Mechanisms of Cell Death in Neurodegenerative Diseases: Fashion, Fiction, and Facts

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Apoptosis has become a most popular concept of cell death. However, the term is now so widely used and employed in such general terms in relation to neurological diseases that its application is very problematic. In addition, with the exception of developmental conditions, there is essentially no evidence of apoptosis fulfilling the criteria of its classical definition in any of the important human neurodegenerative diseases, including Alzheimer's, Parkinson's, Huntington's, Amyotrophic Lateral Sclerosis, and Creutzfeldt-Jakob disease. Importantly, a number of new cell death forms have been described in the literature and there is good reason to pay attention to these emerging concepts as they may provide a rationale for the development of disease-specific therapies.

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Defining Neurodegeneration

Neurodegenerative diseases are characterized by the premature, *primary* death of specific nerve cell populations. This is not disputed. However, confusion exists as to which cell death mechanisms are involved. Specifically, while the role of apoptosis during CNS development is unquestioned, its contribution to neurodegeneration in the adult CNS is controversial. For example, although a relatively large number of papers dealing with "apoptosis" in Alzheimer's disease (AD) have been published (Figure 1), there is no convincing evidence in support of a contribution of apoptotic cell death to the pathogenesis of AD (29). Rather, studies from laboratories with special expertise in tissue analysis suggest that several distinct cell death phenotypes exist in the CNS. Thus, the traditional "necrosis-apoptosis" paradigm appears inaccurate, and in fact, misleading when applied to the human brain and spinal cord. Confusion seems to have culminated with the creation of the term "undead" neuron (21). Lately, previously strong promoters of apoptosis in adult-onset neurodegenerative diseases have begun to revise their views (7), but nomenclatorial uncertainty is so fundamental that research into this aspect of CNS diseases is compromised. More than 55 000 publications in the literature dealing with one or another aspect of "apoptosis" indicate that the problem is an important one.

The following neurodegenerative conditions are at the center of the current cell death debate: Alzheimer's disease, Parkinson's disease (PD), Amyotrophic Lateral Sclerosis (ALS), Huntington's disease (HD), and Creutzfeldt-Jakob disease (CJD). They are listed here in an order of decreasing frequency and will be discussed in relation to relevant hypotheses of neuronal cell death mechanisms. In addition, a *non-neurodegenerative* condition, ischemia, will be considered for comparative purposes (Figure 1). Glio-degenerative diseases, such as multiple system atrophy or cell death following direct nervous system trauma will not be discussed.

Apoptosis: A Term About to Lose its Meaning

"What we can't say we can't say, and we can't whistle it either." F.P. Ramsay's acid variation of the last sentence of Wittgenstein's *Tractatus Logico-Philosophicus* (27) illustrates the importance of the clarity of terms. The term "apoptosis" in its original and generally accepted meaning refers to a morphological phenomenon (11). The characteristics of an apoptotic cell include chromatin condensation, nuclear fragmentation (pyknosis), and cell shrinkage. Eventually, the cell breaks into small membrane-surrounded fragments, or apoptotic bodies, which are cleared by phagocytosis without inciting an inflammatory response (11, 17).

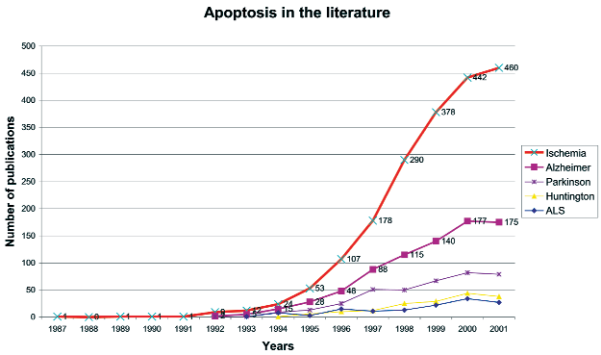

Figure 1. This figure illustrates the development of the "apoptosis" literature (PubMed database). All articles contain the terms "apoptosis" and/or "apoptotic".

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Designation	Disease condition	Characteristics	References
Abortosis	Alzheimer's disease	Elevated expression caspases 8 & 9 but control levels of downstream apoptosis effectors (i.e. caspases 3 and 7) Absence of nuclear chromatin clumping and apoptotic bodies	(23, 26)
Aposklesis	Parkinson's disease, Retrograde response to axotomy	Nucleus and nucleolus well preserved, absence of significant chromatin condensation and nuclear fragmentation Slow degeneration of neurones, over a period of weeks	(6, 19)
Autophagy (incl. granulovacuolar degeneration)	Parkinson's and Alzheimer's diseases Normal neuronal development and following axotomy	Pyknosis of nucleus (may bleb or segregate) Endocytosis and blebbing of cell membrane Abundant autophagic vacuoles; ER, mitochondria and Golgi dilated Golgi has greatly enhanced nucleoside diphosphatase activity Occasional and late heterophagic elimination	(1, 3, 7)
Dark degeneration	Huntington's disease Animal model of Huntington's disease Drosophila model of Huntington's disease	Nuclear and cytoplasmic condensation Clumping of chromatin Before condensation there is transient swelling of mitochondria and Golgi network Ruffling of plasma membrane Invagination of the nuclear membrane (No fragmentation or blebbing of the nucleus or cytoplasm) TUNEL negative	(9, 36)
Ischemic cell death	Global ischaemia, stroke	Condensation of nuclei, enhanced eosinophilia of the neuronal cytoplasm, delayed activation of caspase-3	(4, 14)
Paraptosis	In vitro (293T and transgenic Apaf-1 null mouse) embryonic fibroblasts	Cytoplasmic vacuolation predominantly from ER Mitochondrial swelling late in neuronal degeneration No autophagic vacuoles Process inhibited by actinomycin D and cycloheximide Pathway mediated by caspase 9 and Apaf-1 independent Caspase inhibitors ineffective Absence of nuclear fragmentation, apoptotic body formation, cellular blebbing & chromatin condensation	(32)
Sick neurones	Parkinson's disease Alzheimer's disease Huntington's disease Amyotrophic lateral sclerosis	Degenerative changes in size, shape and morphology of the neuronal soma and dendrites Cellular shrinkage, cytoplasmic and nuclear condensation. Nuclear deformities	(10)

Table 1. Proposed forms of neuronal cell death (non-apoptotic, non-necrotic). Note: This table is not all-encompassing.

Typically, the apoptotic cell death process has a very rapid time course and is complete within a few hours. While the occurrence of apoptosis in developing human brain tissue has been confirmed (34), there is no proof of functionally significant apoptosis in adult human brains (10). A main reason for the present confusion is that the term apoptosis is used by many authors in a much wider sense than originally proposed and that it has become synonymous with "non-necrotic" cell death.

For more than a century the term necrosis has been used in English, French, and German to describe the "mortification of tissue" until its use became more specific during the 1980s to refer to one of the cell death pathways (3). Ultrastructurally, necrosis is defined by the loss of cytoplasmic membrane integrity, swelling and disintegration of cytoplasmic organelles, including mitochondria, dispersion of ribosomes from the endoplasmic reticulum (ER) and dilation, fragmentation and vesiculation of ER cisterns and the Golgi apparatus. In contrast to apoptosis, the necrotic cell death process has an extended duration with a time course of days or even weeks similar to other cell death phenotypes (Table 1).

The Problem of TUNEL Labeling

Confusion in the identification of neuronal cell death mechanism has arisen as a result of the use of non-ultrastructurally based molecular in situ methods to detect apoptosis, notably the *terminal transferase-mediated dUTP nick-end labeling* (TUNEL), and alternative DNA fragment *in situ end-labeling* techniques (ISEL). However, TUNEL or ISEL positive labeling is not necessarily a marker of apoptosis. TUNEL represents a staining method which has proven useful for the in situ detection of DNA fragmentation. This is what the technique does and not more. However, there are numerous reports in the literature equating TUNEL positivity with apoptosis, and this is where much of the confusion arose and still arises. However, it has been convincingly demonstrated that TUNEL labeling is not specific for apoptotic cells and does not even necessarily label cells that are committed to die (33). TUNEL positivity has been detected in AD cortex, PD nigra, HD striatum, ALS spinal cord, and CJD cortex although a convincing demonstration of the corresponding structural phenotype has not been achieved. In spite of these facts, the topic of apoptosis in neurodegenerative diseases has attracted the attention of the science tabloids and has been "developed" by in vitro researchers as well as some clinical laboratories independent of correlating tissue pathology.

It is clear that a molecular definition of cell death has to be the final goal of all cell death analyses but the damage done by an inappropriate use of terms, and be it morphological ones, cannot be overestimated as words represent thoughts, and we cannot do without them.

Molecular Markers of Apoptosis

Much of our understanding of the genetic regulation of cell death is based on experimental work conducted by H.R. Horvitz' group on the nematode Caenorhabditis elegans (5). Several regulators of a developmentally very important "programmed cell death" pathway have been described including CED-3, CED-4, and CED-9. Work on mammalian systems has evolved around this concept, and pro- as well as anti-apoptotic molecules have been identified (12, 20). Apoptosis can be viewed as one form of programmed cell death and appears to be the most common manifestation of physiological cell death during development.

In mammals, apoptosis is regulated by the bcl-2 family of proteins, the adapter protein "apoptosis protease-activating protein" (Apaf-1) and caspases, a family of aspartyl-specific cysteine proteases. Caspases can serve both as transducers and executioners of cell death (28). The typical morphology of apoptotic cells is dependent on the action of these enzymes. There are now more than 10 human caspases, which can be divided into functional groups. The CED-3-like caspases can have initiator and effector functions that are directly involved in apoptosis (29).

Cell Death in Neurodegenerative Diseases

Alzheimer's disease. Alzheimer's disease is one of the most common neurodegenerative illnesses. Consequently, research into the molecular basis of cell death in this genetically heterogeneous group of disorders has attracted considerable attention. There are numerous claims that a postulated apoptotic cell death pathway plays an etiological role in AD. However, although at least 7 different caspases (caspases 1, 2, 3, 6, 8, 9, and 12) have been implicated in regulating neuronal cell death in response to beta-amyloid exposure, the direct involvement of caspase-dependent neuronal apoptosis in AD pathogenesis remains uncertain (29). Importantly, there is again a lack of morphologically convincing apoptotic neurones in thoroughly studied AD brains. As pointed out by Roth in his excellent review article (29), inferring outcome simply on the basis of increased expression of certain genes is unsound. This is illustrated by the upregulation of cell cycle-associated genes in AD brain: clearly, aberrant expression of cyclins in AD neurones cannot be taken as evidence that neurones in AD brain are actively undergoing mitosis. Therefore, alterations in the expression of genes, which is observed in association with apoptosis, in some AD neurones does not imply that AD neurones are committed to apoptotic death. Other forms of cell death such as autophagy or aposklesis may in fact be more relevant (*vide infra*).

Parkinson's disease. In the case of Parkinson's disease, a number of groups have consistently failed to confirm the occurrence of apoptosis in the Parkinsonian substantia nigra (for review, see 6, 7). These results were obtained on relatively large series of cases. However, there are anecdotal claims based on a few patients by other workers who have described rare apoptotic neurones in the Parkinsonian nigra (7). However, essentially all of the latter reports are based on TUNEL labelling or indirect visualisation using confocal microscopy. The respective patient sets were also heterogeneous. Furthermore, the frequency with which supposedly apoptotic cells were found in PD by these workers (up to 5%) is incompatible with the slow time course of a chronic neurodegenerative disease. Results

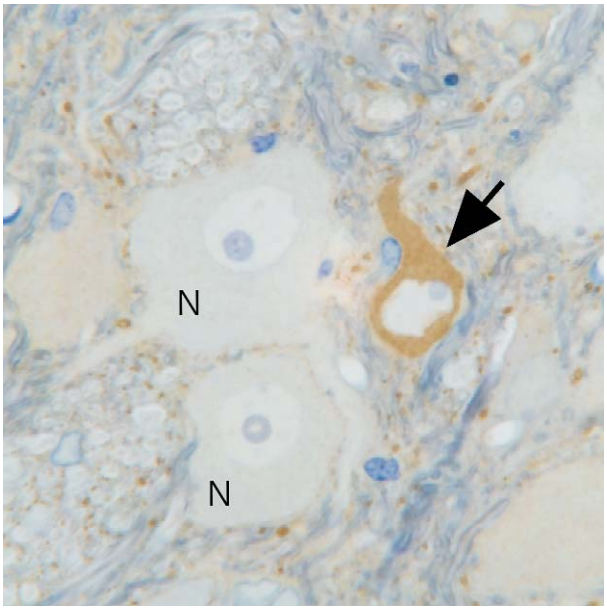

Figure 2. Example of slow, non-apoptotic cell death of an alpha-synuclein expressing motor neurone (arrow) following axotomy in the adult rat (cf. 19). N, Regenerating motor neurones. Primary magnification: ×40.

from animal models have also been used as an argument in support of the occurrence of apoptosis in PD, but it is clear that models represent a weak argument when discrepant with human pathology. Furthermore, much emphasis has been placed on the detection of caspase-3 in the substantia nigra of patients who have died with Parkinson's disease but the correlation described between the degree of neuronal loss in dopaminergic cell groups affected in PD and the percentage of caspase-3 positive neurones in these cells in control subjects (7) is far from convincing. The situation is thus quite analogous to that in the AD field. Even if caspase-3 were to play a role in nigral cell death in Parkinson's disease it does not necessarily imply that an apoptotic death process is involved at all. In fact, it would seem a safe prediction that some of the diverse cell death pathways which are now being described in the literature may share some common mechanisms. For instance, it is already apparent that mitochondria play a key role in various forms of cell death. In fact, mitochondria have been termed the "cellular centres of death control" (35). Thus, there is increasing evidence against apoptosis playing a relevant role in the selective loss of dopaminergic neurones in Parkinson's disease.

Amyotrophic lateral sclerosis. The validity of the concept of an "apoptosis-necrosis continuum" as proposed by some authors (24, 25) suffers with every unique cell death phenotype reported in the literature. ALS represents such an example. While we agree with the author of one of the most popular studies claiming apoptosis in ALS (16) that there are similarities between the chromatin condensation seen in motor neurones undergoing degeneration in ALS and the clumping of chromatin so typical of apoptosis, we cannot concur with their conclusion that neuron death in amyotrophic lateral sclerosis "is apoptosis." One of several reasons is that there is no fragmentation of the neuronal nucleus. It is important to note in this context that the interpretation of the findings of this group are strongly influenced by experimental results obtained using a sciatic nerve avulsion model (18). However, upon critical analysis of the results of this paper (18), it seems to support the opposite of what is claimed, ie, even following the severe challenge of nerve avulsion, apoptosis of axotomised motor nerve cells in an adult motor nucleus is a very rare exception rather than the rule. The authors state explicitly that the frequency of TUNEL-positive motor neurones was not commensurate with the loss of motor neurones as predicted by cell counts in Nissl stained sections (18). This is in full agreement with our own findings. Apoptosis *can* be induced in *newborn* animals by simple peripheral nerve transection but only a few weeks later motor neurones are rather resistant to this type of injury and do *not* undergo apoptosis anymore (19); relatively small numbers of nerve cells which do die following axotomy in the adult die very slowly and over a period of weeks. They show signs of "withering," or aposklesis (Figure 2).

Huntington's disease. The mechanism of cell death in Huntington's disease has been studied in great detail. In cultured cells, intranuclear huntingtin has been shown to induce the activation of caspase-3 and the release of cytochrome c from mitochondria, causing the cells expressing intranuclear huntingtin to undergo apoptosis (13). However, findings in transgenic animals and in human brain tissue of HD patients, which are ultimately relevant, are at variance with these observations. Turmaine et al (36) reported that dying neurones in situ characteristically exhibit neuronal intranuclear inclusions, condensation of both the cytoplasm and nucleus, and ruffling of the plasma membrane while maintaining ultrastructural preservation of cellular organelles. These cells do not develop blebbing of the nucleus or cytoplasm, apoptotic bodies, or fragmentation of DNA. Furthermore, nerve cell death occurs over a period of weeks but not hours. Degenerating cells of simi-

lar appearance were found in the same regions in brains of patients who had died with HD. The authors therefore suggest that the mechanism of neuronal cell death in both HD and their transgenic mouse model of HD by neither apoptosis nor necrosis. Instead, the authors refer to the process as "dark degeneration."

Creutzfeldt-Jakob disease. The claim that apoptosis plays a pathogenetically relevant role in CJD is essentially based on the detection of TUNEL staining as well as new markers for molecules known to be involved in the apoptotic process (37). However, as outlined above the detection of individual molecules that may be involved in one or another aspect of apoptosis does not necessarily imply that true apoptosis is occurring. Strong evidence against apoptosis in prion diseases stems from the fact that there is a striking lack of apoptotic morphology in nerve cells in the brain regions affected by spongiform change.

Cerebral ischemia. There are an enormous number of publications on the possible role of apoptosis in cerebral ischaemia (Figure 1). However, it is now evident that the dominant mechanism of cell death following cerebral ischaemia is not apoptosis but necrosis (4, 8). It is helpful in this context to consider the pathophysiology and sequence of events leading to irreparable damage of brain tissue as a result of insufficient perfusion. Apoptosis as a process depends on a functioning energy metabolism in cells that are fated to die (21). In other words, apoptosis—unlike necrosis—requires significant amounts of cellular energy, which is in keeping with the fact that apoptosis represents a programmed form of "active" cell death. Yet, this also means that *reversibility* of the disturbed perfusion in ischemic tissue is a key requirement for apoptosis to manifest itself (8). Since re-perfusion may not happen effectively, the number of nerve cells undergoing apoptosis-like changes in ischemic brains is small, and "apoptotic" features are essentially confined to the penumbra of an infarct.

More Ways to Die: Relevance of Cell Death Mechanisms for the Treatment of Neurodegenerative Diseases

There is growing acceptance that the strict adherence to the dichotomous view of cell death does not adequately accommodate the diverse range of morphologies observed in degenerating neurones (3, 24, 25). This inadequacy is readily apparent in descriptions of cell death during development (2, 22, 31) and some forms of ischemia-induced cell death (15). As a result, novel descriptive terms have been devised to more accurately describe the various cell death types which occur in neurodegenerative disorders. Examples include aposklesis (6), paraptosis (32), abortosis (26), and, the more traditional, autophagia (29) (Table 1). Difficulties in defining forms of neuronal cell death have also arisen from the fact that the vast majority of studies have been undertaken in the fields of cancer and immunology (3), with many investigators assuming that these cell death features are the same for neurones (19).

Finally a word of caution. Research on Alzheimer's disease strongly indicates that synaptic changes play an important role in disease pathophysiology. In other words, neuronal dysfunction rather than actual death of the affected nerve cells may be primarily responsible for the devastating clinical symptomatology of the disease (cf. 30). The same may hold true for the other diseases discussed here.

New treatment concepts focusing on cell death mechanisms will have to take the emerging multiplicity of cell death pathways into account.

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