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Elevated Levels of Fragmented DNA Nucleosomes in Native and Activated Lymphocytes Indicate an Enhanced Sensitivity to Apoptosis in Sporadic Alzheimer's Disease

Specific Differences to Vascular Dementia

Anne Eckert[a, d] Manfred Oster[c] Richard Zerfass[b] Michael Hennerici[c]
Walter E. Müller[a, d]

Departments of [a]Psychopharmacology, and [b]Psychiatry, Central Institute of Mental Health, and
[c]Department of Neurology, Klinikum Mannheim of the University of Heidelberg, Mannheim, and
[d]Department of Pharmacology, Biocenter, University of Frankfurt, Germany

Key Words
Alzheimer's disease · Dementia · Cerebrovascular disorders · Lymphocytes · Oxidative stress · Apoptosis

Abstract
Apoptotic cell death is thought to be the most likely mechanism of cell death contributing to neurodegeneration in Alzheimer's disease (AD). Here, we provide evidence that in sporadic AD cases the vulnerability of peripheral cells to undergo apoptosis is increased compared to non-demented elderly controls and, very importantly, to patients with subcortical vascular encephalopathy (SVE) as another, but demented control group. Quiescent 'native' and 'activated' lymphocytes from AD patients that were predisposed to commit apoptotic cell death by priming the cells with interleukin-2, are shown to accumulate apoptosing cells to a significantly higher extent in spontaneous and in oxidative stress-induced in vitro apoptosis. Our results demonstrate robust differences in cell death sensitivity between AD and vascular dementia. In none of the conditions investigated, lymphocytes from SVE patients were significantly different from non-demented controls. The comparable findings of a higher extent of apoptotic features in neurons and in peripheral blood cells of AD patients are remarkable and may suggest a rather general modulation of apoptotic mechanisms by the disease, which even can be picked up at the level of peripheral lymphocytes under specific in vitro conditions.

Introduction

Degeneration and death of neurons in brain regions involved in learning and memory processes and the associated deposition of amyloid β-peptide (Aβ) are features defining Alzheimer's disease (AD). A growing body of evidence indicates that apoptotic cell death contributes to neuronal cell death in AD [1, 2], although some questions remain to be answered. Apoptotic events are relatively rare at any given moment, apoptosis may coexist with other forms of cell death, and apoptotic cell bodies may be

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Dr. Anne Eckert
Department of Pharmacology, Biocenter
Johann Wolfgang Goethe University Frankfurt
Marie-Curie-Strasse 9, D–60439 Frankfurt (Germany)
Tel. + 49 69 798293 77, Fax + 49 69 798293 74, E-Mail A.Eckert@em.uni-frankfurt.de

rapidly phagocytosed by neighboring glial cells. Nonetheless, in situ data derived from postmortem brain tissue indicate that DNA fragmentation is markedly increased in brain cells of AD patients compared to controls [1, 3]. DNA fragmentation is one of the last and crucial events to occur during the apoptotic cell death cascade. Furthermore, experimental models in vivo and in vitro, some of which reproduce the genetic defects responsible for neurodegeneration in hereditary AD, have provided additional indications. Thus, in vitro studies demonstrate that the peptide Aβ and its fragments induce cell death in neuronal cell cultures [4] by exhibiting classic features of apoptotic degeneration [5]. One possible mechanism initiating apoptosis could be the free radical generation by the peptide leading to lipid peroxidation and oxidative stress [6]. A similar apoptotic pathway has been detected in cultured cortical neurons after brief exposure to direct oxidative damage (H_2O_2) [7]. Moreover, genetic risk factors might also enhance the cellular vulnerability for apoptosis. This is amplified by findings indicating that cells transfected with mutant genes of familial forms of AD show an enhanced vulnerability to apoptosis [8, 9].

Apoptosis seems to be a program that is regulated by several ubiquitous genes in many cells. Although a series of different stimuli can initiate the apoptotic pathway, events of apoptosis occur in a fixed sequence in all different cell types [2]. This is also the case for lymphocytes, where apoptosis plays an important physiological role. Moreover, we could previously demonstrate that similar oxidative stressors that induce apoptosis in neurons are also active in human lymophocytes [10]. Thus, the lymphocyte represents a valuable peripheral system to study alterations in ongoing mechanisms in the apoptotic program in individual humans in general and in AD patients in specific.

Our hypothesis suggests that in AD, genetic and other risk factors, which occur in the same pattern in many other cells, are specifically disturbed not only in brain cells, but may also be present in peripheral cells like lymphocytes. Indeed, first evidence indicates an enhanced cellular vulnerability to apoptosis in lymphocytes from patients with sporadic AD, who make up the vast majority of AD case [11].

On the basis of this evidence, we wanted to address the question of whether sensitivity to the induction of cell death is specifically altered in AD patients and whether this biochemical parameter is able to distinguish AD patients from patients with other types of dementia, such as vascular dementia, which is the second most common type of dementia in the western world, after AD [12]. Therefore, we included in our present study a well-characterized group of patients with subcortical vascular encephalopathy (SVE) [13]. Very interestingly, specific differences in intracellular calcium signalling have been already reported between AD and SVE [14]. Intracellular calcium is not only one of the most important second messengers in signal transduction but also an early event in the signal cascade of cell proliferation and cell death, respectively.

Subjects and Methods

Subjects

Twenty patients (13 men and 7 women) with 'probable' (n = 11) or 'possible' (n = 9) AD according to the diagnostic standards of the 'National Institute of Neurological and Communicative Disorders and Stroke' and the 'Alzheimer's Disease and Related Disorders Association' (NINCDS-ADRDA criteria) [15] were studied. The patients were recruited from an ongoing longitudinal study [16]. The mean age was 69.8 ± 9.0 years (range 59–83 years). The majority of cases exhibited moderate dementia. The clinical dementia ratings ranged from mild to severe (mean 2.1 ± 0.98, range 0.5–4). The Mini Mental State Examination (MMSE) score ranged from 0 to 24 (mean 10.5 ± 9.2). Of the 20 AD cases, 11 had early-onset AD, in whom dementia developed before the age of 60 years (average age at onset 53.3 ± 4.3), and 9 had late-onset AD, in whom onset was after the age of 60 years (average age at onset 73.2 ± 5.8 years). Familial AD (FAD) patients could be excluded by patient history and by gene analysis searching for missense mutations of presenilin 1 (PS-1), which cause early-onset autosomal-dominat FAD the most frequently, using cDNA from total RNA extracted from cultivated mononuclear blood cells by polymerase chain reaction [17].

Blood cells from 21 patients (12 men and 9 women) with subcortical vascular encephalopathy (SVE) [13, 18–20] following the diagnostic criteria for research studies (NINDS-AIREN) [21] were used as a second group of demented patients, to elucidate differences in pathogenesis. Patients underwent a structured medical and neurological examination as well as neuropsychological interviews following a research protocol with particular emphasis on the presence of motor and gait disturbances, urinary incontinence, memory and attention disorders, frontal release signs, and aspontaneous episodes. All patients recruited showed evidence of memory impairment with at least one deficit in cognitive domains (e.g. abstract thinking, language, orientation, flexibility or personality changes), or isolated functional impairment unrelated to physical deficits. Furthermore, dementia was diagnosed only on the basis of combined information from the initial and follow-up studies. All patients who entered this study had repeated follow-ups, with confirmation of the entry diagnosis for at least a 2-year period. Several standardized test procedures, including the Structured Interview for the Diagnosis of Dementia, Brief Assessment Interview, and the Nuremberg Ageing Inventory, were used to exclude patients with other psychopathological diseases, in particular a significant mood disorder and patients with degenerative dementing diseases. Moreover, care was taken to avoid inclusion of patients with mixed forms of dementia by strict adherence to the NINDS-AIREN and NINCDS-ADRDA criteria [15, 21]. Essential differences consisted of focal neurological find-

ings, stepwise versus progressive decline in cognitive functions, presence or absence of lacunar cortical infarction and white matter lesions on MRI scans. The mean age was 72.1 ± 7.8 years (range 55–85 years). MMSE ranged from 15 to 24 (mean 21.6 ± 2.9).

Blood cells from 15 non-demented individuals of similar age (mean age 69.9 ± 5.7 years, range 60–79 years, 9 men and 6 women) were used as a control (average MMSE 29 ± 0.5).

The majority of the patients and the controls were treated with drugs mainly for cardiovascular disease, but then did not receive medications which are known to interact with lymphocyte functions. Subjects with acute infections or immunological alterations according to the SENIEUR protocol [22] were not included in the study. The study was approved by the responsible ethical committee, and written informed consent was obtained from all subjects or, where appropriate, from their caregivers.

Cell Separation

Peripheral blood lymphocytes were separated from heparinized blood by centrifugation on Ficoll-Hypaque for 400 *g* for 40 min as previously described [23].

After depletion of monocytes, cells were investigated directly (native cells) or after short-term culture (activated cells) as indicated. For short-term culture, lymphocytes were resuspended in RPMI-1640 (Dutch Modification, Sigma, Germany) supplemented with 5% heat-inactivated fetal bovine serum, 100 units of penicillin/ml and 0.1 mg streptomycin/ml. The lymphocyte suspension was adjusted to 5×10^5 cells/ml and treated with 5 µg/ml concanavalin A for 48 h at 37 °C and 5% CO_2, washed with 10 mg/ml α-methylmannoside, and cultured again for 48 h with 50 units of human recombinant interleukin-2 (IL-2, Roche, Germany) according to the method of Lenardo [24] to predispose to apoptosis. Native or activated cells were washed with RPMI-1640, transferred into 24-well plates (5×10^5 cells/well), and incubated with a cell-death-inducing agent to induce apoptosis or incubated in the absence of inductor to study spontaneous in vitro apoptosis.

Quantitative Measurement of Cell Death

The detection of histone-associated DNA fragments in one immunoassay demonstrating the internucleosomal degradation of genomic DNA during apoptosis was used. The assay is based on the quantitative sandwich enzyme immunoassay principle using mouse monoclonal antibodies directed against DNA and histones, respectively. This allows the specific determination of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. Cytoplasmic fractions (lysates) and cell culture supernatants (5×10^4 cells/200 µl) were collected at different incubation times, frozen at −80 °C for 1 week and analyzed by ELISA (correspond to 5×10^3 cell equivalents). Following the protocol for cytochemistry of the Cell Death Detection ELISA^{PLUS} (Roche, Germany), nucleosomes were photometrically visualized at 405 nm against blank (reference wavelength 492 nm). Absorption values (calculated for 5×10^3 cells) of each ELISA were standardized to the positive controls (positive control = 1.0).

Induction of Apoptosis

Based on recent findings [25], lymphocytes were incubated with 2-deoxy-*D*-ribose (d-Rib, Sigma, 50 mmol/l) for 0–48 h. Incubation led to a marked increase in cells undergoing apoptosis analyzed by propidium iodide staining using flow cytometry [10]. The time-dependent detection of cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes) after incubation of lymphocytes with d-Rib (50 mmol/l) confirms the flow-cytometric findings (data not shown). Four hours after incubation of activated lymphocytes with d-Rib, nucleosomes became detectable in the cytoplasm of apoptotic cells. The maximum level was reached after 6–12 h. In contrast, no nucleosomes were detected in the supernatant during the first 12 h after cell death induction indicating that DNA fragmentation occurred prior to plasma membrane lysis discriminating between apoptosis and necrosis. A similar pattern of cell death was detected for induction of apoptosis in native cells, but maximum levels of nucleosomes were reached later [10].

Statistics

Statistical analyses (ANOVA) were carried out with the SAS package (SAS Institute, Cary, N.C., USA).

Results

Susceptibility to Apoptosis of Native Mature Lymphocytes

Freshly isolated lymphocytes from AD patients showed significantly enhanced basal levels of DNA fragmentation when compared to elderly non-demented controls and SVE patients, respectively ($p < 0.01$, ANOVA; fig. 1). Thereby, lymphocytes from AD patients exhibited a threefold increase in absorption values compared to the lymphocytes from control subjects and a twofold increase in absorption compared to SVE patients (mean values of absorption ± SEM: AD 0.20 ± 0.03; controls 0.06 ± 0.01; SVE 0.1 ± 0.03; calculated for 5×10^3 cells in each case). This defect itself appears not to be sufficient to lead to a pathological leukopenia in AD patients, since the absorption values correspond to only about 3% apoptotic cells of AD lymphocytes under baseline conditions as detected by flow cytometry [26].

Furthermore, we determined the enrichment in nucleosomes in native cells depending on the culture period in the absence or presence of a cell-death-inducing agent (fig. 2). Significantly elevated levels of nucleosomes (corresponding to a higher portion of fragmented DNA) were found in lymphocytes from AD patients undergoing spontaneous in vitro apoptosis (fig. 2A) compared to non-demented controls and SVE patients over the whole time period (0–48 h; $p < 0.05$, ANOVA).

We additionally determined the enrichment in nucleosomes in cells as a function of the time in tissue culture in the presence of d-Rib (50 mmol/l; fig. 2B) to study cell death induced by oxidative stress. As expected, higher absorption values were observed than in our experiments on spontaneous apoptosis in lymphocytes from patients and controls, respectively (fig. 2A). Again, elevated levels

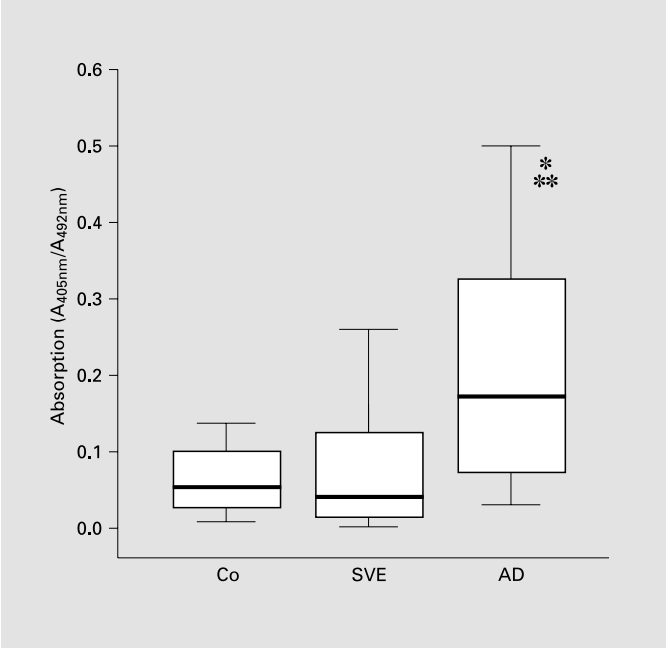

Fig. 1. Basal levels of nucleosomes in native freshly isolated lymphocytes from SVE patients (n = 21), from AD patients (n = 18), and from elderly non-demented controls (Co; n = 14) quantitated by *CDD* ELISA^{PLUS}. Apoptotic nucleosomes (indicated by absorption values referring to 5 × 10^3 cells per well performed in triplicates) were significantly enhanced in lymphocytes from AD patients compared to non-demented controls and SVE patients, respectively ($p < 0.01$, ANOVA, Bonferroni's post-hoc test * $p < 0.05$ vs. SVE patients, ** $p < 0.01$ vs. elderly controls). Data are presented as a box and whiskers graph (the box extends from the 25th percentile to the 75th percentile, with a line at the median, the whiskers extend above and below the box to show the highest and lowest values; medians: controls: 0.05; SVE: 0.04; AD: 0.17).

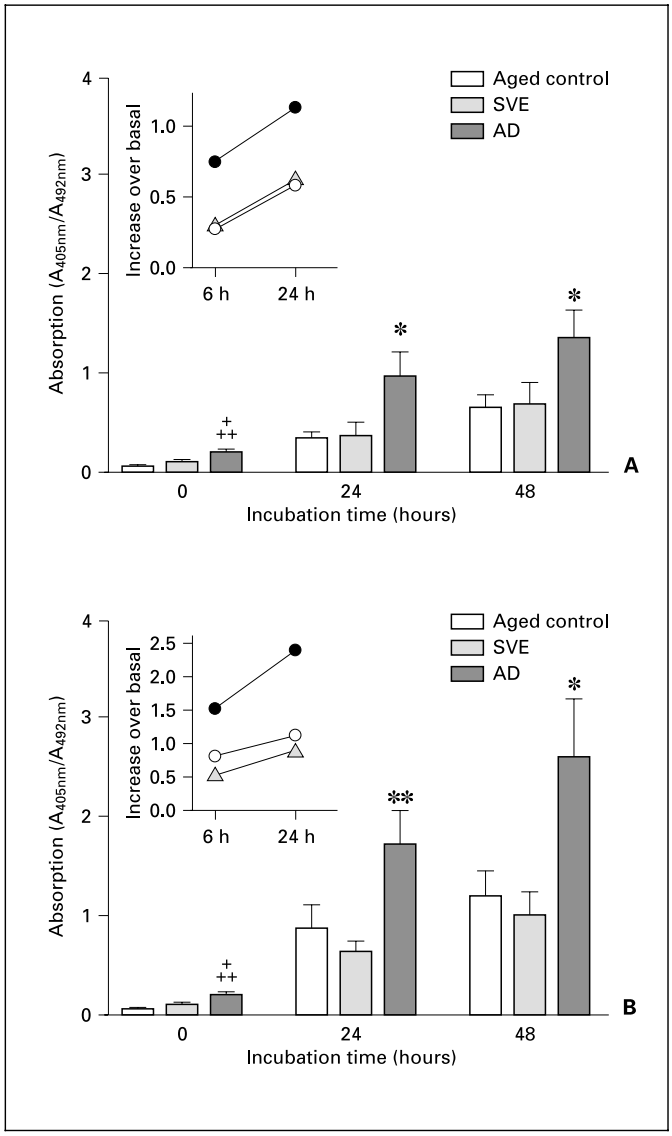

Fig. 2. A Enrichment in nucleosomes in native lymphocytes from SVE patients (n = 21), from AD patients (n = 18), and from elderly non-demented controls (n = 14) undergoing spontaneously apoptosis quantitated by *CDD* ELISA^{PLUS} (total amount of nucleosomes). Data are means ± SEM. Apoptotic nucleosomes (see legend to fig. 1) were significantly elevated in AD compared to elderly controls or SVE patients over the whole period of incubation ($p < 0.05$, ANOVA). Inset: Increase in nucleosomes over basal levels at time point 0 h. **A**, **B** ● = AD; ▲ = SVE; ○ = elderly controls. **B** Enrichment in nucleosomes in native lymphocytes from SVE patients (n = 21), from AD patients (n = 18), and from elderly non-demented controls (n = 14) undergoing activation-induced (d-Rib, 50 mmol/l) apoptosis quantitated by *CDD* ELISA^{PLUS} (total amount of nucleosomes). Data are means ± SEM. Apoptotic nucleosomes (see legend to fig. 1) were elevated in AD compared to elderly controls or SVE patients over the whole period of incubation ($p < 0.01$, ANOVA). Inset: Increase in nucleosomes over basal levels at time point 0 h. * $p < 0.05$ vs. SVE and controls, $^{+}$ $p < 0.05$ vs. SVE, ** $p < 0.01$ vs. SVE, $^{++}$ $p < 0.01$ vs. controls; Student's t test.

of nucleosomes, e.g. a higher portion of apoptotic cells, were found in lymphocytes from AD patients over the whole time period (0–48 h; $p < 0.01$, ANOVA).

In none of the conditions investigated, lymphocytes from SVE patients were significantly different from non-demented controls. In addition, no differences were found in levels of nucleosomes between the early- and the late-onset AD group and between female and male patients or controls. Moreover, there were no significant correlations between the scores of severity of disease and levels of nucleosomes, respectively (data not shown).

Susceptibility to Apoptosis of Activated Lymphocytes

Lymphocytes which are undergoing proliferation can be triggered to programmed cell death by IL-2. IL-2 is the most critical determinant in this process, and activated

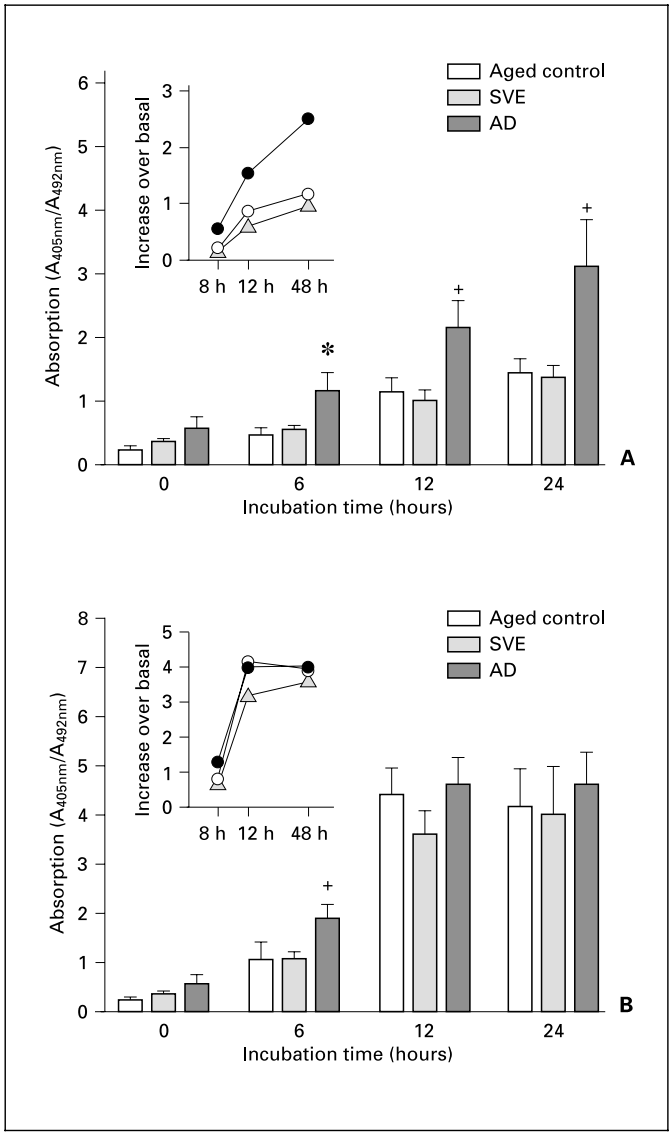

Fig. 3. A Enrichment in nucleosomes in activated lymphocytes from patients with SVE (n = 8–13), AD (n = 10–11), and from elderly non-demented controls (n = 7–10) undergoing spontaneously apoptosis quantitated by *CDD* ELISA^{PLUS} (total amount of nucleosomes). Data are means ± SEM. Apoptotic nucleosomes (see legend to fig. 1) were significantly elevated in AD patients compared to elderly controls or SVE patients over the whole period of incubation ($p < 0.05$, ANOVA). Inset: Increase in nucleosomes over basal levels at time point 0 h. **A**, **B** ● = AD; ▲ = SVE; ○ = elderly controls. **B** Enrichment in nucleosomes in activated lymphocytes from patients with SVE (n = 8–13), AD (n = 10–11), and from elderly non-demented controls (n = 7–10) undergoing activation-induced (d-Rib, 50 mmol/l) apoptosis quantitated by *CDD* ELISA^{PLUS} (total amount of nucleosomes). Data are means ± SEM. Apoptotic nucleosomes (see legend to fig. 1) were significantly elevated at the time point 6 h in AD (* $p < 0.05$ vs. SVE, Student's t test) before reaching a plateau level of nucleosomes. Inset: Increase in nucleosomes over basal levels at time point 0 h. * $p < 0.05$ vs. SVE and controls, $^{+}$ $p < 0.05$ vs. SVE (Student's t test).

lymphocytes have been shown to be more sensitive to apoptosis than non-treated 'native' cells [24].

Accordingly, in our experiments basal levels of nucleosomes as well as levels of nucleosomes after stimulation with d-Rib were always elevated in activated lymphocytes, which were primed to apoptosis by IL-2 treatment followed by IL-2 withdrawal compared to 'native' cells. In addition, the time to get maximal increase in apoptotic features in short-term tissue culture was reduced.

In contrast to freshly prepared native cells (fig. 2), short-term lymphocyte cultures showed a high portion of cells undergoing spontaneous apoptosis per se (fig. 3) corresponding to about 10% apoptotic cells as detected by flow cytometry [10]. Basal absorption values at time point 0 h were three to four times the values from native lymphocytes (mean values of absorption ± SEM: AD 0.58 ± 0.18; controls 0.24 ± 0.06; SVE 0.37 ± 0.06; calculated for 5×10^3 cells in each case), but did not differ significantly among the groups.

However, significantly elevated levels of apoptotic nucleosomes were found in activated lymphocytes from AD patients undergoing spontaneous in vitro apoptosis compared to non-demented controls or SVE patients at 6, 12, and 24 h (fig. 3A).

We also investigated the enrichment in nucleosomes in activated cells as a function of the incubation time in the presence of d-Rib (50 mmol/l; fig. 3B) to study oxidative stress-induced cell death. Significantly higher levels of apoptotic features were found in AD lymphocytes at 6 h, just before maximum levels of accumulated nucleosomes were obtained, indicating a faster turnover of the apoptotic pathway in AD patients (fig. 3B).

Again, lymphocytes from SVE patients did not significantly differ from lymphocytes of non-demented controls.

Discussion

Our results indicate that the susceptibility to apoptosis is significantly enhanced in lymphocytes from AD patients compared to non-demented controls and patients with vascular dementia. Quiescent native as well as short-term-cultured activated lymphocytes from AD patients accumulate apoptosing cells to a significantly higher extent in spontaneous and in oxidative stress-induced apoptosis. We are not aware of any other studies demonstrating robust differences in cell death sensitivity between AD and vascular dementia. As already mentioned, lymphocytes from SVE patients show reduced calcium response

after mitogenic stimulation [14]. The relationship between impaired calcium signalling and sensitivity to the induction of cell death is not clear, but might explain why in our experiments the sensitivity to apoptotic cell death is even slightly lower than DNA fragmentation levels in non-demented control subjects after activation-induced cell death, and might be the reason, as demonstrated by others [27], that lymphocytes from patients with multi-infarct dementia show an altered sensitivity to the inhibition of cell proliferation compared to AD patients.

Most of our data are compatible with the assumption that mainly the sensitivity or vulnerability to apoptosis is elevated, rather than the maximum number of cells, which can be triggered to undergo apoptosis for a specific condition. In addition, we demonstrated that AD lymphocytes are clearly more vulnerable to oxidative stress. Consistent with findings of Barbieri et al. [25], d-Rib, which interferes with the glutathione metabolism provoking oxidative stress [28], induced apoptosis in these cells.

Apoptosis is thought to be the most likely mechanism of cell death contributing to progressive neuronal loss in AD. Several reports indicate that DNA fragmentation, which represents an important and typical apoptotic feature, is increased in postmortem brain sections of AD patients [1, 2, 29]. Among other things, AD is pathologically characterized by the presence of extracellular senile plaques with amyloid deposits, the main component of which is Aβ. Aβ is located partially within the transmembrane region of the amyloid precursor protein (APP) and proteolytically cleaved from APP before secretion [30]. In rare cases of FAD, mutations have been detected within the APP gene. In the majority of autosomal dominant FAD cases, mutations in a gene on chromosome 14 have been found (presenilin 1, PS-1). The highly homologous gene PS-2 on chromosome 1 is also affected in a small number of FAD cases. Both PS-1 and PS-2 have been linked to apoptotic cell death. This has been shown in cells transfected with wild-type or mutant constructs encoding for human PS-1 or PS-2 [8, 9, 31] and in cells derived from PS-1 transgenic mice [32]. In addition, first evidence is provided that expression of mutant APP (e.g. V717F) induces apoptosis in transfected PC12 cells [33]. Both missense mutations (APP and PS) also lead to an enhanced production of the elongated Aβ fragment Aβ1–42(43) [34], the main component of the neuritic plaques. Enhanced cellular vulnerability and/or elevated production of neurotoxic Aβ1–42(43) might contribute to the early onset and the rapid progression of AD in those FAD cases.

The present findings clearly show an enhanced vulnerability to apoptosis in clearly defined cases of sporadic AD. The enhanced vulnerability of lymphocytes and presumably other cells to apoptosis in those patients might be due to a dysfunction of apoptosis-relevant genes or regulating mechanisms, respectively, lowering the threshold of sensitivity to the induction of apoptosis. Similar mechanisms together with the increased Aβ load might be the cause for the enhanced apoptotic cell death found in brains of sporadic AD patients. In agreement with this assumption, changes in the regulation of genes associated with apoptosis (*c-jun*, *bcl-2*, *p53*) and the involvement of caspases in the neurodegenerative process have already been detected in AD brains [3, 29, 35–37] as well as in neuronal cells of APP/Aβ mutant transgenic mice [38]. As already mentioned above, increased vulnerability to cell death appears to be a pathogenic mechanism of PS-1 mutations in AD [32]. In a very recent study, we could demonstrate that lymphocytes from PS-1 mutant transgenic mice exhibited an elevated sensitivity to apoptosis compared to cells from wild-type PS1 mice or non-transgenic littermate controls in a pattern similar to lymphocytes from AD patients [39]. Our findings indicate that increased susceptibility to cell death contributes to the pathogenic mechanisms of sporadic as well as FAD with PS-1 mutations in vivo, which even can be picked up at the level of peripheral lymphocytes under specific in vitro conditions. Furthermore, data on lymphocytes from young and elderly non-demented controls show that AD-induced changes are different from the effects of aging [40].

Our results are furthermore in accordance with findings that oxidative stress damage to DNA in AD brain and, in parallel, in AD lymphocytes is increased [41, 42], and that skin fibroblasts from AD patients show enhanced susceptibility to free radicals [43]. These defects may result in an increased production of reactive oxygen products and may participate in a cascade of events possibly leading to the enhanced basal levels of DNA fragmentation in lymphocytes from AD patients. Consistent with our findings of elevated DNA fragmentation in activated lymphocytes from AD patients after IL-2 exposure are recent results that natural killer cells show enhanced cytotoxic response to IL-2 in AD [44]. The interpretation of these data should not be an oversimplification in so far as that they appear only to be related to changes in immune system function in AD, e.g. enhanced IL-2 production, as postulated in some studies. It is important to consider that a similarly increased IL-2 production has been shown in vascular dementia patients [45] besides the upregula-

tion of other cytokines [46]. However, our vascular dementia patients do not show an enhanced sensitivity to apoptosis. Finally, impaired apoptosis has been demonstrated in lymphocytes of patients with multiple sclerosis, that represents an autoimmune disease [47, 48].

Acknowledgments

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References

1 Adamec E, Vonsattel JP, Nixon RA: DNA strand breaks in Alzheimer's disease. Brain Res 1999;849:67–77.
2 Cotman CW: Apoptosis decision cascades and neuronal degeneration in Alzheimer's disease. Neurobiol Aging 1998;19:S29–S32.
3 Anderson AJ, Su JH, Cotman CW: DNA damage and apoptosis in Alzheimer's disease: Colocalization with c-Jun immunoreactivity, relationship to brain area, and effect of postmortem delay. J Neurosci 1996;16:1710–1719.
4 Pike CJ, Walencewicz AJ, Glabe CG, Cotman CW: Aggregation-related toxicity of synthetic beta-amyloid protein in hippocampal cultures. Eur J Pharmacol 1991,207:367–368.
5 Forloni G, Chiesa R, Smiroldo S, Verga L, Salmona M, Tagliavini F, Angeretti N: Apoptosis mediated neurotoxicity induced by chronic application of beta amyloid fragment 25–35. Neuroreport 1993;4:523–526.
6 Behl C, Davis JB, Lesley R, Schubert D: Hydrogen peroxide mediates amyloid beta protein toxicity. Cell 1994;77:817–827.
7 Whittemore ER, Loo DT, Cotman CW: Exposure to hydrogen peroxide induces cell death via apoptosis in cultured rat cortical neurons. Neuroreport 1994;5:1485–1488.
8 Deng G, Pike CJ, Cotman CW: Alzheimer-associated presenilin-2 confers increased sensitivity to apoptosis in PC12 cells. FEBS Lett 1996;397:50–54.
9 Guo Q, Sopher BL, Furukawa K, Pham DG, Robinson N, Martin GM, Mattson MP: Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid beta-peptide: Involvement of calcium and oxyradicals. J Neurosci 1997;17:4212–4222.
10 Eckert A, Cotman CW, Zerfass R, Hennerici M, Müller WE: Lymphocytes as cell model to study apoptosis in Alzheimer's disease: Vulnerability to programmed cell death appears to be altered. J Neural Transm Suppl 1998;54:259–267.
11 Eckert A, Cotman CW, Zerfass R, Hennerici M, Müller WE: Enhanced vulnerability to apoptotic cell death in sporadic Alzheimer's disease. Neuroreport 1998;9:2443–2446.
12 Amar K, Wilcock GK, Scott M: The diagnosis of vascular dementia in the light of the new criteria. Age Ageing 1996;25:51–55.
13 Hennerici MG, Oster M, Cohen S, Schwartz A, Motsch L, Daffertshofer M: Are gait disturbances and white matter degeneration early indicators of vascular dementia? Dementia 1994;5:197–202.
14 Eckert A, Oster M, Förstl H, Hennerici M, Müller WE: Impaired calcium regulation in subcortical vascular encephalopathy. Stroke 1997;28:1351–1356.
15 McKhann G, Drachman DA, Folstein M, Katzman R, Price DL, Stadlan EM: Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA work group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 1984;34:939–944.
16 Förstl H, Besthorn C, Geiger-Kabisch C, Sattel H, Schreiter-Gasser U: Psychotic features and the course of Alzheimer's disease: Relationship to cognitive, electroencephalographic and computerized tomography findings. Acta Psychiatr Scand 1993;87:395–399.
17 Sandbrink R, Zhang D, Schaeffer S, Masters CL, Bauer J, Förstl H, Beyreuther K: Missense mutations of the PS-1/S182 gene in German early-onset Alzheimer's disease patients. Ann Neurol 1996;40:265–266.
18 Lang EW, Daffertshofer M, Daffertshofer A, Wirth SB, Chesnut RM, Hennerici M: Variability of vascular territory in stroke. Pitfalls and failure of stroke pattern interpretation. Stroke 1995;26:942–945.
19 Schreiner A, Pohlmann-Eden B, Schwartz A, Hennerici M: Epileptic seizures in subcortical vascular encephalopathy. J Neurol Sci 1995; 130:171–177.
20 Gass A, Oster M, Cohen S, Daffertshofer M, Schwartz A, Hennerici MG: Assessment of T2- and T1-weighted MRI brain lesion load in patients with subcortical vascular encephalopathy. Neuroradiology 1998;40:503–506.
21 Roman GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Amaducci L, Orgogozo JM, Brun A, Hofman A, Moody DM, O'Brien MD, Yamaguchi T, Grafman J, Drayer BP, Bennett DA, Fisher M, Ogata J, Kokmen E, Bermejo F, Wolf PA, Gorelick PB, Bick KL, Pajeau AK, Bell MA, DeCarli C, Culebras A, Korczyn AD, Bogousslavsky J, Hartmann A, Scheinberg P: Vascular dementia: Diagnostic criteria for research studies: Report of the NINDS-AIREN International Workshop. Neurology 1993;43:250–260.
22 Ligthart GJ, Corberand JX, Fourmier C, Galanaud P, Hijmans W, Kennes B, Muller-Hermelink HK, Steinmann GG: Admission criteria for immunogerontological studies in man. The SENIEUR protocol. Mech Ageing Dev 1984; 28:47–55.
23 Eckert A, Hartmann H, Müller WE: Beta-amyloid protein enhances the mitogen-induced calcium response in circulating human lymphocytes. FEBS Lett 1993;330:49–52.
24 Lenardo MJ: Interleukin-2 programs mouse alpha beta T lymphocytes for apoptosis. Nature 1991;353:858–861.
25 Barbieri D, Grassilli E, Monti D, Salvioli S, Franceschini MG, Franchini A, Bellesia E, Salomoni P, Negro P, Capri M: *D*-Ribose and deoxy-*D*-ribose induce apoptosis in human quiescent peripheral blood mononuclear cells. Biochem Biophys Res Commun 1994;201: 1109–1116.
26 Schindowski K, Leutner S, Gorriz C, Frölich L, Maurer K, Eckert A, Müller WE: Altered apoptosis in lymphocytes from sporadic Alzheimer's disease patients. Soc Neurosci 1999;25: 850.
27 Nijhuis EWP, Oostervink F, Hinloopen B, Rozing J, Nagelkerken L: Differences in dexamethansone-sensitivity between lymphocytes from patients with Alzheimer's disease and patients with multi-infarct dementia. Brain Behav Immun 1996;10:115–125.
28 Kletsas D, Barbieri D, Stathakos D, Botti B, Bergamini S, Tomasi A, Monti D, Malorni W, Franceschi C: The highly reducing sugar 2-deoxy-*D*-ribose induces apoptosis in human fibroblasts by reduced glutathione depletion and cytoskeletal disruption. Biochem Biophys Res Commun 1998;243:416–425.
29 Su JH, Deng G, Cotman CW: Bax protein expression is increased in Alzheimer's brain: Correlations with DNA damage, Bcl-2 expression, and brain pathology. J Neuropathol Exp Neurol 1997;56:86–93.
30 Haass C, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, Lieberburg I, Koo EH, Schenk D, Teplow DB: Amyloid beta-peptide is produced by cultured cells during normal metabolism. Nature 1992;359: 322–325.

31 Wolozin B, Iwasaki K, Vito P, Ganjei JK, Lacané E, Sunderland T, Zhao BY, Kusiak JW, Wasco V, D'Adamio L: Participation of presenilin 2 in apoptosis: Enhanced basal activity conferred by an Alzheimer mutation. Science 1996;274:1710–1713.
32 Guo Q, Sebastian L, Sopher BL, Miller MW, Ware CB, Martin GM, Mattson MP: Increased vulnerability of hippocampal neurons from presenilin-1 mutant knock-in mice to amyloid beta-peptide toxicity: Central roles of superoxide production and caspase activation. J Neurochem 1999;72:1019–1029.
33 Zhao B, Chrest FJ, Horton WE Jr, Sisodia SS, Kusiak JW: Expression of mutant amyloid precursor proteins induces apoptosis in PC12 cells. J Neurosci Res 1997;47:253–263.
34 Cai XD, Golde TE, Younkin SG: Release of excess amyloid beta protein from a mutant amyloid beta protein precursor. Science 1993; 259:514–516.
35 Nagy ZS, Esiri MM: Apoptosis-related protein expression in the hippocampus in Alzheimer's disease. Neurobiol Aging 1997;18:565–571.
36 Kitamura Y, Shimohama S, Kamoshima W, Ota T, Matsuoka Y, Nomura Y, Smith MA, Perry G, Whitehouse PJ, Taniguchi T: Alteration of proteins regulating apoptosis, Bcl-2, Bcl-x, Bax, Bak, Bad, ICH-1 and CPP32, in Alzheimer's disease. Brain Res 1998;780:260–269.
37 Zhu SG, Sheng JG, Jones RA, Brewer MM, Zhou XQ, Mrak RE, Griffin WS: Increased interleukin-1beta converting enzyme expression and activity in Alzheimer disease. J Neuropathol Exp Neurol 1999;58:582–587.
38 LaFerla FM, Hall CK, Ngo L, Jay G: Extracellular deposition of beta-amyloid upon p53-dependent neuronal cell death in transgenic mice. J Clin Invest 1996;98:1626–1632.
39 Eckert A, Schindowski K, Leutner S, Czech C, Müller WE: Enhanced vulnerability to cell death in lymphocytes from PS-1 mutant transgenic mice. Soc Neurosci 1999;25:426.
40 Schindowski K, Leutner S, Müller WE, Eckert A: Age-related changes of apoptotic cell death in human lymphocytes. Neurobiol Aging, in press.
41 Mecocci P, MacGarvey U, Beal MF: Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann Neurol 1994;36:747–751.
42 Mecocci P, Polidori MC, Ingegni T, Cherubini A, Chionne F, Cecchetti R, Senin U: Oxidative damage to DNA in lymphocytes from AD patients. Neurology 1998;51:1014–1017.
43 Tesco G, Kim TW, Diehlmann A, Beyreuther K, Tanzi RE: Abrogation of the presenilin 1/beta-catenin interaction and preservation of the heterodimeric presenilin 1 complex following caspase activation. J Biol Chem 1998;273: 33909–33914.
44 Solerte SB, Fioravanti M, Severgnini S, Locatelli M, Renzullo M, Pezza N, Cerutti N, Ferrari E: Enhanced cytotoxic response of natural killer cells to interleukin-2 in Alzheimer's disease. Dementia 1996;7:343–348.
45 Huberman M, Sredni B, Stern L, Kott E, Shalit F: IL-2 and IL-6 secretion in dementia: Correlation with type and severity of disease. J Neurol Sci 1995;130:161–164.
46 Elneihoum AM, Falke P, Axelsson L, Lundberg E, Lindgarde F, Ohlsson K: Leukocyte activation detected by increased plasma levels of inflammatory mediators in patients with ischemic cerebrovascular diseases. Stroke 1996;27: 1734–1738.
47 Macchi B, Matteucci C, Nocentini U, Caltagirone C, Mastino A: Impaired apoptosis in mitogen-stimulated lymphocytes of patients with multiple sclerosis. Neuroreport 1999;10:399–402.
48 Pender MP: Genetically determined failure of activation-induced apoptosis of autoreactive T cells as a cause of multiple sclerosis. Lancet 1998;351:978–981.