Immune Escape Variants from Antibody-Based Therapeutics against COVID-19

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The accelerated SARS-CoV-2 evolution under selective pressure by massive deployment of neutralizing antibody-based therapeutics is a concern with potentially severe implications for public health. Escape variants associated with mAb and COVID-19-convalescent plasma (CCP) therapy manifest different type of mutations. For monoclonal antibodies (mAb), most mutations are single amino acid replacements in the receptor binding domain (RBD) domain, while most variants eliciited in patients treated with CCP exhibited amino acid deletions. In fact, it is noteworthy that RBD mutations were relatively rare in CCP escape variants.

SARS-CoV-2 COVID-19 convalescent plasma viral clearance

1. Introduction

The SARS-CoV-2 spike protein is the target of neutralizing antibody (nAb)-based therapeutics. Control of the COVID-19 pandemic is being hampered by continued evolution of SARS-CoV-2, which includes mutations in the spike protein that can affect immunogenicity and antibody-mediated neutralization. Evolutionary modeling suggests that SARS-CoV-2 strains harboring 1–2 deleterious mutations naturally exist, and their frequency increases steeply under positive selection by monoclonal antibodies (mAb) and vaccines [1]. In 2% of COVID cases, SARS-CoV-2 variants with multiple mutations occur, including in the spike glycoprotein, which can become the dominant strains in as little as one month of persistent in-patient virus replication [2]. While mutations can occur as a natural phenomenon of SARS-CoV-2 RNA replication and editing, the pace of mutagen emergence can also be affected by small-chemical antivirals (e.g., remdesivir [3] or molnupiravir [4]). Since antibody-based therapies targeting the spike protein would also put selective pressure on SARS-CoV-2, it is reasonable to assume that widespread deployment of nAb-based therapeutics could accelerate spike immune escape by selecting for variants that resist neutralization.

Mutations that confer in vitro resistance to therapeutic anti-spike mAbs have been characterized with various methods and are informative about treatment-emergent immune escape. Deep mutational scanning (DMS) predicts protein expression, ACE2 binding, and mAb binding [5]. The method was first deployed with yeast display libraries [6], then evolved to phage display libraries (https://jbloomlab.github.io/SARS-CoV-2-RBD_MAP_clinical_Abs/) [7] and finally mammalian cell surface display [8]. nAb binding is common within the fusion peptide and in the linker region before heptad repeat (HR) region 2. The complete escape maps forecast SARS-CoV-2 mutants emerging during treatment with mAbs and allow the design of escape-resistant nAb cocktails. A complete map of SARS-CoV-2 RBD mutations that escape bamlanivimab and its cocktail with etesevimab has been generated [9][10].

Although DMS was also applied to polyclonal antibodies in COVID-19-convalescent plasma (CCP) [11], the problem is much more complex, such that it is almost impossible to identify escape mutations in CCP or vaccinee-elicited sera, given the huge heterogeneity in antibody response among CCP donors and vaccinees, respectively. In vitro, continuous passaging of SARS-CoV-2 in the presence of a CCP unit with nAb titer >1:10^4 led to ΔF140 spike mutation at day 45, followed by E484K at day 73, and an insertion in the N-terminal domain (NTD): these accumulating mutations led to complete immune escape [12]. Similarly, K417N, E484K, and N501Y mutations were selected when pseudotyped SARS-CoV-2 was cultured in the presence of vaccine-elicited mAbs [13]. Although some have speculated that the large-scale use of CCP for COVID-19 could have played a role in the emergence of variants, there is no evidence for such an effect and the most likely explanation for the regular emergence of variants has been the huge number of affected individuals since each infection case provides a natural opportunity for variant creation [14].

2. Immune Escape Variants from Antibody-Based Therapeutics against COVID-19

Escape from nAb-based therapeutics provides a crucial demonstration that these immune therapies target protective antigens, which the pathogen actively evades. Hence, the emergence of neutralizing-resistant variants in individuals receiving mAb and CCP provides powerful evidence for their antiviral activity. This evidence is independent of reduction in viral load, which has been reported with mAbs given early in disease but have been an inconsistent finding in randomized controlled trials (RCT) of CCP for COVID-19 [15].

Obtaining the frequencies for this phenomenon from case series is not possible due to the high risk of selection biases, which would yield unrealistically high frequencies. In contrast, RCTs with their control groups are the suggested reference. With bamlanivimab, resistance was reported in 7% of patients, regardless of dosage (700/2800/7000 mg) versus <1% in patients treated with placebo [16][17]. Apart from registration trials, the largest case series to date evaluated the impact of mAbs on the nasopharyngeal (NP) viral load and virus quasi-species of mAb-treated patients using single-molecule real-time sequencing after bamlanivimab alone (4 patients), bamlanivimab/etesevimab (23 patients) and casirivimab/Imdevimab (5 patients) [18]. To date a single case of immune escape has been reported for the non-overlapping REGN-COV2 cocktail, and accordingly hamster models and clinical trials showed no emergence of variants [19]. Since mAb therapy by definition targets only a single epitope within the RBD, it is unsurprising that escape mutations observed after in vitro and in vivo selection by these mAbs were single amino acid substitutions localized almost exclusively to the RBD, as expected from in vitro studies with single mAb, but largely prevented by non-overlapping mAb cocktails [20].

In contrast to mAb therapeutics, immune escape under CCP has not been investigated in RCTs. Hence, evidence exclusively stems from case series and case reports [21] and is further complicated by exposure to multiple CCP units from different donors, each one having a polyclonal response at differing titers and affinity. Unfortunately, nAb titers were very rarely determined or reported, precluding correlation between the emergence of resistance and subneutralizing CCP doses. Overall, it seems that escape variants from CCP selection have not been reported as commonly nor emerged as fast, e.g., none of the eight recipients of hematopoietic stem cell transplantation or

chimeric antigen receptor T (CART) lymphocytes who were treated with CCP and tested SARS-CoV-2-positive for 2 months showed significant mutations compared to the original strain [22]. A review of the spike protein changes associated with resistance after CCP therapy reveals that most of them had in-frame amino acid deletions in a flexible region that is partially solvent exposed and forms a β strand: plasticity may contribute to the structural permissibility of the identified deletions. The NTD is a flexible region that can be affected by immune escape via either insertions (causing additional glycosylation sites [12]) or recurrently deleted regions (RDR) ΔHV69–70 (RDR1), ΔLGVY141–144 and ΔD146 (RDR2), ΔI210 (RDR3) and ΔAL243–244 (RDR4) [23]: RDR1, RDR2 and RDR4 correspond to NTD loops N2, N3 and N5, whereas RDR3 falls between N4 and N5.

Deletions of amino acids from a protein structure generally result in greater structural changes than single amino acid changes, since these reduce the size of the protein and can trigger changes that propagate through the whole structure. Furthermore, the mechanism for the emergence of deletion variants appears to be very different from the single amino acid changes that are frequent from error-prone RNA replication and could involve deletions from RNA editing. Since CCP targets a large number of epitopes in the spike protein while mAbs target a single epitope, these molecular differences parallel what is expected from their respective selection pressures in the sense that escape from polyclonal preparations requires larger antigenic structural changes than escape from mAbs. In contrast to escape mutations selected for by mAb therapy, CCP selection yields point mutations throughout the spike protein. This reflects the vast antigenic surface area covered by the polyclonal antibodies within CCP. Escape mutations would be theoretically selected for on the basis of the most potent antibodies present in a particular CCP unit, which may vary markedly from donor to donor, which could explain the generally divergent evolution of SARS-CoV-2 in the presence of CCP. However, residues 141–144 and 243–244 are the sites of mutations or deletions in several cases, indicating these sites may offer effective escape from CCP derived from many donors, possibly by triggering a large-scale conformational rearrangement, as discussed above. As RBD binding antibodies are often neutralizing via ACE2 receptor occlusion, it is interesting that only 23% of CCP case studies identified the escape mutations within the RBD. This suggests that antibody binding to other sites on the spike protein may have additional mechanisms of neutralization (i.e., by preventing conformational change after ACE2 engagement), or that additional antibody mediated immune responses (e.g., ADCC) are equally important as direct neutralization to the antiviral response to SARS-CoV-2.

Nothing can be inferred about the fitness of an emerging mutant in the absence of selective pressure, but it is of interest that one variant with the E484K mutant that emerged after bamlanivimab therapy was able to infect multiple household contacts [24]. In vitro, several mutants showed similar infectivity to the wild-type strain but resistance to different CCP donors [25]. In one instance of immune escape associated with CCP, a variant with D796H mutation manifested modestly reduced sensitivity to neutralization by CCP that was associated with reduced infectivity, which was only partly compensated by ΔHV69–70 [25]. Even if immune escape in registration trials has been a rare phenomenon, it should be considered that in real-world practice, mAbs targeting of the SARS-CoV-2 spike protein is being reserved for use in high-risk (immunocompromised) patients. Considering the huge size of the pandemic, the likelihood of immune escape becomes relevant, raising the possibility that rare variants with enhanced fitness could drive the next pandemic waves. Notably, several mutations have recurred in VOC and VOIs (e.g., E484K found in Beta and Gamma, E484Q found in Delta, or ΔLHR244–246 [26] found in VOI

lambda), raising the possibility that such variants emerged during the treatment of patients (iatrogenic variants), but such inference will likely remain very hard to prove. E406W mutation, which causes resistance to REGN-COV-2, has never been reported in GISAID, and other E406 mutations remain exceedingly rare (worldwide, 318 cases of E406Q, 41 cases of E406D, and 2 cases each from USA for E406G, E406A, E406K, and 1 case of E406V out of 4,410,787 sequences deposited in GISAID as of 13 December 2021). The same is true for sotrovimab resistance, with E340 and P337 mutations exceedingly rare to date (E340K in 159 sequences worldwide, P337R in 18, P337L in 195, E340A in 105, E340G in 36, P337H in 44, P337T in 90) (source: Outbreak.info). Similarly, Q493R, which causes resistance to bamlanivimab + etesevimab, had only been reported in 244 sequences and Q493K in 138 sequences, before becoming one of the hallmark mutations of VOC Omicron. L452R, which causes resistance to regdanvimab, also became prevalent first in VOI Epsilon and then in VOC Delta (source: Outbreak.info). Lack of fixation of those mutations facilitates the imputation that these require mAb selective pressure and/or effective infection control techniques in the care of those patients to prevent spill over to the general population.

Within-host variation (so-called “quasi-species swarm”) is a natural phenomenon which has been reported for SARS-CoV-2 in immunocompetent patients and ultimately facilitates the persistence of infection. Among 33 patients having positive NPS PCR for an average of 18 days, Voloch et al., observed a distinguishing pattern of mutations over the course of the infection mainly driven by increasing A→U and decreasing G→A signatures, including spike mutations (V362L, T553I, H655Y, A688V, S691F, S884F, V1176F). G→A mutations are driven by the RNA-editing enzyme activities typical of innate immunity [27]. Nevertheless, several covariates can facilitate immune escape.

Immunosuppression has been postulated to be an accelerator for viral evolution. Actually, **Table 1** shows that very few case reports have detailed intraclonal (within-host) evolution in patients receiving immunosuppressive treatment, and, in the absence of nAb-based therapeutics, spike mutations rarely occurred [22].

Table 1. Intrahost variation in spike sequence detected in immunocompromised patients not receiving nAb-based treatments.

Age/Sex (Identifier)	Condition	Antiviral Treatments	SARS-CoV-2 Strain	Spike Mutations	First Detected at Day	Outcome	Ref
47/F	diffuse large B cell lymphoma (rituximab plus polychemotherapy)	n.a.	B.1.1.163	Y453F, ΔHV69–70, S50L, ΔLGVY141–144, T470N, and D737G	120	negative PCR on day 132	Bazykin et al. [7]
61/F	diffuse large B cell lymphoma stage IVB	remdesivir for 10 days, high-dose steroids for 7 days	B.1.1.401	V3G, S50L, N87S, A222V, ΔLTTRTQLPPAYTN18–30 and ΔLGVY141–144	164	negative PCR at day 197	Borges et al. [28]
3/F (1)	B-cell acute lymphoblastic leukemia	n.a.	20C	silent I410I (22792:C/A)	27	negative PCR at	Truong et al. [29]

Age/Sex (Identifier)	Condition	Antiviral Treatments	SARS-CoV-2 Strain	Spike Mutations	First Detected at Day	Outcome	Ref
	(chemotherapy)					day 91	
2/M (3)	B-cell acute lymphoblastic leukemia	remdesivir for 5 days	20C	V483A and E484Q	139	negative PCR at day 196	
				V70P, ΔLGV141–143, N440K	162		
37/F	advanced HIV and antiretroviral treatment failure	dexamethasone	B.1.1.273	E484K	6	negative at day 233	Karim et al. [30]
				K417T and F490S	71		
				L455F and F456L	106		
				D427Y and N501Y	190		
80/M	chronic lymphocytic leukemia and hypogammaglobulinemic	remdesivir days 213–230, REGN-COV-2 day 265	B.52	L179	58	negative PCR day 311	Kavanagh Williamson et al. [31]
				S255F, S477N, H655Y, D1620A, ΔHV69–70	155		
40/M	autologous hematopoietic stem cell transplant due to a diffuse large B-cell lymphoma	IVIg	B.1.128	ΔLGV141–143 → ΔLGVY141–144		negative PCR on day 196	Mendes-Correa et al. [32]
n.a./n.a.	transplant recipient [3]	remdesivir	n.a. [35]	S13I, T95I, E484G, F490L, ΔLGVY141–144, ΔLHRS244–247, and ΔSPRRARSV680–687	n.a.	n.a.	Weigang et al. [33]
n.a./n.a.	18 B-cell non-Hodgkin lymphoma	44% CCP 37% remdesivir	n.a.	n.a.	requested	n.a.	Lee et al. [34]

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