Caspases, Apoptosis, and Alzheimer Disease: Causation, Correlation, and Confusion

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Abstract. Extensive neuron loss occurs in Alzheimer disease (AD) brain and some authors have speculated that dysregulation of apoptotic death pathways is etiologically responsible for the disease. Apoptosis is regulated in mammalian cells by a family of cysteine proteases called caspases. 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 amyloid β (Aβ) exposure in vitro, in animal models of neurodegenerative diseases, and in AD brain itself. Despite this seemingly impressive array of data implicating caspases and apoptosis as etiologic factors in AD, the direct involvement of caspase-dependent neuronal apoptosis in AD pathogenesis remains uncertain. Alternative explanations for some findings, contradictory experimental observations, and lack of morphologically convincing apoptotic neurons in the vast majority of AD brains has led to the revised hypothesis that apoptosis-associated molecular events cause neuronal dysfunction in the absence of, or prior to, neuronal death. Unfortunately, this new view renders the term “apoptosis-associated” functionally meaningless since it bears no relationship with apoptotic death and fails to focus scientific investigation on the molecular insults that trigger the “apoptosis-associated” response in AD neurons. On balance, an etiologic role for caspases in AD is far from proven. It remains possible, however, that caspase-dependent neuronal death contributes to AD neuron loss and thus, caspase inhibition offers some hope for extending AD neuron survival so that other agents, targeting upstream events, may delay or reverse primary AD pathology.

Key Words: Autophagy; Bcl-2; Cathepsin D; Neurodegenerative disease; Programmed cell death.

INTRODUCTION

Apoptosis is an evolutionarily conserved type of cell death that is defined by specific cytological features including chromatin condensation and margination, nuclear pyknosis and fragmentation, cytoplasmic membrane blebbing, cell shrinkage, and formation of apoptotic bodies (1). In the nematode Caenorhabditis elegans, apoptosis is regulated by a linear death pathway involving EGL-1, CED-9, CED-4, and CED-3 (2). Mammalian homologues of these molecules consist of pro-apoptotic Bcl-2 family members, anti-apoptotic Bcl-2 family members, Apaf-1-like molecules, and the caspase family, respectively (3, 4). CED-3 activation represents the commitment point to apoptotic cell death in C. elegans and caspase activation in mammalian cells is largely responsible for the cytological features that characterize apoptotic cells (5). The significance of apoptotic cell death in normal nervous system development has been recognized for many years and recent studies of mice with targeted gene disruptions have helped define the specific molecular pathways regulating naturally occurring cell death (6). More recently, scientists have recognized that apoptotic death pathways may also be involved in disease processes in the adult organism where too little (e.g. neoplasia) or too much (e.g. hypoxic-ischemic tissue damage) cell death causes significant pathology (7). In neurodegenerative diseases, selective and sometimes extensive neuron loss occurs and it is therefore not surprising that many authors have speculated on the etiological significance of apoptosis in neurodegenerative diseases. Indeed, some recent statements suggest that a role for apoptotic cell death in neurodegenerative diseases in general, and AD in particular, is proven. For example, “Unregulated apoptosis underlies many pathological conditions, including neurodegenerative diseases” (8) and “The loss of hippocampal neurons by apoptotic cell death is a prominent feature of Alzheimer’s disease” (9). In this manuscript, I review evidence for apoptotic death in AD and focus on the possible role of caspases in AD pathogenesis.

Apoptosis and AD

It is unequivocal that extensive neuron death occurs in the AD brain and, with possible rare exceptions, these neurons are not replaced. Intuitively, these facts suggest that neuron death causes dementia in AD; however, an important question is whether neuronal dysfunction precedes neuron loss and is responsible for the neurological symptoms of AD regardless of subsequent neuronal death. There are at least 4 generally accepted neuropathological “hallmarks” of AD: mature senile plaques, neurofibrillary tangles, decreased synaptic density, and neuron loss. Senile plaques and neurofibrillary tangles are easy to identify in routine AD brain sections and have been the focus of the vast majority of AD research since their description almost 100 yr ago. Despite this interest, senile plaque density is only weakly correlated with AD symptomatology and disease progression and although neurofibrillary tangles correlate more closely with these parameters, they affect far too few neurons to directly account for all AD neuronal dysfunction and cell death.
(10, 11). Several studies indicate that both decreased synaptic density (12) and neuron loss (13) are highly correlated to clinical dementia in AD leading to a “chicken and egg” dilemma since synapse loss is an expected outcome of neuron death, and neuron death, possibly via decreased post-synaptic neurotrophic support, is a potential result of synapse loss (14).

A few studies have simultaneously examined synapse and neuron loss in AD brain and suggest that synaptic changes precede frank neuron loss. Rueger et al (15) suggested that synaptic loss and neuronal atrophy rather than cell death per se are important for AD. Neuronal atrophy in the absence of significant cell loss may also occur in several regions of the AD brain, including the nucleus basalis of Meynert (15). Messenger RNA levels for synaptophysin, a synapse-related protein, were reported to be decreased approximately 50% in neurofibrillary tangle-bearing hippocampal neurons (which showed no evidence of an apoptotic morphology) (16). Synaptophysin protein immunoreactivity is decreased significantly in early-stage AD brain (17), suggesting that altered synaptic protein expression occurs prior to neuronal death. Ultrastructural examination of tangle-bearing neurons in AD brain suggests gradual progression of cellular dysfunction not rapid cell death (18). Similarly, several studies have indicated decreased glucose metabolism in the temporal cortex of AD patients very early in the course of the disease, supporting the concept that dysfunction precedes death (15, 19, 20). The resolution of the synapse loss or cell death controversy is far from academic and has been nicely summarized by Swaab et al (21). “For patients currently suffering from AD it does not seem to make much difference whether neurons are present but inactive, or whether these neurons are lost altogether. It does, however, make a huge difference as far as the search for etiological factors and therapeutic strategies is concerned…” Thus, it is critical to determine if and when neuronal apoptosis occurs in AD brain.

Evidence for apoptotic death in AD brain is largely of 5 types: 1) in situ detection of fragmented DNA by terminal deoxytransferase (TdT)-mediated deoxyuridine triphosphate nick end-labeling (TUNEL or in situ end-labeling; ISEL) techniques; 2) patchy neuron loss in the absence of neuronal necrosis; 3) increased expression of pro-apoptotic molecules; 4) experimental data indicating apoptosis promoting effects of Aβ in vitro and/or increased apoptosis susceptibility of neurons with AD-related genetic mutations; and 5) detection of activated caspases and/or their cleaved substrates in AD brain.

In most cells undergoing apoptosis, extensive endonuclease activity is present and DNA is cleaved into a characteristic pattern of oligonucleosomal fragments. Approximately 10 yr ago, several techniques were developed for in situ detection of fragmented DNA, and in some experimental systems these techniques appeared to be specific for identifying apoptotic cells (22). Su et al (23) examined AD and control brains with a TdT-end labeling method and found extensive staining of neuronal nuclei in AD but not control brain sections. They also reported that numerous neuronal nuclei exhibited characteristic apoptotic features and concluded “apoptosis may be a fundamental mechanism leading to neuronal cell death in AD.” Other investigators have similarly observed increased in situ end-labeling in AD brain sections (24) although some have not (25). It is now very clear that these in situ labeling techniques are not specific for apoptotic nuclear events, are subject to artifact, and do not necessarily label cells that are committed to die (26). The sheer magnitude of labeling reported in AD brain is inconsistent with these techniques being specific markers of apoptosis and most investigators now agree that TUNEL reactivity in AD specimens is likely to represent sub-lethal DNA damage and thus, is of no value in determining the presence or absence of apoptosis in AD neurons. The morphological observations of Su et al that both tangle-bearing and non-bearing AD neurons exhibited apoptotic nuclear features have not been replicated by others (27), suggesting that the light microscopic criteria used to classify nuclei as “apoptotic-like” must be stringent. Considering the relatively brief period during which a cell appears apoptotic and the chronicity of the neurodegenerative disease process, the number of apoptotic neurons in AD brain at any given time may be quite low (28). Since definitive identification of apoptosis requires ultrastructural examination, it may prove difficult to unambiguously document apoptosis in AD brain. Such a search for apoptotic “evidence” is further complicated by the fact that postmortem AD brain tissue will probably be the required starting material and if apoptotic neurons are identified, non-AD causes of apoptotic neuronal death (e.g. perimortem hypoxic-ischemic injury, infectious agents, metabolic disturbances) must be excluded. On balance, previous publications on in situ detection of DNA damage and apoptotic-like morphological changes in AD brain neither support nor refute a role for apoptosis in AD pathogenesis.

A second piece of evidence for apoptotic cell death in AD that has been cited is “patchy” or “spotty” loss of neurons in AD brain such that healthy and dying cells are intermingled (29), which contrasts with necrotic cell death where groups of dead cells would be expected to occur. This argument can be immediately dismissed since in addition to apoptosis, a variety of other “non-necrotic” death pathways have been described, including autophagic, paraptotic, and heterophagocytic cell death (30, 31). Thus, neuron loss in the absence of necrotic death provides no evidence for apoptotic death. Autophagic cell death has been repeatedly observed in neurodegenerative diseases and disease models and, in light of increasing...
evidence for lysosomal abnormalities in AD, may prove relevant to the demise of neurons in AD brain (32, 33).

The third argument for apoptotic cell death in AD pathogenesis is that many apoptosis-associated molecules are upregulated in AD brain. Increased expression of prostate apoptosis response-4 (Par-4) protein and messenger RNA in AD brain, and its association with neuronal apoptosis in vitro has been cited as “the strongest evidence to date that neuronal death in AD may be due to apoptosis” (34). Similarly, increased expression of pro-apoptotic Bcl-2 family members, and decreased expression of an anti-apoptotic gene, NCKAP1, have been considered evidence for apoptosis in AD (29, 35). These alterations would be expected to increase the susceptibility of AD neurons to apoptotic stimuli, yet these changes in expression, in and of themselves, tell us nothing about the fate of AD neurons. Using the same logic that equates apoptotic death with increased expression of pro-apoptotic molecules or decreased expression of anti-apoptotic molecules, one could argue that increased expression of Bcl-2 and Bcl-XL, anti-apoptotic Bcl-2 family members, which has been observed in AD brain (36), indicates that AD neurons are healthy, viable, and able to resist apoptotic stimuli—an argument that no one is likely to make. The unsoundness of inferring outcome strictly on the basis of increased expression of genes associated with a biological process can be further illustrated by considering the upregulation of cell cycle-associated genes in AD brain (37). Aberrant expression of cyclin B-1, cyclin D, cdk4, and cdk5 in AD neurons is not evidence that neurons in AD brain are actively undergoing mitosis. Rather, increased expression of cell cycle-associated genes in AD neurons provides evidence for a perturbed cell. In total, alterations in apoptosis-associated gene expression occur in AD neurons but these changes may reflect sub-lethal responses to AD-associated stressors and do not imply that AD neurons are committed to apoptotic death.

The fourth argument for apoptosis playing an important role in AD comes from studies demonstrating that Aβ induces neuronal apoptosis in vitro and that AD-related genetic mutations in experimental animals increase apoptosis susceptibility. The significance of Aβ in AD pathogenesis has recently been reviewed in detail (38), so I will only briefly summarize the relationship between Aβ and neuronal apoptosis. Aβ is a potent neuronal apoptosis inducer in vitro and its death promoting actions are clearly regulated by Bcl-2 and caspase family members. However, the relevance of these observations to human AD is questionable (39). Particularly relevant to the possible effects of Aβ in vivo in human brain has been the failure to consistently observe significant neuronal apoptosis in various transgenic mouse models of Aβ overproduction, despite sometimes dramatic changes in caspase density and/or structure (40). These observations argue against a direct neurotoxic effect of Aβ on neurons in vivo, at least in mice, and suggest that if Aβ contributes to AD symptomatology and disease progression, it does so independently of neuronal apoptosis.

Mutations in presenilin 1 and 2 are associated with familial AD and these mutations have been reported to increase neuronal apoptosis susceptibility in vitro (41). However, some investigators have failed to demonstrate increased apoptosis sensitivity in mutant PS1 expressing neurons in vitro (42) and overexpression of mutant PS1 in transgenic mouse brain has led to inconsistent results (43). Chui et al (44) reported neuron loss in the absence of amyloid plaque formation in 2 lines of mutant PS1 transgenic mice whereas Takeuchi et al (45) found no neuron loss or Aβ deposition in mutant PS1 transgenic mice. Transgenic mice expressing both mutant PS1 and the Aβ precursor protein (APP) Swedish mutation (sw) showed increased Aβ deposition, but still no neuron loss (43, 45). Finally, the apolipoprotein E (Apo E) allele, e4, predisposes humans to AD. Apo E deficiency has been reported to affect synaptic integrity and Apo E4-dependent amyloid deposition and neuritic degeneration occurs in transgenic mouse models but significant neuron loss has not been reported (46, 47). In total, clear and convincing evidence that Aβ and/or AD-related genetic alterations lead to AD through enhanced neuronal apoptosis is not available.

Caspases and AD

Caspases are critically involved in apoptotic death. They can serve both as initial transducers of apoptotic stimuli and final executioners of death. Determining which, if any, caspases are expressed and activated in AD brain would significantly clarify the possible role of apoptosis in AD pathogenesis. Several articles have now reported caspase activation in either AD brain or AD-related experimental models. If convincing, such studies would provide strong evidence of a role for apoptosis in AD. There are 14 mammalian caspases and 12 human caspase orthologues which can be subdivided into 2 major groups, interleukin 1β converting enzyme (ICE)-like, and CED-3-like (7). The ICE-like caspases (e.g. caspase-1, caspase-4, caspase-5, and caspase-12) are involved in the proteolytic processing of cytokines and, with the possible exception of caspase-12, any effect they have on neuronal apoptosis in vivo is likely indirect via their modulation of inflammatory responses. CED-3-like caspases are directly involved in apoptosis and they can be further subdivided into initiator caspases (e.g. caspase-2, caspase-8, caspase-9, and caspase-10) that become activated early in the apoptotic cascade and typically catalyze the activation of the second CED-3 subgroup, downstream effector caspases (e.g. caspase-3, caspase-6, and caspase-7).
Caspases exist in cells at baseline aszymogens and although some unprocessed caspases (e.g. caspase-9) have weak protease activity, full caspase activity requires cleavage of the proenzyme into large and small subunits, which together form the active caspase. Activation can occur autonomously or through transactivation by other caspases. There are 2 major pathways of caspase-dependent cell death, the extrinsic pathway in which binding of a death ligand (e.g. Fas ligand) to its cell surface death receptor (e.g. Fas) triggers caspase-8 activation and the intrinsic pathway in which mitochondrial release of cytochrome c into cytosol facilitates activation of caspase-9 through an interaction with Apaf-1 in an energy-dependent fashion (7). The intrinsic and extrinsic pathways converge at the level of effector caspases. If effector caspase activation is sufficiently large, apoptotic cell death will follow. Not surprisingly therefore, caspase activation is regulated by a complex system of molecular checks and balances (48). Pro- and anti-apoptotic members of the Bcl-2 family can impact at multiple levels in the caspase cascade, although they may also have caspase-independent effects. A family of endogenous caspase inhibitors (inhibitors of apoptosis proteins [IAPs]) can modulate the catalytic activity of cleaved caspases and the IAPs themselves can be inhibited by SMAC/Diablo, a protein released from damaged mitochondria (48). This complex system of caspase regulation provides a measure of safety such that weak apoptotic stimuli and/or low levels of caspase activation do not prematurely commit a cell to the ultimate cell fate decision, death.

At least 7 caspases have been implicated in neurodegenerative diseases and/or AD. From the ICE family of caspases, both caspase-1 and caspase-12 have been studied. Caspase-1-deficient mice show some resistance to hypoxic-ischemic brain injury and overexpression of a dominant-negative caspase-1 mutant slightly delays, but does not prevent, premature death in mouse models of amyotrophic lateral sclerosis and Huntington disease (49, 50). Interestingly, the transgenic mouse model used to examine caspase-1’s involvement in Huntington disease (R6/2) is not associated with apoptotic neuronal death. Turmaine et al examined several transgenic mouse models of Huntington disease and concluded that “neuronal death found in the brain in HD is by a process that is morphologically and biochemically distinct from apoptosis” (51). Therefore, the ability of the dominant negative caspase-1 transgene to delay premature death cannot be attributed to an anti-apoptotic effect of caspase-1 inhibition on neurons. Caspase-1 mRNA levels were reported to be increased in AD brain extracts (52); however, if caspase-1 plays a role in AD, it is likely to act indirectly on neurons through inflammatory cells and cytokine production, not by promoting direct neuronal apoptosis.

Caspase-12 has recently emerged as a possible mediator of neuronal apoptosis. Caspase-12 is localized to endoplasmic reticulum (ER) and may regulate apoptosis in response to ER stress (53). Caspase-12-deficient cortical neurons displayed approximately half as much cell death as wild-type neurons following in vitro exposure to Aβ(1–40) suggesting that caspase-12 may be involved in Aβ neurotoxicity. Caspase-12 does not exist in humans and therefore cannot itself play a role in AD. There is approximately 50% identity between caspase-12 and human caspase-4 and caspase-5. A role for caspase-4 and/or caspase-5 in AD has not been reported. If caspase-4 and/or caspase-5 are found to be localized to neuronal ER, they may be potential mediators of apoptotic death and worthy of future investigation in AD pathogenesis.

Initiator caspases are strategically located in the apoptotic cascade between apoptotic stimulus receipt and effector caspase activation. Thus, they serve to integrate pro-death stimuli and transduce the death signal to downstream killer molecules. Several initiator caspases have been implicated in AD. Caspase-2 deficiency, either by targeted gene disruption or antisense down regulation, protects several neuronal subtypes from Aβ (1–42)-induced apoptotic death in vitro (54). However, in vivo studies of caspase-2-deficient mice led Bergeron et al to conclude that “the function of caspase-2 is not essential for neuronal cell death under pathological conditions” (55). Caspase-2 mRNA and protein levels were reported unchanged in AD brain (36, 52). Shimohama et al found an approximate 50% increase in caspase-2 protein on immunoblots of the particulate fraction from extracts of AD frontal cortex compared to controls (56). This study of caspase-2 protein was complicated however, by the fact that the antibody used to detect caspase-2 was not activated caspase-2-specific, the increased caspase-2 immunoreactivity in AD extracts was limited to the particulate fraction yet the majority of the caspase-2 immunoreactivity was found in the cytoplasmic fraction, and there was no difference in caspase-2 levels between control and AD extracts in the cytoplasmic fraction. Finally, the control samples were obtained from patients with a mean postmortem interval that was twice as long as that of the AD patients, which could have produced increased postmortem degradation of caspase-2 in the control samples relative to the AD samples. In total, a role for caspase-2 in AD has not been established.

Caspase-8 activation after engagement of death receptors by their ligands is a crucial early step in the extrinsic apoptotic pathway leading to effector caspase activation and death. Caspase-8 has been reported to mediate Aβ-induced neuronal apoptosis in vitro (57), to be elevated in immunoblots prepared from the caudates of Huntington disease patients, to be essential for Huntington disease-related neural degeneration in several
in vitro models (58), and to possibly be involved in proteolytic processing of APP (59). Several other observations, however, suggest that caspase-8 is not critically involved in AD and a role for caspase-dependent neuronal apoptosis in Huntington disease has also been questioned (32, 51). Caspase-8 immunoreactivity was not detected in synaptosomal preparations from control or AD AD brain (60), and the levels of both caspase-8 zymogen and cleaved "activated" caspase-8 fragments were reported to be unaltered or actually decreased in Western blots of AD brain homogenates compared with controls (61). Finally, direct microinjection of recombinant activated caspase-8 into human primary neurons in vitro did not produce apoptosis, suggesting that caspase-8 is not a potent apoptosis-inducer in human neurons (62). In total, caspase-8 involvement in AD is unproven.

Caspase-9 plays an important role in mammalian nervous system development and is the dominant initiator caspase in the intrinsic apoptotic pathway (6). Caspase-9 activation triggers caspase-3 cleavage in most, if not all, neuronal cells examined experimentally. Caspase-3 activation has been extensively examined in AD and AD models, however, caspase-9 itself has not. A recent study indicated that caspase-9 is capable of generating a cytotoxic peptide fragment from APP and, although this fragment was undetectable in whole brain homogenates of AD brain, it was detectable in synaptosomal preparations of AD frontal cortex (60). Similarly, an immunoreactive band consistent with cleaved caspase-9 was found in synaptosomal preparations from 5 of 5 AD brains but only in 1 of 5 control brain preparations. The significance of these observations is difficult to know since the proteolytic activity of cleaved caspase-9 may be inhibited by binding of endogenous IAPs (48). Thus, cleaved but inactive caspase-9 could have been detected in synaptosomal preparations. Caspase-9 activation of caspase-3 in the intrinsic apoptotic death pathway requires both cytochrome c and Apaf-1 and alterations in these 2 molecules were not found in AD brain (61). There is currently insufficient data to reach a conclusion about caspase-9 and AD.

Effector caspasas, caspases-3, -6, and -7, are largely responsible for the cytological changes that occur during apoptotic cell death and in some, but not all neuronal death paradigms, effector caspase activation represents the commitment point to death. Caspase-7 is expressed at only low or undetectable levels in the brain and is unlikely to play any role in AD. Caspase-3 and caspase-6 are found at relatively high levels in the nervous system and several studies have suggested they may be important in AD pathogenesis. The striking neurodevelopmental phenotype observed in caspase-3-deficient mice and the lack of obvious neurodevelopmental abnormalities in caspase-6-deficient mice would suggest that caspase-3 is more likely to be involved in the regulation of neuronal apoptosis. However, these 2 caspases have non-redundant roles in cellular protein degradation during apoptosis (63), may interact in some neuronal apoptosis models (64), and caspase-6 may compensate for caspase-3 in cells lacking either caspase-3 or caspase-9 (65). Interestingly, recombinant activated caspase-6 is a more potent apoptosis inducer than activated caspase-3 when microinjected into human primary neurons (62). Thus, caspase-6 represents a potentially interesting caspase to study in AD pathogenesis.

Desjardins and Ledoux failed to detect caspase-6 mRNA in human brain (52). However, caspase-6 proenzyme and its activated fragments have been detected by others in control and AD human brain extracts (66). Too few cases have been examined to determine if caspase-6 activity is specifically increased in AD brain. Activated caspase-6 may be involved in APP processing (59) and caspase-6 inhibition was able to block the apoptotic death promoting effects of the APP interacting protein APP-BP1 in primary neuronal cultures (67). Recently, Raina et al reported caspase-6 immunoreactivity associated with extracellular senile plaques in AD brain sections; however, caspase-6 immunoreactivity was not increased in AD neurons (68). The findings of LeBlanc and colleagues that recombinant activated caspase-6 may increase human primary neuron vulnerability to oxidative stress and produce a protracted form of neuronal death (62, 66) suggests that additional studies of caspase-6 are necessary before any conclusions about caspase-6 and AD pathogenesis can be made.

Of all the caspases, caspase-3 has received the most attention from AD investigators. Evidence for a role of caspase-3 in AD pathogenesis has included 1) caspase-3 activation in ABβ-deleted neuronal cultures, 2) increased levels of caspase-3 expression in AD brain, 3) immunodetection of activated caspase-3 and/or caspase-3 cleaved substrates in AD brain sections, 4) caspase-3 proteolysis of APP and involvement in ABβ peptide formation, and 5) synapse-associated caspase-3 activation in a process referred to as "synaptic" or "neuritic" apoptosis.

Caspase-3 activation occurs following in vitro exposure of neurons to ABβ, and some investigators have found that broad-spectrum caspase inhibitors can prevent, or significantly delay, ABβ neurotoxicity. However, caspase-3-deficient telencephalic neurons were not protected from ABβ (1–40)-induced death indicating that caspase-3 does not mediate ABβ neurotoxicity in vitro (39). Similarly, caspase-3-deficient neurons have not shown significant protection from a variety of other death-inducing insults; however, caspase-3 deficiency does affect the cytological appearance of dying neurons such that typical apoptotic nuclear condensation and fragmentation is not observed (5). These in vitro studies bear little relevance to in vivo neurodegeneration and this is illustrated by our analysis of caspase-3 activation in APPsw transgenic mice (69).
These mice show extensive Aβ deposition yet fail to exhibit activated caspase-3 immunoreactivity either in brain sections or extracts. These experimental animal studies do not definitively address the relationship between Aβ and caspase-3 activation since significant species and/or mouse strain dependent effects can be observed both in the effects of transgenic overexpression of APP and in apoptotic responsiveness.

There are conflicting reports on caspase-3 expression in AD brain. Increased neuronal caspase-3-like immunoreactivity was found in AD brain sections (9, 70) and immunoblots revealed increased caspase-3 immunoreactivity in both cytosolic and particulate fractions of AD versus control brain (56). However, LeBlanc et al found no difference in caspase-3 protein levels on Western blot analysis between control and AD brain extracts (66) and Engidawork et al found a significant reduction in caspase-3 reactivity in immunoblots prepared from AD brain compared to controls (61). Caspase-3 immunostaining was reported to be equivalent in control and AD brain sections (68) and Desjardins and Ledoux found no difference in caspase-3 mRNA levels between control and AD brain (52). Thus, no consistent change in caspase-3 mRNA or protein has been observed in AD brain. The apoptosis promoting effect of caspase-3 is due to the enzymatic activity of its 2 cleaved subunits that are generated by initiator caspase proteolysis. The analysis of cleaved caspase-3 subunits, activated caspase-3-like immunoreactivity, and downstream caspase-3 substrates in AD brain has also lead to conflicting results.

Cleaved caspase-3 subunits were found on Western blot analysis to be either undetectable in AD brain or significantly decreased in AD versus control brain extracts (61, 66). Similarly, immunohistochemical studies of AD brain sections with an antiserum that recognizes activated caspase-3-like immunoreactivity, as well as activated caspase-6 and caspase-7, identified only rare immunoreactive neurons (69). We examined sections of hippocampus and frontal cortex from 11 autopsy cases of AD and sections from surgically biopsied cortical tissue from an additional 3 cases of AD for activated caspase-3-like immunoreactivity. Activated caspase-3-like immunostaining was found in the hippocampus of 9 AD patients but was exclusively present in neurons with the features of granulovacuolar degeneration (Fig. 1, left panel). These neurons lacked cytological features of apoptosis. No immunoreactivity was found in cortical neurons, neurofibrillary tangle-bearing neurons, or associated with senile plaques. Granulovacuolar degeneration also occurs with aging and we observed activated caspase-3-like immunostaining in hippocampal neurons with features of granulovacuolar degeneration in 2 of 10 control elderly patients (>70 yr old). Stadelmann et al obtained identical results; they found activated caspase-3-like immunoreactivity in a large percentage of hippocampal neurons.
undergoing granulovacuolar degeneration in all 9 cases of AD examined (71). Excluding neurons with granulovacuolar degeneration, they found a total of 4 activated caspase-3-like immunoreactive neurons in 25 sections from these 9 AD cases. Jellinger and Stadelmann reported “Morphometric studies of the numbers of neurons in hippocampus and entorhinal cortex showing both strong cytoplasmic labeling for activated caspase-3 and the characteristic histologic changes of apoptosis in 4 AD brains (1 in 1,100 to 5,000 neurons) revealed only 1 single labeled cell” (28). The authors concluded “activation of caspase-3 does not have a significant role in the widespread neuronal death that occurs in AD.” Su et al recently reported activated caspase-3-like immunoreactivity in neurons, astrocytes, and blood vessels in normal human brain and associated with neurofibrillary tangles and senile plaques in Alzheimer disease brain (72). In my experience, such extensive immunoreactivity for activated caspase-3 is only observed with concentrations of primary antiserum that lack specificity for activated caspase-3, as evidenced by immunostaining of caspase-3-deficient mouse brain tissue (unpublished observations). The presence of activated caspase-3-like immunoreactivity in neurons undergoing granulovacuolar degeneration is interesting and suggests that activated caspases may be sequestered in autophagic vacuoles. Autophagic neuronal death has been observed in a variety of neurodegenerative diseases and lysosomal abnormalities are readily demonstrated in AD brain. Additional studies will be required to examine the interaction between apoptotic and autophagic death pathways. In total, these biochemical and immunohistochemical studies suggest that neuronal caspase-3 activation and apoptosis are extremely rare events in AD brain.

In contrast to the above-cited observations, several authors have reported increased detection of caspase-3-cleaved substrates, including processed APP in AD brain. These cleaved products have been found associated with senile plaques and neurofibrillary tangle-bearing neurons (73, 74). It is difficult to reconcile the appearance of these products with the lack of increased caspase-3 activity as assessed by other methods. One possible explanation for these conflicting reports could be that the level of activated caspase-3 existing in AD neurons is below the detection limits of the commonly used assays, i.e. activated caspase-3 specific antibodies, decreased caspase zymogen and increased large and small subunits on Western blots, and detection of caspase-3-like enzymatic activity in extracts. However, it is sufficiently large to cleave endogenous caspase-3 substrates, which then accumulate to quantities detectable with product-specific antibodies. Alternatively, the immunoreactive “caspase-cleaved” products may not be specifically generated by caspase proteolysis. A variety of other intracellular proteases such as calpains and cathepsins (which like caspases are cysteine proteases) show increased activity in AD brain (Fig. 1, right panel) and may be involved in the processing of endogenous caspase-substrates (75). For example, α- and β-calpains, PARP, and tau may be cleaved by both caspases and calpains. Additional studies are needed to resolve this issue.

As data has accumulated and the weight of evidence indicates that apoptosis is an uncommon event in AD brain, there has been a shift in the formulation of the “apoptosis causes AD” hypothesis. In the absence of reproducible evidence for increased caspase activity in AD brain, the occurrence and possible significance of caspase-cleaved APP in AD is unclear.

Based on the extensive synaptic abnormalities that occur in AD brain and in vitro studies suggesting that caspasases may be selectively activated in synaptic compartments, several authors have proposed that synaptic or neuritic “apoptosis” may contribute to AD pathology independently of neuronal cell death. Mattson et al reported that exposure of synaptosomes to Aβ or other neurotoxins could induce caspase-activation, mitochondrial depolarization, phosphatidyl serine exposure, and Par-4 production and speculated that activation of “synaptic apoptosis” could play a role in neurodegenerative synapse loss (77, 78). Ivins et al reported that localized Aβ exposure to distal neurites caused neuritic degeneration with biochemical and morphological features of apoptosis and proposed that in AD brain Aβ could activate apoptotic pathways locally in neurites and cause a loss of neuronal connectivity (79). These changes presumably could occur without caspase activation in neuronal cell bodies and resultant apoptotic cell death. That synaptic degeneration and neuritic abnormalities occur in AD brain is well documented; however, there are several reasons to doubt that selective synaptic and neuritic caspase activation is a significant pathological process in AD. First, using a variety of in vitro and in vivo techniques, Finn et al were unable to replicate the studies of Mattson et al and Ivins et al (80). Finn et al concluded that “a neuron contains at least 2 molecularly distinct self-destruction programs, one for caspase-dependent apoptosis and another for selective axon degeneration.” Second, neither we (69) nor others (28, 71) have detected activated caspase-3-like immunoreactivity in association with Aβ deposits in experimental animals or in human AD brain sections. Similarly, activated caspase-3-like immunoreactivity is not observed in abnormal neurites or in neurofibrillary tangle bearing neurons in AD brain, nor
have we ever observed specific activated caspase-3-like immunoreactivity in mouse brain unassociated with apoptotic cell death. Third, Par-4 levels have been demonstrated to be elevated in AD brain and to be translationally regulated locally within synaptic compartments (81). Par-4 expression is not apoptosis-specific but if “synaptic apoptosis” was occurring in AD brain, one would expect to find extensive Par-4 immunoreactivity in abnormal synaptic structures and neurites in AD brains. Guo et al compared the distribution of Par-4 immunoreactivity in AD brain sections with that of PHF-1 (an antibody that recognizes hyperphosphorylated tau) and reported “PHF-1 immunoreactivity was present in dystrophic neurites associated with neuritic plaques, whereas these structures showed little or no immunoreactivity with the Par-4 antibody” (82). Thus, if components of the classic apoptotic death pathway can be selectively activated in synapses or neurites without cell death resulting, there is no evidence that such a process occurs in AD brain.

Overview

If apoptotic cell death is significant for AD pathogenesis, there are at least 3 possible levels of involvement. Primary involvement would mean there is an abnormality in the apoptotic death pathway itself, which leads directly or contributes substantially, to neuronal apoptotic death. In this scenario, there is neither an increase in apoptotic death stimuli in AD nor neuronal dysfunction prior to death. Evidence for such a primary process would include identifiable genetic mutations leading either to loss of function in anti-apoptotic molecules such as Bcl-X₅, or increased activity of pro-apoptotic molecules such as Bax or caspase-3. To my knowledge, no mutations in Bcl-2 family members, caspases, Apaf-1-like molecules, IAPs, or SMAC/Diablo have been found associated with AD. Mutations in caspase-10 underlie autoimmune lymphoproliferative syndrome type II (83) and mutations in caspase-8, bax, and apaf-1 have been found in some neoplasms. Interestingly, the gene for neuronal apoptosis inhibitory protein is partially deleted in patients with spinal muscular atrophy and may contribute to the severity of the disease indicating that mutations in apoptosis pathway genes can cause a neurodegenerative disease (84).

Apoptosis could be involved secondarily in AD if an increase in apoptotic stimuli leads directly to “appropriate” activation of apoptotic death effectors and neuronal dysfunction results from apoptotic death. There is a great deal of support for increased apoptotic stimuli in AD brain ranging from oxidative stress to decreased neurotrophins. In familial AD, APP, PS1, and PS2 mutations may directly or indirectly increase apoptotic stimuli, which then engage the apoptotic pathway. However, as reviewed earlier, neuronal dysfunction in AD may precede extensive neuron loss and what neuron loss does occur in AD brain is not convincingly apoptotic. Thus, activation of apoptotic death effectors is unlikely to secondarily cause AD.

Following a review of the literature, I favor the view that apoptosis is involved in AD in a tertiary fashion. In AD brain, there is an increase in apoptotic stimuli that leads to sub-lethal increases in pro-apoptotic molecules and compensatory changes in anti-apoptotic molecules and cellular processes which maintain neuron survival. This adaptive response to acutely sub-lethal stress is highly regulated and results in structural and biochemical changes that ultimately manifest as neuronal atrophy and dysfunction. If the pro-death stimuli persist, neuronal apoptosis and/or other morphological and biochemical types of death will ultimately occur; yet it is neuronal dysfunction, not neuronal death that is initially responsible for AD symptomatology.

If this view of AD pathogenesis is correct, caspases are involved only in the last stage of the disease process. Caspase activation is not the initiating stressor, apoptosis is not “dysregulated” in AD, and neuronal dysfunction does not result directly from caspase-dependent apoptosis. Caspase inhibitors however, could prove valuable if they delay neuronal death such that other pharmacological agents targeting the primary disease process can act to decrease stress levels in AD neurons. Unfortunately, the primary stressors in AD pathogenesis are still ill defined and may represent a combination of Aβ, oxidative stress, age-related metabolic alterations, and/or neurotrophin abnormalities. Hopefully, the next 10 yr of research will focus on defining the primary events in AD pathogenesis, the molecular pathways controlling all types of “regulated” neuronal death, not just apoptotic death, and developing pharmacological agents to intervene at the earliest possible stage of AD pathology.

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