

Responses to Participants' Questions

The overarching goal of the R3 Seminars is to catalyze a shared understanding of the research being conducted by the scientific stakeholder community within the RECOVER Consortium. The R3 Seminars and the Q&As typically feature highly scientific material intended for researchers and clinicians. For other audiences interested in these topics, a link to the National Library of Medicine's MedlinePlus medical dictionary is provided at the end of the Q&As as a resource to help in understanding the scientific terminology.

This document provides responses* to questions raised by webinar participants related to the following presentations at the R3 Seminar *Mechanistic Pathways of PASC: Session 2 — Viral Persistence and Viral Reservoirs* held on January 24, 2023:

- **Presentation 1: *Impacts of Persistent Viral Infections on Human Health: Implications for COVID-19 and PASC***
Tim Henrich, MD
- **Presentation 2: *SARS-CoV-2 Persistence and Relevance in PASC***
Sindhu Mohandas, MD
- **Presentation 3: *Models of SARS-CoV-2 Viral Persistence***
Mehul Suthar, PhD
- **Discussant: Adolfo Garcia-Sastre, PhD**

* Responses may have been edited for clarity.

All Presenters: Questions and Responses

Q. Is Dr. Mohandas's data on duration of viral shedding based on the presence of viral RNA or viral antigen?

Response:

Dr. Mohandas: The data are based on both. There are multiple studies that looked at viral RNA, as well as viral antigen with the spike and the nucleocapsid. Much of the data are based on these studies looking at all remnants of viruses. A lot of the retrospective studies and the studies that I mentioned in the beginning would be primarily RNA.

Q. Evidence of replication component virus was not seen beyond day 12 in the autopsy study that attracted attention late last year, but positivity by PCR test was widespread at the late time points. Does this favor the viral remnant theory over the viral persistence with ongoing

replication theory, and could transcribed subgenomic remnants lead to autoimmunity through molecular mimicry?

Response:

Dr. Henrich: The fundamental question is whether there is ongoing replication in these tissues over time or are there viral remnants, whether nucleic acids, proteins, etc.? The answer is somewhat complicated. I agree that it's hard to find replication in tissues over time. I should mention that especially from the gut, it's challenging to get replication of a competent virus; for example, in HIV over chronic infection. We know that it's in the gut. We know from antiretroviral therapy (ART) research it can replicate in the gut, but it can be very difficult to find or grow a virus in the gut. It depends on what tissues you're looking at and it has to do with the local environments, which facilitate viral replication within those specific tissues. This is where some of the live models can be very helpful as well in parsing out some of these questions.

Obviously, ongoing replication has implications. For example, are things like protease inhibitors or nucleoside analogs and the antivirals going to help? One of the best ways to do this is simply to try it: conduct a study where you interrupt potential replication and see if there's any clinical or biomarker improvements in disease. It's a simple yet complicated experiment at the same time to do, but it's certainly where we need to be going and I know that RECOVER is going in that direction as well.

If you have persistent protein, it can have toxic effects. They can trigger pattern recognition, and you can have ongoing immune dysfunction and inflammation, even without frank or high levels of replication in various tissues. These things aren't well understood and we need further study.

Q. How does vaccination impact the rate of PASC in breakthrough infections?

Response:

Dr. Mohandas: The studies that have reported are mixed on this issue. Some studies show that when vaccination occurs, it doesn't of course prevent all breakthrough infections. But when breakthrough infections occur, vaccination reduces (but does not eliminate completely) the chances of PASC occurring. When PASC occurs, the total number of symptoms that patients who have been vaccinated in the past—as compared with patients who are not vaccinated—tend to have is a much smaller number of some symptoms and they are of lesser duration.

It's not an answer that will solve all problems, but it is possible that it may change the course of PASC to be shorter and with less symptomatology. Again, this is something that needs more research. What does vaccination do to patients who already have PASC? How does it change the course of PASC in these patients? It's a very good question and one that needs to be studied.

Dr. Henrich: If you envision that there is protein, like spike protein, that's circulating in tissues, direct antivirals that are targeting replication life cycles might not be the best. But if you somehow boost neutralization, or binding to those circulating antigens, that's where vaccine studies could be helpful; although the results have been mixed.

In the anecdotal studies, some people get better and some people get worse. Monoclonals are an option, but then a lot of the neural strains aren't neutralized very well from those monoclonals. That's also becoming a challenge. It really needs more study.

Q. Has viral sequencing been done to show how the virus has been changing over time in Long COVID patients?



Response:

Dr. Henrich: That's a challenging question because to do that you need a longitudinal study in PASC. You need to get sequences after acute infection, during acute infection, and then farther out as well. To my knowledge, there haven't been large systemic studies looking at potential tissue-based evolution of virus; and especially studies after people stop shedding from their nasopharynx or stool. Once they stop, which most people do, especially if they're not immunosuppressed, then you need to go through tissues longitudinally to look for that type of evolutionary change. That would be a fascinating study: if there is tissue persistence, is there a change? For example, has it escaped from immune pressure? Now, you have these kind of areas and deeper tissues of immune-privileged sites that might allow persistence in some shape or form, if there's been such an evolution away from that kind of immune response, and there has been some type of quasi-check. These would be difficult but very important studies to do.

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- CDC information: Information for the general public and for healthcare providers about Post-COVID Conditions: <https://www.cdc.gov/coronavirus/2019-ncov/long-term-effects/>
- For medical/scientific terminology: <https://medlineplus.gov/healthtopics.html>