THE IMPORTANCE OF NATURALLY ATTENUATED SARS-CoV-2 IN THE FIGHT AGAINST COVID-19

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The current SARS-CoV-2 pandemic is wreaking havoc throughout the world and has rapidly become a global health emergency. A central question concerning COVID-19 is why some individuals become sick and others not. Many have pointed already at variation in risk factors between individuals. However, the variable outcome of SARS-CoV-2 infections may, at least in part, be due also to differences between the viral subspecies with which individuals are infected. A more pertinent question is how we are to overcome the current pandemic. A vaccine against SARS-CoV-2 would offer significant relief, although vaccine developers have warned that design, testing, and production of vaccines may take a year if not longer. Vaccines are based on a handful of different designs (*1*), but the earliest vaccines were based on live, attenuated virus. As has been the case for other viruses during earlier pandemics, SARS-CoV-2 will mutate and may naturally attenuate over time (*2*). What makes the current pandemic unique is that, thanks to state-of-the-art nucleic acid sequencing technologies, we can follow in detail how SARS-CoV-2 evolves while it spreads. We argue that knowledge of naturally emerging attenuated SARS-CoV-2 variants across the globe should be of key interest in our fight against the pandemic.

As expected for any RNA virus (*2*,*3*), over time, individuals are infected with SARS-CoV-2 variants that typically display some degree of genetic drift, compared with the first isolates of the virus obtained in Wuhan (*4*). Indeed, this genetic drift permits SARS-CoV-2 variants to be tracked as they spread around the globe (*5*). We know from studying viral evolution that genetic drift, in particular derived from genomic deletions, will almost inevitably attenuate the pathogenicity of viruses given enough time (*2*). It is entirely plausible that, at present, some individuals, by chance, have already become infected with attenuated versions of SARS-CoV-2. Since self-seclusion of the symptomatic and those at high-risk has been prompted by many authorities, there may have been, concomitantly, selective advantages for potential attenuated variants of SARS-CoV-2 to spread over non-attenuated variants. Fortunately, COVID-19-related hospitalizations and fatalities have decreased several weeks after lock-down measures were installed in various countries. Clearly, the reduction in transmission of the non-attenuated virus has been key in these successes. It cannot be excluded, however, that competition of attenuated strains with the non-attenuated SARS-CoV-2 has also played a role in curbing demand on the health care systems.

Reasoning along these lines, competition of attenuated variants with the non-attenuated SARS-CoV-2 may explain certain particularities in the course of the epidemic at different locations around the globe. Although currently available data on mortality for the SARS-CoV-2-caused pandemic (*6*) should be interpreted with caution, it seems that case fatality rates tail off even in locations with less effective containment measures. Furthermore, effects of founder events may shed light on the atypical dynamics of some local epidemics, which pair high numbers of infections with low mortality rates. For example, if many of the first local spreaders of the virus were infected with attenuated strains, the spread of the non-attenuated virus may have been blunted from the onset, due to competition. Mathematical modeling may provide support for these hypotheses in further detail, in particular after more reliable data becomes accessible.

Currently available sequences for SARS-CoV-2 isolates from across the globe confirm that genetic drift is occurring (*3,4*), although association between these genetic alterations and severity of clinical outcome are sparse. The only hint at attenuation comes from an observation in Singapore, where some SARS-CoV-2 isolates turned out to have a 382-nucleotide deletion in ORF8 of the viral genome (*7*). This finding is of particular interest, because a deletion of 29 nucleotides appeared in ORF8 during early spreading of SARS-CoV-1 in 2003. This 29 nucleotide deletion was demonstrated to attenuate viral replication when introduced in an infectious clone generated by reverse genetics (*8*). Likewise, the SARS-Unique Domain in the Nsp3 protein of SARS-CoV-1, which is partially conserved in SARS-Cov-2, has been linked to evasion of host immune recognition, suggesting that the loss of this domain could result in reduced virulence (*9–11*). Based on these examples and others (*12*), one would expect that attenuated variants of SARS-CoV-2 would emerge as the pandemic unfolds.

We argue that sequencing efforts, thus far, likely underestimate the emergence of attenuated SARS-CoV-2 variants. Most of the isolates that have been analyzed are derived from individuals that sought medical care, and, hence, are unlikely to have acquired any features leading to reduced virulence. Instead, currently existing attenuated SARS-CoV-2 variants should be searched for in the large pool of infected individuals that remain asymptomatic. An interesting aside from an evolutionary perspective is that lock-down and other countermeasures may have accelerated local enrichments of particular SARS-CoV-2 variants. In any case, should particular viral variants be linked to various levels of virus attenuation (or aggressiveness), such findings would have implications for diagnostics, prognostics, disease support management, and eventually, what type of pandemic management policies would be required.

With present-day sequencing prowess, it is feasible to sequence hundreds of thousands of viral genomes, starting from the raw material collected for diagnostic Q-PCR testing. For this, worldwide

sequencing platforms could dedicate time and workhorse instruments for sequencing virus-positive samples. We argue that given testing capacity is expanding, it should extend also to samples from asymptomatic individuals. Sample identification of positively-tested individuals would allow determination by follow-up of who was and remained asymptomatic. All viral sequences at centralized sequence repositories then should be annotated for symptomatic or asymptomatic clinical outcomes and other relevant medical parameters, such as age, gender, and co-morbidities (in accordance with appropriate patient privacy legislation). The next step would be to score for specific genetic determinants of the virus, in particular deletions, that are enriched within the pool of isolates derived from confirmed asymptomatic populations but absent in isolates from symptomatic COVID-19 patients.

The focus should be on those genetic determinants of SARS-CoV-2 isolates that would correlate with asymptomatic outcomes and that would be spread at relatively high frequencies. It is key that these potentially attenuated sequences are also found in individuals who otherwise would be at high-risk of developing severe symptoms from COVID-19, such as 60+ year-old individuals with comorbidities that are considered to be risk factors. These individuals could then be approached to be tested for having undergone seroconversion and, if so, for consistent and prolonged asymptomatic status. Statistics should support whether these individuals would be, at least for some time, protected from reinfection with non-attenuated SARS-CoV-2, with a confidence assessment at least comparable with the current standard for phase III clinical trials concerning a typical designer vaccine. In other words, the pandemic, itself, already may have inadvertently performed a ‘natural’ clinical trial, which, for regular vaccine development, is by far the most time-consuming step.

On the whole, the current global crisis is of a magnitude such that it is feasible to identify currently existing attenuated SARS-CoV-2 variants and to assess their prevalence. Such an effort would be instrumental not only to better understand the evolution of SARS-CoV-2 as it spreads among

humans, but also to predict the dynamics of the current and, possibly, also future pandemics (*13*). Knowledge of SARS-CoV-2 attenuation might also have relevance for developing safer and more effective anti-SARS-CoV-2 vaccines. Live attenuated anti-SARS-CoV-2 vaccines would entail some unknown degree of risk and may cause unpredictable outcomes, for instance, due to recombination events (*14*), in particular if the non-attenuated virus would remain wide-spread. Indeed, designer vaccines have replaced live attenuated vaccines these days because of safety concerns (*1*). We, nevertheless, argue that determination of naturally circulating attenuated SARS-CoV-2 variants is an urgent matter.

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