Mutations (red) in the spike protein (green) of SARS-CoV-2 variants that affect host receptor (light gray) or antibody (dark gray) binding could impair immunity.

**INSIGHTS**

**PERSPECTIVES**

**VIEWPOINT: COVID-19**

The emerging plasticity of SARS-CoV-2

The evolution of SARS-CoV-2 poses challenges for vaccines and immunotherapies

By Kevin D. McCormick, Jana L. Jacobs, John W. Mellors

Viruses evolve as a result of mutation (misincorporations, insertions or deletions, and recombination) and natural selection for favorable traits such as more efficient viral replication, transmission, and evasion of host defenses. Newly selected traits may be linked in unpredictable ways and raise concern that virus spread and evolution could result in greater virulence (disease severity). The limited diversity of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reported during 2020, ascribed to the 3’-5’ exoribonuclease proofreading function of nonstructural protein 14 (nsp14), led to the view that vaccines based on a single sequence of the viral spike (S) protein, which mediates host cell entry, would likely generate immune protection to all circulating variants (1). However, variants of SARS-CoV-2 with mutations in S have emerged around the world, posing potential challenges for vaccination and antibody-based therapies. The continued spread of SARS-CoV-2 creates the opportunity for accumulation of additional consequential mutations in S and throughout the viral genome.

Although SARS-CoV-2 shares high sequence homology with SARS-CoV, which caused the 2002–2004 SARS outbreak, the coronavirus family is diverse in both sequence and in host receptor preference. For example, SARS-CoV-2 and a “common cold” human coronavirus, HCoV-NL63, both recognize angiotensin-converting enzyme 2 (ACE2) as the host cell receptor, but SARS-CoV-2 and HCoV-NL63 belong to different coronavirus genera and have major sequence and structural differences in the receptor-binding domain (RBD) of S, sharing <30% sequence homology (2). This diversity in S indicates that coronaviruses have broad potential to tolerate changes in both sequence and structure without substantial loss of function. This may partially explain why coronaviruses can undergo zoonotic transmission and suggests that the full evolutionary potential of SARS-CoV-2 has yet to be revealed.

The S protein comprises two subunits: S1, which contains the RBD, and S2, which mediates virus–host cell fusion. Antibody-neutralizing epitopes are scattered throughout S but are mostly concentrated within the RBD. Despite the potential for plasticity, after nearly a year of spread (from December 2019) to >100 million people, there was limited evidence for evolution of SARS-CoV-2 S. The only notable evolutionary event was the D614G (Asp614Gly) substitution in S1, which increases ACE2 affinity, leading to higher infectivity and transmissibility. Viral sequences deposited in public databases were mostly obtained from the upper respiratory tract during acute infection, before major immune responses have occurred. Such sequences might not capture the effect of within-host immune selection on viral diversification.

Extensive intrahost evolution of SARS-CoV-2 has been reported in at least five individuals with protracted infection because of immune impairment from therapy for hematologic malignancies or autoimmunity (3–7). They had active SARS-CoV-2 infection for an average of 115 days before clearing the infection or succumbing to COVID-19. Each patient also had at least one convalescent plasma (CP) treatment (intravenous transfusion of blood plasma from a donor who has recovered from COVID-19) and/or monoclonal antibody therapy. Some of these individuals were shedding high titers of SARS-CoV-2 variants with mutations, raising concern that vaccine efficacy could be compromised.

The continuing spread of SARS-CoV-2 presents the opportunity for further selection of the virus and potential emergence of variants that evade immune responses and therapies. This raises the challenge of developing vaccines and immunotherapies that can provide broad and sustained protection against the virus.

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Division of Infectious Diseases, University of Pittsburgh School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA. Email: jwm1@pitt.edu
Tyr mutations were seen in two out of five infections (3, 5–7); the N501T (Asn→Thr) or N501Y (Asn→Tyr) mutations were seen in two out of five infections (5, 6); and the E484K (Glu→Lys) and Q493K (Gln→Lys) mutations in the RBD of one infection also arose in antibody-resistant viruses after in vitro selection.

These reports preceded the detection of three major circulating variants—B.1.1.7, B.1.351, and P1—which all contain at least eight single, nonsynonymous nucleotide changes, including E484K, N501Y, and/or K417N (Lys→Asn) in the ACE2 interface of the RBD (shown in the illustration). There are also various deletions in the amino (N)-terminal domain (NTD) of S1 in B.1.1.7 and B.1.351 (see the figure). Although most of the mutations in these variants were observed in a minor fraction of SARS-CoV-2 sequences during the first year of the pandemic, including K417N, E484K, and N501Y, there is no evidence to suggest that these variants were created through sequential addition of each substitution during interhost transmission.

Because only a few SARS-CoV-2 mutations were in circulation during most of 2020, it is likely that the three major variants are the result of selective pressures and adaptation of the virus during prolonged individual infections and subsequent transmission. All the case reports of individuals with extensive intrahost SARS-CoV-2 evolution indicated that they had been treated with suboptimal neutralizing antibodies (that is, the CP treatment did not neutralize the entire virus population). Whether or not antibody therapy played a role, it is likely that the same variants or variants containing new mutations will continue to emerge in different geographic locations as the result of intrahost selection and subsequent transmission. Indeed, other variants have been reported with multiple mutations in S1, including the lineages B.1.526 (detected in New York) and B.1.429 (which originated in California) containing a substitution in the RBD that is distinct from other variants; and B.1.525 and A.231 that are thought to have originated in Nigeria and Uganda, respectively (8) (see the figure).

The individual phenotypic effects of the mutations in S1 are incompletely understood, but some initial clues are emerging. Substitution at position Asn505 with Thr or Phe increases affinity for ACE2 binding (9), and Tyr505 increases infectivity and virulence in a mouse model (10). Some circulating variants may have reduced sensitivity to neutralizing antibodies that bind to the RBD directly (attributed to triple substitutions of key amino acids in the RBD at the ACE2-binding interface: Lys417, Glu484, and Asn505) or to the NTD (conformational changes in the NTD are required for ACE2 attachment). More studies to correlate viral genotype and phenotype are needed.

It is possible that mutations that reduce neutralizing antibody binding, such as E484K, may require compensatory mutations that restore infectivity, such as N501Y. There appears to be convergent association of mutations such as the triple RBD mutation (Lys417, Glu484, and Asn505) that evolved in two distinct lineages (B.1.351 and P1). Moreover, E484K was also recently detected with N501Y in the B.1.1.7 lineage (11). Δ69–70 in S1 doubles the infectivity of SARS-CoV-2 pseudo-virus, implying that the deletion may have been required to compensate for a mutation, D796H (Asp→His), that reduced antibody neutralization sensitivity at a cost to viral fitness (7). The role of compensatory mutations is also supported by the emerging B.1.525 lineage that has both E484K (reduction in antibody sensitivity and Δ69–70 (compensatory increase in infectivity).

It is not yet known whether the complex mutational patterns observed in SARS-CoV-2 variants are linked on the same viral genome or represent mixtures of different variants within the same patient. Studies evaluating the linkage of these mutations in individual SARS-CoV-2 genomes using single-genome amplification and sequencing, as has been used to characterize genetic diversity of HIV-1 and other viruses, are needed to accurately assess the infectivity and phenotype of individual variants. A case report of intrahost SARS-CoV-2 evolution showed that SARS-CoV-2 can evolve multiple distinct lineages within the same individual (6).

Several studies suggest that the major circulating variants have reduced neutralizing sensitivity to CP and plasma from recently vaccinated individuals. For example, CP from individuals who were infected with the B.1 lineage (D614G-containing SARS-CoV-2) had varying reductions in neutralizing activity against live virus isolates of the B.1.351 lineage. Additionally, vaccine-elicited antibodies have reduced neutralization of pseudo-virus containing the triple mutation in S1 (K417N, E484K, and N501Y) of the P1 and B.1.351 variants (12). Pseudovirus bearing the deletions and mutations found in the B.1.1.7 lineage also showed reduced neutralization sensitivity, but titers of antibody were suf-
(which produce neutralizing antibodies), there are numerous additional vaccine-induced responses of the innate and adaptive immune systems that may protect against infection and further viral immune escape. Conversely, there are uncharacterized mutations outside of S that could facilitate SARS-CoV-2 immune evasion.

The growing evidence for the emergence of immune escape mutations in protracted SARS-CoV-2 infection and for multiple, rapidly spreading variants should raise broad concern and action. Reducing the spread of SARS-CoV-2 is most likely to prevent further selection of immune escape variants. This will require a coordinated and comprehensive global vaccination and prevention strategy. Partial roll-out and incomplete immunization of individuals leading to suboptimal titers of neutralizing antibody could promote selection of escape variants that negatively affect vaccine efficacy. Increased genotypic and phenotypic testing capacities are essential worldwide to detect and characterize SARS-CoV-2 variants that may emerge from selection by natural or vaccine-mediated immune responses. Infections that occur among vaccinated individuals should be aggressively evaluated for the mechanisms of breakthrough. The explosive, global spread of SARS-CoV-2 and the devastation it has wreaked is a stark warning of the potential worldwide to detect and characterize SARS-CoV-2 variants and phenotypic testing capacities are essential to global vaccination and prevention strategies.

REFERENCES AND NOTES

3. V. A. Avanzato et al., Cell 183, 1901 (2020).

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IMMUNOLOGY

Unusual T cell receptor in opossum

The structure of a marsupial T cell receptor illustrates the emerging trend of noncanonical antigen binding

By Michael F. Criscitiello

B and T lymphocytes of the vertebrate adaptive immune system have structurally, genetically, and evolutionarily related receptors for antigen recognition that initiate immune responses with notable specificity and memory. In general, the antigen binding sites of these receptors are evolutionarily conserved, yet a few very different immunoglobulin (Ig) structures have been characterized from shark, camels, and cow B cells. On page 1383 of this issue, Morrissey et al. (1) reveal the structure of an opossum T cell receptor (TCR) that also eschews the vertebrate norm. This marsupial TCR is the latest in an emerging trend of small, projecting structural domains that are used for antigen recognition by the adaptive immune systems of some species, and it might have therapeutic potential.

From sharks to man, vertebrates have two varieties of T lymphocytes for orchestration of immune responses through cytokine secretion and direct killing of infected or cancerous cells. T cells of αβ and γδ lineages differentiate in the thymus where they rearrange genetic loci encoding either an αβ or a γδ heterodimeric TCR for antigen recognition. More than a decade ago, a fifth TCR chain in the older nonplacental mammals (including platypus, echidna, and marsupials), called TCRμ, was discovered (2). Although most similar to TCRδ, TCRμ is encoded at a distinct locus and was predicted to have two variable domains at its membrane-distal amino terminus. Little is known of the T cells that express TCRμ.

Morrissey et al. greatly extend our understanding of TCRμ and the cells that express it. Although the TCRμ chain was found not to be expressed in the peripheral blood mononuclear cells in opossums, nearly as many γδ T cells were found in the spleen as αβ-expressing T cells. In addition to confirming TCRγδ as the heterodimeric partner of the TCRμ chain, single-cell RNA sequencing analysis showed that most of the γδ T cells use the CD8αα⁺ homodimer, although some expressed neither the CD4 nor the CD8 TCR coreceptor. The more common CD8αβ⁺ heterodimer is used by cytotoxic T lymphocytes. CD8αα⁺ function is largely unknown, although in humans it can be an inhibitory coreceptor on natural killer cells (3). Functional studies will have to determine if TCRγδ signaling is inhibitory or more regulatory in nature.

Reaching for antigen

TCRγδ is part of a growing trend of T and B cell antigen receptors with reach. Most vertebrate antigen receptors bind antigen with six CDRs, three contributed by V domains of each partner chain (heavy and light chains, TCRα and β or TCRγ and δ). TCRμ is the latest to break from this canon.

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