

# Problems of cell death in neurodegeneration and Alzheimer's Disease

Kurt A. Jellinger<sup>a,\*</sup> and Christine Stadelmann<sup>b,\*\*</sup>

<sup>a</sup>*Ludwig Boltzmann Institute of Clinical Neurobiology, Vienna, Austria*

<sup>b</sup>*Department of Neuroimmunology, Brain Research Institut, University of Vienna School of Medicine, Austria*

Progressive cell loss in specific neuronal populations is a pathological hallmark of neurodegenerative diseases, but its mechanisms remain unresolved. Apoptosis or alternative pathways of neuronal death have been discussed in Alzheimer disease (AD) and other disorders. However, DNA fragmentation in human brain as a sign of neuronal injury is too frequent to account for the continuous loss in these slowly progressive diseases. In autopsy cases of AD, Parkinson's disease (PD), related disorders, and age-matched controls, DNA fragmentation using the TUNEL method and an array of apoptosis-related proteins (ARP), proto-oncogenes, and activated caspase 3, the key enzyme of late-stage apoptosis, were examined. In AD, a considerable number of hippocampal neurons and glial cells showed DNA fragmentation with a 3- to 6-fold increase related to amyloid deposits and neurofibrillary tangles, but only one in 2.600 to 5.650 neurons displayed apoptotic morphology and cytoplasmic immunoreactivity for activated caspase 3, whereas no neurons were labeled in age-matched controls. Caspase 3 immunoreactivity was seen in granules of cells with granulovacuolar degeneration, in around 25% co-localized with early cytoplasmic deposition of tau-protein. In progressive supranuclear palsy, only single neurons but oligodendrocytes in brainstem, around 25% with tau-inclusions, were TUNEL-positive and expressed both ARPs and activated caspase 3. In PD, dementia with Lewy bodies, and multisystem atrophy (MSA), TUNEL-positivity and expression of ARPs or activated caspase 3 were only seen in microglia and oligodendrocytes with cytoplasmic inclusions in MSA, but not in neurons. These data provide evidence for extremely rare apoptotic neuronal death in AD and PSP compatible with the progression of neuronal degeneration in these chronic diseases. Apoptosis

mainly involves reactive microglia and oligodendroglia, the latter occasionally involved by deposits of insoluble fibrillary proteins, while alternative mechanisms of neuronal death may occur. Susceptible cell populations in a proapoptotic environment, particularly in AD, show increased vulnerability towards metabolic or other noxious factors, with autophagy as a possible protective mechanism in early stages of programmed cell death. The intracellular cascade leading to cell death still awaits elucidation.

**Keywords:** Alzheimer's disease, Parkinson's disease, programmed cell death, apoptose-related proteins, activated caspase-3

## 1. Introduction

Neurodegenerative disorders such as Alzheimer disease (AD), the most common type of dementia, and Parkinson disease (PD), the most frequent movement disorder, are morphologically characterized by progressive neuronal loss. In AD, loss of cortical neurons and synapses is accompanied by extracellular deposition of amyloid $\beta$  ( $A\beta$ ) in senile plaques and cerebral vessels, and cytoskeletal changes with deposition of paired helical filaments containing hyperphosphorylated microtubule-associated tau-protein forming neurofibrillary tangles (NFT), neuropil threads, and neuritic plaques [1]. In PD or brainstem type of Lewy body disease neuron loss in substantia nigra is accompanied by widespread occurrence of intracytoplasmic Lewy bodies (LB) formed from fibrillary  $\alpha$ -synuclein and hyperphosphorylated neurofilament protein [2], and frequent additional cortical LB in dementia with Lewy bodies (DLB) [3]. In multiple system atrophy (MSA) and progressive supranuclear palsy (PSP), multisystemic degeneration is associated with intracytoplasmic inclusions (ICG) containing  $\alpha$ -synuclein [4] and tau-protein differing from that in AD related to mutations

\*Corresponding author: K.A. Jellinger, Ludwig Boltzmann Institute of Clinical Neurobiology, O. Wagner Hospital/B-Building, Baumgartner Hoehe 1, A-1140 Vienna, Austria. E-mail: kurt.jellinger@univie.ac.at.

\*\*Present address: Department of Neuropathology, Charité, Berlin, Germany.

in the tau gene [5,6]. The causes of cell death and their pathogenic relationship to the morphologic disease markers in these and other neurodegenerative disorders are still unknown. Recently, apoptosis, a specific form of gene-directed programmed cell death (PCD) [7, 8], has been implicated as a general mechanism in the degeneration of selective neuronal populations [9,10], since apoptosis is induced by exposure of neuronal cultures to A $\beta$  peptide, the amyloidogenic cleavage product of amyloid  $\beta$ -protein precursor (A $\beta$ PP) [11–15], with selective increase in cellular A $\beta$ 42 related to apoptosis but not necrosis [16,17], and neurotoxins inducing experimental parkinsonian syndromes [18,19].

In AD brain, increased expression of both proapoptotic (c-Jun, c-Fax, Bax, p. 53, APO-1/Fas-DC95) and antiapoptotic proteins (Bcl-2, Bcl-X) [20–25], and in the MPTP model of parkinsonism, changes of antiapoptotic proteins Bcl-2, Bcl-X [26], and participation of prostate apoptosis response-4 (Par-4) related to Fe<sup>2+</sup>-induced mitochondrial dysfunction [18] – similar to an experimental Huntington disease model [27] – have been observed. They have been related to cell death in human PD [19], where upregulation of Bcl-2 in basal ganglia [26] without changes of Bax and Bcl-X have been reported [29,30]. Histochemical studies for demonstration of fragmented DNA as a sign of programmed cell death (PCD) by terminal deoxynucleotidyl transferase dUTP and labeling (TUNEL) and related methods have revealed large numbers of neurons and glial cells in postmortem AD brain [31–34], with co-expression of apoptosis-related proteins (ARPs), like c-Jun, Bax and Bcl-2, but decreased levels of Bcl-2 in tangle-bearing neurons [20,22,35]. There have been conflicting reports on the incidence of DNA fragmentation in PD as well as in other neurodegenerative disorders [10,36–42]. In order to further elucidate the enigma of PCD, we performed extensive studies in post mortem brain tissue of several neurodegenerative disorders.

## 2. Material and methods

Brain tissues from 9 cases of neuropathologically confirmed cases of AD, all fulfilling the CERAD criteria of definite AD [1] and Braak stages 5 or 6 [43], 5 cases of confirmed PD, 3 cases each of DLB (criteria by McKeith et al. [3]), PSP, MSA [4], and 7 age-matched controls without brain diseases were investigated. Brains were fixed in buffered formalin and blocks from multiple areas were embedded in paraffin.

Since tissue pH levels of less than 6.4 as a result of ante mortem hypoxia may affect the preservation of RNA after death [44], cases with long agonal state of hypoxia were excluded.

Immunohistochemistry was performed on 5  $\mu$ m deparaffinized sections according to the avidin-biotin-peroxidase complex (ABC) and alkaline phosphatase-anti-alkaline phosphatase (APAAP) methods using diaminobenzidine (DAB) and Fast Red (TR) salt, respectively, as chromogens. Primary antibodies against c-Jun/AP1, ASP, bcl-2, Bax, p53 protein, Bcl-X, CD 95 (Fas/Apo-1), non-activated and activated caspase 3 (using an affinity purified rabbit polyclonal antiserum reactive against human activated caspase 3 (CM-1) (IDUN Pharmaceuticals, La Jolla, CA) [45], against several heat-shock proteins, PHF-tau (AT-8), A $\beta$  (4G8), and  $\alpha$ -synuclein were used [46,47]. The expression of these substances was not influenced by postmortem delay. Control sections were incubated without primary antibody. In situ terminal deoxynucleotidyl transferase (TdT)-mediated incorporation of dioxigenine-labeled nucleotides (TUNEL method) was used to detect DNA fragmentation [48]. Post mortem delay up to about 24 hours did not significantly influence the numbers of TUNEL positive nuclei [31,33], whereas archival length in 10% buffered formalin that also can affect TUNEL labeling in postmortem human brain [49,50] was comparable in all examined cases as well as in controls.

## 3. Results

Compared to controls, DNA fragmentation in AD brain was about 50 fold increased in neurons and 25 fold in glial cells, mainly microglia and oligodendroglia, only 28% of all degenerating cells representing neurons [31]. However, only exceptional hippocampal neurons displayed the typical morphology of apoptosis, i.e. a reduction in cell size, chromatin condensation and the formation of apoptotic bodies [7], or showed diffuse cytoplasmic expression of either ARPs or caspase 3 (Fig. 1(A)). No CM-1 immunoreactive neurons were found in aged controls. Most of the TUNEL-positive neurons were seen in the medial temporal allocortex. Only 13 to 50% (mean 28%) of the degenerating neurons were located within or next to A $\beta$  deposits, but these were 5.7 ( $\pm$  0.8 SD) fold more than without contact to plaques. NFTs involved a mean of 41% (range 18 to 66%) of all degenerating neurons, which means a 3 ( $\pm$  0.5 SD) fold increased risk of degen-

Table 1

In situ tailing and immunohistochemical results in Parkinson's disease (PD), Progressive Supranuclear Palsy (PSP), Alzheimer's disease (AD), and Controls (CO)

Method/antibody applied	PD			PSP *)		AD °)	CO °)
	SN-Neurons	Microglia	Lewy body	Neurons	Oligos		
In situ Tailing (TUNEL)	0	+	–	+ (~0.1%)	++	+(1.1±0.4%)	+(0.02±0.01%)
<i>Immunohistochemistry (ARPs)</i>							
c-Jun/AP-1 (ASP 1)	+ <sup>a</sup>	+	–	±	+	±	+ <sup>a</sup>
c-Jun	+ <sup>b</sup>	+	–	++	+	++	± <sup>b</sup>
Bcl-2	± <sup>b</sup>	+	–	+	++	++	± <sup>b</sup>
Bcl-X	++	+	–	++	+	++	++
p 35	±	±	–	±	+	+	–
CD 95	–	±	–	±	+	+	–
<i>Immunohistochemistry (Heat-shock) and others</i>							
α B-Crystallin	–	–	+	±	+	±	±
HSP 27	–	–	–	–	–	±	±
HSP 65	–	–	–	++	–	++	++
HSP 70	–	–	–	+	–	±	±
HSP 90	–	–	–	++	–	++	++
PHF/Ubiquitin	–	–	+	±	+	++(NFT)	±(NFT)
Ubiquitin	–	–	+++	+	–	++(NFT)	±(NFT)
PHF-Tau (AT-8)	–	–	–	++	++	+++	±(NFT)
α-synuclein	–	–	+++	–	–	–	–
Activated caspase 3 (CM1)	–	±	–	±(<0.1%)	+	±(02–0.05%)	–

– no labeling; ± exceptional cells labeled; + few cells labeled; ++ many cells labeled;

\*) brainstem; °) Stadelmann et al. AJP 155 (1999) (hippocampus);

<sup>a</sup>) nuclear granules; <sup>b</sup>) cytoplasmic granules; NFT = neurofibrillary tangles

eration compared to non-tangle bearing neurons [31]. Morphometric studies of the numbers of neurons in hippocampus showing both strong cytoplasmic labeling for activated caspase 3 and the characteristic histological changes of apoptosis (Fig. 1(A)) in 3 AD brains revealed only one single labeled cell among 2.600 to 5.650 counted neurons (Table 1). On the other hand, activated caspase 3 was detected in granules of  $55 \pm 10\%$  of neurons showing granulovacuolar degeneration (GVD) (Fig. 1(B)) that was present in around 12% of subicular and CA1 neurons in AD, while they were only found in a few cells in controls. These data were recently confirmed by Marcon et al. [51] showing occasional neuronal caspase-3 immunoreactivity in sporadic AD, A $\beta$ PP-FAD, and GVD but not in PS-FAD and frontotemporal dementia (FTDP-17) cases in the absence of TUNEL staining and apoptotic morphology in these cells. Caspase 3 immunoreactivity was restricted to the granules in GVD and not present in other cytoplasmic components, e.g. lipofuscin or NFTs. Double-staining with the tau-antibody AT-8 revealed fine granular cytoplasmic expression of hyperphosphorylated tau suggesting a “pre-tangle” stage [52] together with CM-1-immunoreactive granules in  $26 \pm 5\%$  (range 2–91%), whereas neurons involved by NFTs did not express caspase 3 (Fig. 1(B)). GVD was much more frequent in AT-8 positive ( $78 \pm 7.7\%$ ) than in tau-negative neurons

( $21.7 \pm 7.8\%$ ); GVD granules were not labeled by the AT-8 antibody. None of the neurons with caspase 3 immunoreactive GVD showed nuclear alterations indicative of apoptosis [46].

In PSP, only single neurons in brainstem tegmentum (about one among 1050) were TUNEL-positive indicating DNA fragmentation (Fig. 1(F)), with moderate expression of c-Jun and some heat-shock proteins, much less of ASP-1 and Bcl-2. Only one of five such neurons showed co-expression with the antibody AT-8 decorating hyperphosphorylated tau inclusions (fibrillary tangles), while neurons in SN, basal ganglia and pontine nuclei were all TUNEL-negative. On the other hand, a number of oligodendroglial cells in brainstem tegmentum and pontine basis were TUNEL-, ARP- or CM-1 positive indicating apoptotic cell death (Fig. 1(E), Table 1). About 25–30% of these oligos contained tau-inclusions (coiled bodies) (Fig. 1(G)), while no signs of apoptosis were detected in astroglia with tau-positive inclusions.

In PD brains, all displaying severe loss of melanized neurons in the ventral and caudal parts of substantia nigra zona compacta (SNZC) with gliosis and variable numbers of subcortical LBs, and in DLB showing additional cortical LBs in cingulate, frontal, and temporal cortex, nigral neurons revealed a loose and finely granular nuclear chromatin structure. Only exceptional

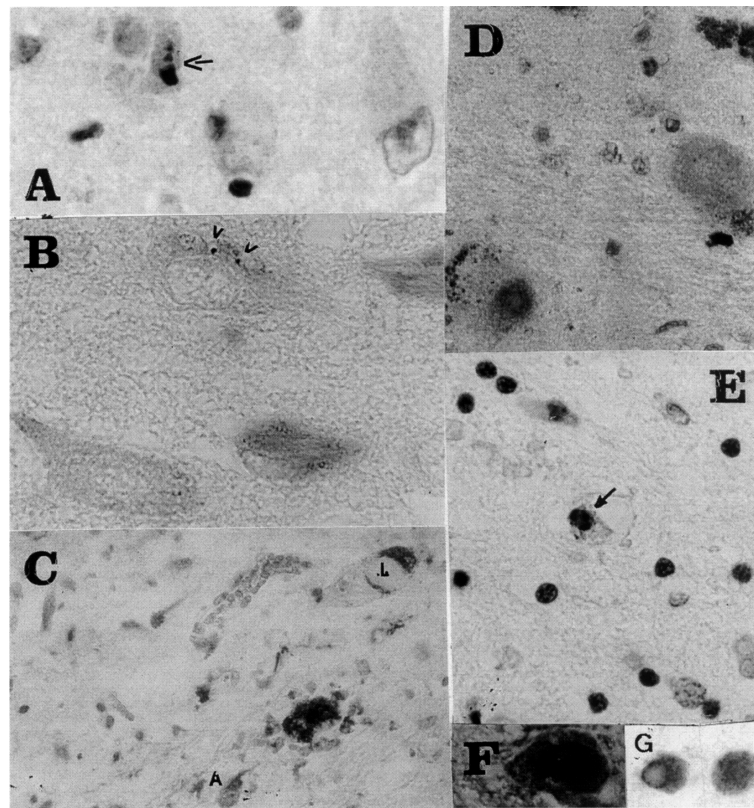


Fig. 1. *A-B*. Hippocampus of 65 year old female with sporadic Alzheimer's disease (Braak stage 5). (A) CA1 neuron immunoreactive for activated caspase-3 (yellow) with condensed, fragmented nucleus and shrunken cytoplasm indicative of apoptotic cell death (arrow). Other neurons showing no nuclear changes are negative for CM-1 antibody ( $\times 1,000$ ). (B) Neurons in subiculum. One shows granules of granulovacuolar degeneration immunolabeled with the CM-1 antibody (arrowheads), while other cytoplasmic compartments remain unstained but slightly express AT-8 antibody (blue) suggesting finely granular accumulation of hyperphosphorylated tau protein. Two other neurons with neurofibrillary tangles express AT-8 (blue) but are negative for CM-1; one unaffected neuron (right bottom) is unlabeled for both antibodies ( $\times 1,000$ ). *C-D*. Substantia nigra zona compacta of 73 year old female with Parkinson's disease. (C) Nigral neurons and Lewy body (L) are all TUNEL negative; reactive microglia and one astrocyte (A) are TUNEL-positive (blue) ( $\times 500$ ). (D) Nigral neurons with mild nuclear (left) and extremely mild cytoplasmic (right) expression of c-Jun/AP 1 antibody (APAAP, FR,  $\times 6000$ ). *E-G*. Pontine nuclei of 57 year old male with PSP. (E) Many oligodendrocytes show positive TUNEL-reaction with negative expression of CM-1 antibody, with positive expression of CM-1 in granulocyte (center) ( $\times 250$ ). (F) Single tangle-bearing neuron in pontine tegmentum showing positive TUNEL-reaction (blue) ( $\times 500$ ). (G) Double-labeling of two oligodendrocytes in pontine basis with CM-1 antibody (brown) and AT-8 (blue) indicating coexpression of tau-positive cytoplasmic inclusions and activated caspase 3 ( $\times 800$ ).

neurons showed a reduction in cell size and clumping of nuclear chromatin resembling apoptosis [53]. However, not a single among 1080 counted melanized neurons in SNZC and in locus coeruleus with or without LB in PD, DLB or controls showed DNA fragmentation. On the other hand, varying numbers of microglial cells and a few astrocytes were TUNEL-positive (Fig. 1(C), Table 1). Melanized neurons of SNZC of PD, DLB, and controls showed mild to moderate expression of c-Jun, finely granular reaction of C-Jun/AP1 in the nucleus and of ASP in cytoplasm (Fig. 1(D)), weak Bcl-2 and Bax immunoreactivity in cytoplasm, and rather strong expression of Bcl-x with no differences between neurons with or without LBs and between PD/DLB

and controls. There was no neuronal expression of p53, CD95 (Fas/APO-1) or CM-1 in any of the investigated brains, while reactive astroglia and microglia expressed Bcl-2, Bax,  $\alpha$ -B crystallin and, less, Bcl-x. LBs were all negative for the examined ARPs and activated caspase 3, but showed strong expression of ubiquitin,  $\alpha$ -synuclein, and less of  $\alpha$ -B crystallin, while axonal spheroids and neuritic axons ("Lewy neurites") were strongly immunoreactive for ubiquitin,  $\alpha$ -B crystallin, with mild decoration by Bcl-X and CM-1. In MSA, TUNEL-positivity and expression of ARPs and CM-1 were only seen in microglia and oligodendroglia with  $\alpha$ -synuclein/ubiquitin positive ICGs but not in nigral or other neurons (data not shown).

#### 4. Discussion

Apoptosis has been proposed as a major pathway of neuronal degeneration in a variety of disorders including AD [9,20–25], PD [19,28,39,42,54–57], MSA [38], frontotemporal dementia [41], Huntington's disease [37,39], Werdnig-Hoffmann disease [40], while other recent studies did not confirm its presence in AD [59], PD [30,37,47,53,61,62], PSP [38,47], Pick's disease [62] or amyotrophic lateral sclerosis [63,64].

Mutations of the presenilin (PS) 1 gene in familial early onset AD have been shown to sensitize neuronal cells to apoptosis [65] and render neurons from mutant mice susceptible to various inducers of cell death [66–68]. Cleavage of PS 1 and 2 proteins generates anti-apoptotic C-terminal fragments [67–69]. Transfection of neuronal cells with mutants of the A $\beta$ PP gene causes DNA fragmentation [70], while A $\beta$ -peptide induces apoptosis-related changes in synapses and dendrites [11] that are potentiated by inhibition of NF $\kappa$ B [71], and a novel A $\beta$ PP mutation increases A $\beta$  peptide levels and induces apoptosis [72]. In sporadic AD, DNA fragmentation may accompany tangle formation but is less correlated with the amyloid (plaques) load [31,73,74], while in familial AD related to PS 1 mutations, no correlation between DNA fragmentation and the severity of A $\beta$  deposits or NFTs was found [75]; the majority of TUNEL-positive neurons, however, showed ultrastructural features of cell death by necrosis [76]. The relationship between presenilins and cellular damage has recently been discussed by Lucassen [77]. The preferential activation of caspase 3 in APP related FAD but not in PSI-FAD, suggests that caspase 3 may be involved in APP processing and A $\beta$  formation *in vivo*, while its absence in FTDP-17 cases argues against a role of this enzyme in tau cleavage and tangle formation [51].

Neuropathological studies in AD brains and, less, in PD point towards a disturbed balance of pro- and anti-apoptotic proteins indicating the presence of a proapoptotic environment [20,22–24,26,33–35], and incomplete cell cycle activation in postmitotic AD neurons possibly leading to their elimination by apoptosis [9,77,78]. Recent studies suggest that the development of A $\beta$ -plaques in the brain may cause damage to axons, and the abnormally prolonged stimulation of the neurons to this injury ultimately results in cytoskeletal alterations that underly neurofibrillary pathology and neurodegeneration [58]. A further argument for apoptotic cell death was the significantly elevated number of cells with DNA fragmentation in AD brain compared to

normal controls [31–33,50]. However, the incidence of TUNEL-positive neurons in AD is significantly higher than could be expected in a disease with an average duration of 10 years plus [79,80]. Recent data provide evidence for apoptotic neuronal death in hippocampus, the most severely involved area in AD brain, with an extremely low frequency compatible with the slow progression of neuronal degeneration and the clinical latency of this disease. This appears realistic in view of the short duration required for the completion of apoptosis and the protracted course of AD. On the other hand, the significantly increased incidence of cells with DNA fragmentation together with the "proapoptotic" phenotype of neurons in AD brain in comparison to age-matched controls indicates that AD neurons may be more vulnerable to hypoxia and other pathogenic factors [31,46,75,77,79,80]. The variable correlation between neuronal PCD with A $\beta$  deposits and NFTs in AD suggests that these two hallmark lesions may not be the only causative factors of neuronal death [31,75].

The negative findings in neurons of PD, DLB and MSA are at variance with previous studies [42,54–57], but are in agreement with recent results [30,38,47,60,61]. Only in rare PD cases, occasional neurons with a reticular TUNEL labeling of nuclei in the absence of classical criteria of apoptosis were recorded [53,60]. These data are supported by the fact that no significant differences in the expression of pro- and antiapoptotic proteins are found in SNZC neurons in PD and DLB versus controls [28,47,60]. On the other hand, in human PD substantia nigra, significantly increased levels of caspase 1 and 3 and of tumor necrosis factor receptor R1 (TNF-R1, p55) have been observed [81] suggesting a proapoptotic environment, while the percentage of neurons expressing activated caspase 3 was significantly higher in PD brain than in controls suggesting that its activation precedes and is not a consequence of apoptotic cell death [42,82]. In human subjects with parkinsonism following MPTP exposure and survival times between 3 and 16 years, signs of active, ongoing nerve cell loss and clustering of microglia around nerve cells in SNZC were observed [83]. The most convincing finding arguing against apoptotic cell death mechanisms in SNZC neurons in PD; DLB, and MSA is the absence of expression of activated caspase 3, the central effector enzyme of the terminal apoptotic cascade [9,10,84,85]. In PSP, only exceptionally few neurons in midbrain tegmentum show DNA fragmentation with coexpression of ARPs and tau-immunoreactive inclusions. On the other hand, DNA fragmentation and expression of both ARPs and activated caspase 3 was

seen in activated, iron-loaden microglia in the SNZC in PD, DLB, and MSA brains, and in oligodendroglia in PSP and MSA, several of which showing cytoplasmic inclusions expressing either hyperphosphorylated tau (in PSP) or  $\alpha$ -synuclein (in MSA). In MSA, a distinct cytoplasmic expression of Bcl-2 was seen in oligodendroglial cells with coexpression with ubiquitin in about 25% of inclusion-bearing cells [36,47]. Since oligodendrocytes are generally Bcl-2 negative, its expression in pathologically altered cells in MSA may represent a final repair mechanism of a sublethally damaged cell to avoid cell death via apoptosis by upregulation of this antiapoptotic protein. In MSA, a sporadic synucleinopathy, glial changes and glial-neuronal interactions caused by widespread biochemical modifications of  $\alpha$ -synuclein are considered a fundamental molecular characteristic and early pathogenic event of this disorder [4, 86] and recent studies have demonstrated induction of cell death in cultured neurons by  $\alpha$ -synuclein [87]. Their formation may be the crucial event through which the oligodendroglia-myelin-axon pathway causes neurodegeneration and more widespread myelin degeneration than previously recognized [87]. In Huntington's disease, Portera-Cailliau et al. [88] reported DNA fragmentation in oligodendroglia and suggested it to result either from Wallerian degeneration due to neuronal death or a direct result of genetic IT-15-huntingtin changes, while the pathogenic role of oligodendroglial involvement in PSP appears hitherto unresolved.

TUNEL-positivity and occasional coexpression with tau- or  $\alpha$ -synuclein-positive ICGs in oligodendrocytes suggest that aggregation of insoluble protein filaments in the cytoplasm (NFTs, LBs, tau-inclusions, Pick bodies etc) may contribute to dysfunction or increased vulnerability of the involved cells [2]. However, demonstration of negative DNA fragmentation in SN neurons with LBs [30,47,90], in neurons with Pick bodies [62], limited involvement of NFT-bearing neurons and of inclusion-containing oligodendrocytes by apoptosis [36,46,47], and recent computer models of tangle-bearing neurons in AD suggesting their survival for about 20 years [91] indicate that these inclusions do not predispose a cell to undergo (programmed) cell death. This is in agreement with recent results after chronic inhibition of protein phosphatase 1 and 2 causing dephosphorylation of tau protein and neuronal apoptosis that show different distribution of tau protein and apoptotic neurons, indicating that these cytoskeletal changes have no obvious sequelae for the viability of the involved neurons [92]. Recent studies showed different solubility of  $\alpha$ -synuclein between LBs in DLB

(insoluble) and ICGs in MSA oligodendroglia (soluble) probably resulting from different processing of  $\alpha$ -synuclein [93], which might also influence the viability of involved cells in different ways. Thus, the biologic significance of these cytoplasmic inclusions related to mismetabolism of cytoskeletal proteins and the role they play in neurodegeneration are still enigmatic.

The demonstration of very rare activation of caspase 3 in hippocampal neurons in AD and in brainstem neurons in PSP, with absence of DNA fragmentation and significant upregulation of APRs or activated caspase 3 in SN neurons in PD, DLB and MSA, suggest that variably increased rates of DNA fragmentation in susceptible neurons in these and other degenerative disorders indicate PCD not necessarily via apoptosis, but rather reflect the combined action of deficient DNA repair and accelerated DNA damage within susceptible cells [9,47,62]. Cells with increased DNA damage may show increased vulnerability towards metabolic disturbances and several pathogenic factors, e.g. oxidative stress, mitochondrial damage, etc., inducing a cascade of events finally leading to cell death [94–96]. On the other hand, the demonstration of activated caspase 3 expression in autophagic vacuoles of GVD, of upregulation of antiapoptotic proteins (Bcl-2, Bcl-x) [22–24], of DNA repair enzymes such as Ref-1 [20], and the coexpressed GADD45 protein [97] in AD may indicate possible responses to oxidative stress or attempts to repair damaged DNA and, thus, to prevent cell death [98]. Recent demonstration of elevated casein kinase 1 (CK-1), a member of protein kinases, in both the matrix of GVD bodies and tangle-bearing cells suggest a molecular link between these two lesions in AD [99]. Ultrastructural features of autophagic degeneration [100], i.e. mild condensation of nuclear chromatin, moderate vacuolation of endoplasmic reticulum, and lysosome-like vacuoles but normal mitochondria, were occasionally seen in melanized SNZC neurons of PD brain [55], suggesting alternative mechanisms of cell death. These and other data suggest that cells with DNA fragmentation are injured cells, although not necessary undergoing apoptosis or necrosis, and that activation of caspase 3 does not have a significant role in the widespread neuronal death that occurs in AD, although it may occasionally contribute to the loss of specifically vulnerable neurons in the hippocampus [46]. Alternatively, there may be other cellular mechanisms limiting the activation of the caspase cascade which would suggest that there may be compensatory mechanisms in neurons that respond to various chronic and perhaps accumulating insults in neurodegenerative disorders [9]. Thus,

neuronal death in neurodegeneration may represent a form of cell death that is neither classical necrosis nor apoptosis [14].

In contrast to AD and PSP, both disorders related to pathologic tau-protein deposition in neurons and glial cells, where rare neuronal apoptosis is probably related to a proapoptotic tissue environment inducing enhanced vulnerability of susceptible cell populations to a variety of noxious factors, in PD and related synucleinopathies, neither neuronal apoptosis nor such proapoptotic environment have been detected so far. This suggests that cell death mechanisms other than classical apoptosis may be operative in these chronically progressive disorders, where a final trigger may occur during the terminal period of the patient's life [31,38]. In conclusion, despite considerable progress in the clarification of the mechanisms of programmed cell death [10,19,101–104], the intracellular cascade leading to neuronal death in chronic progressive disorders remains to be elucidated. Understanding of these mechanism may lead to the development of protective strategies and novel approaches for the treatment of these diseases.

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