SPOT LIGHT

COVID-19, cardiovascular disease and mass cytometry

HEMA KOTHARI, PHD

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A lesson in how CyTOF technology can empower flexibility in research

Junior Investigator Hema Kothari, PhD, is one of many researchers who have been able to pivot their research to study mechanisms that influence the risk of severe COVID-19 progression. Kothari is a Research Assistant Professor in Dr. Coleen McNamara's lab at the University of Virginia (UVA) in Charlottesville and focuses on understanding the role of immune cell subsets in cardiovascular disease and other chronic inflammatory diseases.

In an effort to develop approaches that better enable personalized treatments for immunomodulatory and anti-inflammatory therapies, the lab created a mass cytometry-based pipeline for in-depth analysis of human peripheral blood mononuclear cells (PBMC) to identify specific immune phenotypes that associate with disease severity and response to therapy. Work is ongoing using these customized panels and study populations including subjects with rheumatoid arthritis, coronary artery disease and other inflammatory illnesses.

Recently, IL-1β, a proinflammatory cytokine investigated by Kothari, has emerged as a key mediator of the cytokine storm linked to high morbidity and mortality from COVID-19. In addition, blocking the IL-1 receptor with anakrina has entered clinical trials in COVID-19 patients.

Upon the realization that her current study could easily translate to and possibly accelerate COVID-19 research, Kothari refocused her efforts on the specific immune cell subsets targeted by IL-1β and IL-1β-induced signaling pathways in humans.

Cardiovascular problems are a known issue with cytokine storm onset in COVID-19 patients and correlate with disease severity and mortality. Applying her knowledge from cardiovascular research to SARS-CoV-2 activated inflammation, Kothari has initiated a new study that is funded by the UVA Manning Fund for COVID-19 Research. The study's goals are to phenotype PBMC samples collected from COVID-19 patients at UVA by mass cytometry to identify circulating immune cell subtypes that are most responsive to IL-1β stimulation, immune phenotypes that correlate with disease severity, and to develop a customized diagnostic biomarker assay for early identification of those at risk of a cytokine storm for improved patient outcomes.

With high interindividual heterogeneity in response, when some patients get better and some progress to critical stages of the disease, the ability to look at broad intracellular signaling supports a greater understanding of what is occurring upstream of cytokine production. This will allow earlier identification and possible prediction of which patients are at a higher risk of cytokine storm and which are most likely to benefit from anakrina therapy.

Uniquely CyTOF

Kothari works with CyTOF® technology for the majority of her projects because it offers comprehensive immune cell analysis, the ability to more deeply profile immune cell subtypes and the flexibility to adjust and expand panels easily. With flow cytometry or other conventional cell analysis methods, it can be challenging to expand a panel to more than 20 markers. That ceiling tends to be just enough to identify different immune cell types in the PBMC sample but does not allow the opportunity to ask more in-depth questions about what these cells are doing.

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"CyTOF is really helpful because you can identify all cell subsets as well as the specific proteins that you are interested in while avoiding challenges with fluorophore spectrum overlap," explains Kothari. "For example, we were able to use a 37-marker panel in the COVID-19 study. I am interested in looking for cell surface receptors such as IL-1 and IL-6 receptors, intracellular signaling proteins activated downstream of IL-1β and IL-6, intracellular cytokines and other markers across different immune cell types. The power of CyTOF is that you can have a lot of different markers in a panel and analyze them all at once."

Furthermore, CyTOF allows more flexibility in experimental approach as the metal-tagged antibodies used are compatible with harsh cell fixation and permeabilization treatments. The compatibility of CyTOF with methanol treatment is key for performing analyses such as phosphoflow in the high-parameter space, mentions Kothari. Many fluorophores used in high-parameter fluorescence cytometry can be destroyed when cells are treated with methanol.

Further exploration takes many directions

With their CyTOF based approach already in place, the team can easily look for IL-1β-mediated signaling pathways across different immune cell subtypes and identify immune cell targets for IL-1β in humans, for more general cardiovascular implications as well as for COVID-19 specifically. One of the key findings uniquely enabled by CyTOF and advanced computational analysis was the discovery that CCR6-positive T cells are a major target of IL-1β in humans. Next steps could include looking for downstream effects of this interaction and whether it plays a role in IL-1β-mediated inflammatory mechanisms in cardiovascular diseases or in other inflammatory diseases. Kothari would also

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like to take these findings back into murine systems and investigate the impact of CCR6-positive T cells on atherosclerosis.

Kothari is able to expand her investigations in many directions using the lab's current approach involving custom panel development and CyTOF technology to identify mechanisms, predict disease outcomes and characterize therapeutic response.

One therapy does not fit all. With this in mind, Kothari ultimately would like to apply her findings to developing precision and personalized approaches for the treatment of cardiovascular disease.

Learn more about Fluidigm's response to COVID-19 using mass cytometry: **fluidigm.com/singlearticles/covid-19**

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