Invited Review

CROI 2021: Summary of Basic Science Research in HIV and SARS-CoV-2

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The Conference on Retroviruses and Opportunistic Infections (CROI) serves as one of the most highly visible platforms upon which researchers gather to share the most recent findings on HIV/AIDS and, recently, on SARS-CoV-2 research. Research presentations on the novel coronavirus SARS-CoV-2 have become an increasing fixture at the conference since it was first covered at last year's conference. Although CROI 2021 was virtual, the organizers coordinated a seamless platform for presentations and poster sessions that effectively engaged the audience. CROI 2021 had a strong showing in terms of basic science presentations on HIV-1 and on SARS-CoV-2. Highlights included new insights into some of the more elusive steps in the viral replication cycle as well as new findings on immune escape strategies employed by SARS-CoV-2. The new investigator workshop has become a valuable resource that can be used by early stage and established investigators alike to receive state-of-the-art updates on research areas that might be outside their immediate areas of research. The new investigator workshop featured engaging presentations on novel aspects of HIV-1 and SARS-CoV-2 replication, impact of host immunity on HIV-1 and SARS-CoV-2, and approaches to assessing viral reservoir dynamics and strategies for viral reservoir elimination.

Keywords: CROI, 2021, HIV-1, SARS-CoV-2, reservoirs, cure

Virology

Although researchers have gained detailed mechanistic insight into host and viral processes involved in the viral replication cycle, there are some aspects of the replication cycle that remain poorly understood. The symposium, "Navigating to the Nucleus" (Symposium 01), reviewed what is known as well as important new insights into several key steps in the first half of the HIV viral life cycle. The process by which the viral nucleic acid is able to access the host cell nucleus prior to integration is one of the more elusive in the viral replication cycle. Upon virus attachment to receptor and coreceptor molecules, the viral membrane fuses with the host cell membrane to release the viral capsid core into the host cell cytoplasm. The viral capsid core disassociates to liberate the viral genomic RNA that, through the process of reverse transcription, is converted to a cDNA. The steps between entry of the viral core into the host cell cytoplasm and core disassociation are collectively referred to as uncoating.

Until recently, models of retrovirus replication described the uncoating process to involve capsid dissociation as occurring immediately after fusion of viral and host cell membranes. The process of reverse transcription took place within a protein complex that comprised viral nucleic acid and the viral enzymes, reverse transcriptase, and integrase. Reverse transcription was completed in the cytoplasm and nascent cDNA then translocated to the nucleus so that it could integrate within host cell DNA. Complexes of viral nucleic acid and enzymes, often referred to as the reverse transcription complex, have a molecular mass approaching that of the ribosome and a diameter of 61 nm. Therefore, the challenge faced by retroviruses and lentiviruses is how to get a ribosome-sized reverse transcription complex across the nuclear membrane so that they can integrate their genomes into host cell DNA.

Transport across nuclear membranes is regulated through nuclear pores that have an inner diameter of 61 nm. From here on, it appears that lentiviruses and retroviruses have developed different strategies to get their nucleic acids to the nuclear compartment of the host cell. Retroviruses access the nucleus and integrate their DNA in cells that are actively undergoing mitosis. Since the nuclear membrane disaggregates at mitosis, the reverse transcription complex has unhindered access to host cell DNA. In contrast, lentiviruses have the capacity to access the nuclear compartment of nonmitotic cells, so they possess a unique mechanism for getting their reverse transcription complex across the nuclear membrane. In this case, capsid molecules remain part of the reverse transcription and are thought to aid in the process of nuclear uptake. This capacity underscores the ability of lentiviruses to transduce nondividing cells such as macrophages and this has been exploited in the design of lentivirus vectors for transduction of nondividing cells including muscle cells and microglia.

These studies were corroborated in the presentation in the same symposium by Muller of the University Hospital Heidelberg. Muller noted published studies[1] that indicate the diameter of the nuclear pore to be larger than previously suspected and to be of a size that would permit translocation of an intact HIV-1 core (Abstract 19). Collectively, studies reviewed in the symposium indicate that current models of

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lentiviral replication should be revised to reflect a nuclear location for lentiviral uncoating and completion of reverse transcription.

Studies discussed in this symposium also provided fresh insight into the intricacies of nuclear translocation by lentiviruses. Pathak used labeled components of the reverse transcription complex as well as labeled antibody to the nuclear pore protein, POM 121, to allow live cell imaging of the dynamics of nuclear translocation of HIV-1 reverse transcription complexes (Abstract 20). The first surprise was that, in contrast to cellular cargo that translocated the nuclear membrane in seconds, reverse transcription complexes docked at the nuclear envelope for 1.5 to 2 hours, suggesting that the nuclear envelope represents a bottleneck in the process of lentivirus infection.

Through the use of labeled capsid molecules, investigators in the Pathak laboratory were able to image the events around nuclear uptake and capsid dissociation. They observed that docking of reverse transcription complexes at the nuclear pore extended over several hours but viral uncoating (capsid dissociation), occurred rapidly (within 20 minutes). From here on, another 1.5 hours was required for integration of uncoated complexes. Remarkably, the investigators determined that nuclear complexes were largely intact with regard to capsid protein composition and similar to that for intact cores. This suggests that contrary to current models, uncoating did not occur until after the viral core entered the nucleus. Time-of-addition assays with the integrase strand transfer inhibitor raltegravir further demonstrated that reverse transcription was completed before uncoating and that integration occurred shortly after uncoating of viral cores in the nucleus.

These studies were corroborated in a presentation in the same session by Muller of the University Hospital Heidelberg and were summarized in the plenary session by Krausslich (Abstract 14). Muller also discussed published studies[1] that indicate the diameter of the nuclear pore to be larger than previously suspected and to be of a size that would permit translocation of an intact HIV-1 core. Collectively, studies presented in the symposium indicate that current models of lentiviral replication should be revised to reflect a nuclear location for lentiviral uncoating and completion of reverse transcription. It remains to be determined as to what advantage HIV-1 has in adopting such a strategy to maintain core integrity until the point of integration.

The revised model of preintegration events in lentiviral replication and the role of the capsid protein in these events further validate capsid as an attractive target for antiviral drug development. In the same session, Cihlar of Gilead Sciences described an investigational novel small molecule that specifically targets the capsid protein (Abstract 22). Capsid function relies on protein-protein interactions that, from a small molecule inhibitor development standpoint, represent a far more challenging target than enzymatic proteins of the virus. Small molecules that target the capsid monomer as well as compounds targeting capsid hexamers have been described. Those targeting the hexamer, such as PF-3450074 (PF-74), accelerate the assembly of capsids during formation of nascent virion particles. Cihlar and colleagues used an in vitro biochemical capsid assembly assay to assess 3000 analogues of a second-generation lead that emerged from an inhibitor screen.

Lenacapavir was selected on the basis of potency, pharmacokinetic (PK), and off-target profiles. Lenacapavir exhibits low picomolar antiviral activity, essentially making it the most potent small molecule inhibitor for any HIV target. Lenacapavir is active in primary CD4+ T cells and macrophages with a narrow range of potencies against all HIV-1 subtypes. Structural analysis identified the lenacapavir binding site in capsid hexamers. The lenacapavir binding site is conserved across all HIV-1 subtypes, which indicates that there is no preexisting resistance to lenacapavir. In addition, lenacapavir retained full antiviral activity against viruses harboring resistance mutations to other classes of antivirals.

Stepwise passage of HIV-1 in increasing concentrations of lenacapavir promoted the development of N74D and Q67H mutations in capsid. The investigators also assessed emergence of lenacapavir resistance in clinical isolates maintained in the presence of drug in peripheral blood mononuclear cells (PBMCs) to identify additional mutations (such as L56I and M66I) that might impact lenacapavir sensitivity. Although some mutations (such as Q67H/N74D, M66I) reduced lenacapavir sensitivity by several hundred fold, viral fitness was significantly impacted. Consistent with a role for capsid in early steps of viral replication, lenacapavir was found to be more effective in inhibiting early events in viral replication.

Time-of-addition experiments were used to identify the time point at which lenacapavir impacts early events in HIV-1 replication. In the presence of lenacapavir, the abundance of integrated proviruses and 2-long terminal repeat (2-LTR) circles, which are specifically formed in the host cell nucleus, was greatly reduced. In the presence of the lenacapavir analogue GS-CA1, nuclear import of capsid molecules and of viral DNA, was inhibited. The host cell proteins cleavage and polyadenylation specificity factor 6 (CPSF6) and nuclear pore complex 153 (NUP153), have been shown to participate in the nuclear translocation of HIV-1 reverse transcription complexes.[2] The lenacapavir binding site on HIV-1 capsid overlaps the CPSF6 and NUP153 binding sites, and lenacapavir also was independently shown to inhibit the binding of these host nuclear import factors to capsid tubes assembled in vitro.

Lenacapavir has also been shown to stabilize HIV-1 cores, and as such, can impact uncoating and nuclear import that might themselves require structural changes or partial disassembly of the viral core, as suggested by Pathak's studies outlined above. Lenacapavir impacted late stages in virus replication by accelerating the rate of capsid polymerization in a way that leads to formation of poorly organized and misfolded capsid aggregates that leads to the production of aberrant and noninfectious virions. Importantly,

lenacapavir exhibits PK properties that could enable once-weekly oral dosing as well as once every 6 months subcutaneous injections. Lenacapavir represents a remarkably potent and novel capsid inhibitor that targets early and late stages in the HIV-1 replication cycle. As such, it should extend the armamentarium of agents for the treatment and prevention of HIV-1 infection and, in particular, increase treatment options for individuals infected with multidrug-resistant HIV-1 variants.

Viral Reservoirs and Mechanisms of Viral Persistence

HIV-1 establishes lifelong persistence in the host even in the face of overwhelming antiretroviral therapy (ART). Stigma, adherence issues, and implementation challenges, particularly in low- and middle-income countries, have prompted the pursuit of a cure for HIV-1 infection. Given the progress in ART options, particularly with advances in long-acting formulations, it has been argued that a cure HIV-1 is no longer needed. Nevertheless, pursuit of a cure for HIV-1 infection remains an aspirational goal for many HIV/AIDS researchers. Two paths are being pursued. The greatest amount of effort has focused on identification of strategies that achieve HIV-1 remission. Under remission, the virus has not been completely eliminated yet is maintained at undetectable levels and is unable to be transmitted. The more challenging path involves identification of strategies for a sterilizing cure where all vestiges of the virus have been eliminated.

Yu made the case that some elite controllers (ECs) are representative of what might be considered a sterilizing cure (Abstract 57). Elite controllers are rare individuals in whom HIV-1 remains undetectable in the absence of ART. Some ECs owe their status to being infected with defective (eg, *nef*-deleted) viruses and others have mutations in genes that are important cofactors for viral replication (eg, CC chemokine receptor 5 [CCR5]). Recent studies from Yu and colleagues have indicated that in some ECs, the viral reservoirs have become inert. Proviruses in ECs were profiled by individual proviral sequencing, and the integration sites in host DNA were mapped. In the majority of ECs, proviruses were found to be intact and replication competent but were concentrated in nongenic or heterochromatin regions of host cell DNA. This was surprising because a number of studies have demonstrated HIV-1 integrates preferentially within gene-rich regions of human chromosomes. These regions have a relaxed chromatin architecture that allows free access of transcription factors that regulate gene expression. Using single-cell assays in which integration sites and HIV transcriptional activity could be analyzed simultaneously, the investigators concluded that proviruses in nongenic regions had very limited transcriptional activity, akin to "deep latency." Those proviruses also bore epigenetic modifications that limited their transcriptional capacity. In a subset of ECs, intact proviruses were not detected. Collectively, these results paint a picture of what a sterilizing cure might look like.

Processes that led to the establishment of the EC state remain to be defined. It is possible that following initial infection, reservoirs in ECs were limited in size and that cells harboring active proviruses (ie, in gene-rich regions) were selected against and that over time, inactive proviruses in gene-sparse regions predominated. These studies also raise the possibility that, in the absence of transcriptional activity, proviruses are epigenetically and, perhaps, irreversibly silenced.

Host Antiviral Immune Responses and Viral Reservoir Control

The extent to which host antiviral immune responses control viral reservoir dynamics is unclear. Jones presented studies that suggest reservoir cells have intrinsic resistance to host antiviral immune clearance mechanisms (Abstract 41). CD8+ T cells are well recognized as exerting potent antiviral pressure on HIV-1. For example, when antibodies are used to deplete CD8+ T cells in simian immunodeficiency virus (SIV) infected macaques, there is a corresponding increase in plasma viremia. In addition, CD8+ T cell recognition epitopes are the most highly variable in the viral proteome, which demonstrates that HIV-1 is under considerable CD8+ T-cell pressure and continually mutates to escape it. Studies from research groups of Picker, Skinner, and Connick have also suggested the presence of viral sanctuaries in the lymph nodes (see also Abstract 43 by Okoye). CD8+ T cells are excluded from the B-cell follicles in the lymph nodes, and it has been proposed that those sites provide viral sanctuaries from host immune surveillance.[3] These studies have driven the rationale for cure strategies that harness the antiviral activity of CD8+ T cells.

In "shock and kill" approaches to reservoir elimination, CD8+ T cells are expected to provide the bulk of the kill effect following viral reactivation (shock). Jones presented evidence that reservoir cells from individuals on effective ART are resistant to clearance by CD8+ T cells. Published studies from the Jones group have demonstrated that, even after stimulation with strong latency-reversing agents such as ionomycin and phorbol 12-myristate 13-acetate (PMA), latently infected CD4+ T cells from infected individuals on ART were resistant to CD8+ T-cell killing ex vivo.[4] Jones also highlighted prior studies that demonstrated the resistance of infected macrophages to killing by CD8+ T cells[5,6] where resistance was under scored by increased expression of inhibitors of granzyme B, a key enzyme that promotes target cell killing by CD8+ T cells, in infected macrophages.

Gene profiling of reservoir CD4+ T cells that survived CD8+ T-cell killing ex vivo likewise revealed the increased expression of cellular factors that, in the cancer research field, have been shown to play a role in cytotoxic T lymphocyte (CTL) resistance (eg, cellular Fas-associated death domain-like IL-1β-converting enzyme [FLICE] inhibitory protein [c-FLIP]) and that have the potential to influence susceptibility to CD8+ T-cell killing (eg, enhancer of zeste homologue 2 [EZH2]). A number of other host cell factors that were upregulated in reservoir cells need further investigation for whether they play a

role in the resistance of reservoir cells to CD8+ T-cell clearance. These studies may point to the identification of approaches that improve the clearance of infected reservoir cells through "kick and kill"-based strategies.

Extending on the theme of the role of host antiviral mechanisms on viral reservoir dynamics, Hahn examined the sensitivity of rebound HIV-1 to type-1 interferon (Abstract 44). Type-1 interferons are potent effectors of the innate host antiviral response, and some cure strategies have attempted to exploit the antiviral activity of type-1 interferons, but with limited success. To define the impact of endogenous interferons on reservoir dynamics, Hahn examined the interferon sensitivity of plasma viruses before and after initiation of ART and of viruses rebounding after an analytic treatment interruption (ATI). Longitudinal analysis of interferon sensitivity indicated that acute infection isolates were uniformly interferon resistant and that this resistance declined by 2 to 4 orders of magnitude over the first year of infection. The resistance was partially reacquired during disease progression. Interestingly, interferon resistance did not increase in slow progressors but remained elevated in individuals with accelerated disease progression. In individuals initiating ART, the level of interferon resistance was maintained at similar levels to ex vivo outgrowth viruses (using quantitative viral outgrowth assay [QVOA], assessed 1 to 2 years after ART initiation).

Hahn then assessed the interferon sensitivity of rebound viruses post-ATI as well as that of viruses obtained in ex vivo outgrowth assays using pre-ATI and post-ATI CD4+ T cells. Surprisingly, all rebound viruses were interferon resistant, and QVOA viruses pre- and post-ATI were interferon sensitive. ATI also appeared to reseed the reservoirs with interferon-resistant viruses although interferon resistance declined during prolonged ATI. Two closely related QVOA viruses exhibited only 5 nucleotide differences (in the LTR, *pol*, *env*, and *nef*) yet exhibited markedly different levels of interferon sensitivity. This indicated that very few sequence changes are sufficient to dramatically impact interferon sensitivity. Hahn then explored 2 options that might underscore the origins of interferon-resistant rebound viruses. For example, these viruses could emerge from a preexisting reservoir of interferon-resistant viruses or have rapidly evolved from interferon-sensitive variants at sites of viral reservoir reactivation. Recent studies have suggested that macrophages may serve as a source of rebounding viruses.[7] Surprisingly, a number of rebound viruses and a few QVOA assays were able to replicate efficiently in macrophages, but some rebound viruses were not able to replicate in macrophages. This suggests that CD4+ T cells can archive macrophage-tropic viruses and that CD4+ T cells are the main cell type for virus amplification post ATI. It remains to be determined whether macrophages can serve as the origin of interferon-resistant rebound viruses.[8]

As discussed earlier, CD8+ T cells are largely excluded from lymph node germinal centers, and this may afford the virus an opportunity to establish viral reservoirs at these locations. Okoye (Abstract 43) outlined approaches aimed at removing the B-cell follicle barrier. Within germinal centers of B-cell follicles, follicular helper T cells (TFHs) interact with antigen-specific B cells to promote their differentiation into long-lived memory B cells and plasma cells. Access to the germinal centers is tightly controlled by chemokine interactions that permit entry of CXC chemokine receptor 5 (CXCR5)+ CD4+ TFHs but not CXCR5– T cells or antiviral CD8+ T cells. Studies with HIV and SIV have demonstrated that TFHs are highly susceptible to infection and in SIV-infected animals that attain virologic control, SIV replication is restricted to the CD4+ T-cell compartment in B-cell follicles.[9,10] In addition, virions can be deposited on follicular dendritic cells within the follicles.

Several approaches are being explored to reduce reservoir dynamics within the follicles. For example, anti-CD21 reduces the deposition of virions on follicular dendritic cells during acute SIV infection. Others have been exploring use of chimeric antigen receptor (CAR)-T cells or CD8+ T cells that have been modified (eg, to express CXCR5 from a lentivirus vector) to target B-cell follicles. Okoye presented studies that exploit an anti-CD20 antibody to deplete follicular structures. CD20 antibody administrations were able to substantially deplete B-cell follicles, and this was reflected by better control of SIV infection in EC monkeys. Okoye then explored whether more profound depletion would result in more effective viral control. The investigators used anti-CD20 CAR-T cells to achieve almost complete B-cell depletion in most anatomic compartments. Increased B-cell depletion also resulted in better virologic control. B-cell depletion at the time of ART release resulted in a modest and transient level of post-ART control. Anti-CD20 in conjunction with interleukin (IL)-15 was found to promote a more complete loss of B-cell follicles than CD20 alone. However, despite profound depletion of B-cell follicles, there was no delay or early control of rebound SIV. Therefore, although B-cell follicle disruption is able to promote CD8+ T-cell–mediated control of ongoing viral replication, it has limited impact on post-ART rebound.

The presentation by Caskey (Abstract 36) focused on the use of broadly neutralizing antibodies (bNAbs) in prevention, management, and elimination of HIV-1 infection. bNAbs differ from conventional HIV-specific antibodies in that they display remarkable breadth and potency of neutralization against HIV-1. Some modifications can extend the plasma half-life of bNAbs and increase their utility for HIV management, but long-term expression for HIV management and for reservoir reduction will likely depend on vector delivery strategies. Abstracts 160 and 270 presented results with adeno-associated virus (AAV)-mediated delivery of bNAbs. Recombinant AAV offers a highly feasible solution for sustained antibody delivery approaches that overcome the challenges associated with regular antibody administration. AAV is a small DNA virus that has been engineered to lack everything from the AAV genome except for the 2 inverted

terminal repeats required for genome circularization and concatemerization in the nucleus of the transduced cell. This allows transgenes up to 5 kilobase (kb) to be accommodated in the AAV vector.

AAV applications for gene therapy have markedly increased in the past 10 years with the European Medicines Agency approval of alipogene tiparvovec for lipoprotein lipase deficiency and US Food and Drug Administration (FDA) approval for voretigene neparvovec-rzyl to treat inherited retinal disease and onasemnogene abeparvovec-xioi for the treatment of spinal muscular atrophy. These studies under score the remarkable safety profile of AAV and its capacity for long-term transgene expression, as illustrated with factor IX where therapeutic amounts were still being produced 10 years after intramuscular injection.[11]

Martins (Abstract 270) presented results with AAV-mediated delivery of the bNAb 3BNC117 and of the antibody-like molecule eCD4-Ig in the prevention of oral acquisition of simian HIV (SHIV). Following a single intramuscular injection, plasma eCD4-Ig levels in the 12 to 70 µg/mL range and 3BNC117 levels in the 48 to 79 µg/mL range, were sustained. In some animals, antidrug antibodies limited the expression of 3BNC117. Animals that sustained antibody expression were protected from oral SHIV acquisition.

Results with AAV for bNAb delivery in humans were presented in Abstract 160. VRC07 was expressed from AAV8 in 8 HIV-infected volunteers on ART. Durable but low expression of antibody (0.17-1.2 µg/mL) was observed in most individuals. Antidrug antibodies limited the long-term expression of antibodies in some individuals. Although these studies highlight the potential utility of AAV for long-term bNAb delivery, approaches to reduce antidrug antibody responses will be needed to ensure consistent antibody delivery.

SARS-CoV-2

Advances in the approaches for developing potent neutralizing antibodies against HIV-1 (B-cell cloning and profiling) have been adopted to accelerate the development of antibodies for the management of SARS-CoV-2 infection. Bjorkman summarized efforts aimed at developing antibodies with potent spike protein recognition and neutralization (Abstract 3). Closely related coronaviruses that can use the angiotensin-converting enzyme 2 (ACE2) receptor for infection have been found circulating in bats. This raises the possibility of future coronavirus transmissions to humans. Through the use of nanoparticles containing mosaic spike proteins, broadly cross-reactive antibodies are being induced. Such antibodies could form the basis for a pan-coronavirus vaccine that could be employed against future coronavirus introductions into humans.

Kirchoff (in the Program Committee Workshops for New Investigators and Trainees; Abstract 01) overviewed advances in the molecular virology of HIV and SARS-CoV-2. He pointed out that Pubmed contains 190,000 publications on HIV-1 over more than 30 years and 66,000 on SARS-CoV-2 (with 64,000 in the last 12 months), which illustrates an explosive growth in knowledge surrounding these pathogens. The interactive session on SARS-CoV-2 evolution in populations and individuals contained presentations ranging from structures of spike protein variants to selections of neutralization-resistant SARS-CoV-2 variants (Interactive Session 01).

Rolland highlighted the rapid adaptation of SARS-CoV-2 to humans that is driven by mutations in the spike protein, which increase viral infectivity. SARS-CoV-2 is transmitted before it has the chance to mutate. Therefore, virus evolution follows different pressures in the naive versus the nonnaive population. In the naive population, viruses evolve under different selective pressures, but in previously infected or vaccinated individuals, evolution is driven by escape from antibody recognition. Luban discussed how mutations that lead to a more open conformation of the spike protein also lead to more infectious viruses that rapidly outcompete other variants in the population. He discussed results with the D614G variant that, in the context of the B.1.1.7 backbone, increases viral infectivity by 30 fold. That mutation appears to allow acquisition of other mutations that would otherwise compromise viral infectivity.

Bieniasz gave an overview of studies aimed at understanding factors that govern the emergence of antibody resistance in SARS-CoV-2. These factors can include viral determinants such as viral replication fidelity or population size versus transmission factors, the interval during which viral replication and antibody responses may overlap. Although the virus is well conserved at the sequence level, as summarized in Caskey's talk mentioned above, the breadth of SARS-CoV-2 neutralizing antibody responses are also well conserved. Therefore, the virus is encountering the same antibody selection pressures in different individuals. Bieniasz used a pseudotyped virus to analyze the neutralization sensitivity of viruses harboring mutations that are frequently detected in SARS-CoV-2 variants of concern (K417E, E148K, N501Y). In a panel of 17 antibodies cloned from vaccines, which are also typical of the forms found in convalescents, 14 had reduced sensitivity to SARS-CoV-2 envelopes harboring those mutations. In some individuals, virus appears to persist for extended periods and this creates an environment in which somatic hypermutation can drive B-cell maturation and production of antibodies that are effective against resistant variants. Antibodies cloned from individuals early versus late in infection were found to be more active against antibody resistant viruses. Bieniasz also looked at the epitopes in the spike protein that account for the neutralizing activity of convalescent plasma and identified domains including the N-terminal domain (NTD), receptor-binding domain (RBD), fusion peptide (FP) heptad repeat (HR)1, and HR2 domains. These studies highlight the interplay between the virus and the host antibody response that is shaping the virus to escape antibody responses of the host.

As highlighted by Bieniasz, SARS-CoV-2 can persist for longer intervals in some individuals, and this can provide an opportunity for evolution of

antibodies that have higher neutralization activity against SARS-CoV-2 as well as for viral escape. Tokuyama (Abstract 115) presented evidence that SARS-CoV-2 can persist in intestinal enterocytes for up to 7 months after resolution of symptoms. Although SARS-CoV-2 RNA has been detected in fecal samples from individuals long after symptom resolution, it is unclear whether this RNA is the result of an active infection or simply residual transcripts from cells harboring defective viruses. ACE2 is expressed in the gut and particularly in the small bowel. Biopsies from the duodenum and ileum were assessed for the presence of viral nucleocapsid antigen by in situ immunofluorescence and for the presence of the virus by electron microscopy. Viral antigen was detected in 16 of 29 participants. Samples positive for viral antigen also contained SARS-CoV-2 virions when assessed by electron microscopy. The majority of those with evidence of SARS-CoV-2 in small bowel samples exhibited mild signs of intestinal inflammation and detection of viral products did not correlate with gastrointestinal symptoms. Additional studies are needed to determine whether viral persistence contributes to protracted COVID-19 disease symptoms.

All cited abstracts appear in the CROI 2021 Abstract eBook, available online at www.CROIconference.org

Financial affiliations in the past 24 months: Dr Stevenson has served as a consultant for EMD Serono. (Updated June 30, 2021)

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