Comparison of Neurodegenerative Pathology in Transgenic Mice Overexpressing V717F β-Amyloid Precursor Protein and Alzheimer’s Disease

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Overexpression of mutated human amyloid precursor protein (hAPP717V→F) under control of platelet-derived growth factor promoter (PDAPP minigene) in transgenic (tg) mice results in neurodegenerative changes similar to Alzheimer’s disease (AD). To clarify the pathology of these mice, we studied images derived from laser scanning confocal and electron microscopy and performed comparisons between PDAPP tg mice and AD. Similar to AD, neuritic plaques in PDAPP tg mouse contained a dense amyloid core surrounded by anti-hAPP- and anti-neurofilament-immunoreactive dystrophic neurites and astroglial cells. Neurons were found in close proximity to plaques in PDAPP tg mice and, to a lesser extent, in AD. In PDAPP tg mice, and occasionally in AD, neuronal processes contained fine intracellular amyloid fibrils in close proximity to the rough endoplasmatic reticulum, coated vesicles, and electron-dense material. Extracellular amyloid fibrils (9–11 nm in diameter) were abundant in PDAPP tg and were strikingly similar to those observed in AD. Dystrophic neurites in plaques of PDAPP tg mouse and AD formed synapses and contained many dense multilaminar bodies and neurofilaments (10 nm). Apoptotic-like figures were present in the tg mice. No paired helical filaments have yet been observed in the heterozygote PDAPP tg mice. In summary, this study shows that PDAPP tg mice develop massive neuritic plaque formation and neuronal degeneration similar to AD. These findings show that overproduction of hAPP717V→F in tg mice is sufficient to cause not only amyloid deposition, but also many of the complex subcellular degenerative changes associated with AD.

Key words: Alzheimer’s disease; amyloid precursor protein; transgenic; electron microscopy; confocal microscopy; neurodegeneration

The neurodegenerative process in Alzheimer’s disease (AD) is characterized by the progressive and irreversible deafferentation of the limbic system, association neocortex, and basal forebrain (Perry et al., 1977; Hyman et al., 1984; Wilcock et al., 1988; Hof et al., 1990; Palmer and Gershon, 1990; Masliah et al., 1993a), accompanied by neuritic plaque and tangle formation (for review, see Terry et al., 1994). The neuritic plaques in AD contain amyloid β protein (Aβ), which is derived from the amyloid precursor protein (APP) (Selkoe et al., 1988; Selkoe, 1993). Embedded in the amyloid plaque core are dystrophic neurites, as well as reactive astrocytes and microglia (Terry et al., 1964; Terry and Wisniewski, 1970; Dickson et al., 1988; Wisniewski et al., 1989, 1991; Masliah et al., 1991c, 1993b; Frackowiak et al., 1992). The neuritic population in the plaque is mixed, being composed of spherical neurites that contain synaptic proteins and APP (type I), as well as fusiform neurites that contain cytoskeletal proteins and paired helical filaments (PHF; type II) (Dickson et al., 1988; Masliah et al., 1993b, 1994a; Wang and Munoz, 1995). The precise mechanisms by which neuritic plaques are formed and their relationship to the overall neurodegenerative process in AD are not yet clear. Increasing evidence indicates that errantly processed APP derivatives may be involved in the pathophysiological process that leads to neurodegeneration and plaque formation in AD (Sisodia et al., 1990; Golde et al., 1992; Seubert et al., 1992; Mattson et al., 1993; Selkoe, 1993).

To study the events involved in this process in vivo, several transgenic (tg) mice harboring the human APP (hAPP) gene have been developed (Higgins et al., 1994; Mucke et al., 1994; Games et al., 1995a,b). Some of these tg mouse models were designed based on the key observation that a number of APP mutations cosegregate with the familial form of AD and that these patients have, on autopsy, neuropathological alterations that are indistinguishable from sporadic AD (Chartier-Harlin et al., 1991; Goate et al., 1991; Murrell et al., 1991; Clark and Goate, 1993). Furthermore, it has been demonstrated that the APP717 mutations result in an overproduction of the highly amyloidogenic Aβ (1–42) relative to other Aβ peptides (Suzuki et al., 1994). Notably, one APP tg mouse model (PDAPP) was generated recently that exhibits AD-like neuropathology (Games et al., 1995a). This transgene used a platelet-derived growth factor-B chain (PDGF-B) promoter to drive a hAPP minigene (PDAPP) (Games et al., 1995a; Rockenstein et al., 1995) encoding alternatively spliced hAPP that contains the mutation V→F in position 717 that is associated with familial AD (Chartier-Harlin et al., 1991; Murrell et al., 1991; Clark and Goate, 1993). This resulted in an age- and brain region-dependent development of typical amyloid plaques, dystrophic neurites, loss of presynaptic terminals, astrocytosis, and microgliosis (Games et al., 1995a,b). More recent studies
designed to characterize the transgene better have shown that the PDAPP minigene contains three modified hAPP introns that differ from the corresponding authentic hAPP gene introns by large deletions (introns 6 and 8) or insertion of four nucleotides (intron 7) (Rockenstein et al., 1995). Furthermore, PDAPP tg mice had four- to sixfold higher levels of total APP mRNA compared with non-tg mice or humans, whereas their endogenous mouse APP mRNA levels were reduced. This resulted in a high ratio of mRNA encoding mutated hAPP versus wild-type mouse APP (Rockenstein et al., 1995).

To clarify more fully the pathology of the heterozygous PDAPP tg mice at the ultrastructural level and to determine the fine ultrastructural differences and similarities between the tg mice and AD, we studied serially reconstructed images derived from the laser scanning confocal microscopy and electron microscopy.

**MATERIALS AND METHODS**

*Mice and human tissues.* The heterozygous tg mice analyzed in this study were from the previously established line PDAPP-119 (n = 4, age 8 months; n = 3, age 12 months) (Games et al., 1995a). Age-matched non-tg littermates derived from the PDAPP (n = 4, age 8 months; n = 3, age 12 months) lines were used as controls. Mice were derived over several generations from hybrid backgrounds representing combinations of C57Bl/6 × DBA/2J. The right hemibrain was processed for electron microscopy, and the left half was processed for double-immunolabeling/laser scanning confocal microscopy. For ultrastructural comparisons with the tg material, epoxy-embedded blocks from the frontal cortex biopsies of two clinically and histopathologically confirmed AD cases were examined for the present study. These were prepared in the 1960s at the Albert Einstein College of Medicine. The material was fixed in formaldehyde–glutaraldehyde–osmium, processed for double-immunolabeling/laser scanning confocal microscopy. For ultrastructural comparisons with the tg material, autopsy tissue blocks from the frontal cortex of two AD cases from the Alzheimer Disease Research Center (ADRC) at the University of California–San Diego were placed in 2% paraformaldehyde–PBS, pH 7.4, at 4°C overnight and analyzed as described previously (Masliah et al., 1992, 1993b).

*Tissue processing.* Mouse brains were perfused and processed for analysis as described previously (Masliah et al., 1992, 1993b). Mouse and human tissues. The heterozygous tg mice analyzed in this study were from the previously established line PDAPP-119 (n = 4, age 8 months; n = 3, age 12 months) (Games et al., 1995a). Age-matched non-tg littermates derived from the PDAPP (n = 4, age 8 months; n = 3, age 12 months) lines were used as controls. Mice were derived over several generations from hybrid backgrounds representing combinations of C57Bl/6 × DBA/2J. The right hemibrain was processed for electron microscopy, and the left half was processed for double-immunolabeling/laser scanning confocal microscopy. For ultrastructural comparisons with the tg material, epoxy-embedded blocks from the frontal cortex biopsies of two clinically and histopathologically confirmed AD cases were examined for the present study. These were prepared in the 1960s at the Albert Einstein College of Medicine. The material was fixed in formaldehyde–glutaraldehyde–osmium, processed for double-immunolabeling/laser scanning confocal microscopy. For ultrastructural comparisons with the tg material, autopsy tissue blocks from the frontal cortex of two AD cases from the Alzheimer Disease Research Center (ADRC) at the University of California–San Diego were placed in 2% paraformaldehyde–PBS, pH 7.4, at 4°C overnight and analyzed as described previously (Masliah et al., 1992, 1993b).

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**RESULTS**

**General characteristics of the plaques**

Analysis of a total of 250 neurtic plaques in the frontal cortex (layers 2–3 and 5) and hippocampus (pyramidal cell layer of CA1) of heterozygous PDAPP tg mice (n = 7) revealed that at least two general types of plaques could be identified: (1) those containing clusters of dystrophic neurites without an amyloid core (Fig. 1A), and (2) those containing extensive clusters of dystrophic neurites accompanied by one or more amyloid cores (Fig. 1B–D). Approximately 20% of the lesions were devoid of amyloid, whereas the
other 80% were associated with amyloid deposits. At least three subtypes of amyloid-containing neuritic plaques were identified: (1) plaques with abundant dystrophic neurites and scant amyloid bundles (Figs. 1B, 2B,E), (2) plaques with abundant dystrophic neurites, abundant amyloid deposition, and some glial cells (Fig. 1C), and (3) plaques with degenerated electron dense processes, dystrophic neurites, and very dense amyloid deposits (Fig. 1D) surrounded by glial cells. Another notable feature in the PDAPP tg mice was the presence of neurons in close association with the neuritic plaques (Figs. 1A,C, 2B, 3C,D, 6A–C). Similar to PDAPP tg, AD cases also displayed clusters of dystrophic neurites (Fig. 2C,F) with or without amyloid and occasional neuronal cells in close proximity to the neuritic plaques (Fig. 2C). However, AD neuritic plaques contained more cells with ultrastructural characteristics consistent with microglia (data not shown) than the neuritic plaques of heterozygous PDAPP tg mice. In contrast to the ultrastructural findings in the PDAPP tg mice, age- and line-matched non-tg littermates (n = 7) did not show neuritic alterations or amyloid formation. Only occasional hypertrophic astroglial cells were observed in the CA1 region of the hippocampus (Fig. 4D). Consistent with the ultrastructural findings, immunolabeling with the anti-neurofilament antibody (SMI312) showed a well preserved and organized neuritic structure (Fig. 4A) in the non-tg control mice.

**Amyloid deposits in plaques**

Amyloid deposits in PDAPP tg mice were remarkably similar to those observed in AD (Fig. 5) and were usually associated with anti-neurofilament- and anti-hAPP-immunoreactive dystrophic neurites and neuronal cell bodies. In both PDAPP (Fig. 5A–C)
Figure 2. Comparison of neuritic plaques between PDAPP tg mice and AD. 

A, Low-power view of the frontal cortex in control non-tg mice shows preserved neuronal structure. 

B, Low-power view of a classical neuritic plaque in the frontal cortex of a PDAPP tg mouse shows abundant dystrophic neurites (DN), amyloid deposits (A), and an associated cell that displays chromatin clumps beneath the nuclear envelope. 

C, Low-power view of a classical neuritic plaque in the frontal cortex of an AD case that displayed abundant dystrophic neurites (DN), amyloid deposits (A), and an associated cell that also presents some nuclear chromatin aggregation. 

D, Higher-power view of the neuropil in the control non-tg mice shows preservation of the neuritic and synaptic structure. 

E, Higher-power view of the dystrophic neurites in the PDAPP tg mouse shows similar electrodense laminar bodies (LB) to the ones observed in AD. The dystrophic neurites made synaptic contacts (S) and contained abundant small vesicles (V). 

F, Higher-power view of a dystrophic neurite in AD that shows abundant laminar dense bodies and mitochondria. Scale bars: B, 10 μm; C, 1.5 μm.
and AD (Fig. 5D–F), the dense extracellular amyloid consisted of filaments ranging in diameter from 9 to 11 nm that were surrounded by cell membranes that were derived from the neuronal elements in the plaque (Figs. 3D, 5A–C, 6A,B,D,E, 7B, E). Dense-core vesicles (neurosecretory type) ranging in diameter from 85 to 250 nm (Figs. 5A,B, 7C–E) in the cytoplasmic compartment adjacent to the extracellular amyloid fibrils confirmed the neuronal origin of these cellular processes. In addition, the neuronal processes apposed to the extracellular amyloid fibrils contained fine granules (average size of grains was ~5 nm in diameter) (Figs. 5A,C, 7E). Compared with biopsy material from the AD cases (Fig. 5D, E), the plaques in the PDAPP mice (Fig. 5A,C) contained more dense extracellular amyloid bundles and more abundant fine granular deposits in the cytoplasmic phase of the neuronal processes associated with the plaque (Figs. 5A,C, 7D). Consistent with the double-immunolabeling results (Fig. 4B,E,H) and the ultrastructural findings, the amyloid fibrils in the PDAPP tg mice were recognized by the anti-β-amyloid antibody (3D6), as reflected by the labeling of the fibrils by colloidal gold (Fig. 6F). Cellular processes near the amyloid core displayed fine intracel-
lular amyloid fibrils adjacent to the rough endoplasmic reticulum (RER) (Fig. 7A) and coated vesicles (Fig. 7F, G), suggesting the possibility that neurons embedded in the plaque participate in amyloid synthesis. These subcellular organelles associated with intracellular amyloid formation in the PDAPP tg mice were similar to those described previously in amyloid-related cells (ARC) of the AD neuritic plaques (Roher et al., 1988; Frackowiak et al., 1992). However, whereas in AD the extracellular amyloid bundles contained several clear vesicles (Fig. 5D, F) that have been associated with pinocytic functions of the ARCs (Roher et al., 1988), in PDAPP tg mice these were only occasionally observed (Fig. 5B).

**Plaque-associated neuronal alterations**

In PDAPP tg mice, neuronal alterations were characterized by involvement of their processes in plaques (Figs. 3, 6) and by damage to their synaptic terminals (Fig. 8). The neuronal cell bodies in close proximity to the plaques formed neuritic processes that were associated with the amyloid core as well as with the dystrophic neurites (Fig. 6). Serial section analysis of electron micrographs and optical sections revealed that neuronal cell bodies and their processes could be identified in >80% of the neuritic plaques (Figs. 1–3, 6). The neuronal origin of these cells was determined by the presence of the following: (1) immunoreactivity with antibodies against high and intermediate molecular weight phosphorylated neurofilaments (Fig. 3B); (2) dense-core vesicles (85–250 nm in diameter) in the cell body and processes (Figs. 6C, 7D, E); and (3) synapses between adjacent neuritic processes and the neuronal cell bodies (Fig. 8A) or between neurites embedded in the plaque and dystrophic neurites (Figs. 2E, 8D, E). Furthermore, the nucleus of these cells usually presented moderate chromatin aggregation within the nuclear envelope and a prominent nucleolus (Figs. 1C, 6A).

Neurons embedded in the plaque appeared to be actively involved in biosynthesis of proteins as evidenced by the presence of a prominent Golgi apparatus (Fig. 7A) and RER (Figs. 6C, 7A) in the perinuclear region. In addition, the neuritic processes embedded in the plaque contained abundant mitochondria, electrondense bodies surrounded by a membrane (suggesting a lysosomal origin), and dense-core vesicles (Figs. 6C, 7B–E). The neurosecretory vesicles were occasionally distributed along the main neuritic process and were more abundant and enlarged in the distal end of

Figure 4. Comparison of the neuritic and glial components in the PDAPP tg model and AD. Sections were double-immunolabeled and imaged with the laser scanning confocal microscope. Images obtained in the Texas Red channel correspond to β-amyloid (3D6) and in the FITC channel (green) to phosphorylated neurofilament (SMI312) (A–C), GFAP (D–F), or hAPP (8E5) (G–I). A, Non-tg control mice displayed a well organized and preserved neuritic structure. The neuronal cell bodies (N) were not labeled with the anti-neurofilament antibody. PDAPP tg (B) and AD (C) cases displayed significant disruption of the neuritic structure and positive anti-SMI312 immunoreactivity in the dystrophic neurites (arrow) associated with the plaque. D, Non-tg control mice displayed occasional GFAP-immunoreactive astroglial cells. PDAPP tg (E) and AD (F) cases displayed hypertrophic GFAP-immunoreactive astroglial cells in the periphery of the plaque. G, Non-tg control mice were negative with the antibody specific for hAPP. PDAPP tg (H) and AD (I) cases displayed positive anti-hAPP immunoreactivity in the dystrophic neurites (arrow) associated with the plaque. Scale bar, 20 μm.
the process adjacent to the amyloid deposits (Fig. 7B–E). In AD, similar neuronal cells were observed associated with neuritic plaques (Fig. 2C); however, they were less prominent than in PDAPP tg mice.

Other neurodegenerative alterations observed in the PDAPP tg mice that were similar to AD (Masliah et al., 1991a, 1993b) included the damage to synaptic junctions. This was characterized by irregular enlargement of nerve terminals (Fig. 8C, F), fewer synaptic vesicles (Fig. 8C, D), and abnormal accumulation of multilaminar bodies, dense-core vesicles (Fig. 8E, F), and electrodense amorphous material (Fig. 8B). These synaptic alterations were more prominent inside the plaque than in the periplaque region.

**Dystrophic neurites in the plaques**

In addition to the neuritic alterations associated with amyloid formation described in the previous two sections, the neurites in the plaques of PDAPP tg mice also showed characteristic dystrophic changes. These dystrophic neurites contained abundant multivesicular, multilaminar dense bodies (Figs. 1A, 2E, 9A–C) as well as dense-core vesicles and small clear synaptic vesicles (Figs. 2E, 8C–E). Occasionally, synaptic contacts were observed between dystrophic neurites (Figs. 2E, 8E). The ultrastructural characteristics of the dystrophic neurites in the plaques of the PDAPP tg mice were essentially identical to those observed in type I neurites (Masliah et al., 1993b) in the plaques of AD cases (Fig. 2C, F), indicating that neurites in the plaques are probably

*Figure 5. Comparison of the amyloid deposits in the PDAPP tg model and AD. Electron micrographs were obtained from the hippocampal region in the PDAPP tg mouse and from the frontal cortex in AD. Low-power (A) and high-power (B) view of amyloid fibrils (AF) in the PDAPP tg mouse showing dense deposits surrounded by a membrane (open arrows) and the neuronal intracytoplasmic compartment (ICC) containing electrodense granular material and dense-core neurosecretory vesicles (arrow). Occasional poorly defined clear vesicles (arrowheads) were associated with the amyloid fibrils in PDAPP mouse plaques. C, In the PDAPP tg mouse, the cytoplasmic component of neurons close to the extracellular amyloid fibrils (AF) showed the presence of amorphous electrodense material (ED). Low-power (D) and high-power (E) view of the amyloid deposits in AD showed abundant dense fibrils (AF) surrounded by a membrane (open arrows) and a cytoplasmic compartment (ICC) containing dense-core neurosecretory vesicles (arrows) and electrodense granular material. F, Clear vesicles were prominently associated with the amyloid fibrils (AF) in AD plaques (arrowheads). Scale bars: A, 1 μm; B, 100 nm.*
undergoing reactive and neurodegenerative changes (Maslia et al., 1993b). Consistent with previous studies (Maslia et al., 1993b), the type I dystrophic neurites in the PDAPP and AD cases were immunoreactive with antibodies against αAPP (8E5) (Fig. 4, H and I, respectively).

In the PDAPP tg mice, some dystrophic neurites in the plaque contained a dense cumulus of cytoskeletal filaments that was surrounded by multilaminated bodies (Fig. 10A,B). These filaments, ranging in diameter between 8 and 10 nm, were identical to neurofilaments. Consistent with these findings, double-immunolabeling studies confirmed that the dystrophic neurites in the plaques of the PDAPP and AD cases were immunoreactive with antibodies against phosphorylated middle and high molecular weight neurofilaments (Fig. 4, B and C, respectively). They formed a reticular matrix and were observed in both myelinated (Fig. 10A,B) and nonmyelinated (Fig. 9I) axons. Although clearly different from PHF of the type II dystrophic neurites in the plaques of AD cases (Maslia et al., 1993b), they were similar to neurofilament aggregates occasionally observed in the type I neurites of the AD plaques (Fig. 10C,D). No classical AD-type PHF was observed in the dystrophic neurites of PDAPP tg mice (Fig. 10E,F). In addition to the neurofilamentous alterations observed in the neuritic plaques of PDAPP tg mice, detailed ultrastructural analysis revealed the presence of crystalline polygonal structures that were formed by symmetrical tubular elements (Fig. 9D-F).

Other cellular alterations not associated with plaques
In addition to neuritic plaque formation, neurodegenerative changes in neocortical and hippocampal cells of the PDAPP tg mice included nuclear chromatin segmentation, formation of dense intranuclear and intracytoplasmic bodies, and intracellular vacuolization of the membranes (Fig. 11). Some cells contained RER and neurosecretory vesicles, suggesting a neuronal origin. However, in cells with extensive vacuolization it was not possible to identify ultrastructural markers indicative of cellular origin. These alterations were not observed in control non-tg littermates. Neurons of PDAPP tg mice that were not associated with plaques showed no neurofibrillary tangles (NFT) or evidence of intraneuronal PHF formation.
Glial cell alterations

Abundant glial cells were observed around the neuritic plaques (Figs. 1C, 3D,E, 12) as well as scattered in the neuropil of the neocortex and hippocampus of the PDAPP tg mice. The great majority of them contained intermediate filaments, consistent with an astroglial origin (Fig. 12). In both the PDAPP and the AD cases, these glial elements in the plaque strongly immunoreacted with antibodies against the astroglial marker (GFAP) (Fig. 4, E and F, respectively). Microglial cells were observed associated with neuritic plaques containing dense amyloid cores and in which the dystrophic neurites exhibited extensive degeneration characterized by the presence of abundant electrondense material. Compared with AD, neuritic plaques in PDAPP tg mice showed a more prominent astroglial reaction and a less abundant microglial component (not shown) (Terry et al., 1964; Terry and Wisniewski, 1970; Frackowiak et al., 1992).

DISCUSSION

Neurodegeneration in PDAPP tg mice

The present study showed that PDAPP tg mice displayed subcellular neurodegenerative alterations strikingly similar to those observed in AD (Table 1). These include characteristic dystrophic neurites with electrondense multilaminar bodies, disruption of synaptic junctions, and intracellular amyloid and reactive gliosis. Furthermore, plaques in PDAPP tg mice displayed extracellular amyloid fibrils associated with cellular processes containing dense-core vesicles, coated vesicles, RER, and membranous recesses in the neuritic plaques (Table 1).
Whereas neuritic plaques in AD contained spherical (type I) and fusiform (type II) neurites (Masliah et al., 1993b), in PDAPP tg mice the neuritic component was mainly the spherical type (type I). This is consistent with the finding that, to date, no PHF has been found in neuronal cell bodies or dystrophic neurites in the PDAPP tg mice. In fact, the plaques occasionally found in cognitively intact aged humans and macaques (Martin et al., 1994) display the presence of type I (but not type II) dystrophic neurites, amyloid fibrils, and astrocytic reaction, as is the case with the PDAPP tg mice. However, similar to AD, the plaques in the PDAPP tg mice appear to be more abundant and show more widespread neuritic dystrophy than the ones observed in aged humans and macaques. Furthermore, similar to ultrastructural findings in AD (Wisniewski and Terry, 1970, 1973; Wisniewski et al., 1970; Masliah et al., 1993b), the neuritic component of plaques in the PDAPP tg mice contained both dystrophic (i.e., mitochondria, lysosomes, and neurofilaments) and regenerative (i.e., clear and dense-core vesicles, mitochondria, ER, and lysosomes) alterations. Previous studies showed that similar ultrastructural alterations occurred in neurites of vitamin E-deficient rats (Lampert, 1967; Southam et al., 1991) and in grafts of peripheral nerves in the CNS (Campbell et al., 1992). Furthermore, consistent with previous studies in AD (Terry et al., 1964; Gonatas et al., 1967; Wisniewski et al., 1981; Masliah et al., 1991b, 1993b), several of the plaque neurites in PDAPP tg mice contained synaptic vesicles and formed synaptic contacts supporting an axonal role and origin of neuritic dystrophy. The neuritic plaque components associated with dense amyloid deposits were of the degenerative type, because they contained abundant electrodense deposits and multilaminar bodies consistent with alter-

Figure 8. Synaptic alterations in PDAPP tg mice. Electron micrographs were obtained from the hippocampus. A, Neuronal cells associated with the plaques showed synaptic terminals (S) associated with the perikaryon (n, nucleus; arrow, neurosecretory vesicle). B, Presynaptic terminals in the periplaque region displayed amorphous electrodense material (E) in addition to synaptic vesicles (SV). C, D, Some synaptic terminals in the periplaque region showed decreased numbers of synaptic vesicles (1), whereas others were distended and contained abundant vesicles (2) (DN, dystrophic neurites). E, Axonal terminals in the midst of the plaque were distended and displayed accumulations of multilaminar, multivesicular, and electrodense bodies. Dystrophic neurites (DN) made occasional synaptic contacts (S) with neighboring neuritic processes. Other neuritic elements in the plaque were electrodense (EDP). F, Adjacent to the extracellular amyloid fibrils (AF), the axonal terminals were distended and contained abundant dense vesicles (1) or clear vesicles (2). A dystrophic neurite (DN) was also present. Scale bar, 1.5 μm.
ations observed in other animal models of neurodegeneration (Lampert, 1967).

In addition to neuritic plaque formation, neurodegenerative changes in neocortical and hippocampal cells of the PDAPP tg mice included nuclear chromatin segmentation, formation of dense intranuclear and intracytoplasmic bodies, and vacuolization. These alterations are similar to apoptotic changes observed in experimental models after treatment with excitotoxins (Pollard et al., 1994; Portera-Cailliau et al., 1995) or adrenalectomy (Sloviter et al., 1993). In contrast to these experimental animal models, previous ultrastructural studies have not described extensive apoptotic changes in cells in AD (Terry et al., 1964; Terry and Wisniewski, 1970). However, recent studies utilizing the in situ labeling technique have suggested that both neurons and glial cells undergo apoptosis in AD (Forloni et al., 1993; Su et al., 1994; Dragunow et al., 1995; Lassman et al., 1995; Smale et al., 1995), as well as in another Aβ tg animal model (LaFerla et al., 1995).

Cytoskeletal alterations in PDAPP tg mice

Consistent with findings in AD, PDAPP tg mice also showed cytoskeletal alterations characterized by abnormal deposits of neurofilamentous material in neuronal cell bodies, as well as in neuritic plaques. Similar alterations have been described in dystrophic neurites in AD (Wisniewski and Terry, 1973; Gheuens et al., 1991), as well as in rodents and humans treated with antimitotic agents (Wisniewski and Terry, 1967; Schochet et al., 1968; Shelansky, 1969; Nakagawa et al., 1987) and in rabbits treated with aluminum (Terry and Pena, 1965). In addition to the neurofilamentous deposits, PDAPP tg mice displayed the formation of crystals formed by symmetrical tubular elements arranged in an angular manner. These crystals had some ultrastructural similarities to Hirano bodies; however, additional electron microscopic and immunocytochemical studies will be necessary to confirm the origin of these struc-

Figure 9. Neuritic alterations in PDAPP tg mice. Electron micrographs were obtained from the hippocampus. In PDAPP tg mice, the dystrophic unmyelinated (A, B) and myelinated neurites (C) contained abundant electrodense laminar and multivesicular bodies. Some neurites contained fine filaments (10 nm; arrow) and were surrounded by electrodense processes (EDP). Other neurites contained characteristic crystals displaying an array of symmetrically organized tubules (D–F). Scale bar, 2 μm.
Figure 10. Comparison of cytoskeletal alterations between the PDAPP tg mouse and AD. Low-power (A) and high-power (B) views of a myelinated dystrophic neurite in the PDAPP tg containing abundant laminated bodies and filamentous material (10 nm in diameter; arrows). Low-power (C) and high-power (D) views of a dystrophic neurite in AD containing multilamellar bodies and neurofilamentous material similar to the one observed in the PDAPP mouse (arrows). Low-power (E) and high-power (F) views of a dystrophic neurite in AD containing classical paired helical filaments (arrows). Scale bars: E, 5 μm; F, 200 nm.
Hirano bodies are crystallloid structures that preferentially occur in the CA1 area of the hippocampus in AD, amyotrophic lateral sclerosis, Parkinsonism/dementia complex, and other disorders (Hirano, 1994). Because Hirano bodies contain actin, neurofilaments, and tau immunoreactivity, they are considered part of the spectrum of cytoskeletal alterations that occur in AD (Hirano, 1994). These crystals have also been described in rodents treated with colchicine and in aged primates (Hirano, 1994). Taken together, these findings suggest that overexpression of mutated hAPP or Aβ in tg mice is associated with neuritic cytoskeletal alterations. These changes could be secondary to the neurodegenerative process associated with a limited sprouting capacity in partially denervated neurons, consistent with the observation that sprouting neurites expressing GAP-43 and APP also display neurofilament immunoreactivity (Cras et al., 1991; Gheuens et al., 1991; Masliah et al., 1991b, 1993a).

**Figure 11.** Apoptotic-like changes in neocortical cells of PDAPP tg mice. Electron micrographs were obtained from layer 5 of the frontal cortex. A–D. Neuronal nuclei contained chromatin segmentation and condensation. Some cells displayed dense intracytoplasmic or intranuclear inclusions surrounded by a membrane (*). These cells also contained RER and neurosecretory vesicles. E. Other cells displayed, in addition to the chromatin segmentation, cytoplasmic distention and vacuolization. Scale bar, 10 μm.

Another prominent feature in the PDAPP tg mice, occasionally observed in AD, was the presence of neuronal elements in close association with the plaques, suggesting the possibility that extracellular amyloid was derived from these structures. Neuronal processes embedded in plaques contained discrete amyloid bundles associated with their RER, as well as large membranous electrodense bodies, coated vesicles, and dense-core neurosecretory vesicles that were also closely associated with amyloid deposits. Moreover, these subcellular neuronal alterations were more prominent and abundant around the amyloid core. Similar sub-
cellular findings have been reported in amyloid-producing cells in AD (Roher et al., 1988; Wisniewski et al., 1991). The role of the dense-core vesicles in this process is uncertain, but previous studies have shown that these vesicles are abundant in dystrophic neurites of AD (Weiler et al., 1990; Munoz, 1991) and are rich in chromogranin, indicating that these cellular processes are of axonal origin and that neurosecretory granules could be involved in the process of amyloid formation.

The precise sequence of events that lead to plaque formation remains to be resolved. Nevertheless, the findings reported here, together with observations from other animal models (Cork et al., 1990) as well as AD (Masliah et al., 1993b; Heinonen et al., 1995; Masliah, 1995), suggest that plaque formation initiates with local synaptic alterations induced by possible abnormalities in APP processing and Aβ formation. The overproduction of the amyloidogenic Aβ (1–42) peptide that occurs as a result of the APP717 mutation in the PDAPP tg mouse is likely also to be critical to the rapid development of plaques seen in this animal model of AD (Suzuki et al., 1994). These events might then be followed by dystrophic neurite formation and extracellular amyloid deposition. These dystrophic neurites might represent regenerative and sprouting terminals and may eventually degenerate to become electrodense processes associated with plaques containing abundant dense amyloid. Supporting this contention, recent studies in PDAPP tg mice of various ages have shown that synaptic and neuritic alterations in the limbic system occur early and are closely followed by microdeposits of β-amyloid (Games et al., 1995a,b). Neuronal alterations and amyloid deposition in the neocortex appear at a later time point. Further studies in PDAPP tg mice of various ages are underway to clarify the progression of the neurodegenerative changes and plaque formation. An alternative explanation could be that neuronal uptake of extracellular amyloid fibrils, followed by disruption of the subcellular neuronal organelles triggers the cascade of events. However, this seems to

Figure 12. Glial cell alterations in PDAPP tg mice. Electron micrographs were obtained from the frontal cortex. A, Prominent astrocytic cells were observed associated with the neuritic plaques (P). These cells displayed some cytoplasmic swelling and contained abundant intermediate filaments. B, C, Higher-power view of the astrocytic processes in the plaque. D, View of an enlarged astrocyte distant from the plaque. Scale bars: A, 10 μm; B, 2 μm.
In this study, it has been postulated that microglial cells play an important role in plaque formation and are not simply trapped in the midst of the lesions. It has been postulated that microglial cells play an important role in amyloid formation in the AD plaque (Terry et al., 1964; Wisniewski and Terry, 1970; Roher et al., 1988; Wisniewski et al., 1989, 1991; Masliah et al., 1991c; Frackowiak et al., 1992). The neuronal processes embedded in the amyloid core of PDAPP tg mice contained abundant RER, neurosecretory vesicles, electrodense granular material, and coated vesicles, suggesting active synthesis and secretion of amyloidogenic elements rather than phagocytosis. These subcellular alterations in PDAPP tg mice imply that neurons play an important role in plaque formation and are not simply trapped in the midst of the lesions.

Table I. Ultrastructural similarities and differences between AD and PDAPP tg plaques

<table>
<thead>
<tr>
<th></th>
<th>Alzheimer's disease</th>
<th>PDAPP tg</th>
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<tbody>
<tr>
<td>Amyloid fibrils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size</td>
<td>9–11 nm</td>
<td>9–11 nm</td>
</tr>
<tr>
<td>Electron density</td>
<td>Moderate</td>
<td>High</td>
</tr>
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<td>Pinocytic vesicles</td>
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</tr>
<tr>
<td>Dystrophic neurites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dense laminar bodies</td>
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<td>Abundant</td>
</tr>
<tr>
<td>Synaptic vesicles and contacts</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Neurofilament accumulation</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
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<td>Paired helical filaments</td>
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<td>None?</td>
</tr>
<tr>
<td>Cells associated with amyloid formation</td>
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<tr>
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</tr>
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<td>Neurons</td>
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</tr>
<tr>
<td>Neurosecretary granules</td>
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</tr>
<tr>
<td>Rough endoplasmic reticulum</td>
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<td>Abundant</td>
</tr>
<tr>
<td>Coated pits</td>
<td>Yes</td>
<td>Yes</td>
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be a less likely possibility because the ultrastructural characteristics of the cellular processes embedded in the plaque were different from the subcellular alterations previously identified in cells phagocytizing amyloid (Wisniewski et al., 1991; Frackowiak et al., 1992). It has been shown both in vitro and in vivo that cells which phagocytize amyloid contain nondegraded fibrils within phagosomes, whereas in cells which produce amyloid, fibrils appear first in altered RER deep infoldings of cell membranes (Wisniewski et al., 1991; Frackowiak et al., 1992). The neuronal processes embedded in the amyloid core of PDAPP tg mice contained abundant RER, neurosecretory vesicles, electrodense granular material, and coated vesicles, suggesting active synthesis and secretion of amyloidogenic elements rather than phagocytosis. These subcellular alterations in PDAPP tg mice imply that neurons play an important role in plaque formation and are not simply trapped in the midst of the lesions.

In conclusion, this study shows that PDAPP tg mice share several critical subcellular alterations with AD that make them a valuable model in which to study mechanisms of neurodegeneration and plaque formation. Furthermore, marked neuronal overexpression of a mutant hAPP (hAPP717V→F) can result in the accumulation of excess Aβ protein, and this accumulation may contribute to neurodegeneration and plaque formation seen in the PDAPP tg mice.

REFERENCES


