IN BRIEF

HAEMATOPOIESIS

A stromal-cell derived membrane protein that supports haematopoietic stem cells.

Ueno, H. *et al. Nature Immunol.* 31 March 2003 (doi:10.1038/ni916)

The bone-marrow microenvironment has a crucial supporting role in the growth, differentiation and survivial of haematopoietic stem cells (HSCs). Without bone-marrow stromal cells, HSCs cannot be maintained *in vitro*, even when cultured with a cocktail of growth factors. In this study, a retroviral-based signal-sequence trap method identified a new candidate HSC-supporting membrane protein, which the authors have named Kirre owing to its homology with the *Drosophila* myoblast attractant factor *kirre*. Kirre accumulated in the areas of contact between HSCs and stromal cells in culture, and abrogating expression of Kirre using small interfering RNA (siRNA) inhibited the capacity of bone-marrow stromal cells to support HSC proliferation *in vitro*. So, the authors propose that Kirre functions to maintain HSCs in an undifferentiated, proliferative state.

CELL DEATH AND IMMUNITY

Cell death induced by granzyme C.

Johnson, H. *et al. Blood* **101**, 3093–3101 (2003)

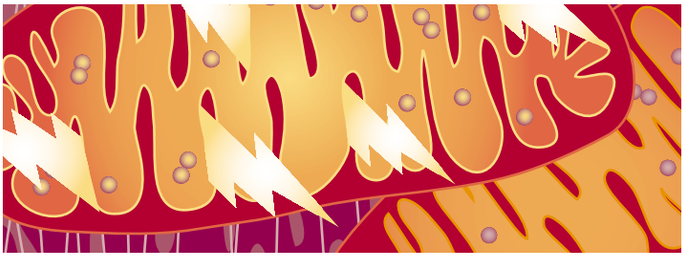
Although much is known about the functions of granzymes A and B, granzyme C has been less well studied. Here, Johnson *et al.* show that mouse granzyme C (which is closely related to human granzyme H) causes cell death with similar kinetics to granzyme B and is of equal potency. Cell death that is induced by granzyme C required its protease activity, resulted in externalization of phosphatidylserine, nuclear condensation and single-stranded but not double-stranded DNA nicking. Granzyme C caused swelling and depolarization of mitochondria, and death induced by this granzyme did not involve caspase activation, cleavage of BID (BH3-interacting domain death agonist) or activation of the CAD nuclease.

T-CELL SIGNALLING

SWAP-70-like adapter of T cells, an adapter protein that regulates early TCR-initiated signaling in T_H2 lineage cells.

Tanaka, Y. *et al. Immunity* **18**, 403–414 (2003)

Here, Tanaka *et al.* describe the identification of SWAP70-like adaptor of T cells (SLAT), a protein that is selectively expressed at high levels by T helper 2 (T_H2) cells but not T_H1 cells. SLAT positively regulated IL-4 expression and negatively regulated IFN-γ expression. After antigen stimulation of the T-cell receptor (TCR) SLAT translocated to the immunological synapse, where it associated with the tyrosine kinase ZAP70. Transient overexpression of SLAT caused reduced association of ZAP70 with TCRζ and interfered with ZAP70, but not LCK, signalling. This study indicates a role for SLAT in the differentiation, activation and/or expansion of T_H2 cells, but further studies are required to elucidate the mechanisms by which it acts.



CELL DEATH AND IMMUNITY

Mitochondria take centre stage

How does granzyme B activate caspases and lead to the destruction of virus-infected or transformed cells? This is clearly an area of considerable interest at present — and some controversy — as in addition to *The Journal of Cell Biology* paper described on page 355, work from the labs of Chris Bleackley and Joe Trapani has also just been published on this subject in *Immunity*.

Cytotoxic lymphocytes mainly destroy target cells by exocytosis of the contents of secretory granules, which include serine proteases, such as granzyme B, and the pore-forming protein perforin. The enzymatic activity of granzyme B is thought to be central to its ability to induce cell death through the activation of caspases, but how it does this is controversial. Previous work has shown that over-expression of BCL-2 — an anti-apoptotic protein, which acts by blocking death effectors that target mitochondria — protects cells against granzyme-B-mediated destruction. This indicates that granzyme B is involved not only in the direct activation of caspases, including caspase-3, but also in the mitochondrial death pathway. Until now, the molecular mechanisms for this were not known.

To investigate this further, Bleackley and colleagues analysed the caspase-3 cleavage profile of BCL-2-overexpressing cells after treatment with granzyme B and adenovirus, which functions as a pore-forming protein to facilitate the release of granzyme B into the target cell. Caspase-3 activation occurs in two steps: an initial cleavage event, followed by autoactivation that is controlled by the self-catalytic activity of the caspase. In BCL-2-overexpressing cells, only partial cleavage of caspase-3 occurred, showing that high levels of BCL-2 prevented subsequent caspase autoactivation.

The authors reasoned that inhibitor of apoptosis proteins (IAPs) might be responsible for this block in caspase activation, and so they tested the effect of overexpressing XIAP on granzyme-induced cell death. Caspase-3 activation was blocked in cells overexpressing XIAP and these cells were protected against the toxic effects of granzyme B. Expression of SMAC/DIABLO, a mitochondrial protein that is released from apoptotic cells and has been shown to displace IAPs from pro-caspases and promote caspase autoactivation, overcame this blockade in both the XIAP-overexpressing cells and BCL-2-overexpressing cells and rendered them susceptible to granzyme-B-mediated destruction.

Data from Trapani and colleagues highlight the importance of the mitochondrial pathway in granzyme-B-induced apoptosis. They showed that the pro-apoptotic BH3-interacting domain protein BID is cleaved by granzyme B and then translocates to the mitochondria. Exposure of Jurkat cells to granzyme B and perforin resulted in release of the pro-apoptotic factors cytochrome *c*, SMAC/DIABLO and HtrA2/OMI from mitochondria. BCL-2 overexpression blocked the release of these pro-apoptotic factors and blocked caspase autoactivation, which resulted in inhibition of granzyme-B-mediated apoptosis of these cells. As above, overexpression of XIAP also resulted in the inhibition of caspase-3 autoactivation and protected these cells from the toxic effects of granzyme B.

These results indicate the importance of the mitochondrial pathway in granzyme-B-mediated cell death. Although granzyme B can directly cause the initial cleavage of caspase-3, for full caspase activation, pro-apoptotic mitochondrial proteins, including SMAC/DIABLO and HtrA2/OMI, are required to block the effects of IAPs, thereby allowing granzyme-mediated apoptosis to occur.

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References and links

ORIGINAL RESEARCH PAPERS Sutton, V. R. *et al.* Caspase activation by granzyme B is indirect, and caspase autoprocessing requires the release of proapoptotic mitochondrial factors. *Immunity* **18**, 319–329 (2003) | Goping, I. S. *et al.* Granzyme B-induced apoptosis requires both direct caspase activation and relief of caspase inhibition. *Immunity* **18**, 355–365 (2003)

FURTHER READING Lieberman, J. The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal. *Nature Rev. Immunol.* **3**, 361–370 (2003)