

## Meeting Report

## Charming to death: caspase-dependent or -independent?

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**The 10th Euroconference of the European Cell Death Organization (ECDO): Paris, October 11–13, 2002.**

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The 10th Euroconference of the European Cell Death Organization (ECDO), coorganized with the Pasteur Institute and the Institut Gustave Roussy, took place in Paris from October 11–13, 2002. One leitmotif of this conference was the debate to which extent cell death depends on the activation of caspases.

In his impressive keynote lecture, Peter Krammer informed the audience that the European Commission decided that Cell Death would not be a prioritized area of research in Europe during the next 4 years, a fact that—although met by general incredulity—may determine Europe's decadence in yet another key area of biomedical research. Peter Krammer insisted on the role of death receptors in a model of murine stroke, in which the simultaneous activation of the CD95/CD95 ligand system (by the *gld* mutation) and the TNF/TNF-receptor system (by knockout of the *tnf* gene) has a major positive effect on animal survival and health, involving either a cell-autonomous effect on neuronal cell survival or a reduction of deleterious lymphoid infiltration into the partially damaged brain areas. In septic patients, both the spontaneous and the TAR-triggered apoptosis of circulating T lymphocytes were found to be strongly increased, and this death was found to be caspase-independent, *ex vivo*. In strict contrast, Don Nicholson reported that caspase inhibition by newly developed caspase inhibitors may have a major life-preserving effect in an animal model of septic shock, a finding that suggests that the proinflammatory function and/or the proapoptotic function of caspases may have a major role in the pathogenesis of the cytokine overproduction syndrome leading to massive cell death of immune, endothelial and epithelial cells in sepsis. Don Nicholson insisted on the observation that caspase inhibitors have differential half-effective doses on distinct apoptosis characteristics such as the cleavage of defined caspase substrates and DNA degradation, implying that very high doses of caspase inhibitors must be used to justify the conclusion that cell death is caspase-independent. He also presented sequence data on the human caspase-12 gene,

indicating that, at least in Caucasians, this gene is inactivated because of a Stop mutation. He concluded that caspase-12 cannot have the same function as a sensor for ER stress and neuronal damage in the human system as in the murine system. This interpretation was contested by Junjin Yuan, who suggested that the true functional human caspase-12 remains to be discovered. Don Nicholson showed that several caspase cleavage products, including caspase-digested XIAP and  $\beta$ -amyloid, exhibit structural similarity with the N-terminus of Smac/DIABLO and may neutralize caspase-inhibitory IAPs, thereby deactivating and accelerating caspase activation. In some cases, activation of noncaspase proteases may be important for the stimulation of the caspase cascade, as reported by Hans-Uwe Simon, who showed that, in neutrophil granulocytes, calpain-1 activation may be critical for the activation of Bax, mitochondrial permeabilization, and consequent caspase activation.

Several speakers concentrated on the mechanisms of the formation of the death-inducing signaling complex (DISC) formed after ligation of death receptors. Milton Werner suggested that both the death domains (DD) and the death effector domains (DED) of FADD engage in the interaction with CD95. Olivier Micheau suggested that TNFR1 signaling involves the production of two different DISCs. The primary complex containing RIP, TRAF2 and TRADD would remain membrane-associated and would activate antiapoptotic NF- $\kappa$ B activation. The proapoptotic secondary complex would be formed upon internalization and/or dissociation of the primary complex from the receptor, accompanied by the association with FADD, caspase-8, -10, and FLIP. The long form of FLIP can actually enhance caspase-8 activation and the caspase-8-mediated cleavage of RIP at the Disc.<sup>1</sup> Patrick Mehlen insisted on the importance of caspases-9 and -3, which are recruited to a receptor-proximal complex when the 'dependency receptors' DCC and UNC5H, two tumor suppressor proteins involved in axon guidance, are not ligated by netrin.

Shigekazu Nagata emphasized the physiological importance of DNases, in particular the cell-autonomous caspase-activated DNase (CAD) and the lysosomal DNase II expressed in phagocytes, for the degradation of DNA in apoptotic cells. Simultaneous inactivation of these two DNases in *Drosophila* leads to activation of the innate immunity, with upregulation of antifungal peptides,<sup>2</sup> suggesting that correct digestion of DNA from apoptotic cells—although irrelevant for cell death by itself—may be important for the maintenance of self-tolerance and/or for the down regulation of inflammatory and immune responses. Nagata insisted also on the importance of the removal of phosphatidyl serine (PS)-exposing cells by lactadherin, a protein that serves as a bridge between PS on the surface of the apoptotic cell and  $\alpha_v\beta_3$  and  $\alpha_v\beta_5$  integrins on macrophages and immature dendritic cells. Sten Orrenius suggested that the post mitochondrial ATP depletion (as opposed to caspase activation) may be responsible for a reduced aminophospholipid translocase activity and consequent PS exposure and that PS, once present on the plasma membrane surface, would be rapidly oxidized.<sup>3</sup> Oxidized PS stimulates phagocytes better than nonoxidized PS. PS exposure may involve the ATP-binding-cassette transporter 1 (ABC1) (Giovanna Chimini). Importantly, liposomes containing physiological PS (which is the L enantiomer), but not control liposomes containing the nonphysiological D stereoisomer, have an immunosuppressive effect *in vivo*, suggesting that engagement of the PS receptor may modulate the immune response in a negative fashion (Valerie Fadok). Indeed, LPS-induced pulmonary inflammation is suppressed by the instillation of PS-exposing apoptotic cells or liposomes. Conversely, masking PS by Annexin V can enhance the immunogenicity of dying tumor cells injected into mice. This deviates the clearance of apoptotic cells from scavenger macrophages to plasmacytoid dendritic cells, with an enhanced production of the inflammatory cytokine TNF- $\alpha$  and a reduced production of the inhibitory cytokines IL-10 and TGF- $\beta$  (Patricia Rovere-Querini).

Junjin Yuan presented a chemical genetic approach to ER stress. By screening a compound library for agents capable of neutralizing tunicamycin-induced killing, she identified an agent, 22P19, which prevents cell death induced by ER stress including brefeldin A, yet is not a general apoptosis inhibitor. This compound apparently can induce an ER-protective reaction, leading to the phosphorylation of eIF $\alpha$  and an upregulation of CHOP and GADD34. Rosario Rizzuto emphasized the role of Bcl-2 as regulator of apoptosis acting, at least in part, at the level of the ER. By increasing the leakage of ER to Ca<sup>2+</sup>, Bcl-2 may reduce the ER Ca<sup>2+</sup> steady-state level, resulting in reduced Ca<sup>2+</sup> spikes upon activation of the IP<sub>3</sub>-gated ER Ca<sup>2+</sup> channels and reduced Ca<sup>2+</sup>-elicited mitochondrial membrane permeabilization, especially in the context of additional stimuli such as ceramide. According to György Hajnócsky, ceramide stimulates the phosphatase PP2a, thereby resulting in the dephosphorylation and mitochondrial translocation of the BH3-only protein BAD. This would potentiate the Ca<sup>2+</sup>-induced, caspase-independent  $\Delta\Psi_m$  disruption and cytochrome *c* release from mitochondria. Ehmadi Alnemri reported the identification of a novel IAP inhibitor that, at difference with Smac/DIABLO and Omi/

HtrA2, does not reside in mitochondria and rather associates with ribosomes. This IAP inhibitor is the processed form of G to S phase transition protein-1 (GSTP1), also called peptide chain release factor-3 (eRF3), and can sensitize to ER stress-induced caspase activation and cell death.

Caspase-independent cell death may involve lysosomes and lysosomal proteases, in particular cathepsins. Marja Jäättelä insisted on the observation that HSP70-like proteins may stabilize lysosomes, perhaps through a physical interaction with the vacuolar H<sup>+</sup>-ATPase. Depletion of Hsp70 with an antisense approach selectively kills tumors while not affecting the survival of nontransformed cells, both *in vitro* and *in vivo*. Selective tumor killing may be explained by an increased vulnerability of tumor lysosomes to permeabilization. In addition, it appears that at least some cancer cell lines express Hsp-A2, a gene from the Hsp70 family which is normally testis-specific. siRNA of Hsp-A2 causes flattening of HeLa or MCF-7 cells with G2/M arrest, suggesting that Hsp-A2 may be an essential tumor protein. Adi Kimchi, showed that DAP-kinase and (presumably downstream of DAP-kinase) DRP-1 can induce membrane blebbing and autophagy in a caspase-independent fashion.<sup>4</sup> A number of stimuli would activate this pathway, in particular antiestrogens (in breast cancer cells), amino-acid starvation and interferon- $\gamma$ . Ceramide causes a reduction of the inhibitory autophosphorylation of DAP kinase, thereby enhancing its pro-death activity. Alicia Tolkovsky showed that sympathetic neurons, once axotomized, deprived of growth factor, and cultured in the presence of the pan-caspase inhibitor Boc-Aspartyl(0-methyl)CH<sub>2</sub>F, die from 'limoktonia', that is macro autophagy-driven destruction of mitochondria. The autophagic removal of mitochondria and consequent cell death are impaired in Bcl-2-overexpressing or Bax-/- neurons. Francesca Doonan showed that photoreceptor apoptosis occurs in a caspase-independent fashion, without cytochrome *c* release in two rodent models of retinitis pigmentosa, contrasting with a model of retinal-detachment photoreceptor apoptosis that is also caspase-independent, yet manifest mitochondrial cytochrome *c* release.<sup>5</sup> Isabelle Lan-Rollin insisted on the possibility that caspase-independent death of rat embryonic cortical neurons induced by camptothecin would not involve AIF (in contrast to a mouse model involving Apaf-1-/- cortical neurons)<sup>6</sup> and rather may be owing to disruption of ATP generation.

Ehmadi Alnemri suggested that the ancient caspase-independent pathway involves the mitochondrial proteins Omi/HtrA2, EndoG, and AIF. Among these proteins, Omi requires trimerization to be active as a proapoptotic serine protease. Ehmadi Alnemri speculated that the caspase activation pathways and caspase themselves, in evolutionary terms, might constitute a relatively recent addition to cell death regulation. Jürg Tschopp suggested that caspase may have evolved to modulate stress or danger signals rather than to induce cell death. To underline this notion, he reminded that caspase activation may be required for positive signals through the T-cell receptor. As a further example of 'positive' effects of caspase activation, he reported the fact that TLR-4 ligation by CD14/LPS can cause the activation of both caspase-1 and -5, within the 'inflammasome',<sup>7</sup> which involves the adapter proteins NALD1 and Pycard/Asc. Cells that have

activated the 'inflammasome' secrete active caspase-1 and -5, in addition to interleukin-1 $\beta$ , thereby preventing these caspases from activating the apoptotic machinery.

Gilles Courtois reported an animal model for incontinentia pigmenti (IP), an inflammatory dermatosis resulting from a mutation in NEMO (also called IKK- $\gamma$ ) the regulatory subunit of IKK. Female mice in which one (X-chromosomal) NEMO gene has been inactivated manifest a similar pathology as patients with IP. A skin-specific knockout of IKK2 also results in a postnatal dermatosis and inflammation, linked to an increased keratinocyte proliferation that is not cell-autonomous and rather appears to be secondary to inflammatory reactions, as indicated by the fact that TNF-R1<sup>-/-</sup> mice fail to develop the dermatosis. Carmen Garrido showed that Hsp27 can interact both with phosphorylated I- $\kappa$ B $\alpha$  and with proteasome components such as PA700, resulting in enhanced degradation of ubiquitinated proteins including I- $\kappa$ B $\alpha$ , enhanced NF- $\kappa$ B activation and reduced apoptosis. This may contribute to the antiapoptotic, oncogenic effect of Hsp27.<sup>8</sup>

Several authors insisted on the link between infection and activation of mitochondrial membrane permeabilization (MMP).<sup>9</sup> Thus, Thomas Meyer showed that porin B from pathogenic *Neisseria* species causes MMP. In contrast, vMIA from human cytomegalovirus (CMV) inhibits apoptosis via a direct effect on mitochondria (Victor Goldmacher). This protein, which is indispensable for viral replication, is also found in primate CMV, with a high degree of conservation in the N-terminal mitochondrial localization sequence and the C-terminal apoptosis-inhibitory domain. Daniela Malide reported that the protein PB1-F2 from influenza virus and in particular a domain containing a cluster of arginines within an  $\alpha$ -helix, similar to the mitochondriotoxic domain of HTV-1 Vpr, targets mitochondria and favors MMP. Importantly, a PBI-F2-deficient virus is 100-fold less lethal for SCID mice than the wild-type virus, implying PB1-F2 in viral pathogenesis. Guido Kroemer showed that the envelope glycoproteins complex from HIV-1 can induce MMP through an indirect pathway that involves transcriptional activation of p53.<sup>10</sup> He also presented a model of Env-mediated syncytium formation resulting into aberrant cell cycle aberration and abortive entry into mitosis. Marie-Lise Gougeon suggested that indirect effects of the virus leading to the downregulation of Bcl-2 might explain the increased susceptibility of CD8<sup>+</sup> lymphocytes to TNFR-induced apoptosis.<sup>11</sup>

In the field of cancer research, Klaus-Michael Debatin presented data implying that the caspase-8 gene is frequently underexpressed in tumor cells and that reactivation of the caspase gene by treatment with the demethylating agent 3'-azacytidine or interferon- $\gamma$  (which does not involve demethylation but rather Stat-1 activation) can re-establish sensitivity to cell death induction via cell death receptors. A peptide derived from the N-terminus of Smac/DIABLO (AVPIAQ), fused to the plasma membrane translation domain of Tat was found to have a major curative effect, when stereotactically

injected with TRAIL into human gliomas orthotopically transplanted into the mouse brain. Klaus-Michael Debatin insisted also on the possible use of betulinic acid, which targets mitochondria, for the proapoptotic treatment of neuroectodermal tumors.<sup>12</sup>

Xin Lu reported that ASPP1 and ASPP2 specifically increase the apoptotic function of p53, by increasing the expression of proapoptotic p53 target genes such as Bax and PIG3, while having no effect on p53-mediated transactivation of p21 or mdm2. Importantly, it appears that 80% of p53-expressing human breast tumors exhibit a reduced expression of ASPP1 or ASPP2 or, alternatively, an increase expression of iASPP, a protein that inhibits the DNA damage-elicited interaction of p53 with ASPP1 or ASPP2. Antisense RNA for iASPP enhances p53-induced apoptosis, while overexpression of iASPP increases cellular resistance to apoptosis induced by cisplatin or UV light. Combined with transformation assays, these data strongly suggest that iASPP is a novel oncogene, while ASPP1 and ASPP2 function as tumor suppressors.

Sandy Zinkel reported that old (>22 months) Bid<sup>-/-</sup> mice develop a chronic myelomonocytic leukemia (CMML), presumably as a result of the increased regenerative potential of Bid<sup>-/-</sup> cells. In addition, it appears that old Bad<sup>-/-</sup> mice spontaneously develop B cell lymphomas of germinal center origin. Such mice also develop, upon  $\gamma$ -irradiation, B- or T-lymphoblastic lymphomas. Thus, BH3-only proteins from the Bcl-2 family may play a role as tumor suppressor proteins. In contrast, thus far, no data are available on the role of Bim in cancerogenesis, although Bim appears to be essential for the negative selection of thymocytes (Philippe Bouillet).

Altogether, the meeting illustrated the rapid progress in the field of programmed cell death, with yields increasingly important insights both for pathophysiology and therapy, in strategic areas such as infectious diseases, stroke, and cancer. The meeting illustrated the important contribution of Europe to this vital research area, yet was overshadowed by a pessimistic note. All the European participants profoundly regret the decision of the European Commission not to prioritize cell death research during the sixth Framework Program.

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