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Commentary

Severe acute respiratory syndrome coronavirus 2 escape mutants and protective immunity from natural infections or immunizations

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field of research: real-time tracking of viruses on a global scale, and trying to predict what mutations and deletions may be relevant. During the global dissemination and long chains of transmission, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has diversified with accumulation of mutations. Many of these mutations are neutral, in the sense that they do not affect any of the properties of the virus, some reflect the geographical dispersal of the virus (founder effects), and some have raised concern because they may allow the virus to evade immunity generated in response to previous infection or enhance transmissibility through mechanisms that are as yet undefined [1]. Increasing population immunity through natural infections and immunizations will increase the selection pressure on the virus and probably increase the evolution of new escape mutants. This brief review examines virus variants and individual mutations of current concern, including evidence for their importance for transmission and pathogenicity.

Introduction

Although coronaviruses have lower mutation rates than other respiratory RNA viruses, the scale of the pandemic has brought the importance of viral evolution for coronaviruses to centre stage. It has long been known that coronaviruses can evolve through acquisition of mutations and through recombination, but knowledge in the field is far behind that of some other viruses with global reach. This pandemic has seen an unprecedented amount of genomic sequencing, which is starting to open up an entirely new

Mutations

It has been estimated that SARS-CoV-2 evolves at a rate of ~1.1 × 10^{-3} substitutions per site per year, corresponding to one substitution every ~11 days [2]. Changes in the SARS-CoV-2 spike protein may alter both host receptor and antibody binding with possible effects on infectivity, transmission potential and antibody/vaccine escape. Although the relevance of the different variants and their combinations can be assessed *in silico*, their actual effects need to be measured and verified both experimentally and by epidemiological investigations. More than 12 000 mutations have already been detected in the SARS-CoV-2 sequence, compared with the reference sequence described at the beginning of the outbreak in Wuhan (hCoV-19/Wuhan/WIV04/2019). Their effects on viral fitness, transmissibility or clinical outcome are still largely

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unknown. Surveillance of mutations has been implemented by different databases and bioinformatics platforms, for instance GISAID [3], the EU COVID19 open data [4], the NCBI SARS-CoV-2 resource [5], Nextstrain [6], and the China Centre for Bioinformation [7]. There are also now three nomenclature systems, developed by Nextstrain [6], the University of Edinburgh PANGO [8] and GISAID [3], respectively, all widely used in the field. Updates of detections of spike gene mutations for each GISAID clade are posted daily on the *CoVariants* website [3,9], together with geographic maps of their proportional distribution [10], and visualized through collaboration with Nextstrain. The current version of the Nextstrain data visualization software allows switching between GISAID and PANGO lineage designations, and the WHO is working on a unifying system for nomenclature.

Three recently detected SARS-CoV-2 lineages (B.1.1.7, B.1.351 and P.1), have been scrutinized because they are unusually divergent and each possesses a unique constellation of mutations of potential biological importance, several of which are in the gene coding for the spike protein (Table 1) [11–15]. In addition, some viral genomes of the B.1.1.7 lineage have been observed that acquired the E484K mutation, which is thought to be linked to immune evasion [15–17].

Cell binding and functional immunity

Mutations that cause conformational changes in the spike protein receptor binding domain, and which affect binding to key host tissue receptors, are expected to impact on infectivity. For the above variants, there is some evidence that the binding affinity is increased, and that there may be a selective advantage increasing transmissibility. A recent study plotted the binding affinity to angiotensin converting enzyme 2 of all receptor binding domain mutations against their incidence in the population and showed a strong correlation between the two [18]. It also showed how further evolution might increase binding affinity even further [19]. Such mutations are also considered to have the most important impact on the effects of neutralizing antibodies. One study found that binding by polyclonal serum antibodies is affected by mutations in three main epitopes in the receptor binding domain [1]. The most important site is E484, where neutralization by some convalescent and vaccinee sera is reduced more than ten-fold by several mutations, including one in emerging viral lineages in South Africa (B.1.351, also named 501.V2) and Brazil (B.1.1.28.1, also named P.1) [1,20]. A study using Varicella-Zoster (VSV) pseudovirus expressing different variants of the SARS-CoV-2 spike protein found that VSV pseudoviruses with spike containing K417N-E484K–N501Y-D614G and full B.1.351 mutations were less easily neutralized with 2.7-fold and 6.4-fold lower neutralizing antibody titres respectively, when compared with the non-variant VSV pseudovirus used in this study [21].

Although some of these observations are reason for concern, the field is very young, and careful evaluation of the effects is needed, for instance on the impact of the emergence of variants on vaccine efficacy. In addition to neutralizing and binding antibodies, SARS-CoV-2 infection also elicits a vast repertoire of T-cell responses and dominant epitopes had the capacity to bind multiple human leucocyte antigen allelic variants [22]. A further study by the same authors found that 'CD4+ and CD8+ T cell responses in convalescent COVID-19 [coronavirus disease 2019] subjects or COVID-19 mRNA vaccinees are not substantially affected by mutations found in the SARS-CoV-2 variants' [23]. A major limitation of that study is that all donors were recruited in California and had no known exposure to the escape variants first found in the UK, South Africa or Brazil. Therefore, we do not know if T-cell responses elicited by previous SARS-CoV-2 infection or immunization with the present first-generation vaccines will be protective against infections with escape mutants.

South African variant B.1.351

The B.1.351 lineage is present in 90% of recent infections for which sequence data were generated in South Africa, and has been associated with increased transmissibility [24]. Press reports indicate that it may also predominate in Botswana, Zimbabwe, Zambia, Namibia and Malawi [25]. An increasing number of cases have been detected with the B.1.351 variant in different parts of the UK, which is believed to represent second- and third-generation cases, as those infected had no known links to South Africa [26]; at the time of writing, B.1.351 variant viruses have been found in more than 50 countries. The Novavax COVID-9 vaccine was reported to be somewhat less effective in preventing infection in a second trial in South Africa, where the SARS-CoV-2 variant B.1.351 is prevalent. In the South Africa trial of over 4400 people, the vaccine was 60% effective in people who were human immunodeficiency virus negative [27], compared with 89.3% effective at preventing COVID-19 in participants in its phase 3 clinical trial in the UK [28]. Protective efficacy was reduced further to 49.4% in South African individuals infected with the B.1.351 variant [25]. In a phase 3 trial including 44 000 people, a single dose of the Johnson and Johnson, JNJ, vaccine showed an overall protective efficacy of 66%. However, the contrasting efficacy of 72% in the US arm of the trial versus 57% in South Africa supports the concept of immune escape/resistance of the B.1.351 variant, as seen following the Novavax vaccine [29]. A recent study from South Africa found that effective neutralization by immune sera after B.1.351 infections inhibited first-wave (non-B.1.351) virus, providing preliminary evidence that vaccines based on variant sequences could work against other circulating SARS-CoV-2 lineages [30]. However, it remains to be seen to what extent emergence of new variants affects vaccine efficacy because results from separate trials are difficult to compare.

Brazilian variant P.1

The P.1 lineage was first discovered in Manaus, Brazil, where it accounted for 42% of genomes sampled in December 2020, having

Table 1
Non-synonymous mutations in the spike protein of variants of concern, in comparison with reference strain hCoV-19/Wuhan/WIV04/2019

Gene	SARS-CoV-2 variants of concern		
	VOC 202012/1 or B.1.1.7	S.501Y.V2 or B.1.351	P.1 or 484K.V2
Spike (S)	**HV 69-70 deletion, Y 144 deletion, N501Y, A570D**, D614G, **P681H, T716I, S982A, D1118H**	L18F, **D80A**, D215G, L242H, L 242-244 deletion, R246I, **K417N**, **E484K, N501Y, A701V**	L18F, T20N, P26S, D138Y, R190S, **K417T, E484K, N501Y,** H655Y, T1027I, V1176F

A report for VOC 202012/1 obtained by the analysis tool CoVsurver is accessible at www.gisaid.org [3]. Variant-defining amino acid mutations are shown in bold [12]. The S.501Y.V2 mutations list is from Tegally et al. [13], in bold only if the mutations are defined as fixed, i.e. present in almost all the samples, and consistently high in frequency across time.

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been absent in samples collected there between March and November 2020 [14]. A study from Brazil found that 76% of the population had been infected with SARS-CoV-2 by October 2020 [31]. The sharp increase in the number of COVID-19 hospital admissions seen in Manaus in January 2021 (3431 for 1–19 January 2021 compared with 552 for 1–19 December 2020) indicates that immunity obtained during infection in mid-to late 2020 was not fully protective [32]. Little is known about the transmissibility of the P.1 lineage, but it shares several independently acquired mutations with the B.1.1.7 (N501Y) and the B.1.325 (K417N/T, E484K, N501Y) lineages first detected in the UK and South Africa, which seem to have increased transmissibility [13]. The variant has been detected in 25 countries.

Vaccine upgrades and the future of immunization against SARS-CoV-2

Moderna is exploring how their vaccine could be updated to incorporate sequences coding for the new variants of the spike protein [33]. Novavax is working on a booster and/or combination bivalent vaccine in response to the B.1.351 variant in South Africa [34]. BioNTech is looking to authorize 'a new version of the Pfizer-BioNTech vaccine that would be better able to head off the variant in South Africa' [25]. Studies estimating the ability of vaccines to reduce transmission of different viral lineages continue to be essential [35]. A challenge will be when to decide to change the vaccine composition, as the dispersal is not uniform globally. Also, regulators are studying the regulatory process needed for vaccine updates.

Conclusion

The global expansion of SARS-CoV-2 and the continued circulation in partially immune populations has led to the emergence of variants with some adaptive changes leading to increased transmissibility and/or decreased sensitivity to neutralizing antibodies. The expanding number of people with immunity to SARS-CoV-2 following natural infection or immunization, and the inequality of access to and/or application of interventions and vaccines is likely to create further immune pressure on the virus. As a result, the strategy by vaccine manufacturers to foresee the need for second-generation vaccines covering the new mutants is reassuring. From a public health perspective, it is likely that we will need repeated immunization rounds, like those already in place for influenza, with annual vaccines tailored to new variants. The challenge with SARS-CoV-2 is the global scale of the immunization need. Therefore, the immunization infrastructure developed for the present programme will probably need to be maintained. In order to have a global perspective on the evolution of SARS-CoV-2, increases in global capacity for viral surveillance are essential to monitor the inevitable appearance and spread of new mutations in different parts of the world. This includes harmonization of the currently confusing nomenclature.

Transparency declaration

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Authors' contributions

All authors contributed equally to the manuscript.

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