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 3: Organ Damage and Reprogramming of Host Tissues and Organs*** held on April 11, 2023:

- **Presentation 1:** ***Understanding the Molecular Basis of Long COVID Using the Hamster Model***
  Benjamin R. tenOever, PhD
- **Presentation 2:** ***Plasma Proteome of Long COVID Patients Indicates Vasculo-Proliferative Disease Associated with Organ Dysfunction***
  Douglas D. Fraser, MD, PhD
- **Presentation 3:** ***Regulatory-Like CD8+ T-Cells Define Long COVID and Autoimmunity Protection***
  James R. Heath, PhD
- **Discussant: James R. Stone, MD, PhD**

* Responses may have been edited for clarity.

All Presenters: Questions and Responses

Q. How frequently does Long COVID affect the liver; specifically, as inflammatory long-term damage?

Response:

Dr. tenOever: Viral RNA can be readily found in the liver, but we never detect infectious virus in the organ from healthy hamsters. If we immune suppress the hamsters, however, we can measure virus from the liver in about half of the animals. In healthy animals, inflammation in the liver is transient and only persists for about 10 days. In immune-compromised animals, inflammation of the liver is extended but, even in this model, it eventually clears – lasting approximately 14 days post infection.

Q. Are there any behavioral changes that would suggest a symptom equivalent to parosmia (smells are detected but are qualitatively incorrect)? This has been a significant issue for humans recovering from smell loss and can persist for many months.

Response:

Dr. tenOever: That's a very difficult question to address using the hamster model. I would imagine that because the biology in hamsters as it relates to the response to SARS-CoV-2 is very similar to what we see in humans that parosmia almost certainly also materializes in a subset of hamsters. It's probably caused by the fact that when that baseline inflammatory signaling is at its peak, the olfactory neurons that are taking in all these responses basically shut off to prioritize antiviral signaling. When the virus-derived inflammatory signal dissipates, those neurons can return to their normal biology, which includes reestablishing various synaptic connections. Given this biology, it seems very plausible that if these connections are altered during this phase, parosmia might result.

Q. Since the flu and COVID can both cause ME/CFS, can the flu be used as a control?

Response:

Dr. tenOever: It's true that flu can cause long-term complications, but it's a much rarer occurrence with flu than what's observed for SARS-CoV-2. We also didn't find any cognitive dysfunction or anosmia in flu-infected hamsters, which suggests, at least in this model, that it was okay to be used as a control.

Q. Would you expect that inhaled steroids for asthma would cause some of the same problems as dexamethasone? What are the implications of prescribing dexamethasone as an immunosuppressant? Would you expect that inhaled steroids for asthma would cause some of these same problems as what you're seeing in your model?

Response:

Dr. tenOever: When we give dexamethasone to people in a clinical setting, it is well after the 7- to 10-day period when they have infectious virus. It would be a bad idea to give someone a systemic steroid when an active infection is happening, because it's going to prevent the immune system from acting on this virus, which is obviously important. We were using dexamethasone to artificially mimic what it would look like if you were immunocompromised in some way, which then takes on a very different biology. So, from an experimental model point of view, it's a very different setting. It does speak to the fact that we should not use steroids in people who are actively infected with virus, but that's also something obvious that a clinician wouldn't do. It's interesting though in the setting of Long COVID in that if we really do feel that some Long COVID individuals do have a latent virus, then the use of steroids would in fact make it worse because you would be providing the opportunity for that virus to flare up again because you've now suppressed the immune system. It seems as though the safest thing to do would be to start with Paxlovid, for example, where if there was any latent activity of the virus, you've

got 5 days to clear it up prior to inducing any level of immune suppression. After Paxlovid treatment, you could try steroids to see if the driving cause of the clinical complication can be reset down to baseline values by simply using steroids.

Q. Do hamsters show "PASC", meaning new symptoms weeks or more after recovery? If so, do you see the same changes in olfactory epithelia and now in other organs, especially the brain?

Response:

Dr. tenOever: Yes, we've used a battery of tests at 5, 7, 14, and 31 days post infection to see how cognitive decline might materialize. We found that both marble burying and forced swim tests showed significant changes in hamsters, but only after Day 14**.**

Q. Would similar mechanisms lead to dysosmia or other changed olfactory function short of anosmia?

Response:

Dr. tenOever: Yes, for sure. Basically, SARS-CoV-2 induces so much antiviral signaling that the transcriptional bandwidth of every cell in the vicinity is needed, which stops their normal function.

Q. Why aren't younger patients being studied? They have a different immune system profile.

Response:

Dr. tenOever: We study hamsters at various ages. There isn't a big difference when comparing young to middle-aged hamsters. Older hamsters show the most dramatic changes, but it's not possible to purchase aged animals. We would have to generate aged hamsters ourselves, which makes such studies prohibitively expensive. It's possible that this biology is a lot less prevalent in the young when the innate immune system is arguably at its strongest, but this isn't something we're able to study using our hamster model.

Q. Is Western University running these results again with the huge RECOVER biobank? It seems like a great opportunity.

Response:

Dr. Fraser: We hope to develop that relationship. We also have a large cross-country initiative already collaborating in Canada.

Q. Have you or others looked at the effects of SARS-CoV-2 on lipid levels, HDL-function in your hamster model? Does it parallel the human disease too?

Response:

Dr. tenOever: We've provided blood samples of our cohorts to many investigators for a variety of "omic" experiments. From what I recall, hamsters also phenocopy the lipid biology that is observed in COVID-19-infected individuals.

Q. How is the "mild COVID" group defined?

Response:

Dr. Fraser: Mild COVID includes acutely ill patients admitted to the ward for oxygen therapy. They required minimal monitoring and interventions. Of the Long COVID outpatients we studied, some had been admitted to the ward (about 30%) or ICU (about 5%) during their acute infection, but the remainder did not require hospitalization for their acute infection.

Q. Dr. Fraser, how are you controlling for additional future exposure to other spike protein variants, such as Omicron?

Response:

Dr. **Fraser:** We just finished all the original samples and now we're focusing on vaccinated as compared with nonvaccinated, different variants of concern, and multiple infections. Stay tuned.

Q. Dr. Fraser, can PASC testing be adapted to help clinicians diagnose ME/CFS?

Response:

Dr. Fraser: Yes, we can adapt our techniques to any health problem. The issue is obtaining sufficient, quality human samples with matching clinical data to conduct the plasma protein investigations, and the cost implications.

Q. How does your work square with the microclot research?

Response:

Dr. Fraser: Neutrophil extracellular traps can contribute to thrombosis, as do activated platelets. Additionally, we published early on that the glycocalyx (little hair-like structures on the interior walls of small blood vessels) are degraded in acute COVID-19. The glycocalyx is antithrombotic by blocking platelet adhesion and liberating nitric oxide. At present, it's unclear if the microvascular glycocalyx has fully recovered after acute infection.

Q. Question on your dexamethasone treatment on the hamsters. I noticed that you found immune suppression resulted in less viral RNA detection in the brain. What do you think is happening in the CNS?

Response:

Dr. tenOever: We too noted that phenotype and I don't have a great answer to explain it. We wondered if it might be caused by the way sustentacular (SUS) cells responded to dexamethasone. If steroid treatment changed their biology in some fashion, you might block replication in the nasal epithelium.

Q. Metformin has been suggested to decrease occurrence of Long COVID. Do your data suggest a mechanism for this observation?

Response:

Dr. Fraser: I don't have an answer for that, except to say that type 2 diabetes proteins are highly represented, so there could be a relationship.

Q. Dr. Fraser, there are reports of reactivated Epstein-Barr virus (EBV) in patients with active disease and in Long COVID patients. Did you see any proteomic signals from EBV? If not, how would you interpret that?

Response:

Dr. Fraser: The patients were not typed for EBV, but they should be in the future and then the groups could be compared.

Q. Could the genetic condition that you discussed during your presentation be mimicked in those without the genetic condition over time, possibly with multiple exposures? Or is the vulnerability to get Long COVID restricted to a population with certain genetic predispositions?

Responses:

Dr. Heath: The genetic predisposition I showed had two components to it. One component was the risk alleles that were associated with lupus, and the second was the exposure of either the risk alleles or the non-risk or reference allele. And it's known in the literature that even the expression of these proteins can be detrimental even if you know the risk allele. The suggestion would be that you don't necessarily need to have the risk allele to have this autoimmune condition; it's going to be a matter of whether these genes are accessible or not. But if you do have the mutation, it's a higher risk. And I would say the mutations are also associated with some of the proteins that one would expect to be associated with immune activation.

Dr. Stone: Have you thought about expressing genetic predisposition as a percentage of the risk? What percentage can be explained by the genetic component? Are you able to do that?

Dr. Heath: Not yet. And I would love to see in the RECOVER study, for example, collecting a whole genome from every study member, which would allow you to do that. Previously, we've seen that the presence of

autoantibodies early in the disease course, even at diagnosis, informs development of chronic conditions later on. Eventually, the autoantibodies sort of disappear. But if I looked at this T-cell story I described, it doesn't explain what the autoantibodies explain. There are multiple etiologies associated with Long COVID and my guess is that there may be a different set of genetic associations. So that's why if you collect the samples and measure the whole genome of the patients, we could think through this. I think that's what would be required.

Q. What are the potential effects of administering an intranasal steroid—such as dexamethasone, which is currently used for asthma—for COVID?

Response:

Dr. tenOever: The levels of steroids used to treat asthma are significantly lower than what was used in the studies I discussed. This theory would need to be formally tested to say for sure, but I suspect inhaled steroid treatment would have only a limited impact on SARS-CoV-2/COVID biology.

Q. Can we distinguish between organ damage caused by acute infection from organ damage resulting from autoimmunity? Would this be useful for either diagnosis, treatment approach, or both?

Response:

Dr. tenOever: We see no evidence that any lasting damage in the hamster model is the product of autoimmunity.

Q. What treatment therapies are you looking into for patients diagnosed with small fiber neuropath caused by Long COVID?

Response:

Dr. tenOever: We're not presently studying any therapies for Long COVID. This research is very cost prohibitive, so such studies require government support, which has been difficult to secure.

Q. How can your model of Long COVID account for worsening baselines months after the acute infection?

Response:

Dr. tenOever: This is a difficult question to answer. I surmise that the virus generates a lot of stable debris that is inflammatory that can continue to engage the immune system even after the virus is cleared.

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