APOPTOSIS

Death theory turned on its head

Apoptosis can be initiated in many ways — two of which are induced by cytokines or stress. Both rely on the activity of caspases; proteases that signal the cell's downfall. Although the final curtain is drawn on the cell in both scenarios, the performance has always been thought to be quite different for the two cases. That was, until a report by Lazebnik's group in *Science*, which implies that both modes of apoptosis can proceed through conceptually similar pathways.

Stress-induced death, which is mediated by stimuli such as DNA damage, depends on the outcome of the balance of activities of BCL-2-family members on mitochondrial integrity; permeabilization of the mitochondrial membrane releases proteins such as cytochrome *c* that can act together with APAF1 to activate caspase-9, as well as other caspase-independent executioners. In this case, mitochondria take on a central role. In cytokine-induced death, however, mitochondria act more passively as 'amplifiers' — caspase activation results from autocatalytic processing, and mitochondria serve merely to amplify the caspase signal. However, data from Lazebnik's group imply that DNA damage induces caspases to control mitochondrial permeability, rather than vice versa.

They chose to study caspase-2 in IMR90E1A cells (cells transformed with the E1A oncogene, which facilitates the activation of caspase-9). Using small interfering RNA (siRNA), they ablated the function of caspase-2 and, to their surprise, found that this inhibited DNA-damage-induced apoptosis to a similar degree as silencing of APAF1 function. As this pointed to a role for caspase-2 in apoptosis, the authors restored the function of caspase-2 to see whether this restored sensitivity to apoptosis by cytotoxic agents. To prevent the ectopically expressed caspase-2 from being destroyed by siRNA, silent mutations — that is, changes in the nucleotide sequence that do not affect the amino acid that is produced — were introduced. Consistent with the highly specific nature of siRNA, expression of this construct was not silenced, and the result was that wild-type caspase-2, but not a proteolytically impaired version, restored sensitivity to apoptosis.

Looking more closely at the mitochondrial aspect, Lazebnik's group saw that, in the absence of caspase-2 expression, cytochrome *c* was not released from mitochondria. Furthermore, even in the absence of proteolytic processing of caspases 3, 7 and 9, caspase-2 was still cleaved, indicating that it is probably activated before them, and that, in these cells at least, it is needed to permeabilize mitochondria. Extending the analysis to five other human tumour cell lines showed that two of these required caspase-2 for cytochrome *c* release, one did not, and two others could not be evaluated due to technical difficulties.

Taking a step further back in the pathway to look at the earliest detectable change, treatment with caspase-2 siRNA prevented the translocation of the BCL-2-family member BAX from the cytoplasm to the mitochondria. This indicates that a crucial function of caspase-2 is to move BAX to mitochondria, presumably inducing subsequent mitochondrial permeabilization.

The implications of this study are widespread — from the possibility that BCL-2-family members sequester molecules that are required for the activation of caspases, to helping us to understand how BAX is activated. And importantly, this study highlights that "even the basic pathways of apoptosis are not sufficiently understood to allow the efficient modulation of apoptosis to a therapeutic end".

Katrin Bussell
Associate Editor,
Nature Reviews Molecular Cell Biology

References and links

ORIGINAL RESEARCH PAPER Lassus, P., Opitz-Araya, X. & Lazebnik, Y. Requirement for caspase-2 in stress-induced apoptosis before mitochondrial permeabilization. *Science* **297**, 1352–1354 (2002)

WEB SITE

Encyclopedia of Life Sciences: http://www.els.net

Apoptosis: molecular mechanisms

IN BRIEF

ANTIGEN PRESENTATION

Granulocyte–macrophage colony-stimulating factor induces an expression program in neonatal microglia that primes them for antigen presentation.

Re, F. *et al. J. Immunol.* **169**, 2264–2273 (2002)

Only a subset of microglial cells in the brain are replenished by the migration of monocytes across the blood–brain barrier. In the brain, microglial differentiation is influenced by cytokines released by astrocytes and neurochemicals released by neurons. Macrophage colony-stimulating factor (M-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) are two cytokines that differentially regulate microglial differentiation. In this study, Re *et al.* assessed gene expression after stimulation of microglia with M-CSF or GM-CSF. Treatment of primary mouse microglia *in vitro* with M-CSF induced the expression of genes that function in tissue organization and remodelling, and in brain homeostasis, whereas treatment with GM-CSF induced the expression of genes that prepare microglia for antigen presentation — such as MHC class II molecules, cathepsins and chemokines — and T-cell stimulation.

T-CELL RESPONSES

Profound defect in T-cell responses in TNF-receptor-associated factor 2 dominant-negative mice.

Cannons, J. L. *et al. J. Immunol.* **169**, 2828–2831 (2002)

Tumour-necrosis factor receptor (TNFR)-associated factor 2 (TRAF2) is an adaptor protein that links members of the TNFR family, including OX40, CD27 and 4-1BB, to downstream signalling pathways. In this study, the authors used TRAF2 dominant-negative (TRAF2.DN) mice, which had been generated previously, to investigate the role of TRAF2-linked receptors in T-cell responses. TRAF2.DN T cells had impaired responses in mixed lymphocyte reactions. In addition, TRAF2.DN mice had defective $CD4^+$ and $CD8^+$ T-cell responses to influenza virus. These results indicate that TRAF2-linked receptors have an important role in secondary T-cell responses.

T-CELL SIGNALLING

Wiskott-Aldrich syndrome protein regulates lipid raft dynamics during immunological synapse formation.

Dupre, L. *et al. Immunity* **17**, 157–166 (2002)

Wiskott-Aldrich syndrome (WAS) is an X-linked immunodeficiency that results from mutations in the Wiskott-Aldrich syndrome protein (WASp). T-cell receptor (TCR) signalling and T-cell proliferation is defective in patients with this syndrome, but the underlying mechanism of these defects is unknown. Dupre *et al.* now show that within seconds of TCR–CD28 activation, WASp is recruited to lipid rafts in normal T cells. Studies with T cells from WAS patients showed that WASp is required for efficient T-cell proliferation, lipid-raft clustering and upregulation of the lipid-raft marker GM1 at the cell surface after TCR–CD28 stimulation. These data indicate that WASp regulates lipid-raft dynamics during T-cell activation, and thereby immunological-synapse formation.