SIGNALLING

Boosting cell signalling

The PIM family of serine/threonine kinases consists of at least three members that can cooperate with the *myc* or *bcl-2* oncogenes to induce the development of lymphoid malignancies. To date, however, the precise function and physiological substrates of PIM-1 have not been well characterized. Now, reporting in the *Journal of Immunology*, Koskinen and colleagues show that PIM-1 phosphorylates NFATc1 (nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1) and enhances NFAT-dependent transcription.

PIM-1 expression is upregulated during T-cell activation. When T cells are activated, AP-1 (induced by protein kinase C and Ras) and calcium-dependent signalling cause the dephosphorylation of NFATc, which translocates to the nucleus and, together with AP-1, activates genes such as interleukin-2 (*IL-2*).

The authors first looked at the role of PIM-1 kinase in signalling for NFAT activation. Jurkat T cells were transfected with a PIM-1 expression vector and a reporter construct containing NFAT binding sites. When the T cells were activated with phorbol 12-myristate 13-acetate and the calcium ionophore ionomycin, endogenous NFAT activity was enchanced by PIM-1. To assess whether PIM-1 targets NFATc in a physiological setting, Jurkat T cells were stimulated and the amount of IL-2 secreted into the medium was measured. PIM-1 transfection of stimulated Jurkat cells significantly enhanced IL-2 production compared with non-transfected cells. Kinase-deficient mutants of PIM-1 were unable to enhance NFAT activity or IL-2 production. Co-immunoprecipitation experiments showed that PIM-1 can physically interact with NFATc1 and is likely to be able to phosphorylate it *in vivo*.

This study provides the first evidence that a kinase can positively regulate NFAT activity — phosphorylation by other kinases inhibits NFATc translocation to the nucleus. The results suggest that PIM-1 acts downstream of Ras to induce phosphorylation of NFATc1 and IL-2 production in Jurkat T cells.

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References and links

ORIGINAL RESEARCH PAPER Rainio, E-M., Sandholm, J. & Koskinen, P. J. Transcriptional activity of NFATc1 is enhanced by the Pim-1 kinase. *J. Immunol.* **168**, 1524–1527 (2002).

WEB SITE

Päivi Koskinen's lab: http://www.utu.fi/research/receptor/projects/koskinen/koskinen.html

TECHNIQUE

Caught in the act

Peptide–MHC-class I tetramers have revolutionized our understanding of T-cell biology by allowing us to track antigen-specific $CD8^+$ T cells (also known as cytotoxic T lymphocytes, CTLs) during the course of an immune response. But does specificity always equate with function? To answer this, tetramers must be used in conjunction with assays of activity; however, currently only one arm of CTL function — cytokine production — can be assessed at the level of the single cell. But now, Luzheng Liu and co-workers at the Emory Vaccine Center have developed a new assay that allows cytotoxic killing to be detected at the single-cell level, in real time.

CTLs induce apoptosis of their targets, a process that takes several hours. The traditional CTL assay measures the bulk release of radioactivity from ^{51}Cr-labelled target cells when they eventually lyse — the end point of the cytotoxic process *in vitro*. But the new assay, which is described in the February issue of *Nature Medicine*, is based on the detection of a very early event in the induction of apoptosis — the activation of caspases.

In the new assay, effectors are incubated with target cells for several hours then a cell-permeable fluorogenic caspase substrate is added. Each synthetic substrate consists of a peptide conjugated to two fluorophores, but the close proximity of the fluorophores quenches fluorescence. When the substrates are cleaved by active cell caspases the products fluoresce and so the condemned target cell can be detected by flow cytometry and microscopy.

But how does the sensitivity of the new CTL assay compare with the ^{51}Cr-release assay? The authors performed the two assays in parallel using splenocytes from mice infected with lymphocytic choriomeningitis virus (LCMV) as effectors and targets pulsed with various LCMV peptides. The fluorescence-based assay was shown to be more sensitive than the ^{51}Cr-release assay, particularly at earlier time points.

A key advantage of the new assay is that the interaction between the target and CTL leading to the delivery of the lethal hit can be visualized directly, in real time, by fluorescence microscopy. In combination with peptide–MHC tetramer staining, this powerful new technique should lead to new insights into the killing process, as well as more accurate measurements of CTL responses.

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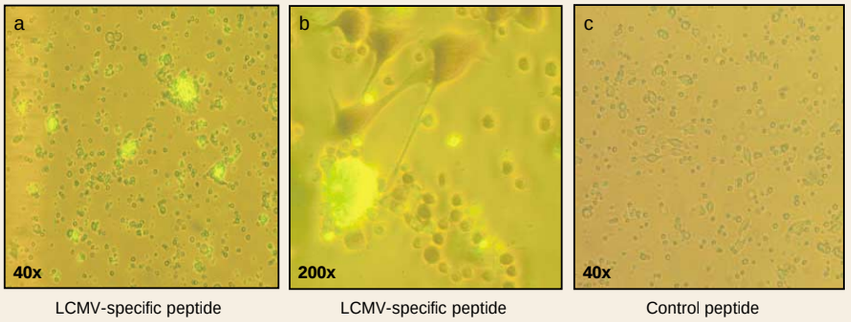

LCMV-specific peptide | LCMV-specific peptide | Control peptide

Microscope images of CTL-mediated killing of target cells detected using the new fluorescence-based assay.

References and links

ORIGINAL RESEARCH PAPER Liu, L. *et al*. Visualization and quantification of T cell mediated cytotoxicity using cell-permeable fluorogenic caspase substrates. *Nat. Med.* **8**, 185–189 (2002).