The stoichiometry of the CD4/Lck complex in T cells

Alexander Morch[1,2], Nicole Ashman[2], Erdinc Sezgin[2], Ana Mafalda Santos[2], Simon J. Davis[2]

[1]Department of Biochemistry, University of Oxford, United Kingdom
[2]Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, United Kingdom

Background

Antigen discrimination describes the mechanism by which T cells differentiate between self and non-self signals in effecting appropriate immune responses. It has recently been proposed that a small fraction of kinase-coupled CD4 molecules explains peptide-MHC specificity in T cell triggering since stable pMHC-TCR engagement is required for kinase recruitment. This is a controversial finding that conflicts with earlier studies. Here, we aimed to resolve this issue by determining the CD4/Lck stoichiometry in T cells using *in vitro* and *in vivo* measurements.

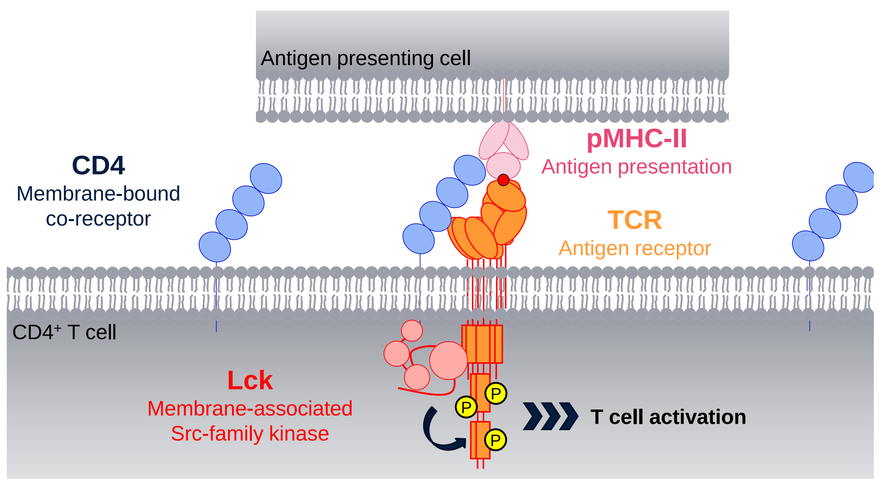

Figure 1: Schematic depiction of CD4⁺ T cell activation.

Results & Discussion

In vitro measurements of CD4/Lck stoichiometry in primary murine T cells were performed using a fluorescence-based pulldown assay in which the relative amount of bead-captured analytes are determined using flow cytometry (**Figure 2**).

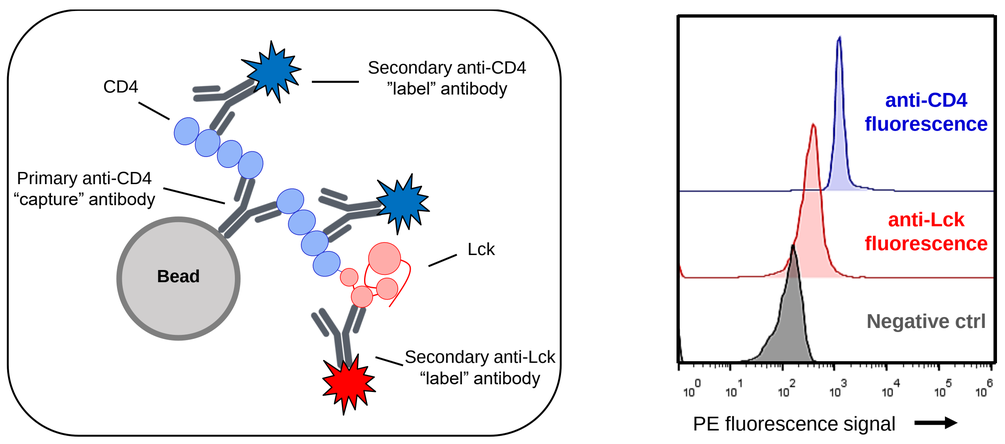

Figure 2: Fluorescence pulldowns. (**Left**) Cell lysates are incubated with anti-CD4 beads before probing with PE-conjugated antibodies to CD4 or Lck at saturating concentrations. (**Right**) Bead fluorescence is then measured using flow cytometry and the ratio between peaks is taken as the CD4/Lck stoichiometry.

By constructing a timepoint dissociation curve, the CD4/Lck complex was shown to be relatively stable ($t_{½}$ ≈ 16 minutes) with an initial stoichiometry of **93%** (sd 25%, n = 21) (**Figure 3**).

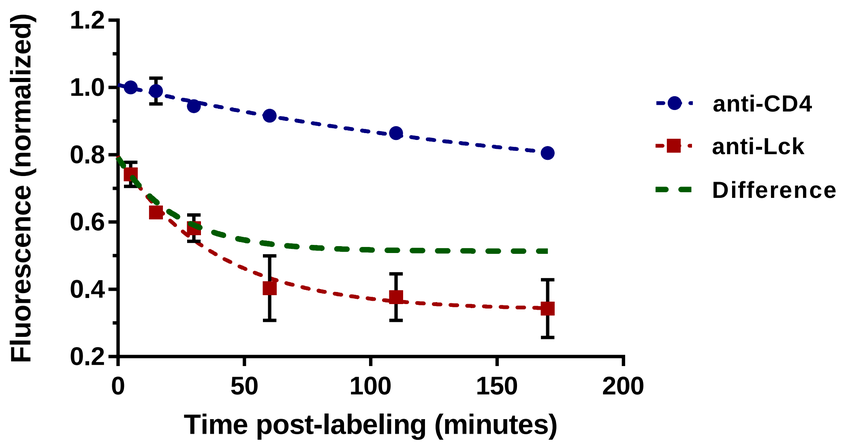

Figure 3: co-IP dissociation curve. By measuring fluorescence at different times post-labeling, dissociation curves were constructed and modeled as one-phase exponential decays (GraphPad, Prism). Bars are median absolute deviations.

To complement this approach, fluorescent versions of murine CD4 and Lck were then transfected into Jurkat T cell lines for imaging protein-protein association on live cells in real time using fluorescence cross-correlation spectroscopy (FCCS). Co-diffusion measurements were performed on wildtype constructs (WT) combined with mutant CD4/Lck constructs lacking a functional interaction motif (mCD4/mLck) (**Figure 4**).

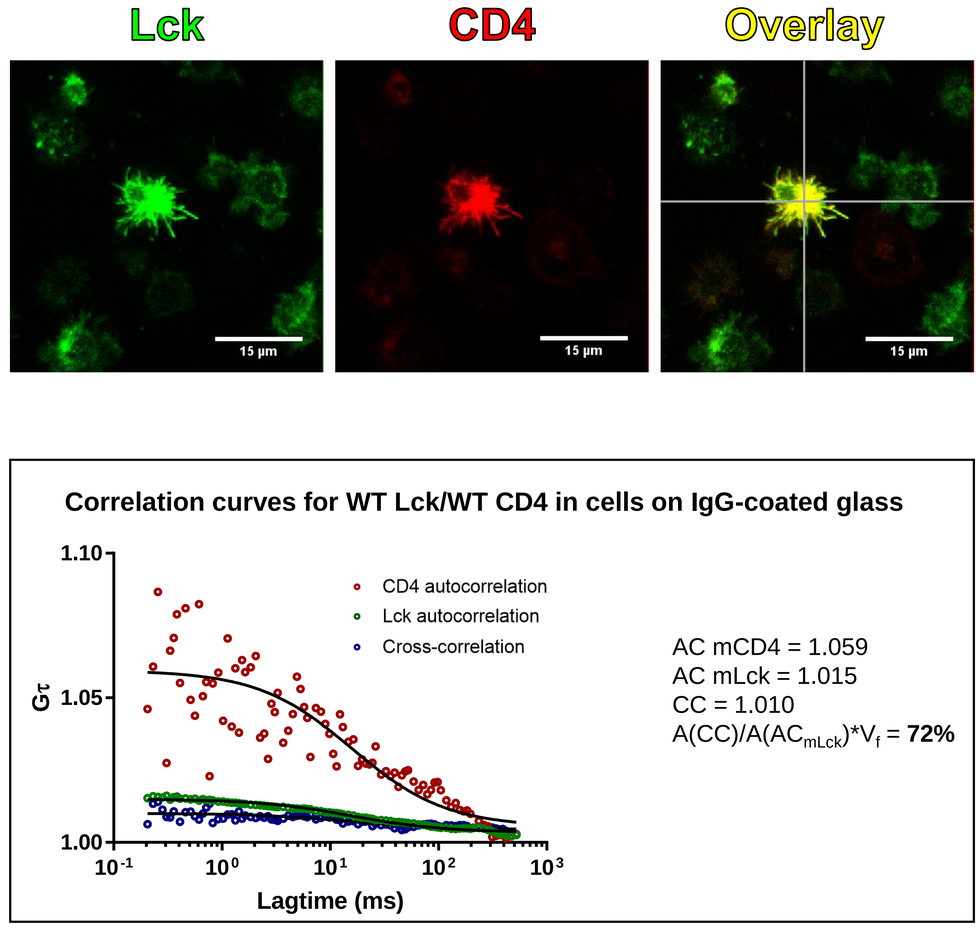

Figure 4: FCCS experiments on Jurkat cells. Cells were dropped onto antibody-coated glass coverslips photon point-measurements performed at 37℃ on 10-20 cells. Correlation curves were fitted using FoCuS software. Representative curves from WT constructs are shown.

Finally, the level of associated CD4 and Lck was found to differ between *in vitro* and *in vivo* measurements of cell lines with WT or mutant constructs. This suggested some contribution to the *in situ* membrane interaction not present in the co-IP (**Table 1**).

	WT-Lck WT-CD4	mut-Lck WT-CD4	WT-Lck mut-CD4
In vitro pulldowns	**77%**	**3%**	**0%**
In vivo imaging	**72%**	**20%**	**32%**

Table 1: CD4/Lck stoichiometries from different methods. Pulldown stoichiometry values measured by co-IP, imaging stoichiometry values measured by FCCS correlation.

Our findings suggest that the previous measurement of CD4/Lck stoichiometry (**6.8%**) underestimates the co-receptor/kinase occupancy in CD4⁺ T cells. This implies that low complex stoichiometry is unlikely to explain kinase recruitment as proofreading step for antigen discrimination. Other kinetic determinants of T cell triggering may include Lck kinase ‘priming’ and the pMHC-TCR dwell time.

Acknowledgements

We would like to thank J. Tanner (OU Biomedical Services) for the provision of murine samples. This project was completed thanks to the Laidlaw Undergraduate Research & Leadership Programme at the University of Oxford.

The MRC Weatherall Institute of Molecular Medicine is a strategic alliance between the Medical Research Council and the University of Oxford