Mitochondria in apoptosis: past, present and future

M. Degli Esposti
School of Biological Sciences, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, U.K.

Abstract
The role of mitochondria in cell death has been increasingly appreciated in the last few years and is now well established in a variety of cellular systems. At present we know that the involvement of mitochondria is regulated by proteins of the Bcl-2 (B-cell lymphocytic-leukaemia proto-oncogene 2) family, which biochemically act by altering the properties of mitochondrial membranes to facilitate the release of apoptogenic proteins like cytochrome c and Smac/Diablo that, once released into the cytosol, are crucial for activating the caspase cascade of cell degradation. The precise mechanism of the pro-apoptotic action is not fully understood yet, but could be clarified in the near future. Instrumental to this clarification is the emerging evidence that CL (cardiolipin), an unusual membrane lipid that is predominantly present in mitochondria, is required for the action of major pro-apoptotic proteins like Bid and Bax. New results obtained in myeloid cells further sustain this possibility and suggest that Bid may be involved in the metabolic cycle of CL re-modelling. In agreement with this postulate, preliminary results indicate a down-regulation of Bid in parallel to the genetic deficiency in CL re-modelling that is associated with a rare human disease, ‘Barth Syndrome’. Intriguingly, this disease is characterized by neutropenia, suggesting a link between myeloid differentiation and cell death (and myeloid lymphoma pathogenesis too). I will project current results and trends towards future investigations on the involvement of CL and mitochondrial membranes in myeloid differentiation, cell death and disease.

Past: different forms of cell death but common involvement of intracellular organelles
The word apoptosis was re-introduced in the medical literature to describe a defined form of cell death following a precise programme, which is evolutionarily conserved and includes ‘death’ proteases, the caspases [1]. However, caspases are not required in all forms of controlled cell death [1,2]. Whereas caspase activation is fundamental for the morphological and biochemical changes in the nucleus, cell death can progress without an increase in caspase activity, often showing diffuse vacuolization of the cytoplasm [1,2]. This kind of death has been observed following physiological stimuli in vivo [2] and variably defined as ‘autophagic’ or ‘necrotic’. In fact, there is a continuum of cell death modalities that depends on the cellular system, time of observation and type of stimuli studied [1].

The extrinsic apoptotic pathway, promoted by death receptors like CD95/Fas, activates both caspase-dependent and -independent reactions that modulate the downstream effects on cell structure and function [2]. Stimulation of death receptors leads to alterations that propagate from the plasma membrane to intracellular organelles, in particular mitochondria [2]. The intensity of mitochondrial involvement depends on a balance between apical caspases like caspase 8 and other effector molecules that are recruited to the cytoplasmic complex of ligated receptors [2]. In most cells, activation of apical caspases is amplified by the release of mitochondrial factors like cytochrome c that activate the apoptosome [2].

Whether or not caspases are engaged, dying cells generally show a proliferation of intracellular membranes, which implies major changes in the metabolism and traffic of intracellular membranes and their lipid constituents [3]. These changes impact on the mutual relationship between organelles, especially mitochondria, endoplasmic reticulum and the endolysosomal compartment, which are intimately connected by membrane contacts and lipid traffic. To study the early events in physiological cell death, I have focused on membrane lipids as if the point of observation was at the mitochondrial surface. Following this approach, novel changes in mitochondrial lipids have been observed; they are presented here in relationship to pro-apoptotic Bid, myeloid cell death and disease.

Present: Bid interacts with CL (cardiolipin) metabolites
How death receptors transmit apoptotic signals to mitochondria was illustrated by the discovery that Bid, a widespread pro-apoptotic protein of the Bcl-2 (B-cell lymphocytic-leukaemia proto-oncogene 2) family, is specifically cleaved by caspase 8 [4]. The C-terminus of Bid (tBid) then moves to mitochondria and strongly promotes the release of

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1email mauro.esposti@man.ac.uk
Changes in CL and Bid during cell death

The metabolic cycle of CL is particularly sensitive to alterations occurring early after cell death induction, since a variety of apoptotic stimuli induce loss of mature CLs (reviewed in [8]). For Fas, CL is rapidly converted into MCL even before caspase 8 activation can be measured [7]. This CL degradation is observed in parallel to an increased association of full-length Bid with mitochondria and also a translocation of ‘intermediate’ Bid bands out of mitochondria (Figure 1 and [7]). These intermediate bands, which have been observed also by others, may derive from tight association of native forms of Bid to CL-related lipids and lysolipids enables Bid to transport CL metabolites between donor and acceptor membranes [3,5,7]. Interestingly, tBid is more effective than full-length Bid and displays an increased affinity towards MCL (monolysocardiolipin) [7]. MCL is the immediate product of CL de-acylation and functions as a key intermediate in CL re-modelling [3,10], a complex process catalysing the rapid conversion of newly synthesized CL (with short and saturated acyl chains) into its mature forms, predominantly containing long and unsaturated acyl chains [3,10]. MCL and other metabolites are located on the outer membrane and extra-mitochondrial compartments, where efficient de- and re-acylation occurs [11,12]. By combining these notions with the novel finding that Bid can transport CL metabolites [5], I proposed that Bid may play an underlying role in the metabolic cycle of CL [3].

Future: clarify how Bid and CL influence myeloid cells

Neutrophils differentiate from myeloid precursors through a process that involves depletion of mitochondria in parallel to the proliferation of vacuoles derived from the endolysosomal compartment forming the characteristic granules [15,16]. Bid might play a crucial role in the traffic of CL metabolites that probably increases during differentiation, in part because endolysosomal vacuoles require CL as the source for a major phospholipid of their membranes, lysosomal phosphatidic acid [8,17]. In the future, this possibility will be tested in detail to reveal how Bid and CL are involved in the life and death of myeloid cells.

References


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