Immunity to SARS-CoV-2 variants of concern**:** Variants show variable escape from vaccine immunity but residual protection may suffice

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Vaccine candidates based on spike, the glycoprotein that is essential for host cell entry by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), were being designed within days of its reported sequence in January 2020. All the vaccines aim to prevent disease primarily (but not exclusively) by eliciting neutralizing antibodies that block spike and therefore prevent the ability of SARS-CoV-2 to infect cells. The 95% efficacy of the BNT162b2 messenger RNA (mRNA) vaccine (from Pfizer/BioNTech) heralded a series of results showing that eliciting neutralizing antibodies to spike strongly correlated with protection from disease in clinical trials of various vaccines. Currently, there is concern about reduced vaccine-induced immune protection to emerging variants that have mutations in the spike protein. On page XXX of this issue, Muik et al. (1) found reduced induction of neutralizing antibodies from BNT62b2. However, there is likely sufficient efficacy remaining to confer protection from symptomatic disease.

Coronaviruses are very large and complex compared to other RNA viruses (around 4 times the size of the hepatitis A virus genome) and their replication fidelity must therefore be higher. Despite this, once a pathogen has been allowed to infect more than 100 million people, it is not a surprise that sequence variants with a selective advantage emerge. Towards the latter part of 2020, just as regulators were granting approvals to a series of vaccines based largely on the wild-type, 'Wuhan' sequence spike antigen, several SARS-CoV-2 'variants of concern' emerged. These variants have potentially enhanced transmission, pathogenicity, immune escape, or a combination of all three.

The first sequences of a variant of concern that emerged in the UK, B.1.1.7 (also called 501Y.V1), emerged in September 2020. It includes 8 amino acid changes within spike. One of these, N501Y (Asn^{501}→Tyr), increases the affinity of spike for its cellular target angiotensin-converting enzyme 2 (ACE2), and together with other less well characterized mutations has resulted in enhanced transmission (recognized since December), and possibly enhanced pathogenicity. Might this variant also escape antibody mediated immunity? Muick et al. examined the ability of immune sera from 40 older or younger 2-dose Pfizer vaccine recipients for neutralization of a pseudotype virus (a safe, surrogate virus engineered to express spike) carrying Wuhan sequence spike or all of the B.1.1.7 spike mutations. The sera had a wide range of neutralizing antibody titers measured against the 'Wuhan' spike, from around 1/50 to around 1/1200. Although there was a significant reduction in geometric mean titers for the younger (though not the older) cohort against the B.1.1.7 variant, the authors argue that, based on our understanding of other respiratory viruses such as influenza virus, an overall reduced titer of some 20% would not be predicted to meaningfully reduce vaccine efficacy. However, such findings confirm that the B.1.1.7 spike mutations impact not only transmission but also, immune recognition.

Another study looked in considerable detail at potential vaccine escape by B.1.1.7 (2). They considered immune sera from 23 vaccinees with a mean age of 82, analyzed at 3 weeks after a single dose of BNT162b2. Using a pseudotype virus carrying spike with all 8 mutations led to reduced neutralization for the majority of sera, by a factor of up to 6-fold. In this older cohort, the ablation of functional neutralization was more striking in those starting with lower antibody titers to the Wuhan sequence spike. A parallel dataset for pseudotype neutralization of Wuhan sequence or B.1.1.7 spike by mRNA-1273 (Moderna) or NVX-CoV2373 (Novavax) vaccinee sera detects a more marginal reduction in activity against the variant (3).

When population variation can mean that people develop diverse neutralizing antibody titers after vaccination, the extent to which a small drop in neutralization endangers protection from symptomatic disease depends to some extent on the immunogenicity of the vaccine and how much margin it leaves for protection. This issue is starting to be addressed by analyses of ChAdOx1 nCoV-19 (University of Oxford/AstraZeneca) vaccinees in the UK (4). Although neutralizing antibody titer against B.1.1.7 was reduced about 9-fold (from a mean of around 1/500 against wild-type virus), this did not affect vaccine efficacy because there was no enhanced susceptibility to infection [determined by

polymerase chain reaction (PCR)] due to the variant among the 499 participants who became infected.

Although the B.1.1.7 variant has had massive impact in exacerbating caseload and severity across many countries, there is even greater concern about variants carrying additional immune evasion mutations, notably the E484K (Glu[484]→Lys) mutation found in the B.1.351 (501Y.V2) variant that emerged in South Africa, the P.1 variant found in Brazil, and sporadic examples from UK sequencing showing E484K on the B.1.1.7 background (5). That the immune evasion mutations, K417N (Lys[417]→Asn), E484K and N501Y, can arise in evolution experiments in vitro involving culture of SARS-CoV-2 in the presence of immune sera, offers caution against sub-optimal vaccination regimens (6).

Concern about the B.1.351 variant derives from analyses of its effects on neutralization activity. The variant shows substantial ablation of any virus-neutralizing activity of therapeutic monoclonal antibodies (mAbs) (7). Preliminary data suggest a reduced neutralizing response in sera from ChAdOx1 nCoV-2 vaccinees, and reduced efficacy in preventing mild-moderate COVID-19 (8). This supports the view that neutralizing antibody titer is the key correlate of protection (CoP). Although analysis based on loss of in vitro neutralizing activity by individual mAbs representing the three dominant classes of epitopes on spike offers strong evidence for immune evasion by the variants, the effect is less pronounced at the level of polyclonal immune serum after convalescence or vaccination. This suggests that the neutralizing repertoire is broader and more resilient than so far documented.

Findings from studies with mAbs offer caution for using these therapeutically, given their vulnerability to loss of individual epitopes and also their ability to drive selection of variants that can evade immune recognition. Of course, the flip side of this argument is that detailed mapping of the neutralizing antibody epitopes in spike can facilitate the design of broadly neutralizing vaccines and mAbs that can target numerous spike mutants (9). It has been posited that SARS-CoV-2 may continue to accumulate mutations that evade immune responses (10). But, as previously explored for other viruses such as HIV, immune evasion often comes at a biological fitness cost to the virus, tending to impose an upper limit to the number of mutations that can be afforded when faced with a broad, neutralizing antibody repertoire (11). Additionally, given that similar mutations arise recurrently in spike, presumably by convergent evolution in geographically distinct isolates, it is possible that the spike variants offering a survival advantage to the virus will be constrained and finite. Furthermore, a spike protein that mutates residues A, B, and C to evade antibodies recognition runs the structural risk of generating a new neutralizing epitope, D. Therefore, a finite number of iterative vaccines could target key variants, but this would not necessarily have to be reappraised annually as with influenza virus vaccines. Seasonal 'common cold' human coronaviruses tend to appear with two-yearly cycles, and recent data for one of these suggests that antigenic drift (mutations that undermine immune recognition) may underly escape from acquired immunity (12).

T cell immunity is likely to feature as an additional CoP against COVID-19 (13). The CD4^{+} and CD8^{+} T cell response encompasses specificity to several hundred epitopes across the entire SARS-CoV-2 proteome, the majority of which are unimpaired in the variants (14, 15). Even those T cell epitopes that are altered in the SARS-CoV-2 variants will in most cases bind to the different human leukocyte antigens (HLA) that present antigenic peptides to T cells, although binding affinities may be altered. It would be helpful to investigate whether T cell immunity is modified by the SARS-CoV-2 variants.

The assessment of variants on neutralization are complicated by the variability of pseudotype assays used in these studies. It would be helpful to have standardized live virus in vitro neutralization assays as internationally comparable reference points. As ever, these are discussions that must be tethered to some sense of CoP values. Although much debated, many researchers have the sense that people with a neutralizing antibody IC50 (half

maximal inhibitory concentration) greater than ~1/100 serum dilution would likely be safe from infection, or at least from symptomatic infection. Given that these are highly potent vaccines often inducing antibody responses with IC50s of 1/1000s, there is hopefully a reasonable safety margin before reduced recognition of variants means that effective protection is lost. Ultimately, the best defense against emergence of further variants of concern is a rapid, global, vaccination campaign – in concert with other public health measures to block transmission. A virus that cannot transmit and infect others has no chance to mutate.

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