SPOT LIGHT

Uncovering immunological mechanisms of protection from infection and vaccination in humans

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Vaccine development is a complex undertaking, involving several stages from discovery, exploration and preclinical studies to phased clinical trials. As a leading researcher who has worked for over three decades in this field, Marcelo Sztein, MD, at the University of Maryland is always looking for ways to improve and accelerate the development of novel, highly effective vaccines.

To start, Dr. Sztein uses mass cytometry as an exploratory tool to uncover immunological mechanisms of protection from infection and vaccination in humans. Mass cytometry can also be used as candidates move through the preclinical stage, helping to identify immunological responses observed in various animal models.

Sztein sees the biggest impact of mass cytometry during the performance of Phase 1 and Phase 2 clinical studies in humans, as a key tool to identify correlates of protection during these early stages of human vaccine trials. During these studies, vaccine performance in humans can only be ascertained by looking at the human immune response. To help address limitations in the number of specimens available—an obstacle inherent to human experimentation—mass cytometry enables the measurement of a high number of parameters that allow assessment of complex immune responses and, hopefully, identify immunological correlates of protection. Similarly, mass cytometry can play a critical role during studies involving the challenge of volunteers with wild-type organisms under closely monitored conditions. Again, mass cytometry might contribute to the identification of immunological responses that correlate with protection from disease.

Sztein is Associate Director for Basic and Translational Research at the Center for Vaccine Development and Global Health and Professor of Pediatrics, Medicine and Microbiology and Immunology at the University of Maryland. One area of work his group focuses on is Salmonella Typhi, the causative agent of typhoid fever. This is an infectious disease of great public health importance, causing 17 million cases and about 200,000 deaths per year worldwide.

Typhoid fever is caused by the Salmonella enterica serovar Typhi bacterium, which is spread through contaminated food and water. Since Salmonella Typhi is a human-restricted facultative intracellular pathogen, efforts to uncover correlates of protection to this pathogen must be performed in humans. Salmonella Typhi is known to elicit a diverse array of cellular immune responses. Yet, it is unknown which of these responses, if any, is relevant for protection against disease. This is a primary goal of vaccine development, and the number one question Sztein and his team are focused on.

The group successfully used mass cytometry in a three-pronged study to examine immune responses elicited during the development of disease and in response to a licensed oral live attenuated vaccine, as well as to evaluate epigenetic changes that could help characterize pathways associated with protection from infection for further vaccine improvement.

Why specific and age-related immune responses matter to vaccine efficacy

To build upon earlier work describing different cell-mediated immune responses (CMI) following exposure to Salmonella Typhi, Sztein and his team focused new efforts1 on T regulatory cells for the first time in volunteers, trying to understand whether they play a role in protection.

Regulatory T cells are an important arm of the immune response, responsible for keeping the

immunological effect of responses in check. Sztein and Monica McArthur, MD, PhD, were interested in identifying whether these cells are specifically activated during typhoid disease. In a collaboration with Andrew Pollard, PhD, and his team at the University of Oxford2, who had re-established a human challenge model for the infection, Sztein and his team were able to evaluate blood samples from challenged volunteers at multiple time points and analyze the samples with mass cytometry.

Notably, only 61% of the challenged volunteers developed typhoid disease. Access to both positive and negative cases presented the team with a unique opportunity to directly compare the

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different responses elicited in volunteers who developed typhoid disease to those who did not. CyTOF® analysis revealed a spike in circulating integrin-α4β7+ T regulatory cells that exhibit the potential to migrate to the gut in volunteers who developed typhoid fever.

"Mass cytometry allowed us to simultaneously study many parameters on these cells, particularly those related to activation and homing," Sztein says. T regulatory cell analysis in addition to functional studies of effector T cell responses on these cells revealed targeted behavior that indicates possible functional suppression of Salmonella Typhi specific responses.

Sztein and Mark Rudolph, PhD, followed up this study with an investigation3 of the differences in CMI between children and adults who are orally immunized with the licensed Ty21a oral attenuated typhoid vaccine to assess the impact of age on the immune responses to vaccination. Studies4 performed in Chile in the 1970s and 1980s showed that the efficacy of the Ty21a vaccine was lower in younger children: 59% in those 5 to 9 years old, 67% in those 10 to 14, and 85% in those 15 or older.

Sztein notes, "To this day, we have a very poor understanding of the human pediatric immune response. This is largely due to limited accessibility, not only of volunteers, but also to limitations in the availability of specimens and low cell numbers, which add to the complexity of performing human pediatrics studies. While the general consensus is that younger children are less likely to develop robust long-lasting CMI responses, children remain a critically underexplored population."

Evaluation of the pediatric response to Ty21a vaccination compared to adults by immunophenotyping and studying specific responses to S. Typhi in blood samples using mass cytometry exposed several remarkable differences. Adults not only have a significantly lower percentage of CD4+ and CD8+ naive cell subsets and significantly higher T effector memory cells than children, they also exhibit more prominent multifunctional responses than children do. And it is this multifunctional response that can have direct implications in protection from infection.

Additional mass cytometry studies on cell responses to both nonspecific stimulation with a staphylococcal enterotoxin B, a superantigen that induces proliferation and cell activation in a large fraction of T cells, and specific stimulation with

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Salmonella Typhi, concluded that indeed children and particularly younger ones are less able to effectively respond to stimulation elicited by Ty21a typhoid vaccination.

A look into epigenetic modifications and their implications using EpiTOF

Sztein and Rosangela Salerno-Goncalves, PhD, pushed mass cytometry a step further to address important questions regarding cell type-specific epigenetic modifications elicited by exposure to this organism and the implications they might have in the gut[5].

The establishment of a unique epigenetic profile is a key molecular process that underlies the differentiation of cells throughout maturation. Post-transcriptional modifications, including acetylation, methylation, phosphorylation and ubiquitination, dictate chromatin structure and function, thereby regulating gene activity and expression.

This regulation of gene expression leads to changes in cell behavior as well as in tissue development. Bacteria-induced epigenetic changes may affect the host cell function by either promoting host defense or allowing pathogen persistence.

However, there are no studies reporting whether exposure to bacterial organisms results in a specific imprinting on immune responses to bacterial infection. Sztein hypothesized that gut colonization by Salmonella Typhi causes epigenetic changes that are cell-type specific. He targeted the terminal ileum, the main segment of the gastrointestinal tract in humans, which exhibits pathology associated with Salmonella Typhi infection and is widely believed to be the favored site of infection.

The team leveraged EpiTOF (epigenetic landscape profiling), a mass cytometry-based analytical approach that enables the simultaneous measurement of epigenetic and immunological markers at the single cell level. The technique was developed by Peggie Cheung6, PhD, and colleagues in 2017 with the goal of using cytometry to study histones and their epigenetic modifications.

This uniquely powerful tool enabled Sztein to create an epigenetic atlas based on chromatin modification profiles in cells isolated from the human terminal ileum following in vitro exposure to wild-type Salmonella Typhi. The data was analyzed with t-SNE and FlowSOM to compare chromatin modifications between and within the different cell types.

Using a 33-marker panel, the team studied post-translational modifications in 11 major subsets concomitantly, including CD3+ T cells, CD4+ T cells and CD8+ T cells, as well as NK, gamma deltas, mucosal associated invariant T cells, NKT, monocytes, macrophages and even epithelial cells. “This is very important for us because with mass cytometry, we could study 8 to 10 million individual cells for epigenetic marks per sample,” explains Sztein.

“Because this is mass cytometry and we collected close to 10 million individual cells, we were able to look at the chromatin profiles of the epigenetic changes in each of the 11 subsets, finding a significant increase in arginine methylation. Again, because this is mass cytometry, we were also able to look at the phenotype of the arginine methylated CD4+ cells, where we see increases in the T effector/memory subsets, but not in either the central memory T effectors or T naive cells,” Sztein says.

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The team concluded that Salmonella Typhi infection results in epigenetic modifications that are cell type- and subset-specific and are very likely to have a major effect in T cell differentiation and gene expression. They also observed increased arginine methylation, which controls the strength of cell signaling via the gamma chain family of cytokines, in CD4+ T effector/memory cells following infection. The possibility that particular epigenetic changes have a major impact on effector cell function is strongly suggested by these early epigenetic investigations.

These studies are the first proof of principle that early epigenetic changes in mucosal cells, isolated from the human terminal ileum, can be directly linked to exposure to a major intestinal bacterial pathogen. Understanding the epigenetic regulators, which determine gene transcription patterns in different cell types or subsets, will be essential in further characterizing the pathways associated with protection from infection that eventually lead to a robust and effective host response.

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Watch Marcelo Sztein's webinar: Uncovering immunological mechanisms of protection from infection and vaccination in humans

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