SPOT LIGHT

CyTOF reveals immune disruption

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CyTOF reveals immune disruption: discovering a hallmark phenotype of COVID-19

As a specialist in the management of systemic autoimmune diseases, Benjamin Terrier, MD, PhD, regularly works with patient samples to learn how the activation state and proportions of immune cell subsets change with disease severity.

Dr. Terrier is Professor of Medicine in the Internal Medicine Department of Cochin Hospital in Paris. Given his in-depth understanding of how the immune system can react to various diseases, confirmation of the novel coronavirus in France prompted a shift in his focus toward the management of COVID-19 infections. Terrier and his team observed patients with progressive worsening of the disease from cytokine storm and questioned the mechanism involved in disease severity. They initiated an investigation of the immunological characteristics associated with disease deterioration consistently observed between 8 and 12 days from the onset of symptoms.

Because of the urgent need for more information about COVID-19 disease progression, the study covered a cross-sectional analysis of patients admitted due to disease worsening around day 10. And while comorbidities and potential autoimmune predispositions are known to correlate to worsening disease, the team specifically examined patients with no or very mild or controlled comorbidities so they could focus on the modifications provoked by the virus itself. A unique phenotype was identified, displaying impaired interferon type I response characterized by low interferon production and activity in severe and critically ill patients. The phenotype was also associated with a persistent blood virus load and an exacerbated inflammatory response.

Terrier collaborates with the Pitié-Salpêtrière Cytometry (CyPS) to apply mass cytometry and the Maxpar® Direct™ Immune Profiling Assay™ in his current studies, and now to COVID-19 immune profiling. “Analyzing the immune response of patients with mild-to-moderate disease, severe disease and critical disease by mass cytometry allows us to cover the innate and adaptive immune responses simultaneously. Not only could we analyze immune cells, but also cytokine production and the transcriptional signatures in these patients. Essentially, we could look at everything in the whole blood,” Terrier explains.

And while sampling whole blood might not be the perfect way to analyze a disease that tends to be localized in the lung, it is clearly a much less invasive approach to collecting samples from patients. Between ongoing collaborations with the CyPS facility and with groups at both the Imagine and Pasteur Institutes working on interferon type I signaling, Terrier was well-positioned for a quick start with an integrated approach.

Easily transitioning CyTOF to COVID-19

"We used the Maxpar Direct Immune Profiling Assay because we wanted a global approach to analyze the different immune cell types from both the innate and adaptive immune response. Most previous works on immune perturbations in autoimmune disease only analyzed the B cell compartment, the T cell compartment or the regulatory T cells. A more comprehensive view is complicated using conventional flow cytometry, involving many different tubes to analyze one sample from a single patient," says Terrier. "So, we wanted to use a more global approach facilitated by mass cytometry."

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Because Terrier had been using mass cytometry for the analysis of perturbations in different autoimmune diseases and associations with disease outcome and response to therapy, he was able to quickly adapt his CyTOF® mass cytometry assays to COVID-19 studies. He recognized that this type of global analysis would align well with similar disruptions of the innate response and apparent abnormalities of the adaptive response observed in COVID-19.

The study included use of the Maxpar Direct Immune Profiling Assay with the addition of PD-1 and Tim-3 immune checkpoint inhibitors to the assay's standard backbone 30-marker panel. The panel was used as is, allowing the team to work quickly to generate quality data, analyze the results and submit a preprint publication within one month. With the success of this panel, the team plans to add at least 12 markers to develop a new panel for further characterization of the cells, including the definition, differentiation and activation status of the immune cell populations.

"Also, a very important advantage to us was the ability to automate our analysis with Maxpar Pathsetter™ software. This is a significant time savings and generates data that is much more reproducible and less impacted by the subjectivity that occurs with manual analysis," he adds.

Expanding current studies with new panels

Terrier notes that further longitudinal follow-up of patients is planned, and additional comorbidity studies are already underway. He and his team are interested in how different comorbidities might affect the observed defect in interferon production across patients with similar severity levels of COVID-19. In addition, the group would like to examine how each cell population assessed differs across disease severity,

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and to better understand the immune system perturbation that was observed.

Terrier points out that getting this global perspective of immune response using mass cytometry worked so well that he plans to expand its use in his current studies on autoimmune disease, adding in new markers to increase immune cell characterization and determine differences between various autoimmune diseases. By generating a baseline signature, it may be possible to identify specific changes occurring in response to therapy and determine predictive characteristics for this response, potentially offering a path to better disease management.

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1. Hadjadj, J. et al. “Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients.” *Science* (2020): 718–724.

Learn more about how the Maxpar Direct Immune Profiling Assay is supporting the response to COVID-19:

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