Integration of IL-2 signaling at the immunological synapse

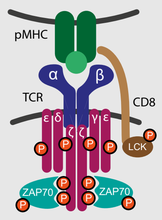
Jesusa Capera[1], Salvatore Valvo[1], Lina Chen[1], Anish Suri[2], Raymond Moniz[2], Michael L. Dustin[1]

[1]The Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom. [2]Cue Biopharma, Cambridge, MA, USA.

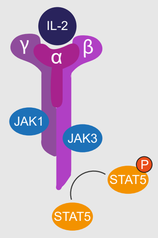
ABSTRACT

T cell receptor (TCR) engagement by antigen presentation results in a stable contact between the T cell and the target cell known as the immunological synapse. Several types of receptor and effector proteins are dynamically organized at the synapse platform to tightly regulate T cell responses to the contact. Interleukin-2 (IL-2) is an essential signal for the proper T cell activation and expansion following antigen presentation. Polarization of IL-2R signalling to the synaptic cleft enhances STAT5 signalling, and trans-presentation of IL-2 at the synapse confers high-affinity IL-2 signalling that promotes priming of naive T cells. However, the role of IL-2 on synapse architecture remains largely unexplored. In addition, while a crosstalk between IL-2R and TCR signalling has been suggested, insight into how these events are coordinated in space and time at the synapse is missing. Here, we analyse the distribution and function of IL-2R at the synapse of different subsets of CD8 T cells. We use a supported-lipid bilayer system to reconstitute the surface of a target cell and generate antigen-specific synapses with CD8 T cells. Using TIRF microscopy, we image the effects of IL-2 on the recruitment of different receptor molecules at the synapse, as well as the spatiotemporal organization of IL-2R relative to the TCR. To further study how IL-2R and TCR signalling are integrated in the synapse, we use a new generation of biologic therapeutics termed Immuno-STATs™ (IST), that are composed of a Fc-formatted peptide-HLA complex and a modified IL-2 with reduced affinity. This provides us with a unique tool to control the segregation or coupling of IL-2R and TCR signalling at the synapse platform. Our data sheds light into the mechanisms of early IL-2 action and improves our knowledge to understand the molecular basis of IL-2 therapeutic strategies.

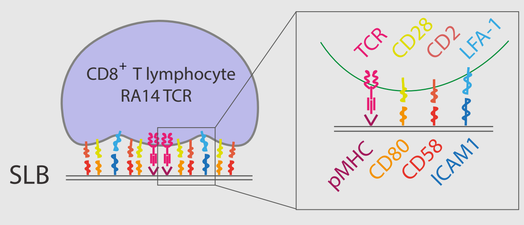
INTRODUCTION



TCR signaling:
The α and β subunits of the TCR recognize the peptide-major histo-compatibility complex (pMHC) with the cooperation of CD4/CD8 molecules. The cytoplasmic tails of the TCR contain ITAM motifs that are phosphorylated upon Ag recognition and initiate a series of signaling pathways that lead to T cell activation.



IL2-R signaling:
High affinity IL2-R consist of three subunits γ, β and α. Binding of IL-2 leads to signal transduction through the Tyr kinases Jak1 and Jak3, which are associated to the γ and β subunits. Jak activation leads to phosphorylation of the transcription factor STAT5, leading to T cell proliferation.



The antigen-specific supported lipid bilayer (SLB) system:
We use a SLB system that mimics the surface of a target cell. The SLB contains a specific pMHC (HLA-A CMVpp65), coactivator molecules CD80 and CD58 and the adhesion molecule ICAM1, which are freely diffusing on the bilayer. Human Ag-specific CD8+ T cells are obtained from peripheral blood. CD8+ T cells are purified and electroporated with mRNA codifying the α and β sub-units of the relevant TCR (RA14). Ag-specific CD8+ T cells are then exposed to the SLB and imaging is performed in live or fixed samples.

RESULTS

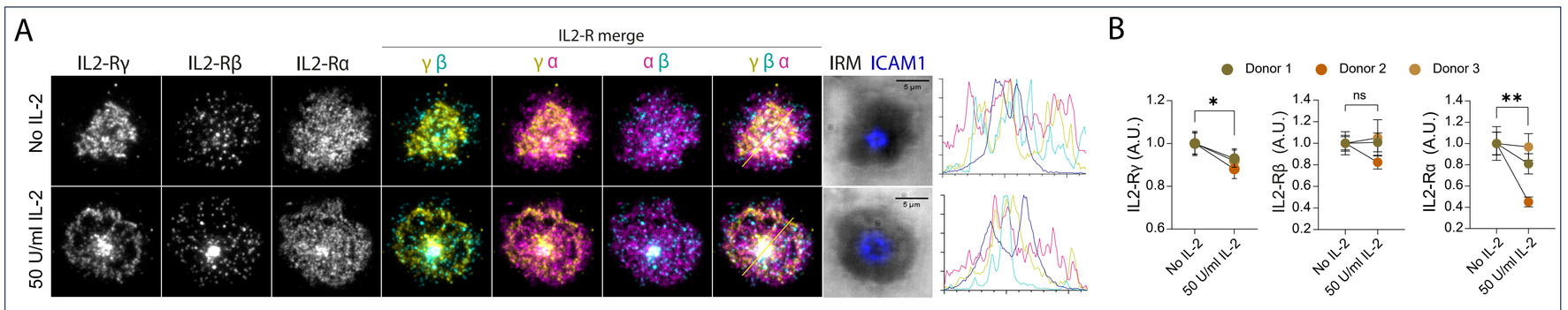


Figure 1. Distribution of IL2-R subunits at the synapse. (A) TIRF images of RA14 CD8+ forming synapses with SLB in the presence or not of IL-2 50 U/ml showing immunostaining of the IL-2R subunits. SLB contained Ag-specific HLA-A, ICAM1, CD58 and CD80. Plots (right) show the intensity line profile for the different proteins. Note the more centralized distribution of the subunits in the presence of IL-2. **(B)** Quantification of the mean intensity values for each IL-2R subunit at the synapse. Data is the mean+/- SE of >50 cells each donor. *pv<0.05, **pv<0.01.

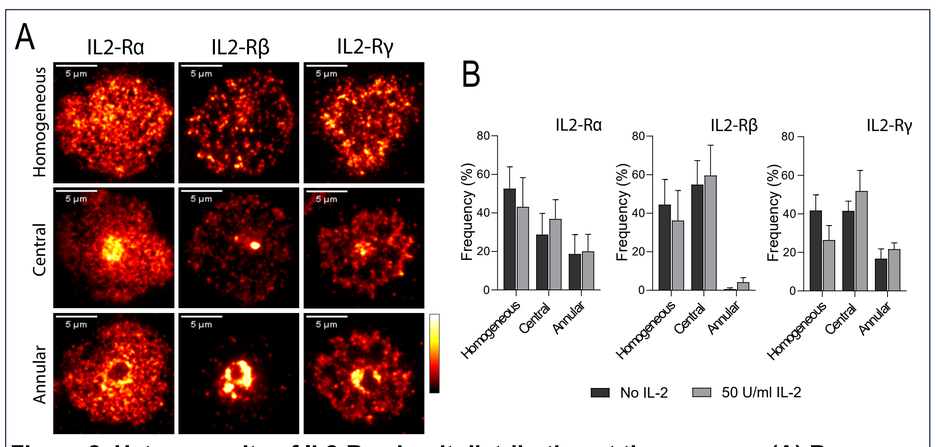
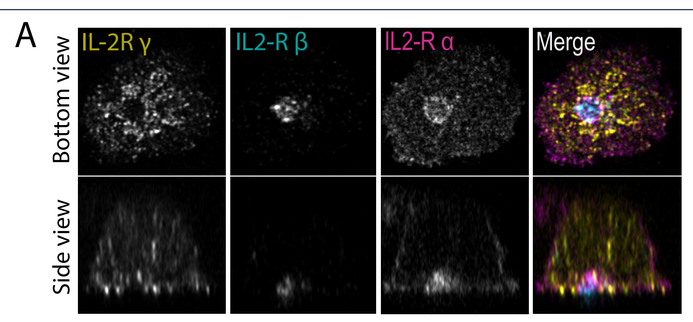


Figure 2. Heterogeneity of IL2-R subunit distribution at the synapse. (A) Representative TIRF images of each IL2-R subunit showing different patterns of distribution at the synapse. Cells were classified into homogeneous, central or annular, based on the radial distribution of the subunit. **(B)** Quantification of the frequency of each phenotype. Data is the mean +/- SE from 3 independent donors.



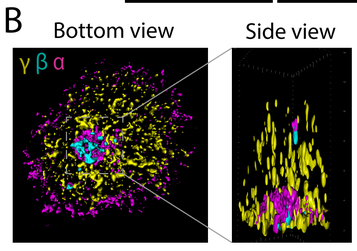


Figure 3. Tridimensional architecture of the IL2-R (A) Representative z-stack Airyscan reconstruction of a CD8+ forming a synapse on a Ag-specific SLB. IL2-R subunits were stained. **(B)** Surface reconstruction of the image in A in the XY plane. Square inset shows the zoom-in area from the side view (XZ plane).

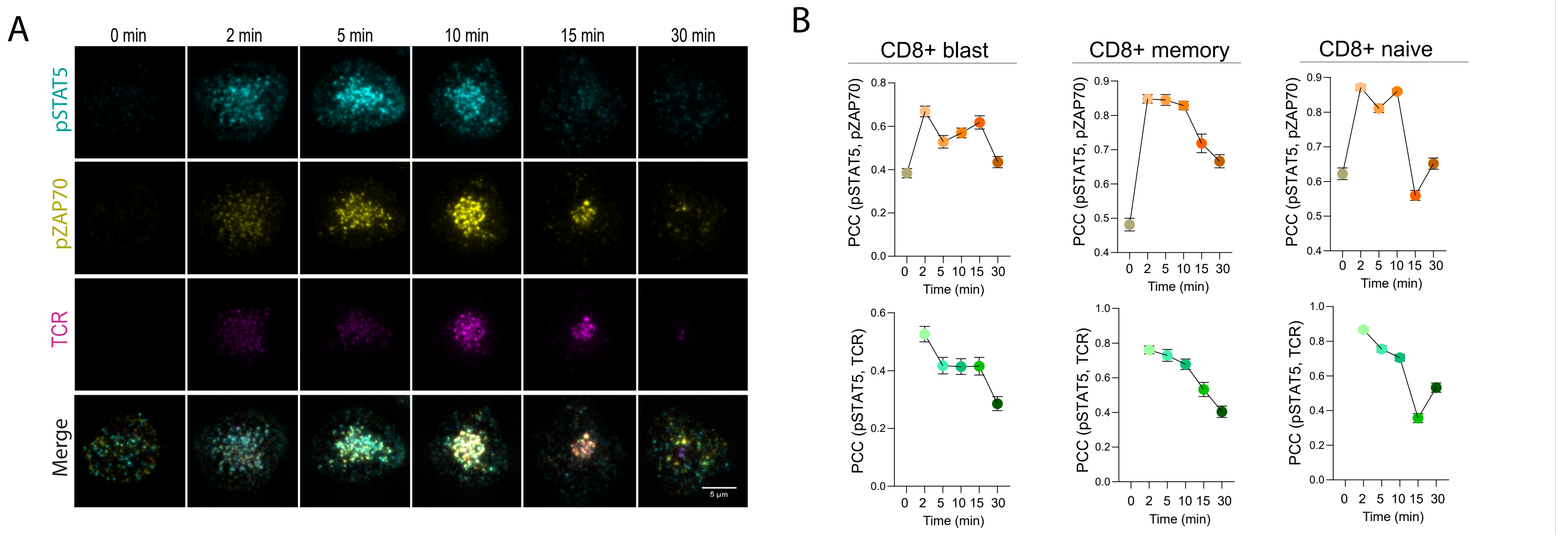


Figure 4. Temporal and spatial correlation between TCR and IL-2R signaling (A) Representative TIRF images of synapses fixed at different time points after exposure to the SLB. Cells were stained for pZAP70 and pSTAT5. Merge brightness was individually adjusted for each time-point for visualization purposes. **(B)** Quantification of Pearson's Correlation Coefficient (PCC) between pSTAT5 and pZAP70 (top) or between pSTAT5 and TCR (bottom) over time. Data is the mean +/- SE of >30 cells.

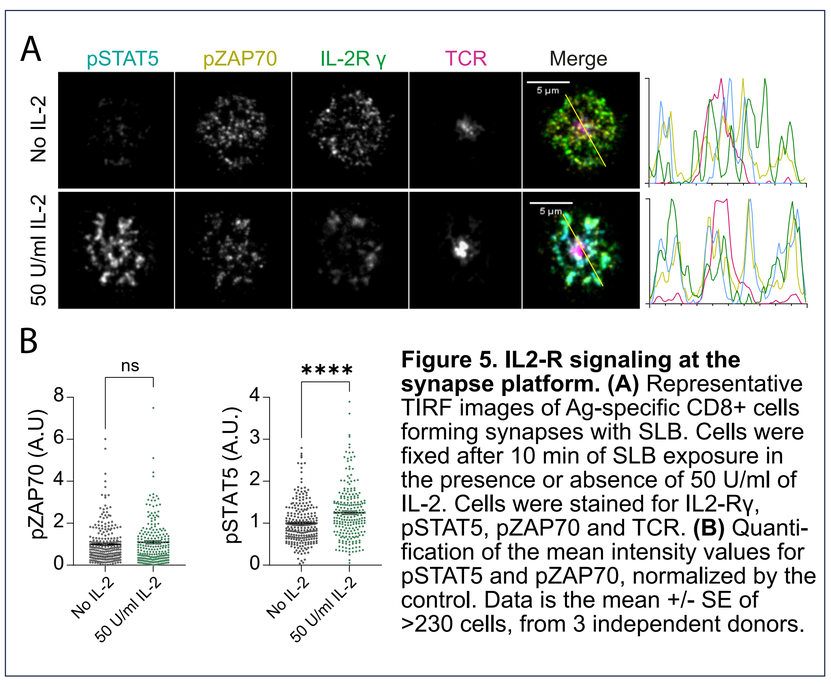


Figure 5. IL2-R signaling at the synapse platform. (A) Representative TIRF images of Ag-specific CD8+ cells forming synapses with SLB. Cells were fixed after 10 min of SLB exposure in the presence or absence of 50 U/ml of IL-2. Cells were stained for IL2-Rγ, pSTAT5, pZAP70 and TCR. **(B)** Quantification of the mean intensity values for pSTAT5 and pZAP70, normalized by the control. Data is the mean +/- SE of >230 cells, from 3 independent donors.

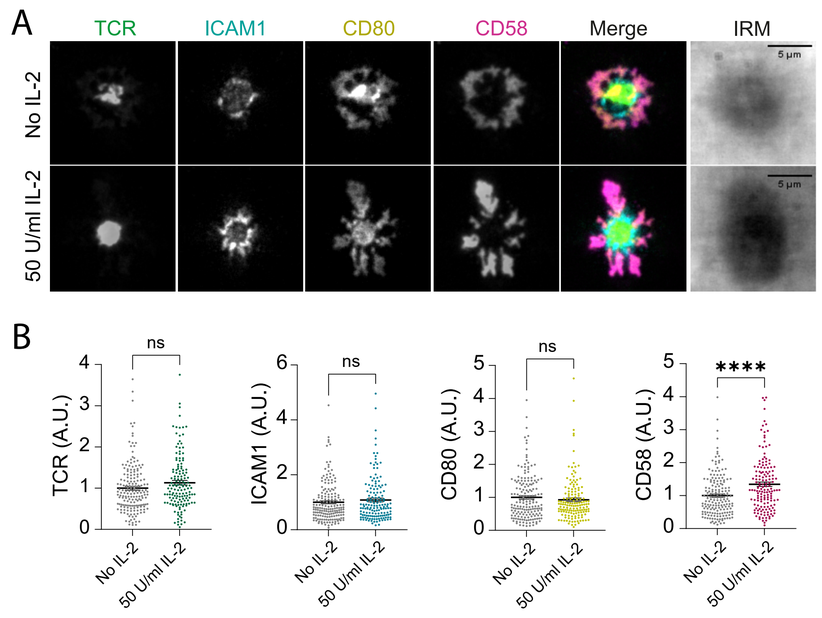


Figure 6. IL-2 effects on synapse architecture. (A) Representative TIRF images of Ag-specific CD8+ cells forming synapses with SLB containing ICAM1, pMHC, CD58 and CD80. Cells were fixed after 15 min of SLB exposure in the presence or absence of 50 U/ml of IL-2 and formation of SMAC compartments was analysed. **(B)** Quantification of the mean intensity values for the proteins on the bilayer, normalized by the control. Data is the mean +/- SE of >150 cells. ****p<0.0001.

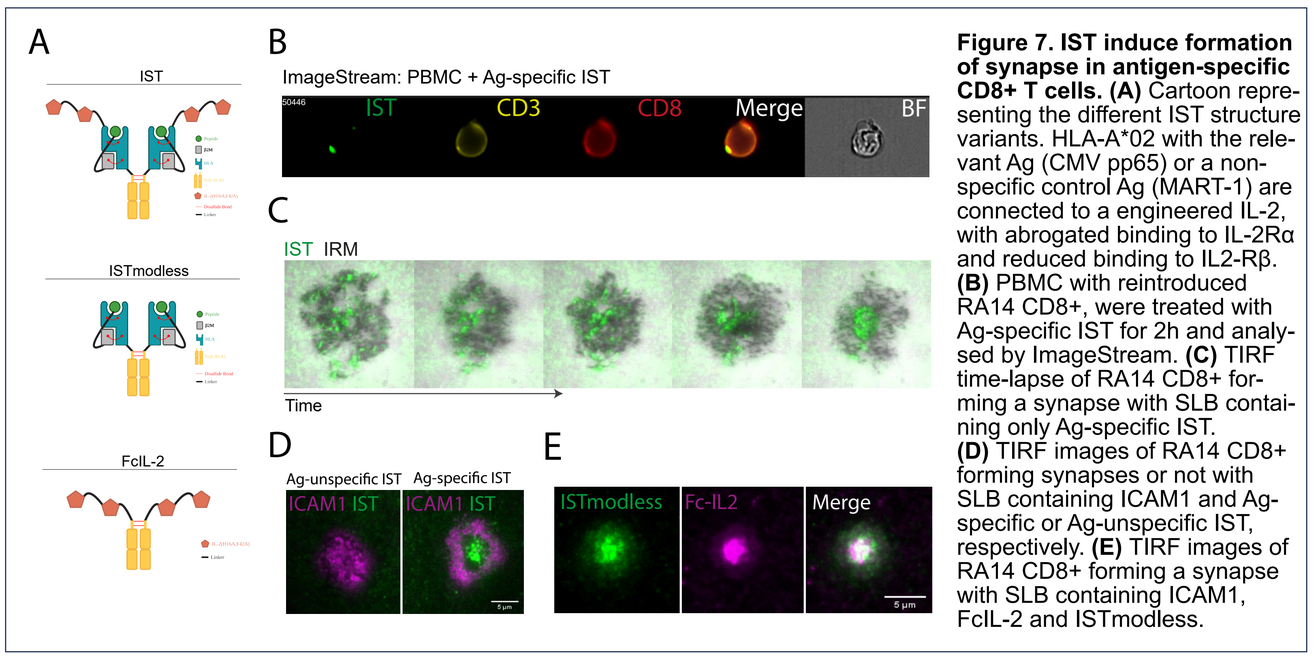


Figure 7. IST induce formation of synapse in antigen-specific CD8+ T cells. (A) Cartoon representing the different IST structure variants. HLA-A*02 with the relevant Ag (CMV pp65) or a non-specific control Ag (MART-1) are connected to a engineered IL-2, with abrogated binding to IL-2Rα and reduced binding to IL2-Rβ. **(B)** PBMC with reintroduced RA14 CD8+, were treated with Ag-specific IST for 2h and analysed by ImageStream. **(C)** TIRF time-lapse of RA14 CD8+ forming a synapse with SLB containing only Ag-specific IST. **(D)** TIRF images of RA14 CD8+ forming synapses or not with SLB containing ICAM1 and Ag-specific or Ag-unspecific IST, respectively. **(E)** TIRF images of RA14 CD8+ forming a synapse with SLB containing ICAM1, FcIL-2 and ISTmodless.

CONCLUSIONS AND FUTURE WORK

IL-2R subunits are recruited to the immunological synapse and show different patterns of distribution within the SMAC compartments. In the presence of free IL-2 in the media, receptor abundance is reduced and we observe internalization of the subunits within the first minutes of the synaptic contact. IL2-R signaling correlates in space and time with the TCR signaling at the synapse. We will use the IST therapeutics to study the effects of coupling TCR and IL2-R signaling at the synapse, as well as the implications of IL-2 trans-presentation.

Acknowledgements:
Cue Biopharma Post-doctoral Fellowship, KTRR Cell Dynamics Platform and Wellcome 100262Z/12/Z