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Deep Immune Profiling in Trauma and Sepsis: Flow Is the Way to Go!*

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High-content immune profiling technologies are transforming the field of predictive medicine. In the critical care unit, predicting which patient is at risk for developing sepsis after a major traumatic injury remains a clinical challenge. A precise understanding of the complex inflammatory response to traumatic injury is crucial for identification of immunologic dysfunction predictive of posttraumatic infection. In this issue of *Critical Care Medicine*, Seshadri et al (1) used a single-cell mass cytometry approach to characterize the phenotype and function of major innate and adaptive immune responses in patients who suffered from traumatic injury. This research breaks ground toward the larger goal of individualized risk stratification based on the deep immune profiling of critically ill patients.

Mass cytometry by time-of-flight (CyTOF) is a high-parameter flow cytometry platform that exploits the sensitivity, dynamic range, and minimal spectral overlap of mass spectrometry to enable the simultaneous interrogation of over 50 parameters on a cell-by-cell basis (2, 3). In their study, Seshadri et al (1) employed two parallel CyTOF antibody panels to analyze longitudinal peripheral blood mononuclear cell samples from 10 patients suffering from traumatic injury (Injury Severity Score > 20) and 10 age-matched controls. The first antibody panel allowed quantification of the abundance of immune cell subsets, and the second, characterization of intracellular expression of over 20 cytokines at the single-cell level.

Seshadri et al (1) should be lauded for the careful design of a complex mass cytometry assay and the use of state-of-the-art methods for sample processing and analysis. Samples were appropriately barcoded, using a mass-tag barcoding method known to significantly reduce

*See also p. 1523.

experimental variability (4). Samples were also normalized to account for changes in instrument detection sensitivity (5). These methodological precautions are imperative when analyzing patient samples with mass cytometry, which, like any antibody-based multiplex platform, is vulnerable to batch effects due to antibody staining, as well as metal isotope- and instrument-specific considerations.

Several themes evolving from analysis by Seshadri et al (1) resonated with known hallmarks of the immune response to trauma (6, 7). For example, analysis of immune cell abundances recapitulated previously reported expansion of monocytes after injury and the concomitant decrease in CD4⁺ and CD8⁺ T-cell frequencies. Similarly, the expression of human leukocyte antigen - antigen D related in monocytes dramatically diminished after injury, consistent with clinically relevant phenotypic changes in these innate immune cells after traumatic injury (8).

The analysis also enabled intriguing discoveries, specifically the functional analysis of T helper 17 (T_h17) cells and natural killer (NK) cell subsets. T_h17 cells are a subset of CD4⁺ T cells that play a critical role at the interface of innate and adaptive immunity, particularly in the host's pathogen defense against bacteria. However, evidences from animal and human studies have linked T_h17-derived cytokines (such as interleukin [IL]-17 and IL-22) to worse outcomes after sepsis (9). Data by Seshadri et al (1) indicates that trauma rapidly induces T_h17 differentiation, followed by the functional capacity to produce IL-17 5 days after injury. Further work will be necessary to determine whether the expansion of T_h17 cells after trauma protects patients from posttraumatic infection or, in contrast, predominantly exacerbates an inflammatory process associated with poor clinical outcomes.

Seshadri et al (1) also find important alterations in NK cell function following traumatic injury. Biphasic changes in the expression of the transcription factor T-bet in NK cells were observed in response to trauma and paralleled the capacity of NK cells to produce tumor necrosis factor- β and interferon- γ . Seshadri et al (1) conclude that trauma induces the rapid recruitment of an immature NK cell population from the bone marrow within 1 day of injury, followed by differentiation into mature NK cells. Interestingly, this NK cell maturation process was delayed in patients who developed infection (four patients) compared with patients who did not. Although the sample size is too small to generalize to a larger population, these results have potential clinical implications for assessing a patient's risk of infection after trauma.

Importantly, the specific immune cell attributes highlighted by Seshadri et al (1) must be viewed as integrated within a much larger network of innate and adaptive responses engaged after traumatic injury. In this sense, immune features (frequencies and intracellular cytokine expressions) described in the article represent only a fraction of the rich dataset made accessible by the high-parameter CyTOF assay. Many more cell subsets could have been phenotyped using the 38-parameter antibody panels and functionally interrogated. Traditional, manual gating strategies are useful for an analysis focused on cell subsets with known immunologic phenotypes. Alternatively, unsupervised algorithms (e.g., Spanning-tree Progression Analysis of Density-normalized Events [SPADE] [10], CITRUS [11], or visual stochastic network embedding [viSNE] [12]) offer the option to agnostically cluster the

single-cell data into phenotypically distinct immune cell subsets. Using one of these deep immune phenotyping strategies, the systematic functional analysis of all gated cell subsets could provide further insight into novel aspects of the human immune response to traumatic injury.

The lack of systematic examination of all immune responses is a limitation of analytical approach by Seshadri et al (1). However, the dimensionality of mass cytometry data and the interconnected nature of single-cell immune responses represent a major analytical challenge. The high-parameter immunologic dataset generated by Seshadri et al (1) therefore provides a unique opportunity for further analyses, which will necessitate the development of new statistical methods adapted to highly correlated single-cell data (13). These novel approaches will be critical in providing a comprehensive survey of the human immune response to traumatic injury and identifying specific immune signatures associated with posttraumatic clinical outcomes.

The implementation of mass cytometry in clinical studies offers unprecedented opportunities to link the single-cell profiling of complex immunologic states to posttraumatic clinical outcomes (14, 15). The work by Seshadri et al (1) paves the way for future studies examining the utility of identified immune responses in predicting posttraumatic, life-threatening infections and sepsis, which may radically change the clinical management of patients suffering from traumatic injury.

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