Optical Manipulation of T-Lymphocyte Activation

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Introduction

Adaptive immunity is conferred when T-cells engage antigen presenting cells (APCs) within a ~70 μm² area, known as the immunological synapse (IS).

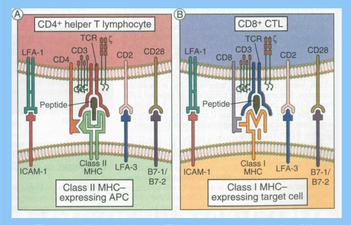

Figure 1: ligands key to T-lymphocyte activation [1]

A variety of co-stimulatory receptors and adhesion proteins (APs) interact with cognate ligands on the apposed cell surface within this interface. For the activation of naïve T lymphocytes, there are two essential pairings: TCR:pMHC in activation due to recognition of foreign peptides and CD28:CD80 in co-stimulatory signaling on T-cells:APCs respectively[1]. Finally, LFA-1 interaction with ICAM-1 is key to proper adhesion (Fig 1).

Background

An increasing body of evidence suggests that the spatial organization of these signaling complexes impacts T-lymphocyte activation and vice versa. Tseng et al. transfected MHC-II I-E^k and ICAM-1 expressing CHO cells to express either full-length CD80 (CD80**FL**) or cytoplasmic tailed deleted CD80 (CD80**TL**) thus compromised downstream signaling [2]. Next, CHO cells presented mouse cytochrome c-loaded I-E^k to activate naïve T-lymphocytes from 5C.C7 TCR transgenic mice. After initial engagement, CD28 on T-cells ligated with CD80. For CD80**FL**-YFP, the two signals segregated (Fig 2a); while in CD80**TL**-YFP, the two pairings co-localized (Fig 2b). The study suggests a link between the degree of activation and the spatial organization of crucial signaling proteins.

Figure 2: CD80 and TCR spatial organization [2].

Motivation

Lymphocyte-specific protein tyrosine kinase (Lck) affects both TCR-CD3 complex and CD 28 signaling. The phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) on CD3 is a well-characterized interaction; then ZAP70 docks onto ITAMs to continue the signaling cascade. While the exact mechanism of CD28-Lck interaction is unknown, literature suggests that Lck is vital to CD28-associated signaling (Fig. 3).

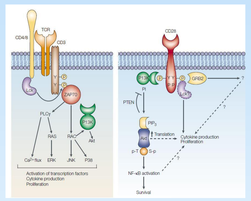

Figure 3: Lck interaction with TCR and CD28 [3].

While Lck activation via phosphorylation (pLck) is established to occur after TCR-engagement, many questions remain. Our group recapitulated the spatial cues on planar surfaces. We observed differential pLck spatial distribution between human and mouse naïve T-cells, which correlates with differences in biological activity.

I am interested in a spatiotemporal study of Lck biological activity. While Lck diffusion is faster in human naïve T-cells, resulting in a more rapid stabilization of CD28-associated biology activities, question remain about the state of the protein.

Goals

- Temporal Control of Lck Activation
  - Photoactivable MHC-II I-E^k
- Reporter of Lck Phosphorylation
  - FRET reporter of pLck activity via ITAM Phosphorylation

Spatial Cues

The spatial cues were recapitulated with microscale patterned planar surfaces via sequential rounds of microcontact imprinting (Fig 4). Because direct stamping of native ligands resulted in their inactivation, robust proteins were chosen to survive the process. To capture CD80, a chimeric protein with a human Fc-region, I utilized anti-hFc antibody. To capture I-E^k-biotin, I stamped strepatvidin.

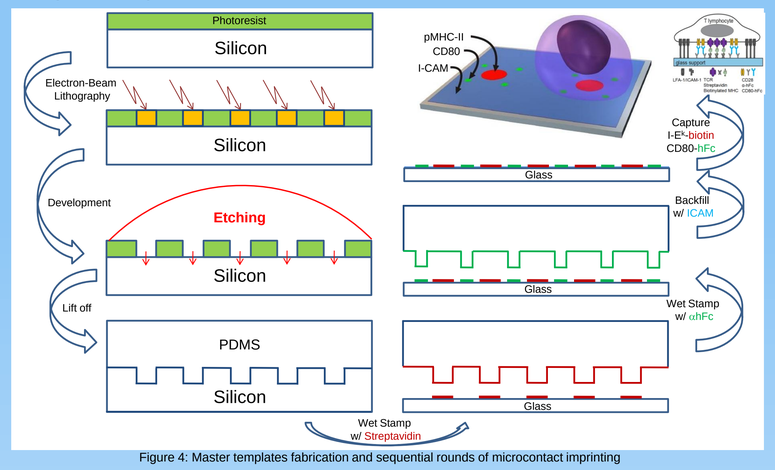

Figure 4: Master templates fabrication and sequential rounds of microcontact imprinting

FRET Reporter of Lck Phosphorylation

The reporter was based on a the CD3ζ ITAM Phosphorylation (ZIP) reporter from Ron Vale's group [5]. ZIP reporter had a full length mouse CD3ζ, eGFP (donor), mCherry (acceptor), and human ZAP70 SH2-domain, a specialized structure to recognize phosphorylated ITAMs (Fig 7).

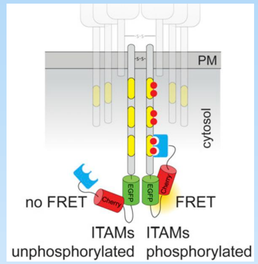

Figure 7: ZIP Reporter [5]

Figure 8: My modification to Vale's ZIP Reporter.

To induce Lck reporter localization with pLck, the full length CD3ζ was replaced with Lck membrane targeting sequence (Lck10: MGCGCSSNPE) and CD3ζ ITAM 3. Examination of eGFP emission and mCherry absorption spectra pin-pointed suboptimal spectral overlap. YPET replaced eGFP; and the T7 promoter was included for *in vitro* transcription of mRNA, for transfection (Fig 8).

Examination of ITAM 3, the pLck target substrate, revealed that the reporter should work in both human and mouse T-lymphocytes, as ITAM 3 is identical in both species (Fig 9).

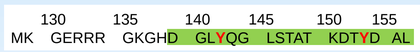

Figure 9: ITAM 3 With two tyrosines for pLck phosphorylation

Photoactivable T-cell Activation

We utilized photoactivable MHC-II I-E^k-biotin to achieve temporal control of Lck activation, as TCR engagement with MHCs induces pLck. Photoactivable MHCs was attained by modifying mouse cytochrome c (MCC), which was loaded on to the I-E^k peptide-binding cleft.

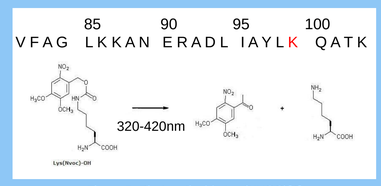

Figure 5: Photoactivable Lys(NVOC)[4].

MCC lysine 99 is essential to naïve T-lymphocyte (from 5C.C7 TCR transgenic mice) recognition of the foreign peptide to initiate cellular activation. By caging the residue via NVOC and then uncaging with 320-420nm light, we exert temporal control over T-cell activation (Fig 5).

To test the biological activity induced by the photoactivable I-E^k, the internal calcium flux was observed via Fluo-4, with an absorption spectrum compatible to photon excitation at 488nm.The calcium-sensitive dye was internalized via an acetoxymethyl ester linker, which was subsequently hydrolyzed by cytosolic esterases, permitting the binding of calcium ions.

The patterned surface capturing CD86-hFc and caged-I-E^k-biotin, was activated with a 350nm light, inducing a typical calcium response (Fig 6).

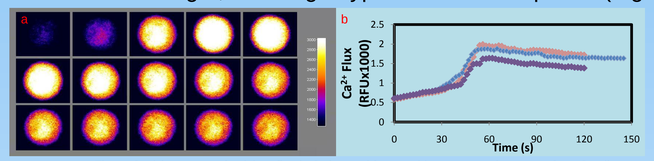

Figure 6: a) heat map of calcium flux in 6s interval frames, b) representative traces from 3 cells

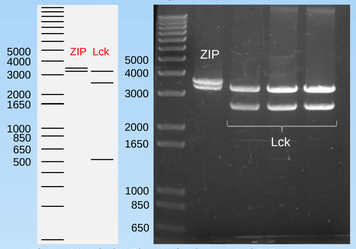

Figure 10: HindIII Digest of Lck – control ZIP

HindIII digest of the plasmid was a preliminary check for Lck10-ITAM3-YPet insertion, followed by sequencing of the reporter (Fig 10). Next, the plasmid was utilized for *in vitro* mRNA transcription, complete with 5'-capping and 3'-poly A tailing. Finally, the plasmid transformed bacteria to produce protein for *in vitro* assay of reporter specificity.

Future Research

- Perfect parameters for mRNA transfection
- Perform DNA plasmid transfection
- Combine transfected cells with the photoactivable MHCs to understand the role of pLck in differential T-cell behaviors in human and mouse systems.

Ultimately, this observation has impact on clinical research especially the area of immunotherapy, where murine models are often utilized in pre-clinical studies.

References

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