SPOT LIGHT

Taking on pathogens, from HIV to SARS-CoV-2

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The critical role CyTOF plays in helping Nadia Roan understand viral persistence and clearance mechanisms

Although treatments for HIV infections keep the disease at bay, neither an effective vaccine nor a cure for HIV currently exists. With a long-standing interest in studying the interactions between immune cells and microbial pathogens, Nadia Roan, PhD, is working to better understand how HIV establishes initial infection and how it can persist in the face of antiretroviral therapy.

Understanding host-pathogen interactions centers on immune responses. In the case of HIV, key cells that orchestrate immune responses are the same ones that are attacked by the virus. These cells, CD4+ T cells, are the main targets of HIV infection. Which of the many different CD4+ T cells are the most susceptible to infection, and the first to become infected? How does HIV manipulate, modify or remodel infected CD4+ T cells to quickly spread, and survive in the form of a long-lived reservoir in the presence of antiretroviral drugs? Answers to these questions not only could help prevent the spread of HIV but could also further our understanding of the role CD4+ T cells play in immune homeostasis.

Roan, an Associate Professor at the University of California, San Francisco, and a scientist at Gladstone Institutes, focuses her research on the mechanisms of HIV transmission and the possibilities of a cure. The research lab headed by Roan uses various *ex vivo* models of HIV infection. By isolating immune cells from blood and various tissues of healthy individuals and exposing the cells to a reporter HIV virus, the lab can characterize cell types, activation status, infection susceptibility and more.

A recent paper from Roan's lab published in *eLife* depicts how a newly infected cell changes its regulation and signaling following HIV infection. Using an *ex vivo* genital infection model and CyTOF® to model HIV transmission through the female reproductive tract, the group found down-regulation of receptors that could disrupt proper signaling and up-regulation of survival factors to help infected cells live long enough to spread the virus to other cells. They further used bioinformatics approaches to predict the original states of infected cells before viral-induced remodeling to define the CD4+ T cells most susceptible to HIV.

Supported by CyTOF technology

In much of her early work Roan used flow cytometry to study immune cell features and responses, but she recently shifted to mass cytometry to more easily characterize and directly compare immune cell subsets. "One common use of mass cytometry is to phenotype all the major immune subsets in a single sample," Roan explains, "without the need to split the sample to be stained by multiple smaller phenotyping panels. And while we do use mass cytometry for that, more often we take advantage of the technology to really dive deeply into one particular cellular subset, such as CD4+ T cells."

Along these lines, the lab has designed multiple T and NK cell panels, as well as panels focused on characterizing expression levels of viral intracellular sensors and restriction factors. One use of a T cell panel designed by the Roan Lab was to specifically study HIV latency during antiretroviral therapy, in particular to characterize the phenotypes of the *in vivo* HIV reservoir. By understanding the phenotypes of cells latently infected with HIV and the mechanisms that allow the persistence of these cells, Roan hopes that novel approaches can be designed to target these cells as a strategy to cure HIV.

"The high-dimensional nature of CyTOF datasets allows various pseudotime analytical approaches, which can be used to predict cell states prior to cellular changes, including that caused by viral reactivation."

HIV persists in infected individuals on antiretroviral therapy due to a small reservoir of long-lived CD4+ T cells, and potentially other immune cells, containing quiescent HIV. Even though HIV researchers have known for quite some time about the existence of this reservoir, no biomarkers that can specifically detect this cell population have been identified. Combining CyTOF based phenotyping of infected cells and predicted precursor cells as determined by single-cell linkage using distance estimation (PP-SLIDE), Roan could trace back the original states of latently infected cells. This was accomplished by establishing an atlas of different CD4+ cell types and then using bioinformatics approaches to identify the original phenotypes of the latent cells induced to come out of latency by reactivation. This approach of charting the *in vivo* latent reservoir was recently accepted for publication in *eLife*.

"The high-dimensional nature of CyTOF datasets allows various pseudotime analytical approaches, which can be used to predict cell states prior to cellular changes, including that caused by viral reactivation," Roan says. "Another advantage of CyTOF technology is the ability to work with fixed cells. This becomes very important in our research, where we collect longitudinal human specimens and then want to analyze them all at once to limit batch effects."

Applying host-pathogen knowledge to SARS-CoV-2

Since Roan and her lab members already had in-depth knowledge about T cell behavior and response to viral infection, early reports of T cell depletion in severe COVID-19 cases intrigued them. "We already know T cells to be very important for viral infections, and in the case of severe COVID-19, these were the ones that were markedly depleted."

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Given the development of a wide variety of T cell-specific panels from their HIV research, the group was already primed to adapt to studying the functional features of T cells specific to SARS-CoV-2. In addition, the team modified an intracellular cytokine stimulation assay they built for identifying cytomegalovirus-specific and HIV-specific T cells, whereby cells responding to peptide pools can be identified and deep-phenotyped using CyTOF technology.

Collaborating with UCSF colleague Dr. Sulggi Lee, MD, PhD, who established a patient cohort, COVID-19 Host Immune Response Tracking Genesis Report (CHIRP), Roan has been able to examine the SARS-CoV-2-specific immune response that occurs in individuals who recover from mild or asymptomatic COVID-19. A better understanding of mild and asymptomatic cases opens the possibility of vaccines or treatments that promote less severe symptoms after contracting SARS-CoV-2.

In a paper recently published in *Cell Reports Medicine*, Roan and colleagues performed longitudinal analyses on a group of convalesced individuals who had recovered from mild SARS-CoV-2 infection and were never hospitalized. They used CyTOF technology and developed a tailored T cell panel to characterize SARS-CoV-2-specific CD4+ and CD8+ T cells and to measure their longevity. The team tested patients up to 69 days post-infection, when they were still able to see a clear population of SARS-CoV-2-specific T cells. Those cells were capable of expanding in response to IL-7, suggesting their ability to homeostatically proliferate.

Ongoing studies by the Roan Lab will compare these mild and asymptomatic cases to more severe cases of hospitalized individuals to define effective vs. ineffective or pathological immune responses against the virus. Longitudinal analyses of these patient cohorts will be an important aspect of the studies to better understand what responses associate with full and rapid recovery. Roan can take advantage of CyTOF enabled platforms to generate these data and directly correlate responses across individuals showing various degrees of symptoms.

With the pandemic still ongoing, the hope is that SARS-CoV-2 will ultimately be easier to manage than something like HIV. “Traditional approaches that are being implemented for SARS-CoV-2 should work in some capacity, even if it’s not lifelong immunity. And the more we understand what we’re dealing with using CyTOF technology and other tools to get as much information as possible, the closer we can get to improved disease management and control,” adds Roan.

Roan hopes that novel approaches can be designed to target these cells as a strategy to cure HIV.

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