Kinetics of molecular interactions across the T cell – APC junction.

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T cell receptor (TCR) interaction with peptide-major histocompatibility complexes (pMHC) is central to adaptive immunity as it allows T cell to discriminate pathogens from self- antigens and trigger adaptive immune responses. We used two mechanical assays to study two-dimensional (2D) kinetics of T cell surface molecules interacting with counter-molecules which were isolated and controlled on the surface of a surrogate antigen presenting cell (APC). Antigen recognition was driven by high 2D affinities and rapid on-rates of TCR-pMHC binding. The CD8 coreceptor synergistically cooperated with TCR to amplify the discrimination process. The cellular environment and molecular organization on the membrane regulate the physical chemistry of TCR–pMHC binding leading to a better match of the wide range of functional responses.

Keywords **— TCR-pMHC binding kinetics, antigen discrimination, TCR initial triggering**

I. BACKGROUND

THE sustained interest in the kinetic analysis of TCR–pMHC interactions stems from a fundamental hypothesis that the interaction parameters have a central role in determining the subsequent T-cell response. Numerous models has been generated to explain the discrepancy between the narrow range of 3D TCR–pMHC kinetics and the broad range of T-cell responses [1,2]. We used *adhesion frequency assay* and *thermal fluctuation assay* to make in situ kinetic measurements across the junctional interface of a T cell and a surrogate APC [3-5]. The surrogate APC is a human red blood cell (RBC) in the former assay and a glass bead attached to the RBC in the latter assay, which were functionalized with ligands of our choosing. The RBC serves as an adhesion sensor to register the molecular interactions.

II. RESULTS

A T cell and a surrogate APC were repeatedly brought into contact for specific time duration and the number of successful adhesions was counted. The fitting of the adhesion frequency versus cells contact time duration curves with the previously proposed model allowed the extraction of 2D affinities and off-rates [3-5].

CD8-MHC and TCR-pMHC bimolecular interactions

In the absence of TCR binding, CD8-MHC interaction has low affinity that is independent of the peptide [3]. In the absence of CD8 binding, TCR-pMHC binding showed much broader dynamic ranges for on-rate and 2D affinity compared to 3D data [4]. The opposite trend was found for the 2D off-rate with the agonist dissociating the fastest [4].

TCR-CD8-pMHC trimolecular interaction

TCR engagement triggers cooperative binding with CD8 and pMHC to increase adhesion (especially for strong ligands), amplifying peptide discrimination [5].

Memory effect or adhesion upregulation

A surprising feature that was observed only for TCR-pMHC interaction is adhesion upregulation in the next adhesion test if adhesion happened in the previous test. This "memory" effect could be related to the binding upregulation on the T cell level or on the TCR microcluster level. It also involves the T cell signaling process.

III. SUMMARY

The TCR-pMHC 2D kinetics is different from 3D kinetics in that intrinsic binding parameters are modulated by cellular environment. The first seconds of TCR-pMHC interaction kinetics measured in situ by our 2D mechanical assays correlate well with T cell responses to a panel of peptides.

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