clinical endpoints. When appropriate, placebo controls may be used in communities or subgroups with very limited vaccine supply (e.g., young people) to determine the likelihood of an infected person progressing to severe disease.[61–62] Randomized trials require additional planning, but where feasible, they can prevent differences in unidentified trial design from confounding research results.[63–64] Viral genotyping of patients with breakthrough infections (during or after the trial) can support a variety of analyses, including evaluating the impact of viral variants on vaccine effectiveness. In randomized, controlled studies, for example, genotyping can also generate information about the specific efficacy of the variants. Countries participating in such trials can evaluate the effective effects of vaccines on local viral strains. If these vaccines have been proven acceptable, safe, and effective, they should prioritize obtaining trial vaccines.[65] In areas where placebo-controlled trials of new vaccines are not appropriate, the use of activity comparators can still produce important results.[66] The development of vaccines that can control emerging variants is the direction that the global society must strive for.

4 | CONCLUSION

Current vaccines are effective against variants of SARS-CoV-2 and effective against severe, critical or fatal diseases caused by SARS-CoV-2 or any variant. Although we are developing a new generation of vaccines for variants, we also vigorously promote the inoculation of existing vaccines.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Xiucui Han designed the current study and was a major contributor in writing the manuscript. Qing Ye was responsible for the conception, the modification, and giving final approval of the manuscript. The authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data sharing does not apply to this article as no new data were created or analyzed in this study.

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