CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Emergence of a Highly Fit SARS-CoV-2 Variant

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Sarbecoviruses have emerged twice in the 21st century, causing a worldwide epidemic and pandemic. The ongoing pandemic of coronavirus disease 2019 (Covid-19), the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused unprecedented disruption of human society. Since its emergence in December 2019, SARS-CoV-2 has spread worldwide, infecting more than 70 million persons and causing more than 1.6 million deaths as of early December 2020. Previous studies have clearly shown that epidemic and pandemic RNA virus spread may select for mutations that alter RNA virus pathogenesis, virulence, transmissibility, or a combination of these,[1] yet this process remains poorly studied among emerging coronaviruses in animals and humans.

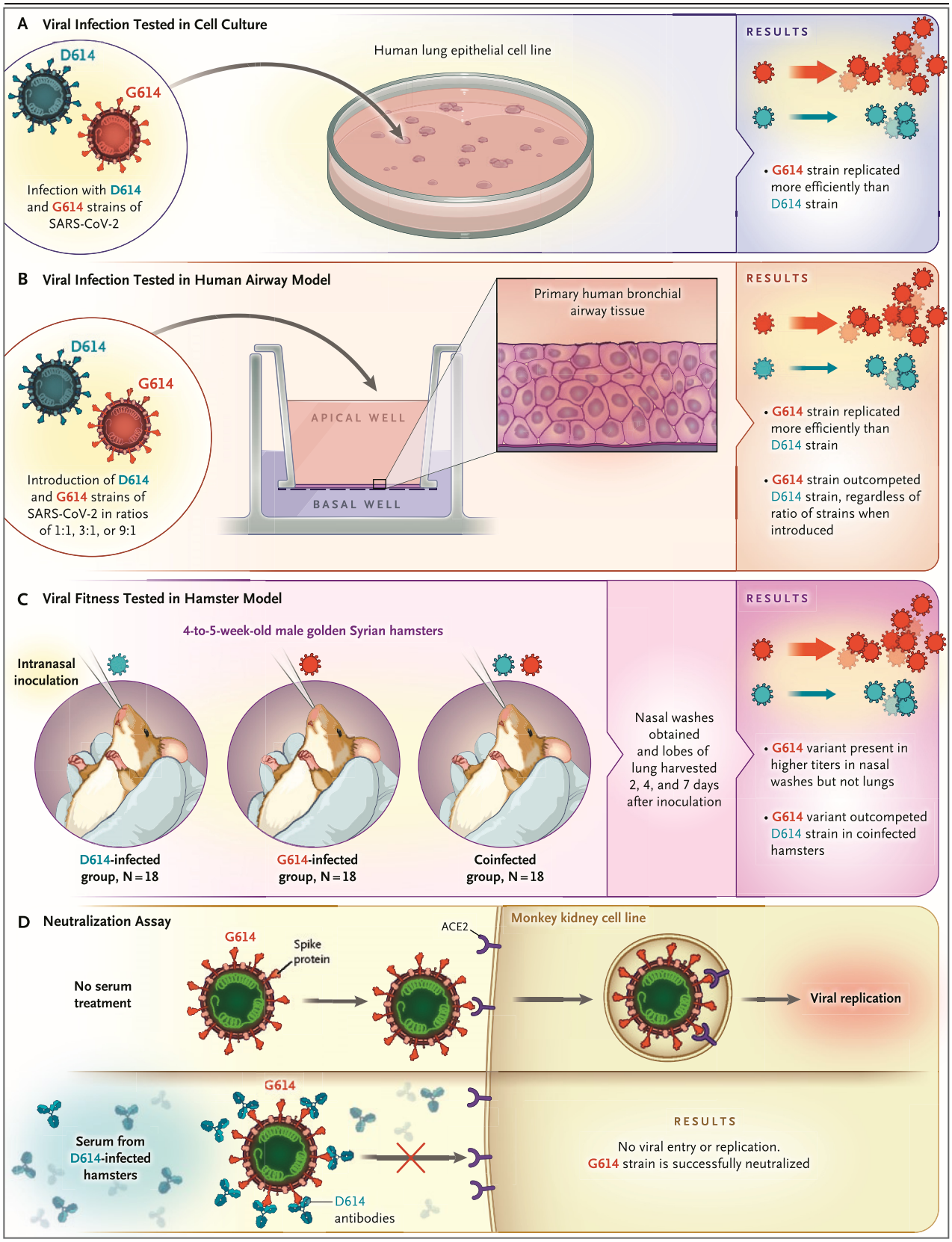
SARS-CoV-2 probably emerged from bats, and early strains identified in Wuhan, China, showed limited genetic diversity, which suggests that the virus may have been introduced from a single source.[2] Early zoonotic variants in the novel coronavirus SARS-CoV that emerged in 2003 affected the receptor-binding domain (RBD) of the spike protein and thereby enhanced virus docking and entry through the human angiotensin-converting–enzyme 2 (hACE2) receptor.[3] In contrast, the spike-protein RBD of early SARS-CoV-2 strains was shown to interact efficiently with hACE2 receptors early on.[2]

However, despite the presence of a CoV RNA proofreading activity that yields high replication fidelity, genetic epidemiologic investigations conducted in late February identified an emerging D614G mutation affecting the spike glycoprotein of SARS-CoV-2 strains from southern Europe; this variant has since spread rapidly and has become the most prevalent genotype worldwide.[4] Patients infected with D614G-associated SARS-CoV-2 are more likely to have higher viral loads in the upper respiratory tract than patients infected with virus strains without the mutation, but disease severity is not affected. Pseudotyped viruses with the G614 form of the SARS-CoV-2 spike protein have been reported to exhibit increased infectivity in continuous cell lines and increased sensitivity to neutralization. In addition, structural analyses have revealed that the RBD of the G614 form of the spike protein is more likely to assume an "open" conformation than the RBD of the ancestral D614 form, implying an improved ability to bind to the hACE2 receptor. However, published reports of isolation of the D614G substitution in an authentic SARS-CoV-2 recombinant live virus are lacking, as are investigations on the effects of the mutation on in vivo replication and pathogenesis.

In a recent study, Plante et al. used reverse genetics to recover isogenic recombinant SARS-CoV viruses encoding the D614G mutation.[5] The G614 variant replicated more efficiently than did the D614 variant in immortalized cells in culture and in primary human airway epithelial cells (Fig. 1A and 1B). Even at D614-to-G614 variant infection ratios of 1:1, 3:1, or 9:1, the contemporary

Figure 1 (facing page). Increased Infectivity of SARS-CoV-2 Bearing the Spike Protein D614G Substitution.

A study recently reported by Plante et al.[5] showed that a variant of SARS-CoV-2 carrying the spike protein D614G substitution results in increased virus infectivity and yield in human lung epithelial cells (Panel A), in primary human airway tissue (Panel B), and in the upper airway of hamsters (Panel C). These data suggest that the D614G mutation results in enhanced transmissibility. In addition, serum samples from D614-virus–infected hamsters can efficiently neutralize the G614 virus from infecting cells (Panel D), which suggests that SARS-CoV-2 vaccines, all of which are based on the D614 variant of the spike protein, will protect against G614 variants of the virus.



G614 strain outcompeted the ancestral D614 strain in primary human airway epithelial cells. The G614 variant also seemed to be more stable than the ancestral strain, which suggests that increased stability may be associated with increased infectivity, although additional investigations will be needed to confirm this finding.

In studies in hamsters infected with D614 or G614 variants, Plante et al. showed that the contemporary G614 variant replicated to higher titers in nasal-wash samples early after infection and outcompeted the ancestral D614 variant (Fig. 1C); these findings suggest increased fitness in a major upper airway compartment potentially associated with enhanced transmission. The SARS-CoV-2 G614 variant did not cause more severe disease than the ancestral strain in hamsters, a finding that supports current findings in humans. The Covid-19 vaccines that are currently being evaluated in clinical trials are based on the original D614 ancestral spike sequence; therefore, the authors used a panel of serum specimens to test whether the G614 variant is as sensitive to neutralization as the ancestral strain (Fig. 1D). Fortunately, the results showed that it is as sensitive to the serum specimens as the D614 strain and thus may allay fears that it could escape vaccine-elicited immunity.

Plante et al. have provided evidence of the genetic and molecular basis for enhanced fitness of the G614 variant over ancestral strains, providing strong support for its role in facilitating global spread. Unlike variants in the SARS-CoV 2003 epidemic strain, those in SARS-CoV-2 may point to new mechanisms that are associated with pandemic spread in human populations. In addition to showing the critical importance of blending genetic epidemiologic studies with empirical molecular virologic studies to understand pandemic virus evolution and spread, the findings raise critical questions regarding the future evolutionary trajectories of the SARS-CoV-2 G614 variant. These questions are especially important at a time when environmental pressures, such as expanding herd immunity, vaccine-induced immunity, antiviral therapies, and public health intervention strategies, may — through selective pressure — promote virus survival and escape. Will these selective pressures drive antigenic variation, promote virus stability and transmissibility, alter virus virulence and pathogenesis, or drive SARS-CoV-2 to extinction or into alternative hosts as reservoirs? Plante et al. articulate a critical need for proactive, rather than reactive, tracking of SARS-CoV-2 and other potential emerging coronaviruses.

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