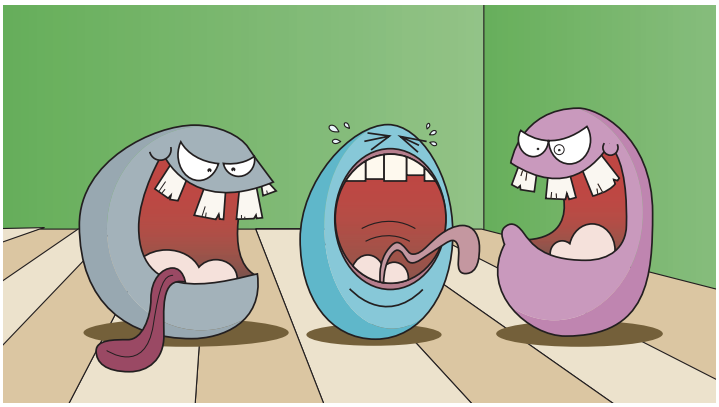
IMMUNOLOGICAL SYNAPSES

Increasing T-cell sensitivity with CD8



The T-cell receptor (TCR) co-receptor CD8 binds non-polymorphic regions of MHC class I molecules and increases signalling from antigen-specific TCRs bound to peptide–MHC complexes in the immunological synapse, but there have been conflicting reports regarding how this is achieved and whether the interaction of CD8 with the TCR–CD3 signalling complex is constitutive or induced by antigen recognition.

This study used microscopic evaluation of FRET (fluorescence resonance energy transfer) between CD8β–yellow fluorescent protein (CD8β–YFP) and CD3ζ–cyan fluorescent protein (CD3ζ–CFP) to look at the interactions between these chimeric proteins in living cells. CD3ζ–CFP and CD8β–YFP were co-expressed in the OT-I T-cell hybridoma, which recognizes ovalbumin peptide (OVA) bound to H2–K^b; the transgenic OT-I cells were then stimulated with antigen-presenting cells (APCs) expressing H2–K^b loaded with OVA or a peptide that is non-stimulatory for the OT-I TCR, such as a peptide derived from vesicular-stomatitis-virus nucleoprotein (VSV) or peptides derived from various endogenous proteins.

As expected, the percentage of transgenic OT-I cells forming conjugates with APCs was greater when the APCs presented OVA than when they presented VSV. However, of the T-cell–APC conjugates that did form, the increase in CD8 concentration in the synapse relative to the rest of the cell membrane was the same when the APCs presented either OVA or VSV. CD8β–YFP recruitment to the synapse, instead, depended on the concentration of H2–K^b at the cell surface of the APC. Therefore, recruitment of CD8 to the synapse is not antigen specific (in contrast to TCR–CD3 recruitment) but is driven by the CD8–MHC-class-I interaction.

The FRET signal between CD3ζ–CFP and CD8β–YFP, however, was only increased after stimulation with OVA-presenting, and not VSV-presenting, APCs. The basal level of CD3ζ–CFP in all synapses was sufficient to yield a FRET signal, indicating that the lack of FRET was not a result of insufficient CFP and that the association between CD8 and CD3ζ is determined by antigen specificity.

Despite the lack of association with TCR–CD3 complexes, CD8 that was recruited non-specifically to the synapse (by MHC class I molecules presenting VSV or non-stimulatory endogenous peptides) increased the T-cell response to OVA — in terms of TCR downregulation and FRET efficiency — when the OT-I cells were stimulated with APCs presenting both OVA and VSV. These data led the authors to propose a model in which the numerous endogenous peptide–MHC complexes at the surface of an APC interact with CD8 in an antigen-non-specific manner to concentrate CD8 and peptide–MHC complexes in the synapse, making a cognate peptide–MHC–TCR–CD3–CD8 interaction more likely and thereby increasing the sensitivity of detection.

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References and links

ORIGINAL RESEARCH PAPER Yachi, P. P., Ampudia, J., Gascoigne, N. R. J. & Zal, T. Nonstimulatory peptides contribute to antigen-induced CD8–T cell receptor interaction at the immunological synapse. *Nature Immunol.* **6**, 785–792 (2005)