The archaeology of apoptosis

R. A. KNIGHT

MRC Toxicology Unit, Hodgkin Building, Leicester LE1 9HN.

SUMMARY

Human death has been recognised as a significant personal and social event for many thousands of years, and classical archaeologists have revealed the changing complexity of rituals associated with it. The study of cell death, however, is a much more recent event, although many of the molecular pathways involved have now been identified, at least in mammalian systems. In studying the loss of cells, the use of the term 'death' is, perhaps, not altogether appropriate both since it carries the cultural resonance associated with bodily death, and because we do not study cell death itself, but rather the processes that lead up to it. Mammalian cell death processes are complex and involve a dynamic equilibrium between death promoting and death inhibiting factors, suggesting that some components of death pathways may have a paradoxical survival function. Since parasites must survive an often hostile environment, they may be a useful model to study whether component molecules of mammalian death pathways originally formed modules of parasite survival strategies, and whether survival and death pathways coevolved.

Key words: Archaeology, autophagy, apoptosis, parasites.

OVERVIEW

I was pleased to see, in the publicity for this meeting, that the use of the word death was relatively restrained. Death is an imprecise term which means different things to different people. For example, outside Africa, the first archaeological evidence of death becoming recognised as a significant event are the earliest known burials, dating to around 60,000 years ago. These were merely internments but, naturally, burial customs evolved over time. Simple burials became covered by increasingly elaborate graves which were often oriented to particular features of the solar and lunar calendars, and to geographical features in the local landscape, and were accompanied with stereotyped assemblages of grave goods. The point I am making here is that, inevitably over a time period long by human standards, a whole set of cultural and other resonances have become associated with the death word which seriously qualify its use as a precise scientific term.

There is another reason for avoiding 'death' in a scientific context. For death is an endpoint, and we either study the processes that lead to that endpoint or, rather like biological archaeologists, study the phagocytic burial processes responsible for removal of cellular corpses. If we study the dying processes that result in death as an endpoint, then surely it is preferable to say that we are studying, for example, apoptosis or autophagy, since these terms largely lack cultural overlay and can be used as more precise definitions of the process we are investigating. But, like most people, I will often lapse into the lazy verbal colloquialism of talking about death when I should use a more exact terminology.

The idea of processes rather than an endpoint is even implicit in the morphological descriptions of dead and dying cells made by microscopists from the middle of the 19th century onwards, since they recognised different structural phenotypes of death. But it wasn't until the morphological was made molecular that the processes themselves could be studied in detail. In relation to apoptosis, it is impossible to overemphasise the contribution of Bob Horvitz and his collaborators, since their identification of the basic molecular programme in *C. elegans* provided a framework for identifying conserved, albeit more complex, homologous routines in higher organisms (Metzstein, Stanfield and Horvitz, 1998). Similarly, characterisation of the 30 or so genes involved in autophagy in yeast has led to the recognition of at least some of the mammalian autophagy genes, although the molecular mechanisms of autophagy in mammals are perhaps less well understood than those of apoptosis. Thus, study of evolutionary primitive organisms can lead to important molecular insights into biological processes in general.

Although I refer to the mechanisms of dying as distinct processes, some would argue that these are merely subroutines of a common basic theme. Indeed, whether a cell dies by apoptosis or necrosis depends, at least in part, on how well oxidative phosphorylation and ATP generation is sustained (Nicotera, Leist and Ferrando-May, 1999). Thus, a catastrophic fall in ATP leads to necrosis; a less dramatic decline to apoptosis. Moreover, if autophagy is sufficiently prolonged, it can lead to death

Corresponding Author: Dr R. A. Knight, MRC Toxicology Unit, Hodgkin Building, Leicester LE1 9HN. Tel: 0116 252 5551. E-mail: r.knight@ich.ucl.ac.uk

Parasitology (2006), **132**, S3–S5.
doi:10.1017/S0031182006000813 Printed in the United Kingdom

by the classical apoptotic pathway rather than autophagic cell death (Baehrecke, 2005). So death processes can be linked, but since they involve distinct molecular mechanisms, I prefer to regard them as discrete processes.

Any cell – from ancestral to specialised mammalian – requires an inherent capacity to adapt to temporary periods of cellular stress. This is perhaps particularly true of highly differentiated end-stage cells such as neurons and cardiac myocytes where adaptive escape by mutation and cell division is not an option. We would expect to see this throughout the evolutionary tree as a fundamental homeostatic mechanism which has been conserved and developed over time. One well established such process is autophagy (Baehrecke, 2005) where, under conditions of extracellular nutrient depletion or growth factor withdrawal, intracellular components are digested in autophagosomes to provide an alternative intracellular nutrient supply. An additional function of the autophagosome is to act as a recycling dustbin for damaged intracellular organelles such as mitochondria. Compromised mitochondrial function, resulting in excess free radical production, has been proposed as one mechanism of ageing and, indeed, the efficiency of the autophagocytic process declines with age (Cuervo, 2004). Although autophagy has been particularly well studied in single-cell organisms, it has only recently been recognised as a normal feature of mammalian systems. For example, it has now been shown to occur in newborn mice in the period between birth and the onset of suckling (Kuma *et al*. 2004). However, if the autophagic stimulus is prolonged and endophagocytosis can no longer maintain metabolic requirements, the cell dies. The molecular switches between autophagic survival and death are not well understood, although the mammalian target of rapamycin as a nutrient sensor seems to be one important factor. But which evolved first – autophagy as an, albeit limited, death sparing process, or autophagy as a death process *per se* – or did these opposing functions coevolve?

Although less apparent, this dialectic between survival and death is also inherent in the apoptotic programme (Yousefi, Conus and Simon, 2003). Indeed, the apoptotic pathway may be regarded as a dynamic equilibrium which keeps the cell alive in the presence of the appropriate positive survival signals, but which defaults towards apoptosis if these are withdrawn, or some more direct injury occurs. Thus, in mammalian systems, in response to a mitochondrial insult, cytochrome c is released from the intermembrane space, and interacts with Apaf-1 and ATP resulting in activation of the initiator cysteine protease, procaspase-9. However, procaspase-9 is also inhibited by the Inhibitors of Apoptosis Proteins (IAPs) and it is only when a second mitochondrial protein, Smac/DIABLO, is released and neutralises the inhibitory effects of IAPs that full caspase-9 activation proceeds (Green and Kroemer, 2004).

A similar set of checks and balances operates at the level of death receptor signalling. Thus, binding of Tumour Necrosis Factor α (TNFα) to TNF-R1 has two results. Firstly, several proteins, including another initiator, procaspase-8, are recruited to the cytoplasmic portion of the receptor resulting in the formation of the Death Inducing Signalling Complex and activation of enzymatically active caspase-8 (Jin and El-Deiry, 2005). In a parallel pathway, the kinase, RIP, also interacts with the bound receptor and increases expression of anti-apoptotic proteins, such as IAPs and anti-apoptotic members of the Bcl-2 family, by an NF-κB-dependent mechanism (Meylan and Tschopp, 2005). When these equilibria become tilted towards apoptosis, the active initiator caspases, 9 and 8, activate downstream effector caspases, such as caspase-3, resulting in the cleavage of substrates important for the maintenance of cellular integrity.

The biological importance of the inhibitory components of the apoptotic pathway is underlined by the presence of homologues of anti-apoptotic Bcl-2 proteins (ced-9) and IAPs (BIR-1) in *C. elegans*.

I have mentioned that the release of some mitochondrial proteins is important for initiating apoptotic signalling in the cytoplasm. There is also cross-talk between the death receptor and mitochondrial pathways mediated by another member of the Bcl-2 family, Bid (Yin, 2000). Bid is cleaved by active caspase-8, and the truncated product translocates to the mitochondria where it inserts into the membrane, causes membrane depolarisation and release of cytochrome c with activation of procaspase-9. Therefore, components of the overall apoptotic programme are coordinated in a similar manner to the linkage between different cell death pathways as described above.

The existence of pro- and anti-apoptotic components intrinsic to the apoptotic programme implies that, provided its perturbation is not too severe, death is not inevitable. A further implication is more clinical – that where cell death is deficient, as in cancer, we can potentially manipulate therapeutically the balance towards death, and where death is pathological and excessive, as in neurodegenerative disease and myocardial infarction, we can tilt things the other way.

However, representing the autophagocytic and apoptotic pathways as dialogues between pro- and anti-apoptotic factors and effects may be oversimplistic. For example, proteins such as the caspases are seen as the bad guys – the 'molecular executioners' of apoptosis – not a phrase I like since it conveys an imagery which, as with the death word, qualifies its meaning – and the IAPs and bcl-2 as good guys. But evidence is emerging that caspases

https://doi.org/10.1017/S0031182006000813 Published online by Cambridge University Press

can be involved in other biological processes which do not necessarily have anything to do with death (Launay *et al*. 2005). For example, caspase-8 has been implicated in cell migration, and caspases-8, 3 and possibly 1 in differentiation, particularly of myotubes. Transient caspase-8 activation is also seen during erythropoietin-driven erythrocyte differentiation, although it is probably not involved in enucleation, and caspase-8 null mice die with congested accumulations of immature erythrocytes. Caspase-1 is an essential component of the inflammasome, a multiprotein structure involved in production of the inflammatory cytokine, interleukin-1β, during the immune response to agents such as bacterial lipopolysaccharide, and caspase-1 has even been proposed to be necessary for macrophage survival during bacterial infection. Therefore, as well as long conserved pro-survival molecules such as IAPs, we now have some faint echoes that even classical pro-apoptotic agents may have, and particularly have had, different functions. So, as with autophagy, we can also ask the same question of apoptosis – does the survival potential in response to cellular stress of the apoptotic amalgam of molecules parallel or even precede its emergence as a cell death programme?

One way, perhaps, to approach this question is to study the ways by which parasites survive. The biological success of a parasite is critically dependent on its ability to resist the defence mechanisms of the hosts in which it resides. Indeed, a parasite survival programme would appear more advantageous than an ability to induce death of host cells or itself, since, if activated prematurely, the latter would compromise completion of that particular phase of its life cycle. Can we identify, therefore, an early parasite which has evolved such an effective survival strategy but which either completely lacks a programmed cell death mechanism, or in which the survival and cell death programmes have common molecular features? Can we also find parasite survival molecules which have become co-opted into death programmes in later organisms? In addressing these questions, we are not strictly in an archaeological situation, since the archaeologist studies artefacts which have remained unaltered over time, whereas the biological archaeologist depends on organisms which have not just survived over millenia but also evolved in response to their own selection pressures over that time. Nevertheless, it may be possible to identify residual footprints of molecules and mechanisms, whose function in parasites is different from that in evolutionary more recent organisms and whose understanding will enhance our ability to manipulate the host-parasite relationship.

REFERENCES

Baehrecke, E. H. (2005). Autophagy: dual roles in life and death? *Nature Reviews in Molecular and Cellular Biology* **6**, 505–510.

Cuervo, A. M. (2004). Autophagy: many paths to the same end. *Molecular and Cellular Biochemistry* **263**, 55–72.

Green, D. R. and Kroemer, G. (2004). The pathophysiology of mitochondrial cell death. *Science* **305**, 626–629.

Jin, Z. and El-Deiry, W. S. (2005). Overview of cell death signaling pathways. *Cancer Biology and Therapy* **4**, 139–163.

Kuma, A., Hatano, M., Matsui, M., Yamamoto, A., Nakaya, H., Yoshimori, T., Ohsumi, Y., Tokuhisa, T. and Mizushima, N. (2004). The role of autophagy during the early neonatal period. *Nature* **432**, 1032–1036.

Launay, S., Hermine, O., Fontenay, M., Kroemer, G., Solary, E. and Garrido, C. (2005). Vital functions for lethal caspases. *Oncogene* **24**, 5137–5148.

Metzstein, M. M., Stanfield, G. M. and Horvitz, H. R. (1998). Genetics of programmed cell death in *C. elegans*: past, present and future. *Trends in Genetics* **14**, 410–416.

Meylan, E. and Tschopp, J. (2005). The RIP kinases: crucial integrators of cellular stress. *Trends in Biochemical Sciences* **30**, 151–159.

Nicotera, P., Leist, M. and Ferrando-May, E. (1999). Apoptosis and necrosis: different execution of the same death. *Biochemical Society Symposia* **66**, 69–73.

Yin, X. M. (2000). Signal transduction mediated by Bid, a pro-death Bcl-2 family protein, connects the death receptor and mitochondria apoptosis pathways. *Cell Research* **10**, 161–167.

Yousefi, S., Conus, S. and Simon, H. U. (2003). Cross-talk between death and survival pathways. *Cell Death and Differentiation* **10**, 861–863.

https://doi.org/10.1017/S0031182006000813 Published online by Cambridge University Press