To the Editor: Long-term sequelae of SARS-CoV-2 infection, referred to as postacute sequelae of COVID-19 (PASC), involve many organs, including the cardiovascular, pulmonary, gastrointestinal, and neurological systems. The basis of PASC is not well understood, but one possible explanation is that the persistence of infectious virus or viral RNA or protein contributes to PASC. This is difficult to assess in patients because human tissue can only usually be assessed for virus or viral products at autopsy or by biopsy. Furthermore, it is not known if some SARS-CoV-2–infected cells survive the acute infection and contribute to long-term sequelae.

To address this issue in mice, we used a lineage-tracing approach that allows longitudinal tracking of previously infected cells over several months (1, 2). For this purpose, two recombinant SARS-CoV-2 expressing cre recombinase and Venus fluorescent proteins were engineered using a previously described BAC reverse genetics system (3) (see Supplemental Methods; supplemental material available online with this article; https://doi.org/10.1172/JCI172659DS1). We used these viruses to infect mice expressing a loxP-flanked STOP cassette in the tdTomato gene driven by the Rosa26 promoter (Ai9 mice). Venus and Cre were linked by a 2A peptide (Venus-2a-Cre [V2C]), which after autocleavage resulted in the release of the two proteins (Figure 1A). V2C was inserted into ancestral SARS-CoV-2 (Wuhan-Hu-1; rSARS2-WH-V2C), which cannot infect laboratory mice but can infect hACE2 […]
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We used this system to assess infection of several organs at 2 dpi as well as at 20 and 60 dpi, when infectious virus was no longer detected. For both experimental systems, we analyzed at least 5 mice and 10–20 slides per mouse. We found virus predominantly, if not solely, in the respiratory tract of all mice, including the lungs and nasal cavity (Figure 1G). Analysis of mice at 2 dpi showed occasional virus-infected cells in the intestine but not in the brain, heart, spleen, or liver (Supplemental Table 2). The intestine was the only extrapulmonary organ in which infected cells could be detected at 2 dpi (Figure 1G and Supplemental Table 2). In the intestine, a common site of SARS-CoV-2 infection in patients (6), virus was detected in occasional, isolated cells, suggesting that infection was nonproductive or, if productive, spread to adjacent cells was inefficient. Consistent with the analyses of acutely infected mice, cells that survived the initial infection were nearly solely found in the respiratory tract at both 20 and 60 dpi (Figure 1, H and I). The lungs, specifically the lung parenchyma, harbored the majority of the surviving cells, while only a few were detected in the nasal cavity and distal airway. Surviving cells in the nasal cavity and lung bronchiole were detected at much lower levels than during the acute phase of the infection (compare Figure 1G with Figure 1H for rSARS2-MA30-V2C–infected Ai9 mice), suggesting that only a small fraction of these cells survived the acute infection. The majority of the surviving cells were located in the alveoli (Figure 1H). Analysis of lung sections at 60 dpi revealed a further decline in numbers of surviving cells compared with those at 20 dpi (Figure 1I). Notably, some of the surviving cells in the alveoli displayed increased branching at 60 dpi relative to 20 dpi (Figure 1H vs. Figure 1I), consistent with lung regeneration. No surviving cells were detected in the intestine (Supplemental Table 3), indicating that none of the infected cells survived the acute infection or they underwent homeostatic turnover. Results were virtually identical in rSARS2-WH-V2C–infected K18-hACE2/Ai9 and rSARS2-MA30-V2C–infected Ai9 mice, with the exception that rare surviving cells were identified in the brains and hearts of the former (Figure 1K and Supplemental Table 3).

All together, this study demonstrates that cells that were previously infected with SARS-CoV-2 survived for extended periods of time only in the respiratory tract, with no evidence of significant survival in other organs. These results, which require validation in humans, suggest that the host response and not persistent virus infection is most important for extrapulmonary sequelae.

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A (kb) 5 10 15 20 25 30

N 5 1a 1b 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

B Nucleus

Cytosol

C Infection Acute (venus*) Death

Survivor (tdTomato*)

D rSARS-2-V2C

rSARS-2-MA30-V2C

E K18-hACE2/Ai9

F K18-hACE2/Ai9 (rSARS-2-V2C) (rSARS-2-MA30-V2C)

G 2 dpi

H Nasal cavity Lung 20 dpi

Lung (alveoli) Lung (bronchiole)

I Nasal cavity Lung (alveoli) 60 dpi

Lung (alveoli) Lung (bronchiole)

J No. tdTomato+ cells per 10 mm²

Mock 20 dpi 60 dpi

P = 0.0007

K Brain Heart 20 dpi

tdTomato DAPI

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Conflict of Interest: The authors have declared that no conflict of interest exists.

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