Endocytosing the death sentence

Gillian M. Griffiths

Sir William Dunn School of Medicine, Oxford OX1 3RE, United Kingdom

A series of recent studies have suggested that endocytosis of the mannose-6-phosphate receptor (MPR)* might play a critical role in delivering the death signal to cells targeted for destruction by the immune system (for review see Barry and Bleackley, 2002). These studies have raised a number of controversial issues regarding the trafficking of proteins from the plasma membrane of the target cell to their substrates in the cytosol. In this issue, Trapani and colleagues examine the death of cells in which endocytosis of the MPR is blocked and show that the death signal is delivered effectively in the absence of MPR endocytosis (Trapani et al., 2002, this issue). How then is the death sentence delivered?

The immune system clears viral infections and tumorigenic cells by regulated secretion of soluble proteins leading to rapid apoptosis of the targets. This “lethal hit” is delivered by either cytotoxic T lymphocytes (CTLs) or natural killer (NK) cells, both of which undergo regulated secretion of specialized lysosomes containing the proteins required to initiate cell death. The key soluble proteins in this pathway are the serine protease granzyme B, which cleaves substrates in the cytosol of the target (initiating apoptosis), and the pore-forming protein perforin, which is required to deliver granzyme B to the target cell cytosol.

When perforin was initially identified in the 1980s, it was found to bear a high degree of similarity to the pore-forming C9 component of complement and, like the membrane attack complex, perforin was shown to be able to insert into lipid bilayers and form 15-nm diameter pores in membranes (for review see Lowin et al., 1995). These findings led to a model of cell mediated lysis involving the formation of a perforin pore at the plasma membrane through which C9 component of complement and, like the membrane attack complex, perforin was shown to be able to insert into lipid bilayers and form 15-nm diameter pores in membranes. This model of cell mediated lysis involving the formation of a perforin pore at the plasma membrane through which endocytosis of the MPR is blocked and show that the death signal is delivered effectively in the absence of MPR endocytosis (Trapani et al., 2002, this issue). How then is the death sentence delivered?

The paper by Trapani and colleagues addresses the role of MPR and the pool of endocytosed granzyme B by using expression of dominant–negative dynamin mutants to block endocytosis. Their results are clear. Target cells are killed equally well whether endocytosis of granzyme B by the MPR is blocked or not, demonstrating that endocytosis by MPR is not necessary for target cell death, and the large pool of granzyme B, which can enter the endocytic pathway, need not play a role in this process. Additionally, the authors reexamine what was until now the most convincing evidence in favor of a role for MPR in target cell death, namely that MPR-overexpressing cells were more rapidly rejected than cells lacking MPR after allotransplantation (Motyka et al., 2000). This study shows that both cell types are completely eradicated. Surprisingly, the same rejections were observed in perforin-deficient mice, demonstrating that cell death was not occurring via the perforin/granzyme-mediated pathway, but rather by antibody-mediated responses, in part against the overexpressed human MPR.

Is there then any role for MPR in the uptake of granzyme B and the delivery of the apoptosis signal? Trapani et al. note that some granzyme B can be taken up into the cell via micropinocytosis and do not completely rule out a role for release of granzyme B from this pathway. But it is also worth outlining the other reasons that MPR binds granzyme B, as well as the current gaps in any argument requiring endosomal disruption as a means of delivering granzyme B to the cytosol. Newly synthesized granzyme B, like lysosomal hydrolases, is sorted to the secretory lysosomes of CTLs and NK cells via the MPR (Griffiths and Isaaz, 1993). Like many of the lysosomal hydrolases, some of the newly synthesized granzyme B is secreted constitutively and can then be taken up by MPR on the cell surface and targeted to the lysosomes by endocy-
The Journal of Cell Biology

156 The Journal of Cell Biology

2001), it is entirely possible that the local concentrations of perforin at this point are indeed very high. One of the problems with studying precisely how these proteins are delivered to the target cell remains the impressive potency of this pathway. Studies on live cell killing demonstrate that very few granules need be secreted in order to destroy a target (Lyubchenko et al., 2001; Stinchcombe et al., 2001), making the task of the cell biologist wishing to follow the pathway of these proteins truly challenging.

Submitted: 21 December 2002
Accepted: 23 December 2002

References


