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COMMENT

The compartmentalized upper respiratory mucosa needs time to rally a sufficient immune force for SARS-CoV-2 clearance

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SARS-CoV-2 viral reoccurrence, which is defined as reliable detection of mature virus or viral products in discharged or recovered individuals with at least two consecutive negative detections of viral genomic ribonucleic acid (RNA) by standard reverse transcription polymerase chain reaction (RT–PCR), raises public concerns about transmission risk and warrants exploration of the underlying mechanism. Currently, recorded viral reoccurrence can be classified into three categories based on viral origin, concentration, and transmission risk. (1) SARS-CoV-2 rebound. This observation first became widely noticed with clinical application of direct antiviral agents (DAAs), such as paxlovid and molnupiravir, two FDA-approved drugs to treat COVID-19 patients who are likely to develop severe symptoms. RNA rebound occurs in individuals who have completed a full 5-day drug intake [1–5]. As viral rebound occurred in several key politicians and scientists, it has led to great attention to antiviral effectiveness and future drug choices. (2) SARS-CoV-2 RNA-positive retest. A positive viral RNA retest among discharged patients has accompanied SARS-CoV-2 transmission since the transition of ancestral SARS-CoV-2 [6] to novel variants [7] and likely will continue with SARS-CoV-2 evolution in the future. It is often neglected worldwide for its extremely low transmission risk. (3) SARS-CoV-2 reinfection. Due to incapacity of viral-specific immunity or waning, some recovered individuals become vulnerable to a second or third infection [8]. Reinfection confirmation that relies on aligning the viral genome sequence from the initial infection (mostly unavailable) with the second infection is technically challenging and thus is excluded from the following discussion.

SARS-CoV-2 viral RNA-positive retest differs from viral rebound in several aspects (Fig. 1A–D). Given that the ancestral SARS-CoV-2 was associated with much lower titers [9], only Delta and Omicron infections are included for comparison.

FIRST IS VIRAL GENOMIC RNA CONCENTRATION AND INFECTION RISK

Both viral RNA-positive retest and viral rebound show equal concentrations of virus in the initial infection [1–3, 5, 7] (Fig. 1A versus c, b versus d). However, positive retesting of viral RNA in nonantivirally treated Delta variant-infected individuals is approximately 100,000-fold lower than that during their initial hospitalization [7]. Interestingly, the kinetics of retesting viral RNA is a kind of "blip", which transiently appears irregularly and unpredictably. Viral RNA can be present intermittently up to several months [7] (Fig. 1C). However, no virus has ever been cultured from residual viral samples, and next-generation sequencing has shown the viral genome to be incomplete. Epidemiologically tracing close contacts has revealed no occurrence of transmission. Thus, retest viral RNA positivity is related to extremely low infectious capacity in the community [7].

In viral rebound, viral loads equal the initial infection [1–5, 10] (Fig. 1D). Importantly, the kinetics of viral rebound are nearly identical to those of a regular and complete acute infection episode starting from an abrupt rise to high peak levels (median Cycle threshold, Ct = 19) and ending with a delayed viral decline [1, 4, 5, 10]. Regarding high viral titers, individuals with viral rebound still shed infectious viruses. Indeed, live virus can be cultured from rebounders [10, 11]. Nevertheless, whether viral rebound causes community transmission is unknown due to a lack of epidemiological information, and infectivity in the real world warrants further investigation.

SECOND IS SYMPTOM SEVERITY AND CLINICAL MANIFESTATIONS

Most individuals with retest virus RNA positivity completely recover from clinical manifestations [7]. All viral RNA retest-positive individuals show improved lung function and nearly "zero" COVID-19-related symptoms, though 4% have cough and tiredness but not requiring further medical treatment or hospitalization. Biomarkers from the initial hospitalization, including demographic characteristics, disease severity, laboratory tests, and underlying diseases, are not able to discriminate vulnerable individuals with a high possibility of viral RNA positivity after discharge [7]. In comparison, patients with comorbidities, organ transplants, and immunosuppressant usage are more likely to experience viral rebound. Almost all SARS-CoV-2 rebounders still have COVID-19-related symptoms related to severe outcomes [2], and approximately one-six of them need further medical treatment in the hospital. Exacerbated health conditions also support that viral rebound is detrimental and should be curbed in a timely manner.

FINALLY, THE TIME AND IMMUNE STATUS OF REOCCURRENCE WERE DETERMINED

A typical SARS-CoV-2 infection, consisting of early incubation, acute increase, short plateau, and significant extended decline to undetectable stages, usually occurs over 2-3 weeks [12] (Fig. 1A). This process provides sufficient antigen exposure duration to the

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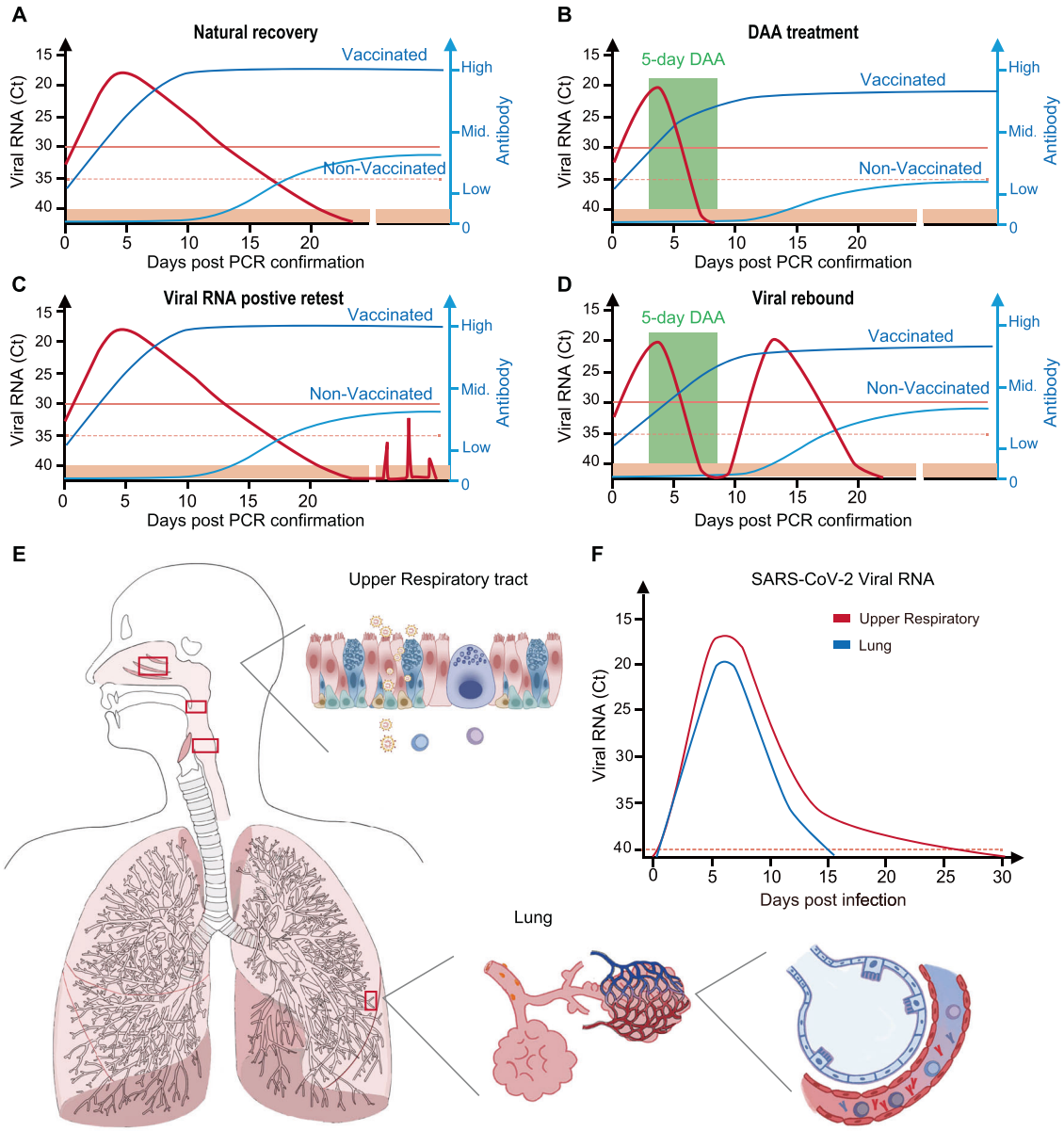

Fig. 1 Kinetics of SARS-CoV-2 RNA titers of viral-specific antibodies in different clinical outcomes. **A** Natural recovery in vaccinated or nonvaccinated populations. **B** Individuals receiving direct-acting antiviral (DAA) treatment, such as paxlovid and molnupiravir. **C** Individuals experiencing viral RNA-positive retesting. **D** SARS-CoV-2 viral rebound in individuals receiving DAA treatment. **E** Illustration of the microenvironment in the mucosa surface of the lung and the upper respiratory tract. **F** Proposed SARS-CoV-2 viral RNA changes between the lung and the upper respiratory tract in vaccinees or individuals given therapeutic antibodies or antivirals. **A**–**D** Red line, SARS-CoV-2 viral RNA. Blue line, viral-specific antibody. Vaccinated, SARS-CoV-2-infected individuals receiving full-dose vaccine prior to infection. Nonvaccinated, vaccine-naive individuals infected with SARS-CoV-2. Cycle threshold, Ct = 30, the cutoff for live SARS-CoV-2 virus isolation. Ct = 35, the cutoff for patient discharge and a noninfectious stage. Ct = 40, the detection limit of viral RNA

host immune system before the immune control capacity matures. Viral RNA-positive retest occurs after natural convalescence when the viral-specific immune response has formed [7], which may require up to several months. Very high titers of viral-specific antibodies and cytotoxic cellular immune responses are generated, both of which can exert strong immune control on virus replication and prevent viral replication to high titers in partially immune-compromised individuals. In contrast, owing to their high potency in suppressing viral replication, direct antiviral agents (DAAs), such as paxlovid and molnupiravir, substantially suppress viral RNA and significantly shorten the whole infection course to within ten days (< 5-day infection + 5-day DAA treatment) [13] (Fig. 1B, D). However, this time course does not allow for full viral-specific immune maturation in individuals with a slow response. Sudden withdrawal of the key controller before backup capacity fully develops will inevitably result in residual virus rebound. In the rebound episode, the virus rebounds to titers similar to those in the initial phase, which suggests that the suppressive immune response is not yet formed [1, 4, 5, 10]. Nevertheless, the real status of viral-specific immunity in the initial infection and rebound episodes is unknown and requires further investigation. In short, a certain duration of viral presentation in vivo seems to be required for the viral-specific immune response to be generated and for the antiviral capacity to rally.

A direct comparison of the occurrence frequency between viral rebound and viral RNA retest positivity is inappropriate, as policies

on COVID-19 patient treatment vary worldwide. Nevertheless, these phenomena have prompted researchers to investigate the underlying mechanisms. 1) Where does the viral genome persist? 2) Why does the host immune system fail to completely clear SARS-CoV-2 viral RNA? 3) Which measure can promote viral RNA clearance?

Single-cell sequencing technology has been used to elucidate the nesting niche for SARS-CoV-2. In a cell culture infection model, a small portion of nasal epithelial cells highly expressing DDIT3 were found to sustain persistently high levels of viral replication for up to four weeks, even in the presence of a robust antiviral response. This suggests that some unique cell types shelter the virus [14] and that innate immunity alone cannot clear it. Viral-specific adaptive immunity is therefore suggested to be critical for viral clearance.

Can a potent immune response prevent virus reoccurrence? SARS-CoV-2 breakthrough infection recalls vaccine-primed memory immune cells in a timely manner to generate high titers of viral-specific antibodies and T-cell immune responses [12, 15], contributing to virus restriction in the lung and pneumonia mitigation and to expedited clinical recovery. However, compared to nonvaccinated patients, vaccination fails to reduce peak viral titers (NC), which occur far before the generation of high concentrations of viral-specific antibodies, suggesting the critical role of the timing of effective immune establishment. Unexpectedly, the same viral RNA-positive retest frequency has been noticed with Delta breakthrough infections [7], even when high concentrations of viral-specific antibodies are present. Additionally, when assessing the clinical effectiveness of neutralizing antibodies (BRII-196/198) against Delta variant infection, a similar frequency of viral RNA-positive retesting was noted in patients with and those without neutralizing antibodies, despite up to three months of antibody persistence in the blood (Journal of Medical Virology in revision).

The viral-specific immune response in the peripheral blood effectively protects the lung from SARS-CoV-2 attack but barely contributes to residual viral RNA clearance in the upper respiratory tract. We postulate that decoupled (or delayed) viral-specific mucosal immunity between the upper respiratory and lower respiratory tracts causes frequent SARS-CoV-2 reoccurrence in the former but significantly mitigates lung damage (Fig. 1E, F). In addition to the mucosal immune response generated per se, the lower respiratory tract can access the endless supply of cytokines, antibodies, immune cells, and antivirals to the alveoli from the bloodstream through the gas exchange process. The lower respiratory tract can form a strong barrier to restrict SARS-CoV-2 replication and expansion with a sufficient supply of viral-suppressing force from the blood and tissue-specific immune response. In contrast, the surface of the upper respiratory tract is a unique anatomical site relatively isolated from the blood. The nasal mucosa is mainly responsible for trapping small foreign particles and humidifying inhaled air. Thus, to avoid frequent inflammation, the immune response in the nasal mucosa is nonreactive to foreign stimuli. What the nasal mucosa surface obtains from the peripheral blood is severely inadequate to compensate for an ineffective local antiviral capacity. The absence of an adequate viral-specific immune response will cause the virus to linger in some specific cells [14, 16] and occasional detection in the upper respiratory tract (Fig. 1F).

Compared with large antibodies and cytokines, small DAA chemicals are better able to permeate the nasal mucosa from the bloodstream and can accumulate to adequate concentrations to inhibit SARS-COV-2 replication. Normally, a five-day DAA treatment with the aid of catching up the viral-specific mucosal immune response is sufficient to clear all virus [13]. The strengthened viral-specific mucosal immune system then acts after DAA is withdrawn. However, when the mucosa fails to rally the immune force in individuals with comorbidities, immune suppression, organ transplants, or other immune-comprising conditions, DAA alone is less powerful in eliminating SARS-CoV-2. In this regard, DAA withdrawal will revive residual virus when host immunity fails to act (Fig. 1D). Extending the treatment course to a natural infection cycle in vulnerable individuals, in theory, will leave adequate time for the delayed viral-specific immune response to mature, thereby avoiding viral rebound.

High titers of novel variants increase the likelihood of viral RNA reoccurrence. Fortifying viral-specific mucosal immunity in the upper respiratory compartment seems vital to eliminate SARS-CoV-2 and avoid viral rebound. As nearly all eligible populations were vaccinated, new generations of SARS-COV-2 vaccines with the capacity to elicit a strong upper respiratory mucosal immune response should be encouraged. Delivering adequate antiviral chemicals and neutralizing antibodies to the upper respiratory mucosa should also be considered, or at least included, and the course of treatment should be extended until the mucosa establishes its defense capacity.

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AUTHOR CONTRIBUTIONS

FL wrote the manuscript, and XZ prepared the figures. XT discussed the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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