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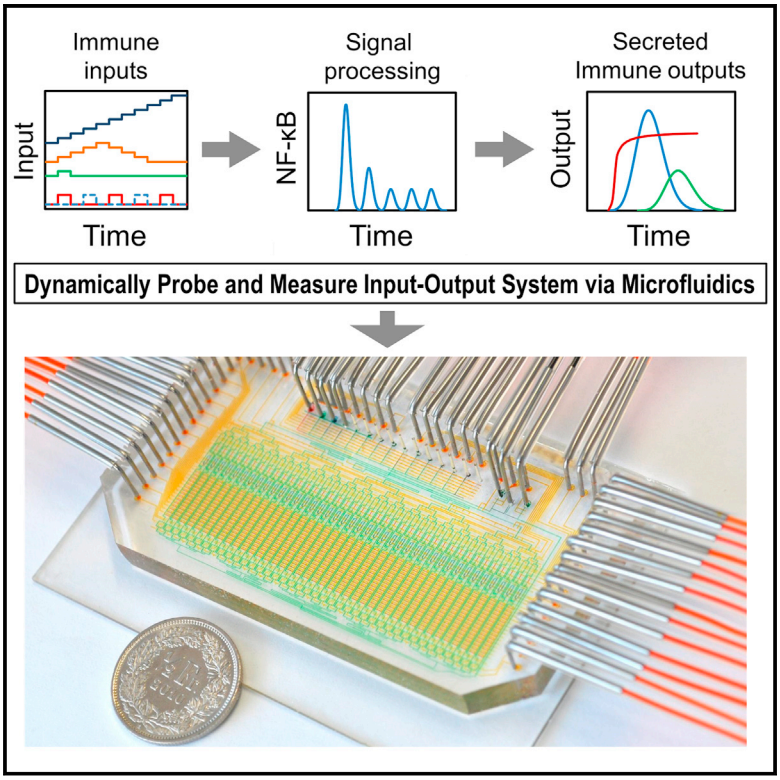
High-Content Quantification of Single-Cell Immune Dynamics

Graphical Abstract



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In Brief

Junkin et al. have developed a method to quantitatively probe single-cell input-output dynamics with an automated microfluidic system. They conduct coupled measurements of transcription factor and cytokine secretion dynamics from the same single cells to enable modeling, which uncovers dynamic and noise-based roles of TRIF in the NF-κB-TNF pathway.

Highlights

- Dynamic stimulation of single immune cells with a versatile microfluidic device
- Coupled longitudinal measurements of NF-κB localization and TNF secretion on the same cell
- Single-cell harvesting, staining, and mRNA quantification on the same device
- High-content dataset, and modeling of TRIF-based noise in TNF secretion

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High-Content Quantification of Single-Cell Immune Dynamics

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SUMMARY

Cells receive time-varying signals from the environment and generate functional responses by secreting their own signaling molecules. Characterizing dynamic input-output relationships in single cells is crucial for understanding and modeling cellular systems. We developed an automated microfluidic system that delivers precisely defined dynamical inputs to individual living cells and simultaneously measures key immune parameters dynamically. Our system combines nanoliter immunoassays, microfluidic input generation, and time-lapse microscopy, enabling study of previously untestable aspects of immunity by measuring time-dependent cytokine secretion and transcription factor activity from single cells stimulated with dynamic inflammatory inputs. Employing this system to analyze macrophage signal processing under pathogen inputs, we found that the dynamics of TNF secretion are highly heterogeneous and surprisingly uncorrelated with the dynamics of NF-κB, the transcription factor controlling TNF production. Computational modeling of the LPS/TLR4 pathway shows that post-transcriptional regulation by TRIF is a key determinant of noisy and uncorrelated TNF secretion dynamics in single macrophages.

INTRODUCTION

Immune cells must coordinate their activity at multiple timescales and mount a finely tuned protective response while avoiding tissue damage. A plethora of signaling molecules, regulatory pathways, and network motifs are employed to distinguish self from foreign and calculate intensity and duration of the immune response. Cells receive time-varying inputs such as changing local concentrations of pathogen or stress-related molecules, utilize pathway dynamics to process signals (Ashall et al., 2009; Batchelor et al., 2009; Nelson et al., 2004; Lahav et al., 2004; Tomida et al., 2012; Kellogg and Tay, 2015; Kellogg et al., 2015), and generate functional dynamic outputs by secreting their own signaling molecules. Dynamic environmental inputs can induce resonant transcriptional dynamics and synergize with intrinsic noise to amplify immune outputs (Kellogg and Tay, 2015). Characterizing such dynamic input-output relationships aids in understanding regulatory mechanisms underlying immunity, enables systems-level modeling to predict outcomes of complex physiological scenarios (Covert et al., 2005; Lipniacki et al., 2004; Lee et al., 2009; Cheong et al., 2006), and would significantly aid drug research and therapeutics (Behar et al., 2013; Cohen et al., 2008).

Understanding and modeling the immune system requires detailed multiparameter and quantitative analysis of its components. Major obstacles in this endeavor are the constantly changing nature of reactions (dynamics), broad timescales of processes from fast (milliseconds) to very slow (days), and ever-present biological noise (Tay et al., 2010; Lahav et al., 2004; Elowitz et al., 2002). Such dynamic variability makes time-dependent quantitative single-cell analysis crucial to understanding how biological systems operate. Measuring of multiple immune regulatory components is thus necessary to gain understanding of dynamic immune functions. Among these are transcription factor (TF) families such as NF-κB, IRFs, STATs, and SMADs that process inflammatory inputs to exercise global control on gene expression (Doupé and Perrimon, 2014; Batchelor et al., 2009; Nathan, 1987; Delgoffe et al., 2011). Live-cell fluorescent microscopy of TF dynamics has recently led to a paradigm change in the understanding of dynamic cell signaling, gene regulation (Nelson et al., 2004; Lee et al., 2009; Lahav et al., 2004; Spencer et al., 2009; Elowitz et al., 2002; Vedel et al., 2013; Lee and Covert, 2010), and contributions of noise (Kellogg and Tay; 2015) to signal processing. Signaling dynamics can influence transcriptional responses as seen with the activation dependence of the master immune regulatory NF-κB pathway upon input frequency (Ashall et al., 2009), or tolerize its response due to repeated pathway activation (Biswas and Lopez-Collazo, 2009). Single-cell NF-κB activation to an increasing cytokine input has also been found to be a digital process with a high degree of cell-to-cell variability (Tay et al., 2010). Dynamic