Review

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Review

Whether SARS-CoV-2 may Become Lethal Again? By Reference to the 1918 Influenza Virus

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Abstract: Omicron variants have higher infectivity, more immune escape but lower risk of severe clinical outcomes than the ancestral variants. However, people are still wondering whether SARS-CoV-2 may become lethal again or whether some highly-pathogenic strain may emerge in the future. Omicron is more selective proliferation in the upper respiratory tract (URT). The temperature of human URT is 33–34°C, and the virus strains other than Omicron cannot replicate effectively at this temperature. While Omicron adapted to the low temperature environment, so its distribution mainly depends on the expression levels of ACE2 in cells. Similar distribution switch from lungs to URT has been observed for the 1918 H1N1 influenza virus, who vanished after 1921 and has been turned to be the seasonal human influenza A viruses. However, some relatively high-pathogenic SARS-CoV-2 strains and influenza-virus strains may still emerge in the future. Dynamic changes in the viral virulence should be monitored constantly.

Keywords: SARS-CoV-2; 1918 H1N1 influenza virus; immune escape; upper respiratory tract; pathogenicity

Introduction

The infections of respiratory viruses induce reactive oxygen species accumulation, causing hyper-immune responses to the viruses, which may have adverse effects on vital organs and result in high pathogenicity and mortality. For example, the avian influenza H5N1 virus may set off a cytokine storm, which leads to the acute respiratory distress syndrome and multi-organ failure (Yuan, 2013). While the coronavirus disease 2019 (COVID-19) also triggers cytokine storms and induces intravascular coagulation, ventilation–perfusion mismatch and life-threatening hypoxemia subsequently (Yuan *et al*., 2021a). High viral load but low clearance rate may be two of the key reasons (Yuan *et al*., 2021b).

So far, five severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants (Alpha, Beta, Gamma, Delta, and Omicron) emerged and became dominant epidemic strains worldwide. Compared to the ancestral variants, the Omicron lineage has the most highly mutations in the spike protein, with more than 50 mutations occurred throughout the genome (Kandeel *et al*., 2022; Tian *et al*., 2022; Viana *et al*., 2022; Wang and Cheng, 2022; Zhou *et al*., 2023). Omicron variants have higher infectivity, more immune escape but attenuated fusogenicity (cell-cell fusion). Although higher binding affinity and more immune escape are usually associated with higher viral replication rate and lower clearance rate respectively, Omicron variants have lower risk of severe clinical outcomes than the ancestral variants (Suzuki *et al*., 2022; Zhou *et al*., 2023). The possible reasons for its lower pathogenicity are discussed in this perspective paper.

Increased binding to the receptor and enhanced immune escape in Omicron

The SARS-CoV-2 viruses have accumulated a lot of mutations to adapt to the human body, which have made the affinity between Omicron and the receptor angiotensin-converting enzyme 2 (ACE2) three times higher than that of the original strain (Cameroni *et al*., 2022; Yin *et al*., 2022). However the stronger affinity to the ACE2 may not be the evolutionary direction. The Alpha strain has a 6.2-fold increased binding affinity to human ACE2 (2.6 times of Omicron; Cameroni *et al*., 2022). However, the Alpha strain is not the circulating one.

Key residue mutations resulted in a sharp decline in antibody titer against Omicron receptor-binding domain (RBD; Cameroni *et al*., 2022; Cao *et al*., 2022a; Mannar *et al*., 2022; Planas *et al*., 2022). Moreover, for the original strain, a large part of the S protein surface is negatively charged or electrically neutral, but its RBD is partly positive-charged. However the surface of the S protein from the Omicron variant is uniformly positive-charged (Yuan *et al*., 2022a). The difference in antigen surface electrostatic distribution may result in charge-related heterogeneity in its corresponding monoclonal antibodies (Vlasak and Ionescu, 2008). Thus, the sharp decline in antibody titer against the Omicron may also be attributed to a change in surface charge of the S protein (Pascarella *et al*., 2022; Yuan *et al*., 2022a). Individuals immunized with mRNA vaccine (against ancestral variants) had more potent neutralizing activity against Wuhan-Hu-1 and retained detectable neutralization against Omicron with a decrease of 21-39 folds. However, reductions of neutralization potency were less pronounced (5 folds) in vaccinated individuals who had been previously infected with ancestral variants (Cameroni *et al*., 2022). Therefore, Omicron may escape the majority of the existing neutralizing antibodies (Cameroni *et al*., 2022; Cao *et al*., 2022a; Mannar *et al*., 2022; Planas *et al*., 2022). However, the viral strain with high immune escape capacity may not necessarily become the epidemic one. Although the Delta lineage exhibited immune escape property similar to the Omicron BA.1 strain (Arora *et al*., 2022), the Delta strain was replaced by the BA.1 variant. Relative to BA.4 and BA.5, BA.2.75 exhibited declined evasion of humoral immunity from BA.1/BA.2 infected convalescent plasma; nevertheless, due to its increased receptor-binding capability and distinct neutralizing antibody escape pattern, BA.2.75 prevailed after BA.4/BA.5 (Cao *et al*., 2022b).

Omicron is more selectively proliferated in the upper respiratory tract (URT)

The increase of binding with ACE2 and higher immune escape capacity did not lead to an increase in its pathogenicity, but the Omicron strain became much milder (Suzuki *et al*., 2022; Zhou *et al*., 2023). This is not due to the reduction in viral replication, but rather its more selective proliferation in the upper respiratory tract (URT; Figure 1; Granerud *et al*., 2022; Salmona *et al*., 2022; Salvagno *et al*., 2022). Omicron patients carried higher viral loads in the nasopharynx and showed more sustained viral shedding, when comparing with the Delta patients (Granerud *et al*., 2022). The viral load of Omicron in nasopharyngeal swabs increased by 8 times compared to Alpha or Delta; while the viral load of Omicron in saliva decreased by 8 times correspondingly (Salmona *et al*., 2022). Nevertheless, there are also contradictory reports showing that nasopharyngeal viral loads for Omicron were similar or lower than other variants (Migueres *et al*., 2022; Sentis *et al*., 2022).

Ancestral variants exhibited reduced viral replication (by 10 times) at 34°C compared to 37°C. Contrastingly, viral loads were either similar or (10 times) higher at 34°C for the Omicron variants (Stauft *et al*., 2023). The temperature of human URT is 33–34°C, and the virus strains other than Omicron replicate less-effectively at this temperature. Omicron replicates poorly in the lungs, which may be due to the higher temperature at 37°C. Nevertheless, Stauft *et al*., 2023 only performed in vitro studies with a non-small cell lung cancer cell line and a monkey kidney cell line. More solid evidences from in vivo studies are still required. Omicron adapted to the low temperature environment, so its distribution mainly depends on the expression levels of ACE2 in cells. In fact, although the expression level of ACE2 in alveolar type II epithelial cells was relatively high, it was much lower than those in nasal goblet cells and bronchial ciliated cells (Figure 1; Viera Braga *et al*., 2019; Hikmet *et al*., 2020; Salamanna *et al*., 2020). Thus, the distribution change of Omicron from the lung to the URT may be explained.

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RNA-dependent RNA polymerase (RdRp) plays an essential role for the viral replication and some mutations may be associated with viral replication in a low temperature (Kim *et al*., 2022). Multiple intra-host single-nucleotide variations have been found in the RdRp coding region of the Omicron variant, such as A1892T, I189V, P314L, K38R, T492I and V57V (Bansal and Kumar, 2022). Although T4685A, N4992N, and G5063S in RdRp were associated with Delta mortality, no mutation in RdRp was found to be significantly associated with Omicron mortality (Saifi *et al*., 2022). SARS-CoV-2 variants containing the P323L or P323L/G671S mutation in RdRp exhibited enhanced replication at 33°C compared to 37°C and high transmissibility in ferrets (Kim *et al*., 2022). Detailed mechanisms for Omicron's adaptation to the low temperature require further studies.

Similar distribution switch from lungs to URT has been observed for the 1918 H1N1 influenza virus

To predict the evolutionary direction of SARS-CoV-2, we may retrospect to another worldwide pandemic, the 1918 H1N1 influenza virus infection. It was estimated that about 500,000,000 people or 1/3 of the world's people were infected with the virus. The number of fatality was estimated to be more than 50,000,000 worldwide (Taubenberger *et al*., 2012; Short *et al*., 2018; Jester *et al*., 2019; Taubenberger *et al*., 2019; Scarpa *et al*., 2020). However, this highly pathogenic strain vanished after 1921 and has been turned to be the seasonal human influenza A viruses (Patrono *et al*., 2022).

The 1918 virus was likely originated from an avian virus, although people were not sure how long the virus had been adapting in mammalian hosts before emerging as a pandemic strain (Patrono *et al*., 2022). Watanabe et al. (2009) constructed a series of reassortants between the highly pathogenic 1918 H1N1 strain and a contemporary seasonal human H1N1 strain, and tested them in a ferret model. And they found that the 1918 virus could efficiently replicate in the lung tissue as well as the URT, but most reassortants and the contemporary human H1N1 virus grew predominantly in nasal turbinates, but only sporadically in the trachea and lungs (Figure 1; Watanabe *et al*., 2009).

A reassortant virus expressing the full 1918 RNA polymerase complex exhibited virulence property in both URT and the lower respiratory tract of ferrets that is similar to that of wild-type 1918 strain. These findings clearly implicated that the viral RNA polymerase is the main determinant of the pathogenicity of the 1918 H1N1 virus (Watanabe *et al*., 2009). Amino-acid mutations in the basic polymerase (PB) have also been shown to be important determinants of transmissibility and the host range (Resa-Infante *et al*., 2011). Whereas avian viruses, generally, replicate at temperatures of 41–42°C (the temperature of bird intestinal tract). For replication in humans, the virus needs to adapt to 33–34°C. The amino-acid substitution E627K in the basic polymerase 2 (PB2) has been suggested to be associated with efficient virus replication in human cells at such lower temperature (Tscherne and García-Sastre, 2011; Herfst *et al*., 2012; Russell *et al*., 2012; Lam *et al*., 2013). Amino-acid substitution S590G or R591Q in PB2 yields a similar phenotype to E627K (Mehle and Doudna, 2009). All the key mutations, E627K, S590G and R591Q, have been found in the 1918 H1N1 strain (Zhang *et al*., 2015).

The hemagglutinin (HA) of human-infective influenza viruses prefer to recognize α-2,6-linked sialic acids (SAs; the human-type receptor), however the HA of avian-infective influenza viruses prefer to recognize α-2,3-linked SAs (the avian-type receptor). Some amino acid substitutions in HA may switch the virus to human-infective influenza (Zhang *et al*., 2015). The α-2,3-linked SAs are mainly distributed on type II pneumocytes with a few number of epithelial cells of the URT; while the α-2,6-linked SAs are abundantly expressed in the URT and secondly in lungs (Figure 1; Yao *et al*., 2008; Kuchipudi *et al*., 2021). The 1918 H1N1 virus (such as the New York variant) bound both α-2,3 and α-2,6 receptors (Stevens *et al*., 2006). The relative lack of avian-type receptor in the upper airway may be another reason why the 1918 virus proliferated both in lungs and in the URT (Watanabe *et al*., 2009) and caused rapid and severe pneumonia, but the seasonal human influenza viruses proliferate mainly in the URT (Watanabe *et al*., 2009) and are usually non-lethal.

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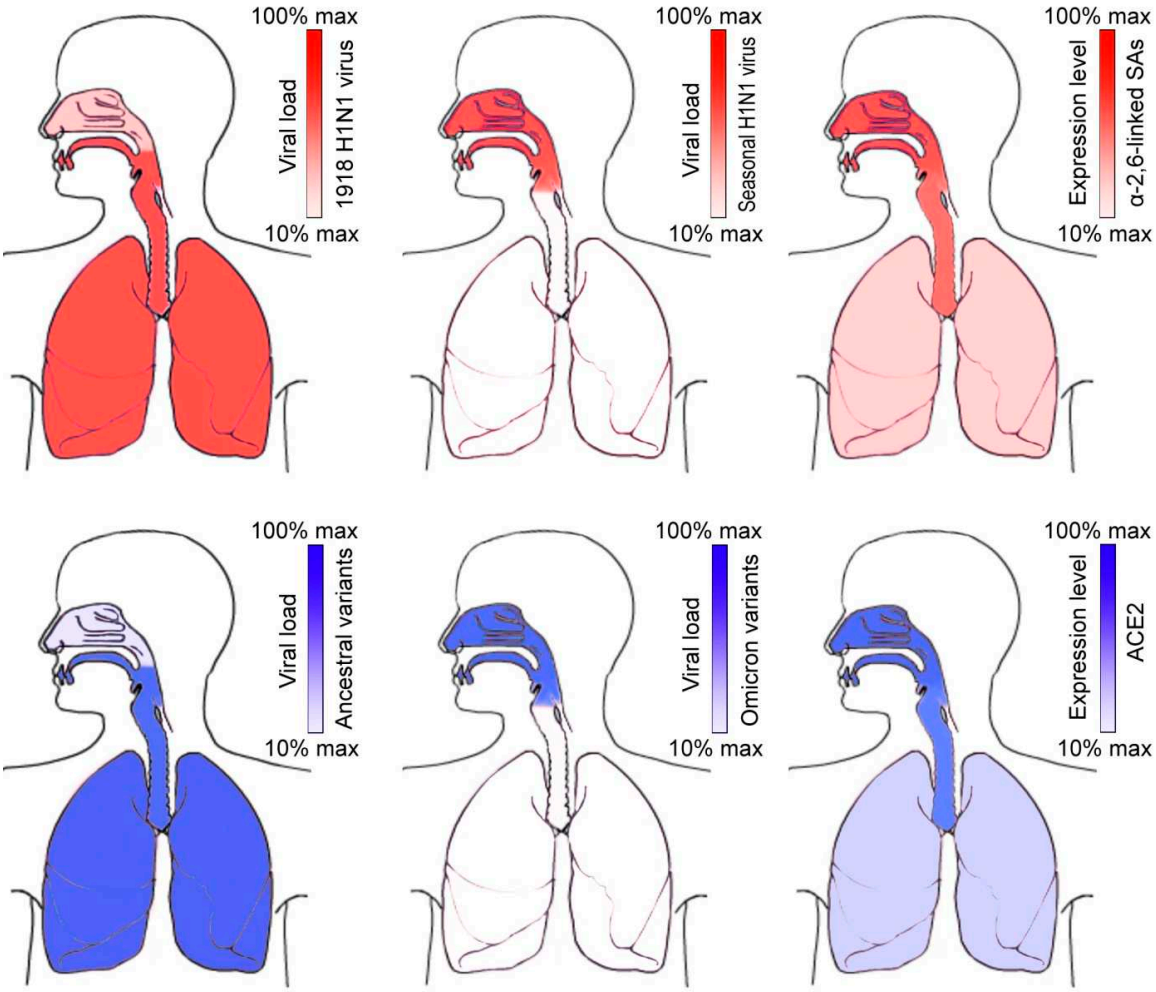

Figure 1. Distribution changes of 1918 H1N1 influenza virus and SARS-CoV-2 after human adaptations.

Putative viral loads of 1918 H1N1 virus, seasonal H1N1 virus, SARS-CoV-2 ancestral variants and Omicron variants in the whole respiratory tract are shown. And the expression levels of α-2,6-linked SAs (H1N1 virus receptor) and ACE2 (SARS-CoV-2 receptor) in the whole respiratory tract are shown.

Future evolutionary direction of SARS-CoV-2

URT infection enables viral shedding via abundant speech droplets (Yuan *et al*., 2020; Stadnytskyi *et al*., 2021). After the emergence of this evolutionary feature of URT distribution, the virus strain with the highest transmissibility would become the epidemic one. For example, the breath emission rate (viral copies in the exhaled aerosols per hour) of cases with BA.5 sub-variant infection was about 40 times higher than that of cases with BA.2 sub-variant (Li *et al*., 2023a). On the other hand, the BA.5 virus showed a significantly higher entry efficiency and enhanced immune evasion than the ancestral wild-type strain or B.1.1.529 (Li *et al*., 2023b). Therefore, BA.5 prevailed after BA.2 and B.1.1.529.

People are wondering whether SARS-CoV-2 may become lethal again or whether some highly-pathogenic strain may emerge in the future. The distribution on the URT also means that the viral loads in lungs are declined, the pathogenicity of the virus is reduced. This may be the evolutionary direction of most airborne viruses after they have infected humans. This evolutionary direction is irreversible, and therefore SARS-CoV-2 may not become lethal again, although new variants may still emerge after Omicron. However, the distribution on the URT does not necessarily mean that the virus

will become further mild. The emergence of highly virulent influenza strains are usually due to the genetic reassortments (Joseph *et al*., 2017). Besides, certain key mutations may greatly enhance the viral replication efficiency and the virulence consequently (Zhang *et al*., 2015; Yuan *et al*., 2022b). Thus, some relatively high-pathogenic SARS-CoV-2 strains may still emerge in the future. Dynamic changes in the viral virulence should be monitored constantly.

Implications from the URT distribution

It is well-known that nose washing (nasal rinses) can reduce URT infections effectively (King et al., 2015; Farrell et al., 2020; Yuan et al., 2022c). Nasal rinses disrupt the viscous surface layer physically, and remove the mucus with its associated particulate matter (viruses). Moreover, the nasal saline increases hydration of the deeper aqueous layer, improves the underlying ciliary beat frequency and reduces local inflammatory factors (Farrell et al., 2020). A recent study showed that nasal irrigation significantly attenuated SARS-CoV-2 Omicron infection, transmissibility and lung injuries in the Syrian hamster model (Yuan et al., 2022c). So we recommend nasal irrigation to susceptible population, especially who just went to a crowded public place or just began to show some mild laryngopharyngeal symptoms. Nasal irrigation in the incubation period may relieve the symptoms, or even that the infected people may become asymptomatic. On the other hand, nasal irrigation would reduce Omicron's viral load effectively and therefore reduce the complication rate and mortality, which is of importance to children and old people, especially to those who have underlying diseases, like chronic obstructive pulmonary disease (COPD).

Nasal vaccines for COVID-19 treatments should also be developed. Efficient delivery of nasal sprays to ACE2-abundant regions is required urgently, especially in the context that some new strains may not be responsive to current vaccines and more refractory to current drug therapies (Xi et al., 2021; Abdoli et al., 2022; Feng et al., 2022).

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