CELLULAR CYTOTOXICITY

CTL self-defence

The granule-exocytosis cytotoxicity pathway, by which cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells secrete perforin and granzymes to kill target cells, is crucial for host defence. But how do cytotoxic cells protect themselves from the damaging contents of their own granules? Work from Pierre Henkart's laboratory, published in *The Journal of Experimental Medicine,* indicates that granule-derived cathepsin B is crucial for the self-protection of cytotoxic lymphocytes.

Previous studies have indicated that cytotoxic lymphocytes have inherent resistance to cytotoxic mediators, including the pore-forming protein perforin, but the molecular basis for this is unknown. The authors of this study reasoned that a granule component would be a good candidate to mediate resistance as it could provide local protection during granule exocytosis. Cytotoxic granules contain perforin, granzymes (serine proteases) and lysosomal enzymes, including thiol cathepsin endoproteases, which can maintain their proteolytic activity in the extracellular environment. Before the perforin pore-forming complex is assembled, perforin passes through an intermediate membrane-associated stage that is highly susceptible to proteolysis. So, does a cathepsin that is expressed on the cytotoxic-cell surface after exocytosis cleave perforin and, in so doing, provide protection for effector cells?

To investigate this, Balaji *et al.* treated CTLs cultured on plate-bound anti-CD3 antibodies (to trigger degranulation) with cathepsin inhibitors. These drugs resulted in rapid T-cell suicide, which implies that cathepsins do participate in CTL self-protection. This CTL suicide does not require the Fas–FasL death pathway, as it occurred normally in CTLs from *gld* (FasL-mutant) mice, but it does require perforin, as it did not occur in CTLs from perforin-knockout mice.

Further experiments with membrane-impermeant cathepsin inhibitors and cathepsin-B-specific inhibitors showed that cathepsin-mediated protection of degranulating CTLs against perforin attack occurs in an extracellular location and that cathepsin B is required specifically. The authors also showed that CTLs express little surface cathepsin B before degranulation, but after T-cell-receptor triggering, the surface expression of this

NUCLEAR TRANSPORT

Two-way traffic

The Smad signalling pathway functions downstream of transforming growth factor (TGF)-β receptors. Receptor-regulated Smad proteins, Smad2 and Smad3, are phosphorylated after TGF-β stimulation, then each forms a complex with Smad4 and translocates to the nucleus. There, together with DNA-binding cofactors, they direct transcriptional activity. Now, the groups of Joan Massagué and Caroline Hill report new insights into the transport of Smads in and out of the nucleus.

Massagué and colleagues focused on Smad2 and showed that it binds directly to nucleoporins CAN/Nup214 and Nup153, which are located on the cytoplasmic and nuclear side of the nuclear pore complex, respectively. They could inhibit Smad2 nuclear import by adding an excess of a CAN/Nup214 fragment that contains the Smad2-binding domain, as well as by using Smad2 mutants with reduced affinity for CAN/Nup214. This indicated to the authors that direct contact with CAN/Nup214 is needed for Smad2 nuclear import.

On the other hand, both nucleoporins might be required for nuclear export, because Smad2 mutants defective in Nup153 and/or CAN/Nup214 binding inhibited this process. The authors propose that CAN/Nup214 and Nup153 act as docking sites for nuclear import and export, respectively.

Using a nucleocytoplasmic shuttling assay, Massagué and co-workers also showed that Smad DNA-binding factor FAST-1 and cytoplasmic retention factor SARA compete with the nucleoporins for Smad2 binding and therefore inhibit nucleocytoplasmic shuttling. TGF-β signalling also inhibits nucleocytoplasmic shuttling, which can be explained by the finding that Smad2 phosphorylation causes a decrease in its affinity for SARA and an increase in its affinity for Smad4. The authors propose that, in unstimulated cells, Smad2 undergoes continuous nucleocytoplasmic shuttling, and that TGF-β-induced Smad2 phosphorylation causes retention of Smad2 in the nucleus by decreasing its affinity for SARA in favour of Smad4.

Hill and colleagues complemented this work by asking what happens to endogenous Smads during active TGF-β signalling. Using an inhibitor that specifically blocks continuous TGF-β receptor signalling, they showed that receptors remain active for at least 3–4 hours after TGF-β stimulation, and that continuous receptor activity is necessary for optimal transcription. However, accumulation of phosphorylated Smad2 and 3 in the nucleus is complete in 30 minutes. So, how do the active receptors influence Smads once they are in the nucleus? And why is continuous receptor activity needed?

To address the first question, the authors used immunofluorescence and showed that after TGF-β stimulation, rather than remaining statically in the nucleus, Smads cycle continuously between the cytoplasm and the nucleus. The requirement for continuous receptor activity became obvious when Hill and co-workers found that Smad2 and 3 are dephosphorylated and dissociated from Smad4 before returning to the cytoplasm. In the cytoplasm, Smad2 and 3 are re-phosphorylated by the active receptors, form complexes with Smad4 and re-enter the nucleus. So, the shuttling capacity of Smad2 and 3 allows continuous monitoring of receptor activity by the signal-transducing Smads.

The model that Hill proposes features a prominent role for a nuclear phosphatase that allows nuclear exit of Smad2 and 3. The race is now on to identify this nuclear phosphatase.

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References and links

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