

Rising to the challenge of COVID-19: Working on SARS-CoV-2 during the pandemic

COVID-19 altered our lives and pushed scientific research to operate at breakneck speed, leading to significant breakthroughs in record time. We asked experts in the field about the challenges they faced in transitioning, rapidly but safely, to working on the virus while navigating the shutdown. Their voices converge on the importance of teamwork, forging new collaborations, and working toward a shared goal.



Noam Stern-Ginossar
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Pivoting our work to SARS-CoV-2

Toward the end of February 2020, when we began to appreciate the gravity and seriousness of the pandemic and how it was unfolding, it felt odd to continue with our normal work on herpesviruses. We decided to try and contribute our share to the global research effort and it was clear where we could potentially have the biggest impact: mapping the coding capacity of SARS-CoV-2. Characterizing the protein-coding potential of any virus is a prerequisite for understanding viral-host interactions, virus antigenic potential, and pathogenesis. Several groups around the world had begun working toward this aim; however, given our expertise in ribosome profiling-based techniques to map translated regions in herpesviruses, I felt we could make a significant contribution. Luckily, our colleagues at the Israel Institute for Biological Research, which is practically next door to the Weizmann Institute, were already working with this novel virus and had established the protocols for cell culture work. So, after getting all the safety approvals, we decided to jump in.

The mode of work was extremely intense and very different from our usual lab work. There was an enormous amount of pressure to get everything done fast and we were working under a real sense of emergency. People set aside their own research and ambitions to become part of a bigger team effort. It was a very intense experience, stressful on the one hand but extremely fulfilling, as we knew our findings would be of broad general interest. More than a year has passed and these intense efforts allowed us to familiarize ourselves with a new virus, which beyond its immediate importance also brought us fascinating new research directions and exciting collaborations. We decided to continue with our SARS-CoV-2 research and are now investigating the molecular mechanisms pertaining to SARS-CoV-2's ability to suppress host protein synthesis and disarm host defenses.



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Working with purpose and urgency

Anxiety. Uncertainty. Lockdown. Early in 2020, these words became synonymous with everyday life. As COVID-19 spread worldwide and we watched our sense of normalcy slip away, it seemed we were doomed to face the same fate in our lab. But our institutional leadership team confronted the looming crisis head-on and implemented robust public health safeguards, including an on-site nasal swab and PCR testing operation. Thankfully, this allowed us to keep our staff safe and avoid the protracted shutdowns seen at many institutions.

In our lab specifically, the initial shutdown did mean a sharp reduction in the number of people coming to campus for a few weeks. But the level of teamwork I saw in my group was amazing. Everyone came together to move projects forward, even projects that weren't theirs, and we were able to avoid losing the momentum in our science. Additionally, my travel commitments disappeared, allowing me to devote all my time and energy to thinking and planning for our current projects.

Regarding our SARS-CoV-2 projects, I was approached by several lab members about working on the virus early in 2020, but I initially told them no, as historically we had not worked with BSL3 pathogens, so we began our investigations of the innate immune response to coronaviruses by evaluating inflammasome activation and cell

death using the related virus MHV (Zheng et al., 2020). However, as time went by and we saw the way patients were dying in the ICU with severe systemic inflammation, we took it personally, and it led us to think of all the fundamental work we have done on innate immunity, inflammasomes, inflammatory disease, and sepsis. It became clear that we must repurpose our knowledge and join the COVID-19 research efforts. We were able to quickly adapt our experimental models, establish new collaborations, and strengthen existing ones to study the inflammation we were seeing in patients and make key discoveries about the molecular mechanism of the cytokine storm occurring. We found that TNF- α and IFN- γ drive inflammatory cell death, PANoptosis, forming the mechanistic basis of cytokine storm; blocking TNF- α and IFN- γ with clinically available monoclonal antibodies prevented mortality in a murine model of SARS-CoV-2 infection (Karki et al., 2021). Because innate immunity acts as the front line of defense against pathogens and can drive cytokine release, we next sought to identify the innate sensors upstream of the cytokine storm. We performed molecular and genetic screens and found that TLR2 is critical for sensing the envelope protein of SARS-CoV-2 and initiating inflammatory cytokine production (Zheng et al., 2021). However, the cytosolic sensors involved are still unknown, marking a critical avenue for future work.

While the pandemic has interrupted productivity around the globe, my team's efforts to work with purpose and urgency, combined with our dedicated institutional leadership at St. Jude, has meant a rapid acceleration in our progress to answer fundamental questions about the innate immune response to this newly discovered pathogen that has changed our lives forever. The COVID-19 crisis is an example of the rising challenges we face in infectious disease, and innovative basic science research is critical to be better prepared for infectious threats in the future.



Craig E. Cameron
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From poliovirus to SARS-CoV-2

The primary model system used in my laboratory is poliovirus. I always thought that if ever I were forced to discontinue my research, it would be the result of global eradication of poliovirus, not a pandemic. What made the prospect of shutting down my research program so frightening was that my program was only becoming functional again. My lab and I relocated from The Pennsylvania State University to University of North Carolina at Chapel Hill School of Medicine (UNC) in September 2019. To make matters even more terrifying, I was now the chair of the department and responsible not only for my own shutdown, but oversight of the shutdown of the labs of my colleagues, whom I barely knew. Looking back now, it was not that bad. My compatriots in leadership and the faculty in my department were all heroes and made the process as painless and efficient as possible.

Without the fear of an imploding department, I was able to work with my team to develop a strategy to expand the umbrella of our research interests to include coronavirus genome replication. UNC boasts a long history of major contributions to “coronavirology,” in large part a reflection of the leadership of Professor Ralph Baric. I have had a long-standing interest in the viral RNA-dependent RNA polymerase as a target for antiviral therapeutics with broad-spectrum activity. Nucleotide analogs, which promote misincorporation or termination of RNA synthesis, are well-precedented antivirals. Coronaviruses can evade most prototypical, nucleotide polymerase inhibitors. This gain of function for coronaviruses reflects the acquisition of a proofreading exonuclease, which is capable of correcting mistakes made by the polymerase, including incorporation of termination-promoting nucleotide analogs.

Over the past year and in collaboration with a “SARS2 Collaborative,” we have worked earnestly to dissect the mechanism and specificity of the proofreading exonuclease. Preventing the ability of this enzyme to make corrections to the viral genome has the potential to make coronaviruses susceptible to the myriad nucleotide analogs that have been described as antiviral agents.



Zhiyong Lou
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Understanding SARS-CoV-2 life in cells

SARS-CoV-2 encodes non-structural proteins that assemble high-ordered replication-transcription complex (RTC). RTC plays a central role in the replication of genome-length viral RNAs, transcription of viral mRNAs, proofreading to maintain replication fidelity, and many other aspects in the virus life cycle. All these key steps are potential targets for antiviral development. Understanding the working mechanisms of SARS-CoV-2 RTCs can provide insight into the virus and form a basis for antiviral drug discovery.

I entered into the structural studies of coronavirus-encoded proteins after the outbreak of SARS-CoV in 2003, when I was a junior PhD student in the laboratory of Prof. Zihao Rao, who is one of the key scientists in the coronavirus research field. Benefiting from the persistence and experience in coronavirus research over the past 17 years, we gave rapid response to the study of SARS-CoV-2 RTCs in the early phases of the pandemic. To contribute to the global effort in urgent antiviral discovery, we quickly determined the atomic cryo-EM structure of the central RTC (C-RTC) composed by nsp12 (RNA-dependent RNA polymerase, RdRp) and cofactors nsp7/nsp8, presenting the molecular details of the key antiviral target, RdRp, and proposing a mechanism for Remdesivir recognition (Gao et al., 2020). After that, we dug deeper into the details of SARS-CoV-2 replication and transcription. We assembled the elongation RTC (E-RTC) by C-RTC with the helicase (nsp13) and determined its structure to elucidate how the helicase unwinds the high-ordered structure in the viral genome as a template for RNA synthesis. We also identified nsp12 NiRAN domain as the key enzyme for the second action of mRNA capping and presented an intermediate state of RTC towards mRNA capping [Cap(-1)'-RTC] formed by E-RTC with single-strand RNA binding protein nsp9 (Yan et al., 2021).

Though the replication and transcription of SARS-CoV-2 inside host cells is becoming clearer, some key questions still need answers. For example, the role of nsp14 and nsp16 in RTC to finalize mRNA capping is unknown. Moreover, because of the remarkably large size of its genome, SARS-CoV-2 employs a proofreading mechanism to maintain the replication fidelity, being exerted by nsp14. How nsp14 concert in both mRNA capping and replication proofreading is one of the top scientific enigmas in coronavirus research over the past 20 years. Furthermore, how RTC localizes in host cells with double-membrane vehicle and how mRNA is released from RTC to host ribosomes need further structural evidence. Notably, all these mechanisms are related to antiviral development. The structural studies on SARS-CoV-2 RTCs will provide more insight into how the virus lives inside host cells and help us discover new antiviral drugs.

Finally, I would like to highlight my greatest appreciation to all my collaborators, not only domestic but also international. Although traveling is restricted by the pandemic, the internet links us all. Collaboration and sharing helps us step forward together in the battle against SARS-CoV-2.



Sara Cherry
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Repurposing with a purpose

Viruses emerge and re-emerge, becoming more and more common due to societal and environmental changes. Surveillance efforts have impacted our ability to detect and characterize these pathogens. Last February, news of an emerging respiratory virus spread across the globe, and this coincided with what was to be my last trip before the lockdown, a fantastic immunology conference in Australia, where for the first time I saw widespread use of masks and heightened fear everywhere. Our underlying interests in emerging viruses and expertise in dissecting virus-host interactions using high-throughput screening approaches prompted us to employ our skills and knowledge to combat this new virus. Despite their own fears and confusion about the reality of a global pandemic and the new reality we all faced, my laboratory team mobilized quickly and rose to the challenge with dedication and grace. Importantly, university leadership also fully supported our endeavors to study SARS-CoV-2 and potentially identify new therapeutics, which was critical to facilitate our work. Refocusing required us to work with a new virus and develop new cell models than those with which we worked

previously. It also required us to perform large-scale drug screening in the BSL3, which is nontrivial. For the first time in years, I was screening drugs in the BSL3 daily myself and continue to perform many of the experiments in the BSL3 regularly. We have also expanded our studies to work with primary-based cellular models of the respiratory tract, which has been both difficult and incredibly rewarding as it is now clear that there are major differences in drug sensitivities in the respiratory tract compared to non-airway models. Our foray into coronaviruses and the respiratory tract has also led us to new collaborations, both within the US and across the globe, and bringing together researchers with diverse expertise has substantially increased the pace of discovery. I am proud of our accomplishments in the last year. We have developed a plethora of assays and models, leading us to screen thousands of drugs to repurpose existing therapeutics to treat COVID-19. We have made fundamental discoveries about SARS-CoV-2 interactions with host cells and have identified drugs that can block infection in model systems. I am hopeful that some of our findings, and those of others, will translate to the clinic and will result in new antiviral therapeutics to treat or prevent COVID-19.



Jonathan Abraham
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Collaboration lights the way

Our laboratory studies the dynamic interplay between virus cellular receptor binding and pathways for antibody neutralization escape. This interplay can drive viral receptor-binding domain sequence diversity, with implications to zoonotic spillover. Given our laboratory's interests, we naturally transitioned to working on SARS-CoV-2. As an infectious diseases doctor in the hospital wards in the spring of 2020, I also saw the virus push our intensive care units past their capacity and our healthcare system to the brink. This experience intensified my desire to contribute scientifically. Our group joined the newly established Massachusetts Consortium on Pathogen Readiness ([MassCPR](#)), which funded our COVID-19 research and helped us build a network of collaborators. The openness of various groups across the world to sharing critical reagents, including model cell lines, plasmids, recombinant proteins, and most importantly, the rapid sharing of knowledge through pre-print servers, has helped research on SARS-CoV-2 move with unprecedented swiftness. Critical unanswered questions in the field are: (1) does one need detectable antibody titers before SARS-CoV-2 infection or are recall T and B cell responses robust enough to be protective? (2) What is the therapeutic window for direct-acting antivirals? (3) What immunological pathways are regulating pathophysiology in advanced disease? Even partial answers to some of these questions would leave us better prepared to help prevent the next pandemic.



Laura Martin-Sancho
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Racing to find a cure

I remember learning about viral pandemics in university and thinking about the challenges of working with novel viruses. Of course, we virologists contemplate the idea of a global viral pandemic and we discuss this at length in grants and in research articles, but how do you respond when confronted by it? It all started in February 2020 as I was completing my postdoc studying respiratory viruses. The lab had been closely following the worrying news coming from China and decided to drop everything else and work full time on SARS-CoV-2. Once we received the virus in March, it was a race against the clock to get the right conditions, the right cells, and the right reagents to propagate the virus to high enough amounts to start testing small compounds for antiviral activity. With a starting material of barely 100 μ l received from BEI Resources, and long lonely hours in the BSL3, I felt a massive relief when I finally saw that the virus was replicating. Soon after, we had optimized experimental conditions and high enough viral yields to begin the essential experiments.

It was a remarkable feeling to have the whole research institute to just the seven of us. It was just me and three other lab members, our PI, Sumit Chanda, and two members of the institute safety department. With nobody around, it felt like we were apocalyptic survivors racing to find a cure. With a non-stop schedule from 8 am to 10 pm in the lab and trying to play catch up with an everyday-evolving literature, we were barely getting any sleep but never felt so energized. I felt so supported and inspired by my family and friends back home in Spain, which was one of the initial pandemic epicenters

in Europe. I felt it was our responsibility to keep going and to make discoveries that could have a meaningful contribution. And so we did. In only a few months, we evaluated thousands of small compounds (initially in collaboration with Hong Kong university, then in house), we identified the innate immune sensor for SARS-CoV-2, and we illuminated the cellular antiviral landscape to SARS-CoV-2. Of course, this wouldn't have been possible without the expertise and assistance from our countless collaborators across the globe. Indeed, a story to tell my grandkids.