

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 seminar participants related to the following presentations at the R3 Seminar *Understanding the Role of the Immune System in PASC* held on November 14, 2023:

- ***From Macro to Micro: Molecular Imaging Reveals Persistent Tissue Immune Activation and Viral Persistence Following COVID-19***
Timothy Henrich, PhD
- ***Clinical and Immunological Correlates of Long COVID***
Andrea Cox, MD, PhD
- ***Immunology of Long COVID***
Akiko Iwasaki, PhD
- **Discussant: PJ Utz, MD**

* Responses may have been edited for clarity.

All Presenters: Questions and Responses

Q. Were participants who received colon biopsies selected because they displayed gastrointestinal (GI) symptoms?

Response:

Dr. Henrich: Participants were not specifically symptomatic from a GI standpoint, but they all had symptomatic Long COVID. But I think that's an important question because it suggests that viral persistence is a main driver or a potential main driver of a lot of downstream immune dysregulation, inflammation, nervous system dysfunction, and other potential Long COVID mechanisms. It's possible that you can have tissue sources of persistence that don't necessarily correlate with symptoms in that direct anatomical region. For example, when we ask about GI symptoms—whether someone has diarrhea or stomach pain or lack of appetite—would these specific and somewhat nebulous symptoms necessarily correlate with viral persistence in specific regions of the gut tissue? It might, but we're not seeing this tight correlation.

I think that's probably because inflammation in the gut can cause systemic inflammation and immune responses, etc. So, viral persistence in one area could lead to other symptoms across the whole body. Where we did see the correlation between specific Long COVID symptom phenotypes and persistent T cell activation on PET imaging was in the lungs. We observed PET lung uptake correlated with pulmonary symptoms. Going forward, we're imaging and performing biopsies for participants with full recovery, no known prior COVID, and different clinical Long COVID phenotypes to do deep tissue dives; not just in the gut but also in bone marrow, lymphoid tissues, etc.

Q. Is there anything in the literature about potential reservoirs for other viruses, such as respiratory syncytial virus (RSV) or influenza?

Response:

Dr. Henrich: That's a fantastic question. We do know that certain RNA viruses or other viruses that we don't typically consider as chronic viral infections—for example, Ebola—can certainly lead to viral persistence in certain anatomical areas. An important question is if more common seasonal respiratory viruses, like RSV and influenza, also have the capacity to persist in tissues long term. I think part of the reason we don't know about tissue persistence of other "acute" viral infection is that that we haven't looked very deeply; SARS-CoV-2 is essentially paradigm shifting in how we think about acute viral respiratory illnesses and persistence over time.

That said, we also don't see the same burden of post-viral syndromes after these infections as we do with COVID-19. We do see people developing post-viral responses, but in terms of the burden it seems to be much higher in the setting of SARS-CoV-2. I think there's something unique about this particular coronavirus (or related group of coronaviruses) that may lead to more chronic infection in deeper tissues. That said, we really haven't done a deep dive in other viral infections. This is a question we're also looking at now: Is there persistent viral either RNA, DNA, or proteins from other virus in those deeper tissues?

Q. The PMN MDSC (neutrophils and polymorphonuclear myeloid-derived suppressor cells) that you have shown are upregulated in Long COVID and in patients with SARS-CoV-2 infection. Do they have a beneficial function by suppressing an immune response and downregulating inflammation?

Response:

Dr. Cox: They have been shown in cancer to be suppressive. They're less well studied in infectious diseases. Data that I didn't show today demonstrate that the virus can directly upregulate LOX-1 within an hour of SARS-CoV-2 exposure. SARS-CoV-2 causes degranulation of neutrophils and upregulation of LOX-1, and these neutrophils suppress T cell proliferation. Interestingly, prolonged exposure to H1N1 influenza did not. With regard to the question, do all these respiratory viruses do the same thing? Pretty clearly not. And that's very consistent with our ex vivo data showing that human beings with severe acute influenza did not have these granulocytic MDSCs.

We do see them in the lungs of people with severe disease, but the problem is we can't look in the lungs in people with mild illness for comparison. It's hard for us to know what's happening in the lungs of people without severe disease because we don't routinely access the lungs. We don't see them in the normal healthy people undergoing intubation for gastroenterological procedures, for example, such as colonoscopies. Whether they're present in the lungs of people with mild illnesses is hard for us to know. But they do appear in the peripheral blood about the time of severe disease. So, cause and effect are very hard to sort out. And you're right, we could say if it's a hyper inflammatory immune response, their production could be beneficial. But they're very strongly associated with a severe disease and also correlate with later death from SARS-CoV-2. They don't seem to be helpful in the acute phase. In Long COVID, that's much more difficult to assess. We do see that they go down with resolution of symptoms and they go up with increases in symptoms. However, it's hard to know cause and effect.

Q. One of the really nice things about your cohort is that it's very diverse. Did you find differences in race or ethnicity across different groups?

Response:

Dr. Cox: Interestingly, we did not. We saw that these cells, MDSCs, are increasing with increases in severity across racial and ethnic groups.

Q. Can you just elaborate about what role metabolism, oxidative stress, and ER (endoplasmic reticulum) stress might play in Long COVID?

Response:

Dr. Cox: We've characterized the metabolic markers on these MDSCs and where we see metabolic mitochondrial membrane dysfunction in is in the acute phase in severe COVID in T lymphocytes. The MDSCs that are present in these patients with Long COVID don't seem to have profound evidence of mitochondrial membrane dysfunction. But ER stress absolutely induces MDSC formation. It could be that in Long COVID tissue damage is also associated with the development of MDSCs. That's also a distinct possibility.

Q. It's clear there are alterations in the endocrine system more broadly in your studies. Could this impact treatment?

Response:

Dr. Iwasaki: What this suggests, first of all, is that we don't know how universally applicable this finding is. We've done it in two different cohorts, but it may depend on the types of symptoms that people are experiencing and the kinds of severity of Long COVID. So, I don't want to say it's a universal fact, but it's what we've observed in two different cohorts. Secondly, this is in fact not a novel observation. If you look at the myalgic

encephalomyelitis/chronic fatigue syndrome (ME/CFS) literature, there have been many studies that looked at cortisol levels and there have been studies demonstrating hypercortisolism in people with ME/CFS. There have been several clinical trials, randomized clinical trials, to look at the impact of supplementing cortisone in patients with these low levels of cortisol. They haven't been able to identify a dose that is well tolerated and is effective yet. It doesn't mean it doesn't exist. But I feel there should be future studies to look at this more carefully. Can we supplement cortisol level in people with low cortisol to a physiological level, not superseding the physiological level to help those with this particular condition? So that's something I would love for physicians to be thinking about, measuring morning cortisol levels and seeing if supplementation might impact their patient care.

Q. As you know, there are multiple studies now very strongly linking Epstein–Barr virus (EBV) to diseases like lupus and more recently multiple sclerosis. Do you care to speculate about whether you think this EBV reactivation is the driving factor and whether these people will go on to develop autoimmunity?

Response:

Dr. Iwasaki: Yes, that's a really striking link there. There are a lot of viral infections that have been linked to autoimmune diseases, but EBV is at the top of that list, as you say. We think there may be a link here because when we do the sex desegregated analysis, as I mentioned, there's EBV reactivation and autoantibodies predominantly seen in female patients with Long COVID and there may be a link between these things when we correlate their levels. So I don't want to say one is causing the other, but there is definitely a close association of these two observations. And because EBV is latent in B cells and epithelial cells, it's possible that the reactivation may induce activation of those B cells, which may be reactive to self-antigens or it could induce de novo activation of B cells that are surrounding or that are being infected by the reactive virus. There are a lot of hypotheses and links that we can make. We don't have a cause-and-effect relationship though yet.

Q. RECOVER now has this beautiful cohort of over 20,000 individuals with serial samples. What potential studies could these RECOVER samples enable?

Responses:

Dr. Iwasaki: I'm very excited to be able to collaborate with investigators of RECOVER to be able to look at so many different things. But one thing I think Dr. Henrich mentioned that's very important is the tissue level analysis, for example, the autopsy biobank that's being developed by RECOVER. And that's going to be an incredible resource for many people, including our own team, to be able to look at what is this viral presence? Is this replication competent virus that's persisting? If so can Paxlovid or monoclonal antibodies target that? And where are these reservoirs? Can we look at the RNA? So that's something that my team would love to engage in when these things become available.

Dr. Cox: Yes, I completely agree. Additionally, Donna Farber's really excellent collection of living organ donors could contribute to our understanding because some things are harder to assess in autopsy specimens where people are no longer alive. I think that's another possible situation in which we can assess tissue reservoirs. Tissue reservoirs remain an important outstanding question. One should contemplate how they would become associated with more symptoms over time. For example, is the viral reservoir growing in size? It seems more likely to me that it may be a combination of immune responses to a viral reservoir that's persistent and possibly changing over time.

Dr. Henrich: I agree obviously with the tissue work, so this is where we've driven all of our recent efforts to design panels to look at post responses in C2 in these various different cohorts and we'd love to look at this in autopsy cohorts and in organ donor cohorts. It's also important to look at specimens prospectively from otherwise healthy individuals with and without Long COVID symptoms because I think that's going to be important going forward. The problem with some of the autopsies is that tissues from people that were in the hospital or chronically ill have other inflammatory markers, making host viral responses difficult to study. But RECOVER is an amazing resource and with just the sheer volume and the types of samples and the type of clinical correlative data that's being collected is unprecedented. We're very excited to be part of RECOVER and it's a great resource.

Q. Is the proteomics data that Dr. Henrich displayed from patient plasma?

Response:

Dr. Henrich: I showed two data slides and I apologize for the confusion. The first slide I showed, which looked at persistent inflammation 8 months after infection in people that were not hospitalized, was RNA sequence data. So, gene expression data in peripheral blood monocyte cells, essentially immune cells that are circulating in the blood, showing that there were altered gene expression profiles. The second slide showed inflammation that correlates with lung uptake on the PET scan is protein expression data from the plasma that looks at circulating proteins that may be produced elsewhere but are quantified in the peripheral circulation.

Q. Do you think that immune biomarkers might be useful for Long COVID surveillance?

Response:

Dr. Cox: This again highlights the power of RECOVER with the huge numbers of people participating. I think that's critical because there is a diverse array of symptoms. There are people who have restrictive lung disease, cardiac dysfunction, and/or autonomic dysfunction as well as many other symptoms. I doubt there's one biomarker of every one of those manifestations, for example. But having a large number of people in RECOVER affords us an opportunity to identify some biomarkers that are associated with different patterns of symptoms.

Dr. Henrich: Absolutely. I think there are going to be correlations between certain immune biomarkers and symptoms. But I would also caution that I don't think there's going to be a simple single test or something that's

clinically validated that is going to predict specific symptoms. This is going to be an iterative approach looking across much larger populations. I don't want to be pessimistic, but biomarker data may have limited utility in terms of clinical management. Applying machine learning techniques and looking at how to integrate these data in a systematic analysis would make sense.

Many of the studies that are being done are research focused and can't be done in the clinical setting. So, we would love to have a specific clinical marker. Dr. Iwasaki has been talking about cortisol levels or other things that we can use that are available to the clinician and in our hands immediately. Although there's certainly potential to understand the pathogenesis and potentially look at how we could combine different biomarkers to become a clinically effective test, we lack that capability right now, unfortunately, in the clinical space. Some of the changes observed in clinically available biomarker assays in Long COVID are subtle and may vary within the population, making individual interpretation difficult.

Q. Were any of the 24 participants vaccinated and/or boosted?

Response:

Dr. Henrich: Yes, all but one of the participants were vaccinated prior to imaging. We excluded participants who were vaccinated within 3 months of imaging whenever possible.

Q. Have there been any findings that are helpful with ongoing sinus inflammation?

Response:

Dr. Henrich: We do see uptake in nasal pharyngeal tissues in many individuals post-COVID regardless of symptoms, although we do not see a lot of fluid or inflammation in the sinuses on the CT scan.

Q. How do we know these changes are specific to COVID and not seen following other infections (such as influenza or EBV) or other acute physiologically stressful event?

Response:

Dr. Henrich: Excellent question! It is possible that other infections lead to increased PET tracer uptake. We excluded people with recent viral or other infections, but there's no guarantee, of course, that there were no subclinical infections. We expect that our pre-COVID controls would also have similar or even higher incidence of viral infections, so it is very important to have these controls in imaging studies. It's now very hard to get controls like this prospectively, however, and this is a major challenge with any Long COVID study.

Q. Why would you look for persistence of spike protein as opposed to nucleocapsid in human tissues? Are you at all concerned that some portion of spike protein may persist due to vaccination rather than natural infection?

Response:

Dr. Henrich: For PET imaging we need to use commercial mAbs (monoclonal antibodies) that are approved for human use, which are all spike proteins. In tissues, we're looking at nucleocapsids proteins and to parse out any potential vaccine spike persistence.

Q. Can the researchers look at 18F full body imaging for ME/CFS that might be similar to PASC? It would be helpful to demonstrate the degree to which ME/CFS is tissue/organ specific.

Response:

Dr. Henrich: We would love to do this. It's a very important question and an important cohort to target PET imaging studies using novel tracers.

Q. It would be helpful if you characterized autonomic function in your "anxiety" patients. Common anxiety assessment tools are unable to distinguish sympathetic activation due to or associated with autonomic neuropathies and truly cognitive/emotional anxiety. A lot of the anxiety being reported in COVID is likely undiagnosed autonomic dysfunction.

Response:

Dr. Cox: We have ongoing studies to further define mechanisms driving these symptoms, including orthostatic vital signs, tilt table testing, and blood tests.

Q. Did you look at histamine (MCAS) in any way in the anxiety/HA/weakness group?

Response:

Dr. Cox: We have not. Thank you for the suggestion.

Q. Can you comment on the interplay of obesity and Long COVID with regard to PMN MDSC – does COVID aggravate these cells in the context of obesity-driven inflammation (or vice versa)?

Response:

Dr. Cox: Excellent question because obesity is a driver of PMN-MDSC as well, but this seems to be an independent risk factor for Long COVID.

Q. What about mitochondrial dysfunction as a root cause of Long COVID?

Response:

Dr. Cox: Great question. We observed mitochondrial dysfunction in acute COVID-19 in T cells that was associated with age: <https://pubmed.ncbi.nlm.nih.gov/33691089/>. However, we have not seen this phenotype in any cell type in Long COVID.

Q. I'm wondering if any of you have correlated autonomic function to immune function, since there is a large body of literature unrelated to COVID demonstrating autonomic modulation of immune function. This inquiry seems to be left out of most Long COVID immune research to date, but it seems important to investigate given the high rate of autonomic dysfunction in Long COVID.

Response:

Dr. Cox: Thank you for this great question. I agree that this is an important aspect of investigation that needs to be pursued in cohorts within deep immune cell phenotyping. We have orthostatic blood pressure measurements. Adding tilt table measurements and other methods to assess this might add to studies of immune cell changes.

Q. Do you have any longitudinal PET scan T cell activation data? And are COVID-infected people without Long COVID negative in the gut for mRNA?

Response:

Dr. Henrich: We're now performing longitudinal imaging and imaging before and after therapeutic antiviral intervention for Long COVID. We're now expanding tissue collections to fully recovered individuals as well.

Q. Couldn't viral persistence be the cause of autoimmunity as well?

Response:

Dr. Henrich: Yes, this could be a trigger for dysfunctional immune responses, including autoimmunity.

Q. Could you comment on the effect of menopause on the gender differences in Long COVID symptoms?

Response:

Dr. Iwasaki: Given that sex hormones correlate with Long COVID symptoms in our study, we're currently examining the impact of menopause on Long COVID.

Q. Do you believe dysbiosis was present prior to infection with SARS-CoV2 or did it occur as a result of Long COVID?

Response:

Dr. Iwasaki: This is a good question that requires a prospective study to see how preexisting microbiome impacts Long COVID development. We don't have insights on this question yet.

Q. Given the immune modulatory properties of estradiol and testosterone, have they been found to be associated with certain inflammatory markers in women and men?

Response:

Dr. Iwasaki: Yes they have and we're finalizing the study and hope to share more of these results soon.

Q. Regarding presence of the spike protein in Long COVID, were any of the subjects treated with Paxlovid during their acute illness? If so, did they still have the spike protein present?

Response:

Dr. Iwasaki: We don't know whether Paxlovid treatment during acute COVID will reduce the spike protein levels. Given the benefit of Paxlovid treatment during acute COVID in reducing viral replication, my guess is that the treatment will reduce the spike protein present.


Q. Does the risk of getting Long COVID increase with people who have autoimmune disorders such as IgAN, diabetes, asthma, and multiple sclerosis?

Response:

Dr. Henrich: Excellent question and more research is needed in forums such as RECOVER.

Q. Do you think PASC will lead to a long-term auto immune disease?

Response:

Dr. Iwasaki: There are studies showing increased risk of developing various autoimmune diseases in PASC: <https://www.nature.com/articles/s41584-023-00964-y>. 

Q. Are microbial reservoirs an essential element of any theory of PASC that includes a chronic immune system response?

Response:



Dr. Henrich: If I understand the question correctly, persistence of virus or viral elements could lead to ongoing symptoms, although further study will be needed in various syndromes and types of viral or other microbial infections.

Q. Is there a “defect” (characteristic) in the immune system responsible for Long COVID, causing the virus not to be cleared entirely from the system, and thus continuing to be a source of inflammatory response?

Response:

Dr. Henrich: We've shown that virus-specific T cell responses are somewhat dysfunctional, exhausted, and dysregulated with other aspects of the adaptive immune response. I feel that looking at immune responses directly in tissues where virus is hiding will provide many answers to this question that we do not yet know for sure.

To Learn More

- Information about RECOVER research and to volunteer for studies: <https://recovercovid.org/research> 
- Frequently Asked Questions about RECOVER and PASC: <https://recovercovid.org/faqs> 

RECOVER RESEARCH REVIEW SEMINAR

UNDERSTANDING THE ROLE OF THE IMMUNE SYSTEM IN PASC

November 14th, 2023

12:00 – 1:30 PM EDT

- 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>