Invited Review

CROI 2022: Summary of Basic Science Research in HIV and SARS-CoV-2

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The Conference on Retroviruses and Opportunistic Infections (CROI) 2022, which was held as a virtual conference, continues to serve as the preeminent forum that features research advances in HIV-1 and its associated coinfections. The conference has extended its area of coverage to include research advances in SARS-CoV-2. As pointed out in the presentation from Hatziioannou in the New Investigators workshop, there has been an explosion in research activity on SARS-CoV-2 that has eclipsed that for HIV-1. In the past 12 months, there were approximately 6600 publications on HIV-1 and approximately 64,000 on SARS-CoV-2. Although these numbers include review articles, they reveal the tremendous response by researchers to the existential threats posed by lentiviruses and coronaviruses. This poses challenges for any conference committee tasked with selecting abstracts for presentation from the large number submitted for consideration. CROI organizers have consistently been able to assemble a program that, through invited presentations, abstract-driven talks, posters, interactive sessions, workshops, and symposia, showcases the most recent research advances.

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Virology

The structure of SARS-CoV-2 is being revealed in great detail. The viral spike protein, which harbors the receptor-binding domain, may display that domain in an open or closed confirmation. By comparison, the coreceptor-binding region on the HIV-1 envelope remains closed until the virus engages CD4. This triggers a conformational change that exposes the coreceptor-binding domain, which allows the envelope to attach to the coreceptor. This mechanism is designed to minimize the vulnerability of the envelope to antibodies to the coreceptor-binding region. A similar mechanism may be used by SARS-CoV-2 to limit exposure of the receptor-binding domain to neutralizing antibodies. The spike protein was found in 2 main isoforms, a more abundant prefusion and a less abundant postfusion isoform. In the prefusion isoform, the spike trimers were found in 3 classes according to the receptor-binding domain orientation: closed, open, and mobile, mostly closed receptor-binding domain conformations. In the open form, the receptor-binding domain was surface-exposed and able to bind angiotensin-converting enzyme 2 (ACE2). Once bound to the receptor, the prefusion spike underwent a structural transition to a postfusion form in which the prefusion trimers are likely shielded from neutralizing antibodies. Understanding what triggers the conformational change will be important to guide the design of vaccines and small molecule fusion inhibitors that target vulnerable epitopes in the receptor-binding domain.

Zoonotic origins of coronaviruses were also discussed by Hatziioannou (Abstract 1). There have been 7 different coronaviruses known to cross from...
animal hosts into humans: 5 beta coronaviruses and 2 alpha coronaviruses. SARS-CoV-2 is most closely related to coronaviruses circulating in bats and has 79% homology to the SARS-CoV coronavirus detected in humans 18 years ago. Although genetic evidence demonstrates homology to natural viruses circulating in bats, there is no definitive picture on the animal origins of SARS-CoV-2 or when and where the first human transmission occurred. Bats are natural hosts of alphacoronaviruses and betacoronaviruses and RaTG13, a coronavirus isolated from the *Rhinolophus affinis* bat in Yunnan province in China, has 96.2% identity to SARS-CoV-2 and has more than 90% identity with SARS-CoV-2 in all open reading frames (ORFs) throughout the genome including the highly variable S and ORF8. More recently, another bat virus, RmYN02, isolated in Yunnan from a *Rhinolophus malayanus* to RaTG13 bat, was found to have 93% identity to SARS-CoV-2 across the genome and 97% identity in the 1 ab gene.

Although first reported in Wuhan, China, in December 2019, a controversial report claims to have detected SARS-CoV-2 in December 2019 in a patient hospitalized for hemoptysis in a hospital north of Paris, France. This would suggest that the COVID-19 epidemic started earlier in France. This result still needs to be confirmed with retrospective analyses of banked samples from diverse geographic areas. These observations suggest the presence of bat reservoirs of SARS-CoV-2. However, the divergence between SARS-CoV-2 and related bat coronaviruses spans more than 20 years of sequence evolution. Therefore, although these bat coronaviruses are likely to be SARS-CoV-2 precursors, SARS-CoV-2 is unlikely to be their direct descendent.

Pangolins have received attention as possible intermediate hosts for SARS-CoV-2. Palm civets and dromedary camels served as intermediary hosts for SARS-CoV and MERS-CoV, respectively. However, in the case of SARS-CoV and MERS-CoV, viruses in the intermediate hosts and humans exhibited over 99% sequence identity. The fact that, outside of the receptor-binding domain, pangolin coronaviruses have no more than 92% sequence identity with SARS-CoV-2 argues against pangolins being directly involved in the SARS-CoV-2 outbreak. Therefore, the picture remains incomplete as to whether an intermediate host played a role in the introduction of SARS-CoV-2 to humans.

The first half of the retroviral replication cycle, encompassing events from viral fusion with the target cell membrane to integration of viral DNA with host cell DNA, has been a subject of strong interest by numerous research groups and this research continues to turn up surprises. After the virus fuses with the cell membrane, the viral core that contains the genomic viral RNA and reverse transcriptase and integrase enzymes that catalyze cDNA synthesis and integration, respectively, is deposited in the target cell cytoplasm. Once in the cytoplasm, classic models of retroviral biology suggest that the core then dissipates to liberate viral RNA that then undergoes reverse transcription. A theme that continues to prevail in the biology of lentiviruses is that viral genes often have additional functions beyond their classic roles in viral replication. This is to be expected since viruses have to achieve many things with a limited repertoire of viral proteins. Research has revealed, in tremendous detail, the processes governing the integration of viral cDNA with host cell DNA. Abstract 52 presented evidence suggesting that the viral integrase, which catalyzes integration of viral cDNA into host cell DNA, is also involved in viral maturation. Within the virion, integrase is contained within the viral core that is deposited in the cytoplasm together with genomic viral RNA. Integrase needs to remain in tight proximity to viral cDNA as it is being synthesized and transported to the nucleus, where it will catalyze integration of the viral cDNA into host cell DNA. The introduction of mutations in integrase had pleiotropic effects on the virion that included effects on viral replication that were not due to defective integration. Some mutations in integrase impact interaction of integrase with genomic viral RNA, and some cause mislocalization of integrase within the virion that leads to degradation of the viral RNA in target cells. It now appears that integrase mutations that perturb its interaction with genomic viral RNA also impair virion maturation. The interaction of integrase with genomic viral RNA appears to be dependent on a positive electrostatic
potential of the C-terminal domain of integrase. Collectively, these studies reveal activities for the integrase protein that extend beyond its role in proviral formation and that center on the interaction between integrase and genomic viral RNA.

As discussed in the symposium “Navigating to the Nucleus” and the oral abstract session “HIV/simian immunodeficiency virus (SIV) Host and Cellular Interactions,” studies suggest that the entire core accesses the nucleus. This would seem unremarkable but for the fact that the ability of lentiviruses to transduce nondividing cells requires that they translocate the core across an intact nuclear envelope. HIV-1 waits until the nuclear compartment has been accessed before uncoating and liberating viral nucleic acids. This appears to be a mechanism to allow evasion of nucleic acid-sensing mechanisms of the host cell that otherwise would trigger an antiviral interferon (IFN) response.

Similar obstacles are faced by coronaviruses as they invade the cell. Presentations in oral abstract session 1 discussed the complex interplay between SARS-CoV-2 and effectors of innate immunity (Abstracts 18-26). Previous studies demonstrated that rare inborn errors in TLR3- and IRF7-dependent type I IFN increase the risk of severe COVID-19 pneumonia and individuals with autoantibodies to type I INF were at increased risk of severe COVID-19 pneumonia. Rapamycin analogues increased cellular susceptibility to SARS-CoV-2 infection by facilitating spike-mediated virus entry (Abstract 22). The extent to which rapamycin analogues enhanced virus infection was correlated with their ability to promote lysosomal degradation of the IFN-induced transmembrane proteins (IFITM)2 and IFITM3 that have previously been shown to inhibit entry of a large number of enveloped viruses. Investigators expanded on recently published work that IFITM3 knockdown actually impaired cell infection by SARS-CoV-2 (Abstract 23). Knockdown of IFITM2 but not IFITM1 significantly reduced entry and replication of variants of concern including B.1.1.7, (Alpha) B.1.351 (Beta), P.1 (Gamma), and B.1.617.2 (Delta). Furthermore, viral infection was inhibited by an antibody to the N-terminus of IFITM2. Abstract 23 discussed data to suggest that innate immunity is a driver of SARS-CoV-2 evolution. The authors tested the sensitivity of early lineages A, B, B.1, and variants of concern lineages B.1.1.7, B.1.351, P1, and variants of concern (B.1.1.7 etc) to various IFNs. IFN sensitivity of the volatile organic compounds (VOCs) decreased relative to that of the ancestral B lineage and Alpha and Delta variants were also more resistant to B.1. This suggests that the evolution of SARS-CoV-2 is accompanied by increasing IFN resistance. It remains to be determined whether the reduction in IFN is a contributor to SARS-CoV-2 evolution or whether increased resistance to IFN is a consequence of SARS-CoV-2 evolution. Data were presented to suggest that antagonizing type I IFN inhibits SARS-CoV-2 replication and inflammation (Abstract 25). Macaques were treated with an IFN-I antagonist prior to SARS-CoV-2 infection which led to significant reductions in SARS-CoV-2 viral loads in upper and lower airways as well as reductions in soluble markers of inflammation. There were also decreased levels of IFN-stimulated genes post-infection in macaques treated with an IFN-I antagonist. Therefore, IFN-I appears to play an important role in the progression of COVID-19. As such, this finding could open up new avenues for development of therapeutic approaches to ameliorate COVID-19.

Abstract 26 presented findings that point to a molecular mechanism underscoring clotting disorders in patients with COVID-19. Abnormal clotting occurs in individuals with severe COVID-19 and also in those who are asymptomatic, and the fibrin clots observed in SARS-CoV-2 infection are difficult to manage due to their resistance to fibrinolysis by plasmin. The authors reported that the spike protein of SARS-CoV-2 bound to fibrinogen and fibrin, which accelerated fibrin polymerization. The authors also injected mice with HIV-1 virions that were pseudotyped with spike and observed thrombo-inflammatory responses including fibrin deposition in the lung, endothelial activation, and macrophage influx. The proinflammatory effects of spike could be blocked with an antifibrin monoclonal antibody. Collectively, this suggests that clotting is a driver of inflammation during SARS-CoV-2 infection as opposed to inflammation being a driver of clotting.
Viral Pathogenesis, Reservoirs, and Cure

An understanding of the reservoirs that sustain HIV-1 persistence in the face of effective antiretroviral therapy (ART) is important for the design of strategies to promote the elimination of those reservoirs. There has been a lot of interest in studying how the viral reservoirs are shaped, perhaps by host immune responses under long-term ART. There is good evidence that persistence of replication-competent HIV-1 during long-term ART is caused by a combination of at least 2 mechanisms. The first is maintenance of transcriptionally silent but intact proviral genomes within long lived CD4+ T cells. These latent proviruses are established early in infection and are believed to confer life-long persistence of the virus. The second is homeostatic proliferation of latently infected cells leading to a stable reservoir through self-regeneration. Clonally expanded proviruses are capable of generating infectious virions in vivo and viruses from clonally expanded proviruses populate rebounding viremia following analytic treatment interruption. There is also the possibility that proviral expression within anatomic reservoirs could be driven by innate immune (inflammatory) responses (eg, microbial translocation, concurrent infections, and antigenic stimulation).

Abstract 68 longitudinally examined the proviral landscape in ART-treated volunteers for approximately 20 years and in individuals on shorter durations of ART (median, 9 years). The frequency of intact relative to defective proviruses was assessed. After long-term ART, there was no significant difference in the frequencies of total or intact proviruses compared with individuals on short-term ART. However, the proportion of clonally expanded intact proviruses was higher in individuals on long-term ART. There was also an increased proportion of intact proviruses in nongenic DNA in those on long-term ART relative to those on shorter ART durations. There were no differences in chromosomal integration site locations for defective proviruses between the 2 ART groups. This suggests that under long-term ART, there is an increased proportion of intact proviruses in nongenic regions, suggesting that immune responses might promote selective elimination of proviruses that, due to their location in genic or heterochromatic regions, are more transcriptionally active and more susceptible to antiviral clearance mechanisms. Prior studies from this group on elite controllers (ECs), also suggested that there is a selection for proviruses in non-genic regions over time. In the majority of ECs, proviruses were found to be intact and replication competent, but were concentrated in non-genic or 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. Those proviruses also bore epigenetic modifications that limited their transcriptional capacity. Collectively, these studies suggest that there is greater selective pressure on proviruses in heterochromatic regions of the chromosome that eventually shapes the proviral population to become inert or in a state of latency that rarely reactivates (ie, deep latency).

The relationship between drivers of cell proliferation and proviral activity was presented in Abstract 69. Integration of viral DNA within chromosomal DNA of the host cell is essential in the replication cycle of retroviruses. These integrated viral sequences then adopt the dynamics and longevity of the cell they occupy. Therefore, when the host cell undergoes mitosis, the chromosomal DNA, as well as the integrated provirus, is duplicated. If the provirus is located close to a host gene that can influence cell cycle and cell proliferation, promoter elements within the provirus can impact the activity of the host gene and promote the division rate of the host cell. This represents a mechanism by which the proviral population can be maintained and expanded. Clonal expansion of proviruses in this way can result in marked over-representation of proviruses. Furthermore, proviruses in more rapidly dividing host cells might also be more transcriptionally active. Abstract 69 examined HIV expression (cell-associated viral RNA) and T-cell clonal expansion in cytomegalovirus (CMV) and HIV-1 antigen-specific CD4+ T cells from infected subjects. The authors determined that cells harboring HIV-1 RNA were larger in clone size
and had a higher proportion of cytotoxic CD4+ T cells. This indicates that drivers of T-cell proliferation promote viral persistence. Because the study employed single cell profiling, the biologic competence of the proviruses could not be assessed so viral RNA served as a surrogate for the reservoir. Although the level to which cell-associated viral RNA can inform on the dynamics of the biologically competent viral reservoirs is unknown, there are several reports demonstrating that the level of cell-associated RNA in CD4+ T cells in ART-suppressed individual predicts the rapidity with which HIV-1 will rebound when those individuals interrupt ART. By extension, therefore, antigens driving clonal T-cell proliferation also drive reservoir persistence.

The majority of reservoir studies have been conducted with circulating CD4+ T cells. However, the CD4+ T-cell reservoirs within various anatomic compartments harbor the largest proportion of the viral reservoir. Abstract 67 examined viral reservoirs in several anatomic locations in samples collected immediately postmortem from individuals who donated their bodies for HIV cure research. Tissue was obtained within 6 hours of death from 2 individuals who were on long-term ART. The viral reservoirs in anatomic compartments (including lymph nodes, gut, liver, spleen, brain, and testes) were characterized for near-full-length viral DNA and cell-associated viral RNA. Highest levels of viral DNA and RNA were found in the lymph nodes, liver, lungs, and spleen. Clonally expanded proviruses were found in several tissues. This indicates that cells harboring clonally expanded proviruses recirculate amongst anatomic sites during long-term ART.

Almost all of the attention on reservoirs of HIV-1 persistence has been focused on CD4+ T-cell reservoirs. However, the CD4+ T-cell reservoirs within various anatomic compartments harbor the largest proportion of the viral reservoir. Abstract 66 examined viral reservoirs in several anatomic locations in samples collected immediately postmortem from individuals who donated their bodies for HIV cure research. Tissue was obtained within 6 hours of death from 2 individuals who were on long-term ART. The viral reservoirs in anatomic compartments (including lymph nodes, gut, liver, spleen, brain, and testes) were characterized for near-full-length viral DNA and cell-associated viral RNA. Highest levels of viral DNA and RNA were found in the lymph nodes, liver, lungs, and spleen. Clonally expanded proviruses were found in several tissues. This indicates that cells harboring clonally expanded proviruses recirculate amongst anatomic sites during long-term ART.

HIV-associated neurocognitive disorder (HAND) is a well-recognized manifestation of HIV-1 infection, especially in untreated infection. It has been suspected that release of neuronotoxic agents from HIV-1–infected microglia are responsible for the neuronal death observed in HIV-1 infection. However, identification of the mechanisms involved as well as the identity of the neuronotoxic agents themselves has been challenging, mainly due to challenges with in vivo analysis and limitations of animal models. Abstract 18 featured results using a brain organoid microglial model to identify processes underscoring HAND. The authors resorted to induced pluripotent stem cells (iPSCs) to derive cerebral and choroid plexus organoids. In both models, microglia were the most predominantly infected cell type following HIV-1 infection. Infection of microglia led to upregulation of the inflammatory chemokines C-C motif chemokine ligand 2 (CCL2) and C-X-C motif
chemokine ligand 10 (CXCL10) that promote migration of T cells and monocytes across the blood brain barrier. ART inhibited HIV-1 replication within iPSC-derived microglial organoids; it did not block chemokine production. Infected microglia also exhibited inflammatory responses involving several members of the S100 family of genes that have been implicated in several neurologic disorders including HAND. These studies allow detailed interrogation of the processes that drive HAND in HIV-1 infection.

Although there is intense interest in revealing the reservoirs that sustain HIV-1 persistence in the face of ART suppression, there is an equally sustained effort to identify strategies that promote elimination of those reservoirs. Most of the strategies being explored center around approaches that enhance removal of infected cells by the immune surveillance mechanisms of the host. Because most of the viral reservoir is likely to be latent and invisible to the immune system, researchers are exploring numerous ways to reactivate the latent provirus so that infected cells can be recognized by host antiviral clearance forces of the host. Abstract 343 presented a reservoir clearance approach that is distinct to most reservoir clearance strategies currently being pursued. The viral reverse transcriptase and protease proteins are initially contained with a GagPol polyprotein. During virus budding from the plasma membrane of the host cell, the protease self-cleaves the polyprotein to liberate itself and other Gag and Pol subunits from the polyprotein. This sequence of events likely prevents premature activation of the protease and within the infected cell where it might catalytically cleave cytosolic proteins. This would likely impact the health of the infected cell and reduce its capacity to generate progeny virions. Protease inhibitors such as indinavir interrupt this process leading to the production of immature, noninfectious virions. If an nonnucleoside analogue reverse transcriptase inhibitor (NNRTI) binds to a newly translated GagPol polyprotein, this results in homodimerization of the polyprotein and premature protease activation. Previous studies have demonstrated that this then leads to nonspecific cleavage of host cell proteins that ultimately result in apoptosis of the infected cell. Abstract 343 presented efforts to identify small molecules that target the NNRTI binding site of HIV-1 reverse transcriptase to promote Gag-Pol dimerization and premature protease activation. A total of 6628 compounds from a library of NNRTI-related analogues that target the NNRTI binding site were assessed for their ability to selectively kill HIV-1–infected cells. A small percentage of these compounds (1.7%) were cytotoxic. These compounds were optimized for specific cytotoxicity on infected cells. One of these compounds (Pyr01) had 1000-fold increased killing relative to analogue Pyr02 that had similar antiviral activity. Pyr01 represents a novel bifunctional NNRTI that serves as a reverse transcriptase inhibitor and that has the ability to selectively kill HIV-1 expressing reservoir cells through premature activation of the viral protease. This line of investigation opens up exciting new approaches with which to promote elimination of viral reservoirs in infected individuals.

All abstracts cited in the text appear in the CROI 2022 Abstract eBook, available online at www.CROIconference.org

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Additional References Cited in Text


