Docket #: S20-110

High-Throughput Force-Dependent Cellular Response Assay Using Spectrally Encoded Smart Beads

Researchers at Stanford have developed a technology that uses biomechanical force to initiate T-cell triggering in a high throughput method, facilitating the exploration of the force- and sequence-dependent landscape of T-cell responses.

Adaptive immunity relies on the ability of T-cells to sensitively discriminate self from non-self, and to thereby detect pathogen infection or malignant transformations. This discrimination hinges on the ability of T-cell receptors (TCR) to recognize specific peptides presented by major histocompatibility complex (MHC) molecules expressed on antigen presenting cells, which triggers downstream signaling events and T-cell effector functions. However, the relationship between TCR binding and triggering is not fully understood. For instance, measured in vitro affinities with a presented peptide do not always correlate with stimulation of T-cell activity, as some of the most potent stimulatory peptides bind with only weak or moderate affinities. Recent evidence suggests that the pN to nN biomechanical forces generated at the TCR-pMHC interface during T-cell immunosurveillance and synapse formation may be critical for sensitive and specific recognition. Distinguishing agonist peptides from non-agonist peptides therefore requires an in vitro assay in the presence of applied loads that quantifies binding and downstream activation. Currently available screening approaches can only quantify peptide binding alone, and not T-cell activation, and they take place in the absence of force and present peptides at artificially high concentrations, thereby reducing their physiological relevance. Several mechanobiology methods exist that can exert well-calibrated shear loads on T-cells interacting with specific pMHCs displayed at low densities, however these techniques typically require expensive equipment, are labor intensive, and are limited in peptide throughput. A peptide screening method that could probe both TCR binding and triggering at a high throughput level is needed.

Stage of Development
Research –

in vitro

Stage of Research
The inventors have developed a novel technology called BATTLES for Biomechanically-Assisted T-cell Triggering for Large-scale Exogenous-pMHC Screening. This technique profiles T-cell signaling responses for thousands of cells interacting with different pMHCs at low densities and in the presence of physiological shear loads within a single experiment. BATTLES displays candidate pMHCs on spectrally encoded "smart beads" capable of applying physiological loads to T cells. T-cells are deposited onto the surface of hydrogel "smart beads" bearing pMHCs that swell upon small changes in temperature. To monitor downstream signaling responses via high-throughput single-cell microscopy, T-cells and "smart beads" are loaded into microcell arrays in the presence of a Ca2+-sensitive dye.

Technology Reference
CZB-165S-PC, Stanford ref. no. S20-110

Applications

- "Smart beads" can apply well-calibrated loads to single T-cells.
- Spectrally encoded "smart beads" allow simultaneous testing of many potential antigenic sequences.
- "Smart beads" can display peptides at low physiological densities to mimic in vivo conditions.
- T-cells can be paired with "smart" beads for high-throughput monitoring of force- and sequence-dependent activation.
- BATTLES can be applied to identify novel peptide agonists.
- Multiplexing applied loads can elucidate "catch" versus "slip" bond behavior in a single experiment.
- Multiplexing pMHC sequences and concentrations can be used to study dose-dependent immunogenicity.

Advantages

- The BATTLES platform is the first mechanobiology tool that recapitulates the physicochemical cues of active force and low monomeric pMHC density.
- BATTLES screens many peptide sequences for their potential to bind TCRs and activate downstream signaling responses in a combined, high throughput format.

Publications

- Feng Y, Zhao X, White AK, Garcia KC, Fordyce, PM. "Structure-activity mapping of the peptide- and force-dependent landscape of T-cell activation." 2022. Nature Methods.

Patents

- Published Application: WO2022094219
- Published Application: 20230384308

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