Regulation of T Cell Receptor Phosphorylation

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***Abstract* — T cells play a central role in mobilising the adaptive immune system. They perform this function by using their T cell antigen receptor (TCR) to detect antigen, in the form of short peptides bound to MHC (pMHC). Binding of pMHC to the TCR results in TCR phosphorylation, which ultimately determines whether T cells become activated. The phosphorylation of the TCR is regulated by the tyrosine kinases Lck and ZAP and by the tyrosine phosphatases CD45 and SHP-1 but precisely how these molecules regulate TCR phosphorylation remains an open and important problem. In this work, we develop a mechanistic model based on known interactions in the literature. We find that the TCR can produce inhibitory signalling. This has implications for the rational design of therapies for pathologies including HIV and cancer.**

***Keywords* — TCR, SHP-1, ZAP70, ITAM phosphorylation, (tandem) SH2 domain, Protein Tyrosine Kinase (PTK), Protein Tyrosine Phosphatase (PTP), Phosphotyrosine (pY).**

I. Introduction

THE TCR is a multi-protein complex comprising CD3 dimers δε, εγ and ζζ, alongside the ligand-interacting αβ heterodimer. The TCR contains 20 phosphorylatable tyrosine which reside as pairs within Immuno-receptor Tyrosine-based Activation Motifs (ITAMs). ITAM phosphorylation is reciprocally regulated by membrane-tethered Src-family kinases (SFKs) Lck and Fyn, and by membrane-integral phosphatases CD45 and CD148. TCR-pMHC ligation leads to an increase in TCR phosphorylation.

Subsequently, fully (doubly) phosphorylated ITAMs constitute binding sites for the tandem SH2 domains (tSH2) of cytosolic zeta-chain associated protein kinase (ZAP70). ZAP70 is activated both allosterically upon TCR-binding, (as its tSH2 are forced to disengage from auto-inhibitory interaction at the PTK domain), and via trans-autophosphorylation [1]. Docked, activated ZAP70 phosphorylates transmembrane Linker of Activated T-cells (LAT). LAT nucleates a signalosome of SH2-containing components of diverse intracellular signaling cascades; downstream effects of which, include changes in gene expression, proliferation and cytokine secretion.

SH2-contaning Phosphatase-1 (Shp-1) is an inhibitory T-cell phosphatase, with ZAP70-mimetic domain structure N-SH2-SH2-PTP-C. The precise mechanism of inhibitory Shp-1 signaling is unknown. There exists empirical evidence for Shp1-mediated induction of TCR dephosphorylation which depends, at least in part, on SH2 domains [2]; Binding of pY-containing ligand by N-SH2 releases its auto-inhibitory, intra-molecular association with PTP, activating Shp-1 by allostery [3]. Motivated by structural data, we hypothesize a model in which (singly or doubly) phosphorylated ITAMs may constitute SHP-1 binding sites.

How ZAP70 and SHP-1 might compete for binding to, and exacting their opposing regulatory effects on, the TCR thus depends on their relative binding affinities and on the balance between singly and doubly phosphorylated ITAMs (I_1 and I_2 respectively).

II. Results / Hypotheses

We are interested in addressing the following questions:

Do inhibitory effectors bind activatory ITAMs?

We identify hypothetical parameter space for which I_1 dominates over I_2, and TCR is predominantly SHP-1 bound.

Why is there multiplicity and pairing of ITAMs?

The extent of ITAM multiplicity correlates with increasing potency, sensitivity and magnitude of response. ZAP70 engagement at paired ITAMs, coupled with its capacity for trans-autophosphorylation augments its activity in a manner which would be restricted on isolated ITAM equivalents [2].

Why do ITAM binding effectors, such as ZAP70, have tyrosines that regulate their activities?

Mathematical modelling indicates that without trans-auto-phosphorylation-based regulation, ZAP70 causes unwanted constitutive basal ITAM phosphorylation.

III. Discussion

The progress of downstream intracellular signaling events associated with T cell activation and ZAP70/SHP-1 regulation of T-cell sensitivity depend on the phosphor-state of the TCR.

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