

Transcript

Sarah Hatcher:

Good afternoon, and welcome to the RECOVER Research Review, or R3 seminar. My name is Sarah Hatcher, and I will serve as the moderator for today's seminar. I'm a research epidemiologist at RTI International, which serves as the administrative coordinating center for RECOVER. The goal of this seminar series is to catalyze a shared understanding of the research within the RECOVER Consortium. Please note that the research presented today is scientific information and is not medical advice. I want to start by thanking everyone who submitted questions in advance. Please submit any additional questions that arise during today's presentation using the Q and A feature in Zoom.

After the presentation, we will answer as many questions as possible. Some questions may also be answered within the Q and A feature in Zoom. An FAQ document will be posted with the recording of the seminar on RECOVERCOVID.org. The FAQ document will include answers for submitted questions relevant to today's presentation. Answers to broader questions about RECOVER are also available in the faqs@RECOVERCOVID.org. Slides for today's presentations are available upon request. Next slide, please. Our presenters today are Dr. Tim Henrich, Dr. Sindhu Mohandas, and Dr. Mehul Suthar.

Dr. Tim Henrich is an associate professor of medicine at the University of California San Francisco School of Medicine. He completed his medical training at Yale University in 2004, his internal medicine residency at Brigham and Women's Hospital in 2007, and his Infectious Diseases fellowship at Massachusetts General Hospital, and Brigham and Women's Hospital in 2009. Dr. Henrich's research group specializes in immunomodulatory, cytoreductive, chemotherapeutic, and stem cell transplantation approaches to HIV one cure. His group is also involved in the design and implementation of novel, nano, and microtechnologies, and PET-based imaging approaches, that characterize viral reservoirs.

Dr. Sindhu Mohandas is an associate professor of clinical pediatrics at the Keck School of Medicine University of Southern California, and an infectious disease specialist at Children's Hospital in Los Angeles, Our Children's Hospital, Los Angeles, where she serves as the co-director of the Immunocompromised Infectious Diseases Program. Dr. Mohandas trained in India and the United Kingdom. She finished her pediatric residency at Brookdale University Hospital, and completed her infectious diseases fellowship at Albert Einstein College of Medicine in New York. Dr. Mohandas's research seeks to better understand the role of host genomics and other risk factors in patients who develop complications after exposure to SARS COV-2, such as multi-system inflammatory syndrome in children, or post-acute sequelae of SARS COV-2 infection. She is one of the principal investigators for RECOVER at Children's Hospital Los Angeles.

Dr. Mehul Suthar is an associate professor in the Department of Pediatrics at the Emory University School of Medicine. He is also a member of the Emory Vaccine Center and the Emory National Primate Research Center. Dr. Sathar received a PhD in microbiology and immunology at the University of North Carolina at Chapel Hill in 2007, and completed a postdoctoral fellowship at the University of Washington. Dr. Suthar's lab at Emory University is focused on understanding the molecular and immunological mechanisms, by which emerging viral infections are controlled by the host. His research program spans several RNA viral infection models, including flaviviruses, alpha viruses, and coronaviruses. More recently, Dr. Sathar has been involved in a large effort to study the antibody response to SARS COV-2 infection and vaccination.

Dr. Adolfo Garcia-Sastre will serve as our discussant today. Dr. Garcia-Sastre is a professor in the departments of microbiology and medicine, and director of the Global Health and Emerging Pathogens Institute of the Icahn School of Medicine at Mount Sinai in New York. He is a principal investigator for the Center for Research on Influenza Pathogenesis and Transmission, which is one of five NIAID Centers of Excellence for Influenza Research and Response. For the past 30 years, his research interests have been focused on the molecular, biology, virus host interactions, innate immunity, and pathogenesis of influenza viruses, and several other RNA viruses, as well as on the development of new vaccines and antivirals. He has more than 600 peer-reviewed publications in these areas of research. In 2017, he was elected as a fellow of the Royal Academy of Pharmacy in Spain, and in 2019, he was elected to the National Academy of Sciences.

Next slide. Today's seminar is the second session on mechanistic pathways of PASC. The topic of today's seminar is Viral Persistence and Viral Reservoirs. Today's speakers will share information about our current understanding of viral persistence and viral reservoirs as they relate to PASC, the gaps in our knowledge about this subject, and how RECOVER will contribute to filling these knowledge gaps. With that, I will hand it over to Dr. Henrich for our ...

Tim Henrich

Okay, thank you very much for the invitation to speak at the R3 Seminar series. Very excited for this. This morning, I'll kick it off talking about the impact of persistent viral infection, not just SARS COV-2, but using HIV and human herpes virus as an example, and the implications both for COVID-19 and the post acute long COVID. A few disclosures.

I like to start saying that I think my perception, I think of our perception of acute viral infections has been changing over the last three years, especially with the recognition that folks recovering from SARS COV-2 infection may have long symptoms months, if not years, after initial presentation, as well as chronic sequelae and physiologic dysfunction that continues. I think that when we think about viral infections, we think about acute viral

infections. Traditionally, here in red, for example, most RNA viruses, and this is from a recent review from Diane Griffin, which is a fantastic review of biology.

Then obviously, there are other latent or persistent viral infections with features of both in many of these, for example, human herpes viruses, HIV, hepatitis viruses, et cetera. I think as we're going through, I think the questions have been asked, can acute respiratory viruses or what we thought initially as acute transient RNA, mostly RNA respiratory viruses, persist in various tissues over the long term or median term, and cause long-term sequelae? This is something that hopefully this seminar series will address head on.

To give you an example, as well as from this recent review, that many RNA viruses or viruses that produce RNA can persist in various tissues, causing various sequelae over time. I'm not going to go through this full list right now, but you get a sense that many different types of viruses, the picornaviruses, alpha viruses, the flaviviruses, phyllo viruses, et cetera, and of course, coronaviruses now, SARS CO2, there can be RNA persistent in various tissues, anywhere from weeks to months, to potentially even longer, in these infections that we would normally consider as acute infections.

There's also consequences as well. For example, joint pain, and certainly with Ebola for example, you can see persistence in the genital tract and potential shedding, and/or transmission even months after initial infection, as well as other post-acute illness and symptoms. This is something that I think is becoming more recognized now that, again, these acute viral infections really can lead to long-term sequelae. They really need to understand what's going on and how we can break this cycle.

Well, viruses don't just persist, but they often cause damage during persistent. Looking at, again John Wary's group and Amit's group, Rafi, Ahmed, and myself about 10 years ago, actually more than that. I think it's spot on that during acute infections, we typically think that we develop a robust immune response. We have a great poly functional memory T-cell response. We have long-lived plasma cells that can make antibodies, and other processes and that this immune response is able to clear these acute viral infections.

In the setting of chronic or potential latent persistent viruses that reactivate from time to time, that you can actually get exhaustion of these adaptive immune responses, both T-cell and B-cell responses. This leads into a cycle of further viral persistent and downstream inflammation and exhaustion within that immune compartment. Again, I think the lines between acute and chronic infection are becoming more and more blurred, as we learn more about SARS COV-2 infection.

This all feeds into obviously, the biology of PASC for long COVID and how we think about this, that clearly there may be multifactorial processes, and it's heterogeneous in nature across people that are recovering from SARS COV-2. I think that if we think about potential persistence of viruses, which I'll show you a few examples with later outside the SARS COV-2 realm, that these can actually lead to quite a bit of the pathology that has been proposed as leading to long COVID. For example, if there's persistent material or virus in tissues, that can break down epithelial barriers, or cause endothelial inflammation. You can also potentially lead towards the exhausted

pathogenic immune dysregulated, response and you could also have reactivation of other latent viral infections that are preexisting, for example, EBV or CMV. Again, very important to think about how chronic viral infections can influence the biology of PASC.

Well, actually interesting that actually, a vast majority of us, by the time we reach middle age, harbor multiple chronic viral infections on our day-to-day basis. As you can see here, kind of the estimated number infected individuals, again, worldwide, and we can just see that tens to hundreds of millions to billions of people are living with various infections. The blue arrows I have are going to be examples I'll talk about briefly, HIV and also EVB, a little bit of CMV as well, and how we can use what we know about these chronic infections, and the methods, and the treatments that we have incorporated. How can we extrapolate this to thinking about persistent SARS COV-2 infection, and what the sequelae may be?

I want to start with HIV. This is my other full time job before March of 2020. We know that, even in the setting of suppressive antiretroviral therapy, that HIV persists. It integrates into cells, and the CD4T cells is a well described reservoir, other myeloid cells as well, and anywhere from tissues to brain, and that this persistent HIV can lead to systemic morbidity, even in the setting of otherwise suppressive or what we would consider suppressive antiviral therapy. Initially, during infection and replication, we can see multiple organ systems involved. The gut, in fact, is one of the largest site of HIV persistence, both during acute infection, but also after initiation of antiretroviral therapy. It's full of lymphoid tissues. You can also see monocyte activation, and this can lead to all sorts of intermediate effects here, summarized by Steve Deeks, again a few years ago, but I think it's still very pertinent as well. What's interesting about HIV is that it really, as I said, it kind of interacts with the gut in a very detrimental way.

For example, on the top left, you see an example of healthy gut. This, again, is from Steve Deek's review, showing that you have an intact epithelial barrier, you have a healthy robust immune response that can counteract the various processes involved with any type of barrier to the outside world. In the setting of HIV infection, where you have either subacute replication, or persistence of viral proteins and RNAs, you can actually break down the tight junctions. You can get epithelial mucosal immune dysfunction, barrier dysfunction, translocation of microbes, altered microbiota, and this can lead to a whole host of different sequelae.

This doesn't just have to happen in the gut, but in other tissues as well in the setting of HIV. Even in the setting of suppressive ART, we know that there is very low level at least RNA and protein production, and it's potentially there might be low level replication in certain tissues, although this is still quite controversial in the setting of some of the research in HIV. Again, we can see all sorts of things happening from this. Again, these are people that have impacted for long period of time, and anywhere from dysfunction of innate immunity, cardiovascular disease, hypercoagulable state, aging, metabolic and immune aging, microbial translocation, et cetera. This is persistent even in people that are on suppressive trial therapy for long periods of time. To give you a

little bit more of a concrete example, what I'm showing on the top left, this is from an article a few years ago, showing persistent markers, the circulating markers of inflammation in people living with HIV.

On the left are folks that have not started antiretroviral therapy, so viremic individuals. You can see that as would be expected in many types of acute viral infections, or viral infections that have chronic replication, we can see an increase in multiple or many varieties of these fibro markers of both inflammation but also apparent continued immune activation as you can see on the left. What's interesting though on the right in red, are individuals after they've been on suppressive therapy for a certain period of time. Even after people start, they suppress their viral load. That's not detectable by clinical assays. CD4 counts return to near normal or potentially normal levels.

You can actually see persistence of these elevated markers. For example, CRP up here as well, IL-6, you name it, TNF alpha. These can remain elevated in certain people over time. Even if we look longitudinally, from before to after people start antiretroviral therapy, in the first year in orange here, this is the change, a percent change. Yes, some of these markers do decrease when people start antiretroviral therapy, and quite a few of them do, but some don't. Some risks don't really decrease, even with the initiation of therapy. As you can see, CRP IL-6, you get a little bit of a decrease, but those contents go across 0%.

After a year suppressive therapy, really, this levels out. These markers of inflammation of immune activation over time remain elevated in the setting that may be leading to some of the morbidity that I showed on the higher slide. In fact, IL-6 itself has been shown in HIV to be a marker of morbidity and mortality in people that have persistent elevations in IL-6. Now I think this is pertinent because we, but many other groups as well, it's not just us, have identified that that folks have in the setting of post-COVID periods, up to four months in this study here in the late recovery period, have elevations of IL-6, TNF alpha, other inflammatory markers as well.

These are folks that have neurologic symptoms related to severe neurologic disease. You can still see that there's elevation months after initial infection. It's not just markers, but there may be a functional component as well. For example, this is data from Matt Durstenfeld, our late cohort here at UCSF, who showed that markers of inflammation are actually associated with lower peak oxygen consumption by exercise testing post-COVID-19. This actually has been shown out to almost eight months now, that there are persistent dysfunctions in this kind of peak oxygen consumption, and that they correlate with inflammatory markers, such as IL-6, ERP, TNF alpha, and again, so the higher that these markers are, that the lower of this peak oxygen consumption. These are associations, but suggest that there's still physiologic dysfunction that happens over time.

Well, working with Mohamed Abdel-Mohsen and Dr. Giron at the University of Pennsylvania, using samples from our cohort found that that markers such as zonulin, which is a marker of tight junction permeability here in A, and beta-glucan, which is a marker of fungal translocation through the gut, are actually increased in people with long COVID symptoms, with PASC, in our link cohort. What's interesting is if you look at beta-glucan here in panel D, that there seems to be a dose effect. People that have more severe or higher number of

symptoms may actually have higher levels of translocation of fungal elements, leading to increased circulating levels of beta-glucan.

Here, we're showing, like in HIV, that even over time up to four months or plus, that you could actually see persistent breakdown in, again, this gut integrity in translocation. What they hypothesize is very interesting that, again, like other illnesses such as HIV, if you have translocation of these elements, things like beta-glucan, can interact with dexins that actually increase NF CAPA-B signaling in myeloid cells, which can make pro-inflammatory cytokines. You can have pattern recognition molecules recognizing and producing inflammatory cascades even over a long period of time.

The big question is, why is there still gut dysfunction months and months after acute infection? One of the thoughts of course is that there's persistent viral infection that may be causing this chronic tissue inflammation. Interestingly, again, going back to Steve Deeks here at UCSF, that there have actually been proposed or studied already anti-inflammatory agents for HIV. Now, this is for people on ART with HIV that have chronic inflammation, but even here, there can be some of the things that you can see here, whether you intensify treatment, or if you're able to reduce any residual replication, those studies really haven't panned out very much clinically, but it's still an interesting idea.

If you can reduce pathogen co-burden, if you can reduce microbial translocation, if you can mute down chronic inflammation that happens from chronic viral persistence, then perhaps many of these symptoms and even potential physiologic sequelae can be reversed or mitigated. I think it's very interesting to see that many of the drugs on this list are actually similar to what folks are proposing in terms of clinical studies in long COVID, in PASC. Well, it's interesting, HIV has similarities as we showed as a chronic viral infection. When you put both HIV and SARS COV-2 together, they may actually have a unfortunate synergistic effect.

Both data from our group but also another group as well, there's more studies coming out now, have basically been showing that people living with HIV, even on stable antiretroviral therapy, actually have a higher incidence or higher prevalence of long COVID or PASC over time. When you look at things like IL-6, that those levels actually may be increased. By adding another viral infection that is known to increase this immune activation and inflammation, you put that on top of another illness, and you get a potential synergistic effect. This is obviously something very important that I think needs to be looked at and studied.

Well, let me switch gears quickly to briefly talk about EBV and some of the human herpes viruses, but I think this also tells us and can be quite indicative of what might potentially be happening in the setting of SARS COV-2 and potential viral persistence. Well, it's interesting that EBV, of course, during acute infection, causes infectious mononucleosis. This is a disease that affects, we say a mono-like illness, where you get everything from pharyngitis, sore throat, enlarged spleen, lymph nodes, lymphadenopathy, you can get fatigue, and loss of appetite. What's interesting is that this fatigue can be profound during acute mono.

You have extremely healthy college athletes bedridden, that you really just can't get out of bed for weeks after becoming infected with mononucleosis. Something's going on that can do this. These symptoms can last up to six months on some individuals, and potentially even longer. It can also increase risk of these macrophage activation syndromes, [inaudible 00:21:49] suggesting that there's an inflammatory component as well with this. EBV is kind of a special beast. It's a very complicated virus, actually, because it can exist both in a latent and a lytic stage. It can have a kind deep latency, but can also go in and out of a lytic stage, and that these latent and lytic stages lead to expression of various proteins that the virus encodes that are actually expressed on the surface of infected cells. For example, and again, I'm sorry for the complicated nature of this, but I just want to summarize to show EBV infection has been known to do quite a bit. Through the expression of these latency mediated proteins and nuclear antigens can actually prevent apoptosis of cells, can induce angiogenesis, which is obviously why EBV has been associated with multiple malignancies, for example, and cause it in many.

It can promote metastasis, it can promote pathogenic B cell trafficking to areas, like the central nervous system, and it can also block immune mediated killings. It can actually modulate the immune response to these infected cells in a detrimental way that promotes viral health, but not human health. Lastly, that especially the nuclear antigens, the EBV nuclear antigens, or EBVNAs, these will pop up later in this talk, can actually lead to autoreactive mimicry, or molecular mimicry, or autoreactive antibodies and autoimmunity. There's been suggestions that EVD may be associated with several listed here at the bottom of rheumatologic diseases.

Again, a lot of this is either speculative, or based on epidemiologic evidence, but I think to really prove that a viral infection is actually causing some type of discreet syndrome, you actually need large studies. This is of course why RECOVER is doing what it's doing. You need these large epidemiological based studies. For example, I think folks may be aware of this recent article in science, where they've basically shown through a longitudinal analysis of 10 million recruits, only 955 developed multiple sclerosis or MS autoimmune processed in the central nervous system.

They basically saw that initially, that the risk of MS increased 32 fold after EBV infection, but not after CMV infection, the herpes virus has formed, and that they also saw inflammation, so NFL or this neuro form light chain marker of neuro inflammation goes up. Now, I know there's been some critiques about this, whether this may be an overestimate, given some of the selection bias or whatnot within this cohort, but I think it does show you that with larger studies, perhaps we can start to see some of these relationships more clearly.

Well, obviously, EBV as we talked about before, there's potential mechanisms that can lead to MS and potentially other immunologic conditions or autoreactive conditions. For example, NMS, through some of these transformations I showed you earlier, B-cells, which in fact, it can actually gain access to the central nervous system. Then you can actually get synthesis of immunoglobulins within the central nervous system, called oligoclonal immunoglobulin bands, or oligoclonal bands, and [inaudible 00:25:07] for MS, when you see it in the CSF on sampling.

You can have B-cell transformation, you can have potential molecular mimicry again, leading to autoimmune processes, and it can also maybe aid in migration of CD8 T cells into the central nervous system, which can further increase that amount of inflammation. I think that altogether that these are plausible mechanisms of how at least EBV can lead to serious long-term effects. Clearly, this may be the case with other viruses as well. Just to give you a quick overview that EBV, we can actually look at antibody profiles, that IGMs come and go very quickly. This viral capsid antigen IgG stays up quite a long period of time and remains elevated lifelong. Can wane slightly, of course, over time, but most people keep very high levels of this EC IgG. There are other IgGs, like early antigen D, or EAD, IgG that kind of come up over months, and then actually can wane. About 80% plus of people can actually go back onto undetectable levels after a few years of infection.

Might be actually a marker of recent viral activity or reactivation of tissue. Then of course, nuclear antigens kind of come up during the establishment of latency and can stay elevated over time, but those may also go up in the setting of reactivation. It's unclear exactly to what extent, but again, these have been associated with autoreactive [inaudible 00:26:29]. A couple of studies. Most recently, I brought a provocative idea that perhaps EBV reactivation probably during acute infection, when there's a large amount of inflammation, cytokine release, et cetera, from SARS COV-2 infection.

Clearly, the paper and pathogens and then in cell as well suggesting that people with long COVID may be predisposed to developing symptoms, if they've experienced EBV reactivation during acute illness. We took a little bit deeper dive on this to look at different symptom clusters over time. We recently published this paper showing essentially, that high levels of either EBV nuclear antigen, these are the ones that have been pegged with auto reactivity or also EBV, EAD IgGs, so kind of recent reactivation, were positively associated with the risk of developing both fatigue and neurocognitive PASC over time, as does HIV as well, going back to what we had seen in our prior observation.

I just want to show that what was very interesting that unlike EBV and HIV, CMV, although CMV has been associated with more severe disease during acute infection, people that carry CMD IgGs or have been infected with CMV about 60% of the population, may actually have a higher incidence of hospitalization or more acute morbidity in the setting of SARS COV-2 infection. What we found over time, and this is four months later, is that if people that have evidence of prior CMV infection, we don't know about reactivation, but prior exposure, seem to be protected from developing neurocognitive and potentially PASC in general as well.

This brings up a complicated interplay that says not all chronic infections are necessarily a bad thing all the time. In fact, there's been some evidence that CMV infection may actually enhance immune response to influenza, again, in younger people, and not necessarily in older people. It really depends on the population you're looking at, and it depends on how that virus interacts with what you're looking at. I should say that there's been more work, and this is again, I think from the Yale group, showing that again, that there is using this general little

general linear model estimate, that persistence of EBV motifs or antigens are also associated in folks that have PASC, as well as some of these other immune dysregulation and markers of inflammation response.

Another way of showing these types of data. Finally, I'll show you, it's interesting, that Joanna Hellmuth, a neurologist, Dr. Hellmuth has been working with our link cohort to look at CSF samples and to really kind of do a deeper dive. It's really found that in people with PASC months after infection tend to have similar levels of white blood cells. They don't necessarily have a pleocytosis elevated, and they have normal levels of glucose and proteins within the central and the cerebral spinal fluid in the CNS.

In people with PASC, nine out of the 13, or almost 70%, actually had oligoclonal banding, similar to what people had seen in MS, whereas none, although again, it's a limited size of the study, small study, without symptoms over time had detection of monoclonal antibodies. The question is what's causing these? What's happening? Is this viral persistent? Is this reactivation of other latent viral infections that pre-existed SARS COV-2 infection? A lot to think about there. I'll just conclude that certainly, many viral infections either persist for some time, even with treatment, in various tissues. Persistent viral proteins, or RNA, or even potentially low level replication, may trigger tissue damage and systemic inflammation.

Sequelae viral persistence includes significant impacts on human health. Again, even in the setting of treatment, for example, HIV. SARS COV-2 may persistent tissues itself for long periods of time. It may lead to the sequelae that we have seen with other viral infection, and may also be synergistic and detrimental over time. I think there's an urgent need to systemically study SARS COV-2 persistence in tissue, in well curated clinical cohorts. I think this is really where we need to be going in order to fully understand and to help drive therapeutics for long COVID.

With that, I'll just acknowledge both those folks here at UCSF and our Link cohort, but also many of our collaborators, both in and outside our institution, who have made these studies possible.

Sarah Hatcher

Thank you, Dr. Henrich. Now, we'll turn it over to Dr. Mohandas for our next presentation.

Sindhu Mohandas

All right. Thank you so much for having me to present here and after Dr. Henrich's excellent talk about the effect of persistent viruses on human health, what I'll do now is focus specifically on SARS COV-2 virus and its relevance and persistence in PASC. A few objectives of my talk today will be to really understand what literature is out there, in terms of duration and distribution of viral persistence, and how this viral persistence is shown to correlate with PASC. The other question is, what does this viral persistence do in the body, and what is its relationship to the immune regulation?

I started with looking at duration of viral shedding. This is a systematic review and a meta-analysis published in 2021, which included 73 studies. Most of these studies were done in the early part of the pandemic. Really, the aim was to understand the viral kinetics, understand viral infectivity, to define early response to the virus. That's primarily duration of infectivity, infection control measures, and the public health policy. We can definitely use this information to further our understanding of viral shedding and persistence, to see if that leads to PASC in any way.

When they looked at these studies that were done, if you see here, the upper respiratory tract had a mean shedding duration of 17 days with a maximum of 83 days. Fewer studies included samples from the lower respiratory tract, stool, like GI tract or serum. They found that the lower respiratory tract had a mean shedding for 14.6 days, with a max of 59 days. In the GI tract, primarily the testing was done from stool or rectal swabs, and there was a mean shedding for 17.2 days. Frequently, they found that this was longer than shedding through the respiratory tract. The max in this group of studies was 126 days.

In the serum, they detected the SARS COV-2 RNA for 16.6 days, with a max of 60 days. What's interesting is in all these, not all studies looked at trying to isolate live virus or replicated virus, but of the studies that looked into this, no live virus was detected beyond day nine of illness. I think that's something important to remember. This is another study which looked at viral shedding. This is a study by Gabriel Atoll in nature, published in 2021. The primary aim of this study was to look at immune response in patients after COVID. They looked at it over a six to eight month duration.

What they really saw was that the immune response, when they looked at IgG and IGM, it decreased with time, as would be expected. The interesting thing that they really found is that the B-cell, B memory cell response, however, seems to have evolved during those six months. Instead of fading away like the IgG and the IGA antibodies, the memory B-cell response actually evolved with the hypermutated response at the end of the six months. This suggested that there is maybe continued stimulation. This continued stimulation could be due to viral persistence. They hypothesize that the viral persistence could be in places like the germinal cells of lymphoid tissues.

In this study, they also looked at of the cohort they had of more than 50 patients, they looked at small bowel by biopsy. Most of the other studies that look at viral persistence in the GI tract frequently looked at stool and rectal swabs, as these are more convenient samples. This study looked at biopsy samples, both upper GI and lower GI. Of the 14 patients who had these biopsies, seven of the patients had persistence of SARS COVID-2 nucleic acids by immunofluorescence, as you can see in these images. The average time to biopsy was four months after onset of COVID-19.

Clearly, viral persistence was noted within the tissue, even four months after COVID-19. One of the things they did note was that this was not associated with tissue inflammation within the GI tract. There was no evidence of vital cytopathic effect. Also, one thing of note that is the 14 patients included in this who received the biopsy

were not patients who had persistent symptoms. They were not PASC patients. It's interesting to note that the viral persistence was noted several months after the initial COVID infection.

The next question comes up as how long is the virus viable? A lot of these studies are finding virus particles within the tissue. Is this viable virus? Of the few studies that have looked at viable virus, if you look at the study on top, the picture, the image by Bullard et al, this study looked at multiple samples, including sputum, nasopharyngeal swab, stool, serum, and urine, and tried to isolate live virus. What they found really is live virus was isolated primarily from the sputum and the nasopharyngeal swab. This was really very early in the course of illness.

From the swab, it was really by day five of illness, and from sputum, it went up to a maximum of day eight. Really no live virus was isolated beyond day eight of illness. This was only from two samples, the sputum and the nasopharyngeal swab. Despite other samples, like stool, serum having really high viral load, live virus could not be isolated from any of these tissues. Again, the figure on the left looks at culture results, and the figure on the right looks at sub-genomic PCRs, which is an indicator for replicated virus.

They found the same thing, that by eight to nine days, evidence of replicated virus is no longer there. Another study by Bullard et al published in CID in 2020 found the same thing, that eight days after onset of symptoms, by culture, virus is no longer isolated. This is a recent study, I think a lot of us are very well aware of this study. This looked at viral persistence at autopsy, and included a wide range of autopsy timings, from if you look at the bottom of the slide, from within days after COVID infection, up to 230 days after COVID infection. The autopsy detected SARS COVID-2 RNA from 84 distinct anatomical locations and body fluids. In this figure, the respiratory samples are on the top, and the samples from the brain are at the bottom. As you move to the right, the number of days after COVID infection increases. As would be expected, a high amount of viral load is seen in the respiratory samples, especially early on, especially when death occurs early on after infection. It's really interesting to note that viral RNA is still persistent in multiple tissues, even 76 days and 230 days after infection.

The vertical bar seen here are indicative of subgenomic PCR, again, which is an indication for viral application and as would be expected, this is also very prominent early on after infection, but it's interesting to see that subgenomic PCR was also positive sometimes in a long duration after the SARS COV-2 infection. They did note that significantly higher burden was of SARS COV-2 RNA was detected in the respiratory samples compared with the non-respiratory tissues. Another thing interesting noted by this study was even though the SARS COV-2 RNA was detected in multiple tissues, corresponding signs of inflammation were not noted in many of these tissues, especially as greater time passed since the initial SARS COV-2 infection.

This is another study, which is an autopsy study, which primarily looked at multi-organ proteomic landscape. It didn't appear that they looked for viral RNA, but they did find that multiple abnormalities persisted in patients, persisted in autopsies, done several days again after the initial SARS COV-2 infections. Fibrosis, coagulative necrosis, interstitial edema was seen in multiple organs several months after SARS COV-2 infection.

They also looked at proteomic factors, and designated them them into these various clusters, and found significant dysregulation within transcription factors and eugenic factors, cytokines, fibrosis markers, coagulation, and receptors for SARS COV-2.

The other thing that's interesting is what we talked about so far was really viral persistence in previously healthy patients, or those who had a normal immune system. Viral persistence in immunocompromised has been found to behave differently. This is a study which longitudinally looked at a single patient who was an immunocompromised patient, and found that the patient presented multiple times with COVID infection. It was really the same virus which persisted in the patient's body. The virus persisted till day 152, and infectious virus was detected by culture as late as day 143. The problem with virus persisting in immunocompromised patients is also that the virus can mutate and evolve within these immunocompromised patients. If you look at this figure, the virus that started out initially had significant mutations by day 143. Is this a mechanism by which the virus evades immunity, which leads it to be more persistent? That is something worth considering. Also, if you look at this figure on the right, the graph on the right, in this patient, they tried to quantify the viral load with the cycle threshold values. The viral load remained extremely high almost throughout the clinical course.

It's important to remember that viruses can persist for much longer in immunocompromised patients, and within these immunocompromised patients there can be an accelerated emergence of variants. This is another study that actually came out of CHLA, which looked at three patients, two pediatric, and one young adult, and showed that ongoing viral replication was noted up to 162 days. An ongoing viral replication was studied via culture, subgenomic PCR, all of which remained positive multiple months after the initial SARS COV-2 infection. Again, noted within these, is especially in patient two and patient three, multiple mutations were noted in regions within the spike gene, which added on and contributed to the mutating virus.

There are much fewer studies on viral persistence in children. The study by Benvari et al is a meta-analysis and a systematic review, which included 12 studies. What they found within the studies, there was persistently positive SARS COV-2 was detected in GI specimens. When I say GI specimens, it's primarily stool and rectal swabs. Again, they found that persistence of SARS COV-2 in GI specimens extended far beyond the respiratory specimens. It lasted up to greater than 70 days after illness onset, and up to five weeks after hospital admission. Another study, which looked at median time to two negative tests in immunocompromised children, this was a retrospective study, and primarily looked at immunocompromised children within various immunocompromised situations, including solid oncology, liquid oncology, transplant, and HIV. Found that the median time to two negative tests was up to 42 days. Again, much later than what we would see in previously healthy children.

This is a study, again, it was in children who underwent a tonsillectomy and adenoidectomy during the SARS COV-2 pandemic. It was really seen that even several months after COVID infection, the germinal centers persisted within these lymphoid tissues. It was also seen that when they looked at S1 RBD specific B-cells, the CD

27 B-cells formed a large number of the reactive cells. The idea was that these cells continue to have some stimulation to persist over this many months, and this could likely be due to viral persistence.

What does viral persistence really mean for PASC? This is a study by Su et al, which looked at early factors that can anticipate PASC. They found that RNAemia during the early COVID infection was one of the predictors or anticipatory factors for PASC. In addition to SARS COV-2 RNAemia, EBV viremia, as you heard Dr. Henrich discuss, as well as type two diabetes, and presence of specific auto antibodies, was predictive of patients who developed PASC.

These are other two interesting studies in looking for biomarkers or predictive or diagnostic features for PASC. The study on top by Swank et al, published in CID 2022, is a very interesting study which looked at spike, the S1 antigen, the whole spike, and the nucleocapsid antigen, and months after diagnosis. Sorry. In the figure on the top, the red shows the patients with PASC, and the blue shows the patients who are control patients who did not develop PASC. As it's clearly obvious, the PASC patients tended to have higher predominance of these antigens. Within the S1 spike and the nucleocapsid, it was really this whole spike antigen which was persistently positive in patients with PASC, up to 12 months after diagnosis.

Interestingly, a study on MISC also found that in children with MISC, the elevated SARS COV-2 antigen, the spike antigen is found during the acute phase when patients have MISC. Does even after starting treatment for MISC, as seen in these graphs here, this can persist for quite some time as well. It is interesting that the SARS COV-2 spike continues to be detected, and continues to be detected in high numbers several months after diagnosis of a SARS COV-2. Could this be a potential biomarker that can be used is something that remains to be seen.

Sorry. Okay, so what are the possible mechanisms? This is, on the right is a nice figure in the paper by Merad et al in the Journal of Science. PASC is a very complex condition, and I expect that the explanation, what we would find for the reason patients develop PASC, is also probably not going to be a simplistic one. Various factors probably contribute to developing PASC, and especially the different subtypes or the different phenotypes of PASC. The factors that could be contributing include viral reservoirs, or viral remnants, tissue damage, or immunity, and dysbiosis.

Clearly, all of this work together to then lead to the eventual phenotype of PASC, where we see increased inflammatory cytokines, lymphocyte activation, and dysregulation. The picture on the left shows a similar kind of hypothesis for neuro PASC, where immune-mediated damage, persistent infection, and the trauma and stress caused by the initial SARS COV-2 infection, can all contribute to damaged neuron, persistent inflammation, and lead to the many symptoms of neuro PASC.

Another really interesting and very debilitating symptom of PASC has been decreased sense of smell, or hyposmia. This study looked at olfactory mucosa in patients with anosmia. They found SARS COV-2 viral particles and signs of inflammation, again, several months after the SARS COV-2 infection, within the olfactory mucosa. The lasting SARS COV-2 presence was seen in multiple cell types within the olfactory neuro epithelium, which included

the olfactory sensory neurons. Within this study, they also went on to do an animal study by infecting hamster, and found that the SARS COV-2 had a specific tropism for the olfactory neuro epithelium, and led to a decrease in the olfactory neuronal cells.

This is another study which, again, recruited patients who had persistent hyposmia, loss of smell, a loss of taste, along with other PASC symptoms. They really found that there was persistent immune cell infiltration and altered gene expression within the olfactory epithelium. The biopsies done in these patients showed that there were fewer olfactory sensory neurons in patients who had decreased hyposmia, and there were interestingly altered immune cell populations.

There was an enrichment in T-cells producing interferon gama. There was an enrichment, as seen in these figures here, of CD 207 positive generative cells, as well as a depletion of M2 macrophages. I think the M2 macrophages is really interesting. M2 macrophages have an anti-inflammatory response, as well as tissue remodeling and healing. This is something really interesting, which could be contributory to the persistent symptoms seen in PASC. This study looked at immune responses, again, longitudinally studying patients, comparing healthy controls, and the perturbations of the immune systems 12 weeks post-infection at different time points: 12 weeks post-infection, 16 weeks post-infection, and 24 weeks post-infection.

If you see the flow cytometry figures here, compared to the healthy control, there was significant perturbation of the immune system at the 12 week mark. This persisted also at the 16 week mark, and decreased somehow at 24 weeks. Also, when you look at the specific markers, there was abnormal or dysregulated immune response, even at the 24 week mark, but clearly, much more prominent at the 12 weeks and the 16 weeks after SARS COV-2 infection. This is a study actually from Dr. Henrich's group, which looked at long-term SARS COV-2 specific immune and inflammatory responses in individuals recovering from SARS COV-2, with and without PASC.

This study included 70 patients, several of them who went on to have PASC. This is really the graphical abstract which was included in the paper. It shows that patients who do not develop PASC have a good CD4 activity which persists, whereas in patients who go on to develop PASC, CD8 responses are lower and waning, especially in the late COVID-19 convalescence phase. They identified lower and more rapidly waning month four, so in specific CDA T-cell responses, particularly with interferon GAMA producing and CD107A positive in those with PASC, they also noted a higher trend to a higher IL-6 in PASC, which Dr. Henrich mentioned in his talk.

Interestingly, they did not note an association between viral shedding, PASC symptoms, or the immune responses. The viral shedding in this study was looked at by saliva, this viral shedding within saliva. One of the postulates was that maybe viral shedding in deeper tissues is what correlates, and maybe that's what needs to be studied in the future. Again, looking at immune responses in a longitudinal fashion, comparing patients with prolonged symptom duration, with individuals who recovered after SARS COV-2. When the immunophenotyping is looked at as a whole, there was really no major difference between patients who recovered, versus those who continued to have persistent symptoms.

When the antigen-specific immune response was looked at, it was obvious that the S protein specific T-cell response, instead of waning, as would be expected in normal infection, tended to persist beyond several months. The same thing was seen with the S protein antibody ability index. The S protein antibody ability was significantly increased in patients who had persistent symptoms, or those who had PASC. All of those points, again, which is kind of a theme through all of this, that there is something which is causing persistent immune stimulation in these patients, and that could be viral persistence.

This is a study which looked at liquid biomarkers or macrophage activators, and circulating spike protein, and to illustrate the biological heterogeneity in patients with post-acute sequelae of COVID-19. In the figure A, comparing patients who never had PASC with those who had ongoing PASC, we again see here that circulating S1 was particularly increased in patients with PASC. Not only was the circulating PASC circulating S1 positive in patients with PASC, the actual number was also significantly increased, compared to patients who never had PASC.

They also found abnormalities of several circulating soluble factors, and again, they noted that the soluble protein, the circulating S1, was not directly correlated with any of these soluble factors. One of the interesting things they did note was the high circulating spike was also associated with a higher S1 IgG and NCP IgG, suggesting that that continues to be an immune stimulation and immune response that occurs in these patients. After reviewing all these studies, I just want to conclude, and also, all of this leads to more questions as well.

What we know is SARS COV-2 viral proteins can persist in various tissues for a long time. It's unclear if there is a direct association between viral persistence and PASC. Most of the studies that are done are from easily accessible samples. Viral persistence in tissues may be more informative. Again, difficult to do, but maybe something that has to be thought of. Immune dysregulation is seen in patients with PASC, is this driven by viral persistence?

Biological heterogeneity in PASC adds a layer of complexity, which has to be thought of while designing studies. Understanding the nature and location of long-term SARS COV-2 persistence and correlation with PASC subtypes could provide us with more insights into the PASC pathogenesis. Thank you, and I'll turn it over to Dr. Suthar.

Mehul Suthar

Thank you very much. I'm going to present on how can we potentially model SARS COV-2 viral persistence using some of the models, tools, reagents that we have in the laboratory that have been developed during the acute infection studies that have been performed over the past few years? I'm going to begin by highlighting the study that Dr. Mohandas presented in her presentation, but I just wanted to touch on a couple of key important points.

One is that this is an autopsy study that was done, mainly from severely infected SARS COV-2 infected individuals. What they were able to find is that the virus seemed to be present in many distinct anatomic locations, primarily within the respiratory tract, but there was quite a bit of non-respiratory tract involvement as well. This

viral presence, or at least the RNA presence, appeared to not be associated with inflammation, which I think tells us something about how the virus persists, and how it's able to suppress that inflammation during its phase of persistence.

Most importantly, and the piece of information that was surprising to me is that they were able to isolate the virus from about 45% of the tissues that were tested. Most of these tissues were from the respiratory tract, different regions within the lungs, the trachea, the upper respiratory tract, but there were also tissues within the intestine, the lymph node, the heart, the thalamus, as well as the eye, that they were able to isolate infectious virus from these compartments.

This demonstrates that not only is the virus present there, the RNA's present, but they're also able to RECOVER a virus from that compartment as well. It suggests that there's tropics for cells in the respiratory and non-respiratory tract. How do we go about modeling this within the laboratory? There's a few different options that we have. First is, if we try, if there's things that we can do to model, just simply in vitro, using cell lines or organoids. What are our options here, especially if we know that there's a given tissue that's being infected?

Is there something that we can look at mechanistically to try to understand more about viral persistence in a relationship with the host innate immune response, which serves the first line of offense against a viral infection. For example, in cultured cell lines, we've used to propagate SARS COV-2 in the laboratory. These cell lines typically express ACE2 and TMPRSS2, either over-expressed, or naturally expressed in lung cancer cell lines or transform cell lines, like CAL3 cells. These are primarily great for studying therapeutic screening or studying virus health interactions. They certainly lack the complexity of a given organ or a tissue.

Whenever you have a transformed cell line, some of those interactions interfere with the innate immune response, allowing for high levels of virus replication, which you normally wouldn't see in the context of a whole organ or within an organism. There's been lots of development over at least the last decade or so on these organoid culture model systems. Over the past few years, several scientists have used respiratory organoids to help study SARS COV-2 infection. This could be from using bronchus organoids, airway organoids, human airway epithelial cultures, these transwell systems that allow for assessing primary cells, multicellular complex of cells that allow for good virus replication, especially within the respiratory compartment.

There's been some studies done in non-respiratory organoids that seem to support SARS COV-2 replication, including some of many of the same tissue compartments that we saw in this previous autopsy study, including intestine, kidney, liver, brain, eye. There's certainly, from one standpoint, these organoids are very useful. Another standpoint, there's also some key limitations that really restrict our ability to really understand long COVID. There's lack of immune cell integration. There's certainly, these organoids tend to be very tissue specific. They may not have all the immune cells, the innate immune or adapt immune cells integrated into that tissue.

There are some individuals that are now engineering these organoids to be able to allow for integration of both these tissue-specific cells, as well as in innate and adaptive immune cells. There's tonsil organoids that people are starting to develop and use in the laboratory. They're certainly heterogeneity between organoids, which is fine, because that's what you typically find in humans. You'd see patient to patient variability. When you're studying a complex disease like PASC, one has to fully understand how the conclusions from those studies can be limited based on heterogeneity.

One of the more important things is lifespan culture. How long can these organoids last for? Are you able to extend them out for one week, two weeks, up to a month or more after infection? One of the limitations I haven't listed here is accessibility. If one wanted to use these in the laboratory, how easily can we develop these in the laboratory if one has not used organoids in the past? Are there core facilities on campus? Those kinds of things that allow us to be able to gain access to these organoids in an easier manner.

There's several in vivo models that have been developed over the last few years to study SARS COV-2. I'm going to quickly go through this because I don't have a lot of time to sort of go through in detail each of these, but these slides will be made available afterwards. There's animal models that exist for studying viral pathogenesis, inflammation, tropism, transmission, vaccine-induced protection, as well as testing antiviral therapies. They can be used to study the effects of age and comorbidities. There's certainly an increasing need to develop animal models to study PASC, looking at persistent viral infection mechanisms of multi-organ sequelae, as well as CNS involvement.

One of the major drawbacks and the limitations of using these animal models is it requires specialized training. Typically, these require ABSL three facilities, facilities on campus, such as enhanced biosafety level containment for animals, institutional support, lots of vivarium staff training, support staff training to be able to support the needs of these animals within these high containment facilities. As well, for studying things like PASC are going to be costly. It requires long-term maintenance. These animals will likely be in a high containment setting for one month up to about six months to a year.

Those daily costs do add up over time. They're not what you'd expect for an acute viral infection. We typically just go for about seven to 14 days after infection. One of the models that I'll highlight here is mice. There's been quite a bit of work done using mice. You can use both inbred mice as well as these K18 human ACE2 transgenic mice, in which ACE2 receptor is overexpressed in mice, for inbred mice, typically, if you have a variant that expresses an N501Y within the spike protein, these variants will readily infect inbred mouse lines, which is great, because it allows for a nice tractable genetic system for one to be able to pick apart the immune system.

There's also mouse adaptive strains that several laboratories have used. The major difference between these two strains is that one allows for replication in the upper respiratory tract, the K18 human ACE2 transgenic mice. Not only will a virus replicate in the respiratory tract, but also extra pulmonary as well with these mice. With

some of the older variants, in the original [inaudible 01:07:23] virus, will succumb to infection, primarily due to CNS involvement. There are also other peripheral tissues that are infected.

Some of the more recent omicron variants seem to be less pathogenic in these ACE2 transgenic mice. In studies that we've performed in my laboratory, using, just an example, of inbred mice infected with an older variant, called the beta variant. This is a naturally occurring variant that has not been modified to be mouse adapted. We see really nice weight loss, up to about 20%, depending on the dose that one provides. It can actually cause some lethality in mice. Virus replication typically peaks around day two to day four post-infection, and this is corresponding with an inflammatory response marked by ISGs interferon stimulated genes, as well as cytokines and chemokines.

The virus is primarily restricted within the large airways. What you can do with these inbred mice especially is that you're able to look at immune cell infiltrates within the lungs. You can perform this neat trick of intravital labeling, where you inject fluorescein labeled antibodies prior to harvesting the tissues, and then you can perform full cytometry once you isolate the cells to see what kind of inflammatory response is occurring. Some of the inflammatory signals that we see in neutrophils, eosinophils, monocytes, macrophages, are all very similar to what we see in acute SARS COV-2 infection in humans.

We could perform single cell RNA seq if you have a TEDx machine in a BSL3 laboratory, and be able to look at gene expression profiles. Ralph Barrack's laboratory has utilized the mouse adaptive 10 strain that is laboratory generated very early on in the pandemic, to be able to help model chronic inflammation and fibrosis in mice. This is correspond with an upregulation of a fibrotic gene, called SPP1, and they believe that this is linked to possibly sustained expression of TGF beta through 30 days post-infection.

In panel H here, these mice seem to have a higher disease score up to 120 days post-infection. It appears that these mice could be used as model systems if one further develops them for studying PASC. In terms of viral persistence, my lab has some unpublished data that we'll probably submit very soon, but we look at the role of CD4 and CD8 T cells, and one of the surprising findings that we made is that when you deplete both CD4 and CD8 in these red circles here, on the bottom right hand side, what we find is that while the virus is controlled within the lungs in these bottom two left graphs, on the right side, in the nasal turbinates in the upper respiratory tract, we can actually get persistent viral infection for up to 30 days.

We now have a model in mice that allows for persistent viral infection, for us to be able to look at what cells harbor this virus, well, how does the virus persist in the face of an innate immune response? What kind of inflammatory response is there out to day 28, as well as are there genetic changes that occur within the virus that we're now studying using this model system? Non-human primates have been extensively used to be able to at least study antiviral therapies and vaccine-induced protection, but there's also been some key studies to look at pathogenesis. These non-human primates can be infected with any of the variants out there. The virus can replicate in the upper and low respiratory tract. Typically, there's a mild to moderate disease. They may lose some

weight loss, they may see differences in respiration, but there is an innate and adaptive immune response that models what we see in humans.

There's a really nice study here at the Emory National Primate Research Center that's trying to now work with the Emory Primate Research Center, the NIH, to be able to help develop a model to be able to study post-acute quality of COVID-19 using, I believe, [inaudible 01:11:27]. This is a study initiated by Mirko Paiardini and Jessica Raper here at Emory University. They're infecting up to about 10 non-human primates to SAR COV-2, collecting a bunch of different samples, and doing some cognitive testing at various time points. They're able to work with our biosafety committee to be able to infect these monkeys and then perform additional analyses at a late time point.

Once these monkeys test negative for viral loads in several of the tissue compartments, these will now be transferred over to the ABSL2, where they can now do more intensive analyses, looking at cognitive testing, food preference, sleep disturbance that really can only feasibly be performed at ABSL2. Lastly, I'll just mention that the hamster model is something that has been used quite a bit, primarily for transmission, but due to lack of key reagents, there's been really a limited analysis done at the immunological side, really hampering our ability to use the hamster model. It's another tool in the laboratory to model SARS COV-2 pathogenesis. I'll just conclude by saying the animal models have the potential to dissect the underlying mechanisms of viral persistence. Small animal models could be used to study recovery from SARS COV-2 infection and chronic inflammation. There is a requirement for specialized facilities, training, support, and certainly, the cost make long-term animal studies difficult.

Certainly, I think the data generated from the RECOVER program, through all the different clinical studies, could be used to support development of animal models and more faith will be recapitulate what's observed for long COVID in humans. I'll stop there.

Sarah Hatcher

I can go ahead and begin with some of the Q and A questions if the panelists are okay with that? Okay. I know that some of our panelists have been answering the questions in the Zoom already, but for the remaining questions, I'll just get started.

We have one question about Dr. Mohandas's slide on the duration of viral shedding, I believe. If this has already been answered in your presentation, just let me know. The question is, is this data on duration of viral shedding based on the presence of viral RNA, or viral antigen?

Sindhu Mohandas

Both, really. There are multiple studies which looked at viral RNA, as well as viral antigen with the spike and the nucleocapsid. A lot of data is based on all of these looking at all remnants of viruses. A lot of the retrospective and the studies which I mentioned in the beginning would be primarily RNA.

Sarah Hatcher

Thank you. We have another question. 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 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 sub-genomic remnants lead to autoimmunity through molecular mimicry?

Tim Henrich

I can help tackle, or Sindhu, if you want to. Either way, I can ...

Sindhu Mohandas

Go ahead.

Tim Henrich

I can start off. Yeah, it's a great question, right? The whole answer, is there ongoing replication in these tissues over time, or are there viral remnants, whether nucleic acids, proteins, et cetera? I think it's a little bit complicated. I agree that it's hard to find replication virus and tissues over time. I should mention that especially from the gut, it's actually challenging to get replication a competent virus, for example, in HIV over chronic infection.

We know that it's in the gut. We know it can replicate in the gut off of ART, et cetera, but it can actually be very difficult to find or grow virus from that compartment. It really depends what tissues you're looking at, has to do with the local environments, what kind of facilitates viral replication within those specific tissues. This is where I think some of the live models and things like that can be very helpful as well in kind of parsing out some of these questions.

Obviously, ongoing replication has indication, will things like produce inhibitors or nucleoside analogs and the antivirals, are those actually going to help? I think that one of the best ways to do is just to try it, to do a study where you actually interrupt potential replication, and see if there's any clinical or biomarker improvement in disease. It's a simple yet complicated experiment at the same time to do, but I think certainly where we need to be going and I know that RECOVER is going that direction as well.

Then the last thought is yeah, absolutely. If you have persistent protein, they can have toxic toxin effects, they can trigger pattern recognition, and you can actually have ongoing, both immune dysfunction, inflammation, even without frank or high levels of replication in various tissues. These are things that really aren't quite very well understood, I think, and still need a lot further study, but I can certainly get others' opinions on that one.

Sarah Hatcher

Thank you. If no other panelists would like to comment on that question, I think Dr. Garcia-Sastre now has connection, and we can hand it over to him to discuss a synopsis of the presentations today.

Adolfo Garcia-Sastre

That has been a great talk. That one that we have heard we heard from Professor Henrich. It is very common for many viruses to cause sequelae, especially those that cause persistence. The evidence that actually, both SARS COV-2 sequelae and the evidence that there is around, looks very similar to what is happening with viruses that persist.

Then we have heard from Professor Mohandas that the evidence that SARS COV-2 persist, at least in expression of RNA or proteins, rather than virus infection, as well as inflammatory responses, suggesting that actually, the main cause of sequelae for SARS COV-2 is the presence of the persistent inflammation, somehow associated with persistence of anti expression, but that this is still unclear whether actually this is the case, how sequelae, and then we have heard about the use of animal models in order to try to address the questions of sequelae.

There are many questions that are still unknown of what can be happening. To me, one thing that is interesting from the point of view, I'm a virologist, it's clear that the sequelae does not represent any advantage for the virus, for which the virus could be selected for, so if we present something like a side effect. To me, and I guess everybody wonders couple of things, but one of them is, with secondary infections, one infection followed by another one, is the secondary infection, the second infection, likely to also use sequelae or not?

Primary infection, in the absence of sequelae, has protect you against second sequelae by secondary infection? I guess vaccination, by this vaccination also preventing from sequelae, even in cases where vaccination is unable to protect against infection, and the person that is vaccinated gets infected doesn't get severe disease. What is the chance the sequelae happen also with vaccination? We can answer this question, we can at least say the vaccination is a great protection against infection. It's a question in general for the panelists. Perhaps, yeah, go ahead.

Sindhu Mohandas

I think the studies that have reported a little bit mixed on this. Really, there are studies to show that when vaccination occurs, it doesn't of course prevent all breakthrough infections, but when breakthrough infections occur in subsequent PASC, so it also reduces the chances of PASC happening, but not completely eliminated. When PASC does happen, the total number of symptoms that patients who have been vaccinated in the past versus those who are not vaccinated, tend to have a much smaller number of some symptoms and duration.

I don't think it's an answer which will solve all problems, but it is possible that it does kind of change the course of PASC to a shorter and with a less number of symptomatology. This is something, again, we need more research in. What does vaccination do to patients who already have PASC? How does it change the course of the PASC in those patients? It's definitely something which it's a good question and which needs to be studied.

Tim Henrich

Yeah. I think if you envision that there is protein, like spike protein that's circulating, or tissue that antivirals, may not be the best ... Direct antivirals that are targeting replication life cycles, but if you somehow boost neutralization, or binding to those circulating antigens, and that's where vaccine studies could be helpful, though it's kind of been mixed.

The anecdotal studies, some people get better, 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 becoming a challenge too. Yeah, but it really needs more study.

Adolfo Garcia-Sastre

Yeah. Thank you. The other thing that it not concern me, but I just wonder, what is known about persistence with respect to sequences? Is there any study to know what the sequences are changing over time, in the case of persistence with SARS COV-2?

I know in immunosuppressed individuals, the sequences are changing, but in those that one can still detect viral RNA, and the one does not detect viral infection, is there any sequences that has been done to know how much the virus is changing over time?

Tim Henrich

I think that's a challenging one, because to do that you need 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. Especially after people stop shedding from their nasopharynx or stool or wherever they may be, once they stop, which most people do, especially if they're not immunosuppressed, then you have to go through tissues longitudinally to look for that type of evolutionary change.

That would be a fascinating study to look, if there is tissue persistence, is there a change? For example, has it escaped from immune pressure, for example? 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 kind of quasi check. Yeah, those would be difficult but very important studies to do.

Adolfo Garcia-Sastre

I have still a lot of questions, but I'll let the audience to continue with a couple of questions. Sarah, you don't mind take over. Thanks a lot.

Sarah Hatcher

Sure, thank you. Unfortunately, we are running out of time, so I'm actually going to close us out if that is all right with everyone. The remaining questions that haven't been answered that are relevant to today's presentation will be answered after the presentation. Thank you to our presenters for sharing your time and your work with us. Thank you to our audience for attending and engaging with the Q and A.

As a reminder, a recording of today's seminar will be available on RECOVERCOVID.org within a few weeks, and we will also, as I mentioned, be posting a Q and A document that has responses to the questions we received today, including those we did not have time to address. Patrick, could you show the slide of the upcoming seminars, please?

The R3 seminars are held on the second and fourth Tuesday of the month from 12 to 1:30 PM Eastern Time. Information on subsequent sessions is forthcoming. This slide shows the future R3 seminar topics in February. The RECOVER team has developed a short three question survey to learn how we can improve our three seminars that should display on your screen now. Please fill out this brief survey before you leave the webinar. That's all we have. Thank you, and have a great day.

Webinar Slides

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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/>