Shock 2023

View Abstract

CONTROL ID: 3908512

TITLE: THE MINNI-CINCI METHOD: A SHORTENED ELISPOT ASSAY TO TEST IMMUNE FUNCTION IN SEPSIS PATIENTS (SPIES)

PRESENTATION TYPE: Oral or Poster

PREFERRED TOPIC: Sepsis

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ABSTRACT BODY:

Introduction: Despite over 100 trials targeted at improving outcomes, sepsis remains a leading cause of death worldwide with no proven immunomodulatory therapies. We posit these therapies failed because they were misapplied to a heterogeneous population of patients that met the criteria for MODS and sepsis, but without diversification based on an understanding of each patient's individual functional immune state (endotype). Stratifying Patient Immune Endotypes in Sepsis (SPIES Study; GM139046) is a prospective, multicenter project to test the utility of ELISpot as a functional bioassay to identify the immunosuppressed endotype and predict clinical outcomes in septic patients. Typical ELISpot protocols take 36-48 h to complete from sample addition to plate reading and data analysis. The objective of this study was to determine the benefit of using a significantly shorter stimulation period in the ELISpot protocol, such that important immune fitness data can be obtained the same day as sample acquisition.

Methods: ELISpot assays were conducted using fresh whole blood or frozen PBMC stimulated *ex vivo* with anti-CD3/CD28 mAb, LPS, PHA, or PMA/ionomycin. Stimulation times were either 2, 3, 4 or 22 h. The number of cells (spot-forming units; SFU) producing either IFNγ or TNFα, spot size, and total cytokine production (spot number times spot size) were determined.

Results: When comparing LPS-stimulated TNFα production, we saw no statistical difference in SFU when cells were stimulated for 3, 4, or 22 h. There was a slight decrease in SFU number with 2 h stimulation. These data suggest the cells making TNFα, presumably monocytes and neutrophils, release this cytokine from premade intracellular stores or rapidly produce it *de novo*, and longer stimulation periods reveal no additional information. Similarly, the number of IFNγ SFU were consistent when anti-CD3/CD28 mAb was used to stimulate T cells for either 4 or 22 h.

Conclusions: The data reveal there are benefits for using a short 2-4 h stimulation to assess immune cell function with the purpose of endotyping sepsis patients. Short stimulation measures the immediate cytokine producing capacity of effector/memory T cells and innate immune cells. The inclusion of multiple ways to stimulate T cells provides the operator the ability to measure extracellular (TCR-mediated) and intracellular activation pathways. Shorter stimulation times can also be combined with automation to permit high-throughput sample testing. Altogether, this modification could help to inform clinicians on the patients' immune status within 24 hours.

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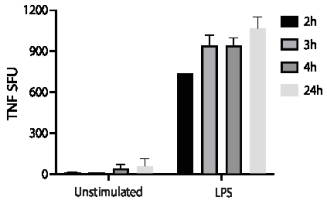
TABLE TITLE: (No Tables)



AWARDS:

Disclosure: NO, there are no relationships to disclose.

Submission Rules Confirmation: I confirm that I have read and complied with the submission guidelines.

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