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CELLULAR CYTOTOXICITY

Complex killing

In the textbook model of cytotoxic killing, granules that contain perforin and granzymes are exocytosed into the intercellular space between effector and target; perforin then polymerizes in the target-cell plasma membrane to form a pore that allows the entry of granzymes, which set the cell-death programmes in motion. The first indication that this might not be the whole story came in 1996, when Christopher Froelich and colleagues showed that granzyme B can be internalized by receptor-mediated endocytosis and then released from endosomes by a perforin-dependent mechanism, in which pores in the plasma membrane that are internalized with the granzymes release the proteases into the cytosol. However, recent investigations from the same group, published in *Immunity*, show unexpectedly that perforin can release granzyme B independent of pore formation — which indicates a completely new model of granzyme delivery.

In this new study, cells that were incubated with concentrations of perforin that were too low for pore formation were still susceptible to granzyme-B-mediated apoptosis. This was the first indication that perforin can deliver granzymes independent of pore formation. Subsequently, immunoprecipitation studies showed that granzyme B exists within granules as a complex with perforin and serglycin (a granule proteoglycan). Exocytosed granzyme B was found to exist solely in macromolecular

complexes with serglycin, with multiple granzyme molecules interacting with a single serglycin molecule. Released perforin, however, exists in both monomeric and serglycin-associated forms.

Together, these data indicated that the pore-independent route might involve the delivery of macromolecular serglycin-granzyme complexes to the cytosol by perforin monomers or serglycin-perforin complexes. But, this needed to be tested — after all, proteoglycans have been shown to inhibit the activity of perforin. First, the activity of macromolecular granzyme B was examined. Although *in vitro* proteolysis of caspase-3 by serglycin-granzyme-B was reduced compared with proteolysis by granzyme B alone, the macromolecular form induced apoptosis more effectively when delivered by perforin or a known endosomolytic agent. Similarly, whereas free perforin was found to form pores more readily, serglycin-perforin was better at delivering macromolecular granzyme B and inducing apoptosis.

On the basis of these findings, the authors propose a new mechanism of granzyme entry that bears a close resemblance to the way in which viruses enter cells (see diagram). After granule exocytosis, cells are exposed to serglycin-granzyme-B-perforin complexes, or serglycin-granzyme-B complexes together with free perforin. Perforin then mediates the release of granzymes from the endosome by disrupting the endosomal membrane. The precise mechanism by which perforin mediates this endosomolytic effect is a key outstanding issue.

Jennifer Bell

References and links

ORIGINAL RESEARCH PAPER Metkar, S. S. *et al.* Cytotoxic cell granule-mediated apoptosis: perforin delivers granzyme-B-serglycin complexes into target cells without plasma membrane pore formation. *Immunity* **16**, 417–428 (2002)

WEB SITE

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