*Commentary*

# Apoptosis: Mitochondria Resurrected?

By Pierre A. Henkart* and Sergio Grinstein‡

*From the *Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, 20892; and ‡Division of Cell Biology, Hospital for Sick Children, Toronto, Ontario, Canada, M5G 1X8*

Although apoptosis research has become a very vigorous field over the last several years, fundamental questions about this type of cell death remain unanswered. Principal among these is whether there is a central core of biochemical steps that are shared by the apoptotic deaths elicited in diverse cell types by different stimuli. For some time the protective actions of Bcl-2 in a variety of apoptotic death systems seemed to provide the most suggestive evidence in favor of this hypothesis (1), particularly because this molecule seems to be related functionally and structurally to the product of the *Caenorhabditus elegans* death-protectant gene *ced-9*. The simplicity of this model can be questioned, since there are a significant number of cases where Bcl-2 expression fails to protect against apoptotic death. However, it has been realized recently that Bcl-2 is only one member of a family of dimerizing proteins that influence cell death. Some, like Bax, enhance death rather than block it (2), revealing a complex balance of agonists and antagonists. Thus, the failure of Bcl-2 to protect against apoptosis in some cases can be rationalized by postulating that there are additional parallel death pathways that are regulated by other species that are related to yet distinct from Bcl-2. It is reasonable to suppose that the molecular targets of the Bcl-2 family members are part of a pathway leading to apoptotic death, which is common from nematodes to man. Bax and its relatives could fill this role.

One model proposed to explain the protective activity of Bcl-2 was that it acts as an antioxidant (3), neutralizing the effects of reactive oxygen species (ROS)[1], which are effector molecules common to various apoptotic death pathways (4). Immunolocalization studies showed that the primary site of intracellular Bcl-2 was the mitochondrial outer membrane. This suggested that Bcl-2 might function to protect cells from ROS generated as a normal by-product of mitochondrial oxidation reactions during the course of ATP biosynthesis. It seemed plausible that the cytoplasmic-facing Bcl-2 could also protect against cytoplasmic oxidants involved in mediating apoptotic death, and it is hard to imagine a more effective way to kill a cell than to burn it! ROS generation associated with apoptotic death has indeed been reported in steroid-treated T hybridoma cells and in the TNF-mediated death of tumor cells (3, 5). Failure to observe ROS generation in other forms of apoptosis can be rationalized if the event were highly localized. Additional supporting evidence for ROS as apoptotic effectors comes from demonstrations that endogenous and exogenous antioxidants can block various types of apoptotic death (4, 6, 7). Nevertheless, although ROS seem to be involved in some examples of apoptotic death, they are likely to be part of upstream signaling events in particular cases, rather than essential elements of a common downstream death-effector pathway blocked by Bcl-2. Thus, glutathione depletion sensitizes HL-60 cells to apoptotic death induced by some agents but not others (8), and antioxidants protect T hybridoma cells against TCR-induced death (9), but not at the terminal stage triggered by Fas-crosslinking (10).

In view of these shortcomings, investigators have pursued the search for apoptotic effectors other than ROS. During the last 2 yr, cysteine proteases with "asp-ase" activity emerged as candidates for common apoptotic effector molecules. The prototype of this family is the *C. elegans* death gene *ced-3*, the mutation of which blocks developmental death in many different cells. Mammalian members of this family, which have homology to IL-1-β-converting enzyme (ICE), are being rapidly described. These proteases are synthesized as precursors, which need to be processed via cleavage at aspartic acid residues to acquire proteolytic activity. One can therefore envision complex parallel or converging proteolytic cascades involved in apoptosis. At this stage, however, it is not clear what controls their activation, nor the nature of the substrates relevant to cell death. Although nuclear substrates such as poly (ADP-ribose) polymerase and DNA-dependent protein kinase may well contribute to the classical apoptotic DNA damage, experiments with enucleated cells indicate that such damage is not required for some forms of apoptotic cell death, like that triggered by Fas (11). The principal evidence that the ICE-like protease family has a common apototic effector function comes from experiments in which protein inhibitors of these proteases have been expressed in a variety of cell types and found to block apoptotic death. The cowpox virus protein CrmA and the baculovirus protein p35 both inhibit ICE and other ICE-like proteases (12–14) (but not necessarily all [15]) and block developmental cell death, as well as that induced by growth factor withdrawal and other agents such as anti-Fas and steroids (16–21). In addition, peptide-based inhibitors of ICE-like proteases block other varied examples of apoptotic

[1]*Abbreviations used in this paper:* ICE, IL-1β–converting enzyme; PT, permeability transition; ROS, reactive oxygen species.

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death (22–25). There are two examples of apoptotic death not blocked by inhibitors of ICE-like proteases, namely, T cell death by IL-2 withdrawal (26) and steroid-induced death of a T hybridoma (27). The significance of these observations is difficult to assess given our incomplete knowledge of the specificity of the protease inhibitors. Overall, ICE-like protease inhibitors seem more generally potent in their ability to block death than does Bcl-2, and one simple way to explain this is to postulate that these proteases act downstream of the site of Bcl-2 inhibition.

The emergence of the ICE-like proteases detracted from the early interest in ROS and mitochondria. The loss of interest was accentuated by several observations. First, some examples of apoptotic death persist when cells are depleted of oxygen to reduce generation of ROS (28, 29). Second, preservation of "normal" mitochondrial morphology has been considered a hallmark of apoptotic death. The third and most important line of evidence came from studies of cells grown under conditions in which mitochondrial DNA replication was suppressed. These cells, termed $\rho^\circ$, lack important components of the respiratory chain, the source of mitochondrial ROS. In such cells, staurosporine triggered apoptotic death, and Bcl-2 expression still afforded protection (30). Together, these lines of evidence argued that mitochondrial-generated ROS are not part of a common downstream apoptotic effector pathway, and mitochondria were thus dismissed, with some relief by many in the field. However, the article in this issue of *The Journal of Experimental Medicine* by Zanzami, et al. (31) brings mitochondria back in the apoptosis limelight under a new context, as potentially important in generating nuclear damage via a pathway independent of ROS. Briefly, these authors postulate that a crucial, common step in programmed cell death is the opening of mitochondrial "megachannels" by the so-called permeability transition (PT), with release of a soluble factor that suffices to trigger nuclear apoptosis. Several lines of evidence support their view: (*a*) a reduction in mitochondrial potential, which is a consequence of the PT, is an early event after triggering apoptotic death in many systems (32, 33); (*b*) isolated mitochondria undergoing PT induce nuclear apoptosis in a cell-free model system (this is significantly simpler than previously described cell-free apoptosis systems); (*c*) atractyloside, an agent that facilitates opening of megachannels, induces apoptosis. Conversely, bongkreik acid, which blocks the PT, impairs the ability of mitochondria to trigger nuclear condensation; (*d*) overexpression of Bcl-2 inhibits the PT and thereby seems to prevent apoptosis. The soluble factor released by mitochondria, preliminarily described in the report by Zanzami et al. (31), does not appear to be an ROS, as it is not neutralized by antioxidants like trolox. Instead, it appears to be a comparatively large (>10 kD), heat-sensitive molecule, likely a protein. It is not seemingly an ICE-related protease, since its action is not blocked by inhibitors of these enzymes. Clearly, the identity of the putative mediator remains an unanswered issue of great urgency.

How can these notions be reconciled with the earlier findings in $\rho^\circ$ mitochondria, which lack mitochondrial DNA? Zanzami et al. (31) point out that, despite the absence of a functional respiratory chain, $\rho^\circ$ mitochondria maintain their permeability barrier and in fact display a sizable inner membrane potential, maintained by glycolytic ATP. Moreover, such mitochondria undergo swelling, indicative of the PT, when treated with atractyloside. Therefore, while production of ROS by the respiratory chain can be ruled out, the opening of megachannels could control apoptosis in $\rho^\circ$ cells, as it is proposed to do in cells with normal mitochondria. Opening of PT and release of the apoptosis mediator represent an attractive model to account for programmed cell death, in that opening of the megachannels could be a convenient site for convergence of multiple signals. The PT is induced and/or facilitated by changes in calcium concentration, redox potential, metabolic state of the cell, and mitochondrial inner membrane electrical potential. All of these have been earlier invoked in the genesis or signaling of apoptosis. Hence, megachannels may provide an effective integrator of multiple measures of cellular status, explaining the seemingly pleiotropic character of programmed death.

How the mitochondrial permeability transition is triggered by the various apoptotic stimuli remains unknown, but one can speculate that ICE-like proteases are involved. If this is the case, the proteases may not be quite as far downstream death effectors as some had imagined. Programmed cell death remains a complex phenomenon, and at present no single scheme can accommodate all the available information, particularly that relating to bcl-2. Nevertheless, in view of the findings of Zanzami and colleagues (31, 34, 35), mitochondria seem to have been resurrected as worthy contenders in apoptotic signaling.

Address correspondence to Pierre Henkart, Lymphocyte Cytotoxicity Section, Experimental Immunology Branch, Building 10, Room 4B17, NIH, Bethesda, MD 20892.

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