Generating Immunological Signatures of Diseases by Multiplex Analysis of Single Cells

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Technical Summary

The activation of T cells is an essential process for coordinating both cellular and humoral immune responses. The release of cytokines is one of several important functions carried out by T cells during either a protective immune response to pathogens or autoimmune responses. Understanding the phenotypes and dynamic functions that indicate effective T cell responses to diseases and clinical interventions has been a central effort to improve immune monitoring. Existing analytical methods can assess the frequencies, magnitude, and number of cytokines produced by individual cells, but few have temporal resolution and abilities to correlate functional profiles with other cell activities, such as proliferation and cytotoxicity. To solve this technical problem, this thesis aims to develop a high-throughput and multiplexed assay to resolve the dynamic functional responses of individual cells following stimulation while minimizing external perturbations that could unintentionally alter their behaviors.

Microengraving is a process that uses a dense, elastomeric array of nanowells to generate microarrays of proteins secreted from large numbers of individual live cells. In this thesis, we improved the sensitivity and multiplicity of microengraving and adapted it to detect cytokine secretions from primary immune cells. We demonstrated that microengraving could provide quantitative measurements of both the frequencies and the distribution in rates of secretion for up to four cytokines simultaneously. The experimental limits of detection ranged from 0.5 to 4 molecules/s for most cytokines in our experiments. These multidimensional measures resolve functional responses by cells exposed to stimuli with greater sensitivity than single-parameter assays. Primary T cells with specific profiles of secretion can also be recovered after microengraving for subsequent expansion in vitro.

The release of multiple cytokines by T cells has been associated with beneficial immune responses. To date, however, time-integrated end-point measurements have not resolved the temporal dynamics of these functions. Here, we used serial microengraving to measure Th1-skewed cytokine responses (IFNγ, IL-2, TNFα) from individual cells after activation ex vivo. The results show that multifunctional cytokine responses are initiated asynchronously but the ensuing dynamic trajectories of these responses evolve programatically in a sequential manner. Furthermore, these dynamic trajectories are strongly associated with the various states of cell differentiation, suggesting that transient programmatic activities of many individual T cells contribute to sustained, population-level responses. The trajectories of responses by single cells may also provide unique, time-dependent signatures for immune monitoring that are less compromised by the timing and duration of integrated measures.

Together, this work demonstrates the utility of quantitative, multidimensional profiles of single cells for analyzing the diversity and dynamics of immune responses in vitro, and suggests new approaches to define functional signatures of immune responses. The multiplexed assay system established in this work and the results could benefit further research on T-cell functions and immune networks. The dynamic single-cell analysis of cellular functional responses should also help evaluate the nature and evolution of intercellular interactions present in other biological systems.