ALZHEIMER’S DISEASE AND AMYLOID: CULPRIT OR COINCIDENCE?

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Alzheimer’s disease (AD) is the largest unmet medical need in neurology today. This most common form of irreversible dementia is placing a considerable and increasing burden on patients, caregivers, and society, as more people live long enough to become affected. Current drugs improve symptoms but do not have profound neuroprotective and/or disease-modifying effects. AD is characterized by loss of neurons, dystrophic neurites, senile/amyloid/neuritic plaques, neurofibrillary tangles, and synaptic loss. Beta-amyloid (Aβ) peptide deposition is the major pathological feature of AD. Increasing evidence suggests that overexpression of the amyloid precursor protein and subsequent generation of the 39–43 amino acid residue, Aβ, are central to neuronal degeneration observed in AD patients possessing familial AD mutations, while transgenic mice overexpressing amyloid precursor protein develop AD-like pathology. Despite the genetic and cell biological evidence that supports the amyloid hypothesis, it is becoming increasing clear that AD etiology is complex and that Aβ alone is unable to account for all aspects of AD. The fact that vast overproduction of Aβ peptides in the brain of transgenic...
mouse models fails to cause overt neurodegeneration raises the question as to whether accumulation of Aβ peptides is indeed the culprit for neurodegeneration in AD. There is increasing evidence to suggest that Aβ/amyloid-independent factors, including the actions of AD-related genes (microtubule-associated protein tau, polymorphisms of apolipoprotein E4), inflammation, and oxidative stress, also contribute to AD pathogenesis. This chapter reviews the current state of knowledge on these factors and their possible interactions, as well as their potential for neuroprotection targets.

I. Introduction

Few illnesses are as devastating as Alzheimer’s disease (AD). Memory progressively fails, complex tasks become even more difficult, and once-familiar situations and people suddenly appear strange, even threatening. Over years, afflicted patients lose virtually all abilities and succumb to the disease. Reflecting an aging population, for most societies, dementia is becoming a major health burden. Because of an aging population, improved diagnosis, and prolonged survival, especially in developing countries, AD is destined to become an ever-increasing socioeconomic burden. Indeed, the Alzheimer’s Disease Association, in 2009 an estimated 5.3 million people in the United States have AD, which is now the sixth leading cause of death in the United States. The incidence will increase to an estimated 7.7 million cases in 2030 and 11–16 million cases in the United States in 2050, and from about 26 million to more than 100 million worldwide, by 2050. These numbers do not include the large number of people with mild cognitive impairment, a significant proportion of whom will progress to AD. AD and other dementias cost Medicare $91 billion per year and Medicaid $21 billion. The total annual costs of AD in the United States are estimated at $148 billion (Alzheimer’s Association, 2009), and the total worldwide costs of dementia disorders have been estimated to exceed US $200 billion (Winblad and Wimo, 2007). While scientists have made significant progress toward understanding what goes awry in the brain when neurons die on a massive scale, a cure for AD (i.e., halting the death of neurons) and related forms of dementia is lacking, and current treatments are limited to modest symptomatic relief. A major hurdle in the development of neuroprotective therapies is due to limited understanding of disease processes leading to the death of neurons.
II. Amyloid and AD

The brains of patients with AD, in addition to showing nerve and synapse loss, are histopathologically characterized by two hallmark lesions—plaques and neurofibrillary tangles (NFTs), the latter being intracellular cytoplasmic lesions composed of hyperphosphorylated forms of the microtubule associated protein, tau, which associate to form insoluble paired helical filaments (Fig. 1)(Goedert et al., 1988; Querfurth and LaFerla, 2010). The core constituent of the senile plaques is a small 4 kDa amyloid β-peptide (Aβ) (Glenner and Wong, 1980). This peptide is generated physiologically following proteolytic processing of a larger membrane-bound precursor protein, known as amyloid precursor protein (APP). Cleavage of APP by β-secretase (BACE1) releases the extracellular soluble APPβ fragment. This is concomitant with the generation of a membrane-tethered C-terminal fragment that is subsequently cleaved by γ-secretase to generate Aβ peptides predominantly of 40 or 42 amino acids in length (Aβ40, Aβ42) (Fig. 2)(LaFerla et al., 2007; Selkoe, 1994; Sisodia and St George-Hyslop, 2002). It is this C-terminal variation that has been most often associated with pathogenicity, with Aβ42 found to be the most toxic form (Selkoe, 2002, 2003). In an alternative nonamyloidogenic pathway, cleavage of APP by α-secretase within the amyloid-β region of APP precludes the release of intact Aβ. In AD brain, Aβ deposits range from diffuse, less-compact plaques to mature neuritic plaques with a dense fibrillar amyloid core, the latter being associated with dystrophic neurites, activated microglia, and reactive astrocytes (Selkoe, 1999). Both lesions occur in areas of the brain associated with cognition, namely, the hippocampus and cortex. Besides these hallmarks, prominent activation of inflammatory processes and the innate immune response are observed (McGeer and McGeer, 2007). In addition to the presence of these two

![Fig. 1. Pathological hallmarks of Alzheimer’s disease brain. Note the senile plaques comprising Aβ and neurofibrillary tangles and dystrophic neurites comprising hyperphosphorylated tau.](image)
classical lesions, AD brain is characterized by synaptic loss and overt neurodegeneration (Whitehouse et al., 1982).

Amyloid deposition can be either within the brain parenchyma (plaques) or in association with the cerebrovasculature (cerebral amyloid angiopathy or CAA). CAA occurs in up to 98% of all AD brains (Rensink et al., 2003) and can lead to bleeding. There are several familial forms of CAA, with the primary ones being the hereditary cerebral hemorrhage with either amyloidosis-Dutch type (Levy et al., 1990) or Icelandic type (Yamada et al., 1996), and the Iowa-type hereditary CAA (Greenberg et al., 2003). There appears to be a significant correlation between cognition and the presence of CAA, indicating that CAA is a major accompanying pathology of AD (Newell et al., 1999).

Like several other proteins associated with neurodegeneration, Aβ has the ability to self-associate, and can form an array of different assemblies ranging from dimers all the way to aggregates of fibrils (Fig. 3) (Haass and Selkoe, 2007; Powers and Powers, 2008). Initially, it was assumed that Aβ toxicity was mediated by fibrils similar to those present in amyloid plaques, but recent data suggest that

Fig. 2. The amyloid cascade. The transmembrane protein amyloid precursor protein (APP) is sequentially cleaved by two proteases, β-secretase (also known as β-site APP cleaving enzyme 1) and γ-secretase, to release various isoforms of the amyloid β-peptide (Aβ). In an alternative nonamyloidogenic pathway, cleavage of APP by α-secretase within the amyloid-β region of APP precludes the release of intact Aβ by releasing soluble APPα and APPβ fragments. The most aggregation-prone Aβ42 isoform associates to form toxic oligomers and deposits in amyloid plaques. Oligomers have acute synaptotoxic effects, whereas amyloid plaques lead to an inflammatory response. The amyloid cascade is thought to trigger downstream tau pathology (see text).
nonfibrillar, water-soluble oligomeric assemblies of Aβ (also known as amyloid β-derived diffusible ligands or ADDLs) may also be important (Dodart et al., 2002; Glabe, 2008; Klein et al., 2001; McLean et al., 1999; Shankar and Walsh, 2009) (Tomiyama et al., 2010). There is a general agreement that synapses—in particular the postsynaptic compartment—are the prime targets of amyloid-β toxicity (Selkoe, 2002; Tanzi, 2005; Walsh and Selkoe, 2004). One of the earliest signs of AD appears to be a reduction in synaptic density (Gonatas et al., 1967; Suzuki and Terry, 1967). Subsequent studies (Cash et al., 2003; Praprotnik et al., 1996) described a marked reduction of microtubules and accumulation of vesicles within both cell bodies and dystrophic neurites, indicative of dysfunctional axonal transport mechanisms. Fast axonal transport is crucial for neuronal function and survival. Because the axon is mostly devoid of biosynthetic machinery, proteins, lipids, and organelles are transported long distances from the cell body, and trophic factors secreted from axonal targets are transported retrogradely from the synapse to the cell body. Multiple neurodegenerative diseases, including AD display defective fast axonal transport as an early pathologic event (Mormino et al., 2009). In particular, axonal transport defects have been found in AD brain (Muresan and Muresan, 2009; Stokin et al., 2005) and in transgenic mouse models of AD (Muresan and Muresan, 2009). A recent study by Decker et al. (2010) shows that Aβ oligomers cause severe axonal transport defects in cultured hippocampal neurons through an N-methyl-D-aspartate (NMDA) receptor-dependent mechanism that is mediated by glycogen synthase kinase-3β.

There appears to be a striking correlation between synaptic loss and cognitive impairment within AD patients (Davies et al., 1987; DeKosky and Scheff, 1990; Hamos et al., 1989; Selkoe, 2002; Terry et al., 1991), which may be more predictive of cognitive decline than either cell death or plaque development. Immunohistochemical quantification of synaptic puncta within AD tissue using markers such as
synapsin I and synaptophysin, revealed a reduction in synaptic density within both hippocampal and cortical regions which correlated with cognitive decline, although differing extents of synaptic loss may occur between different layers of each of these regions (DeKosky and Scheff, 1990; Terry et al., 1991). In APP transgenic mice, synaptic perturbation (loss of synaptic puncta) precedes amyloid plaque deposition (Mucke et al., 2000). It may well be that small Aβ oligomers form intracellularly before being released into the extracellular medium, acting as seeds to accelerate fibril formation (Khandogin and Brooks, 2007; Selkoe, 2003). Other intracellular perturbations, such as multivesicular bodies and aggregated Aβ42 oligomers within both endosomes and along microtubules of neuronal processes have been reported in APP transgenic mice and in cortical neurons cultured from these mice (Takahashi et al., 2004). Aβ42 oligomers accumulate preferentially within neuronal processes and synaptic profiles rather than extracellularly (Walsh et al., 2000). Biochemical analysis of brain indicates that the levels of nonfibrillar forms of Aβ correlate well with synaptic loss and presence of dementia (Lue et al., 1999; Mc Donald et al., 2010; McLean et al., 1999; Tomic et al., 2009; Wang et al., 1999). Shankar et al. (2008) reported that aqueous extracts of human brain contain Aβ assemblies that migrate on SDS-polyacrylamide gels and elute from size exclusion as dimers (~8 kDa), block long-term potentiation (LTP, a synaptic correlate of memory and learning), inhibit synapse remodeling, and impair memory consolidation in the rat. Such species are detected specifically and sensitively in extracts of AD brain, suggesting that SDS-stable dimers may be the basic building blocks of AD-associated synaptotoxic Aβ assemblies (Kuo et al., 1996; Roher et al., 1996). The role of low-n-oligomers of Aβ in the range of dimer to tetramer is also supported by in vitro studies using peptides bearing design mutations which show, for example, that Aβ-mediated neurotoxicity is directly linked to the abundance of mass spectrometry-detected dimers and trimers (Hung et al., 2008) and that aggregation size alone is not the sole determinant of synaptotoxicity and that structure is also critical (Harmeier et al., 2009). More recently, O’Nuallain et al. (2010) generated synthetic dimers of Aβ(1-40) containing cysteine in place of serine, leading to the disulfide cross-linked dimer (AβS26C)2. Freshly isolated (AβS26C)2 did not block LTP whereas (AβS26C)2 solutions that were allowed to form protofibrils did. These data support the idea that Aβ dimers may stabilize the formation of fibril intermediates by a process distinct from that available to Aβ monomers and that such intermediates are potent synaptotoxins (O’Nuallain et al., 2010). Although there might be a single receptor that mediates Aβ toxicity at the postsynaptic compartment, it seems more likely that several postsynaptic receptors are involved, such as prion proteins, α7-nicotinergic receptors, metabotropic glutamate receptors, and in particular, NMDA receptors (Lauren et al., 2009; Shankar et al., 2008; Snyder et al., 2005). Indeed, soluble oligomers of Aβ interfere with NMDA receptor function, inducing abnormal calcium influx and neuronal oxidative stress (De Felice et al., 2007),
and instigate aberrant activation of kinases, including glycogen synthase kinase-3β (De Felice et al., 2008; Hoshi et al., 2003). In the context of the study by Decker et al. (2010) cited earlier, glycogen synthase kinase-3β impairs kinesin-1-based transport in squid axoplasm (Morfini et al., 2002).

Aβ oligomers bind to synaptic sites (Lacor et al., 2007) and reduce the density of spines in organotypic hippocampal slice cultures (Hsieh et al., 2006; Shankar et al., 2007), dissociated cultured neurons (Fig. 4) (Evans et al., 2008; Lacor et al., 2007), and transgenic mouse models (Jacobsen et al., 2006; Lanz et al., 2003; Spires et al., 2005). Consistent with these structural abnormalities, neurons treated with Aβ or that overexpress APP show depressed glutamatergic transmission (Hsieh et al., 2006; Snyder et al., 2005; Ting et al., 2007). More specifically, synthetic Aβ assemblies can inhibit NMDA receptor-dependent but not NMDA receptor-independent LTP (Chen et al., 2002; Zhao et al., 2004) (but see Raymond et al., 2003), a synaptic correlate of memory and learning (Lambert et al., 1998, Shankar et al., 2007, 2008). This result is consistent with reports that synthetic Aβ

![Graph](image)

**Fig. 4.** Aβ42 reduces synapsin puncta in cultured cortical neurons. Cortical neurons at 5 days in vitro were treated for 96 h with Aβ42 prior to fixation and analysis for synapsin I/II-positive puncta. Values are means ± SEM (n = 4). L-8542 (22.25 μM) is an Aβ peptide inhibitor. Modified from J. Neurosci. Methods, 175(1), Evans et al. (2008), Fig. 4, Copyright © 2008 Elsevier B.V., with permission. Synapsin is a good marker for establishment and/or recovery of functional synapses in the central nervous system, and hence, a good indicator of synaptic plasticity. Synapsins are involved in synaptic vesicle interaction with actin microfilaments (Benfenati et al., 1989). Synapsin I is concentrated at presynaptic nerve terminals where it plays a key role in regulation of neurotransmitter release in mature synapses (Chin et al., 1995), and is also involved in synaptogenesis and axonogenesis (Là et al., 1995). Alterations in synapsin expression are observed within the hippocampus of AD patients, in response to perforant path lesioning, and in experimental autoimmune encephalitis (Melloni et al., 1994; Qin et al., 2004; Zhu et al., 2003).
can decrease surface expression of NMDA receptors (Dewachter et al., 2009; Snyder et al., 2005) and increase (Molnar et al., 2004) or decrease (Chen et al., 2002; Raymond et al., 2003) NMDA receptor-mediated synaptic responses. Soluble Aβ oligomers at the pathophysiological levels present in AD brain facilitate hippocampal long-term depression by increasing the activation of NR2B-containing extrasynaptic NMDA receptors, at least in part, by an oligomer-mediated decrease in neuronal glutamate reuptake, thereby inducing glutamate “spillover” to extrasynaptic sites (Li et al., 2009, 2011).

However, issues regarding the subcellular source of Aβ, as well as the mechanism(s) of its production and actions that leads to synaptic loss are poorly understood. One needs to keep in mind that although Aβ is capable of perturbing synaptic transmission and plasticity, such Aβ-mediated processes are subject to activity-dependent modulation. The level of Aβ secretion is controlled by neural activity in brain slices (Kamenetz et al., 2003) and in vivo (Cirrito et al., 2005). In humans, regions of the brain with high resting activity are positively correlated with Aβ plaque load (Buckner et al., 2005). The effects of Aβ may also depend on neural activity. For example, NMDA receptor activation is required for Aβ-mediated spine loss (Shankar et al., 2007) and synaptic depression (Kamenetz et al., 2003). Another confounding factor is the question of whether the Aβ that produces synaptic deficits is generated in pre- or postsynaptic compartments. APP and its derivatives, as well as components of APP-processing enzymes, are found in axons and dendrites (Buxbaum et al., 1998; Koo et al., 1990). In an attempt to address this issue, Wei et al. (2010) isolated the sites of increased Aβ production by selectively expressing APP in pre- or postsynaptic neurons. Using two-photon laser-scanning imaging to monitor the synaptic deficits caused by such dendritic or axonal Aβ, the authors found that either dendritic or axonal Aβ overproduction was sufficient to cause local spine loss and compromise plasticity in the nearby dendrites of neurons that did not overexpress Aβ. In addition, Aβ-mediated synaptic dysfunction could be ameliorated by blocking action potentials (tetrodotoxin-sensitive), NMDA receptors, or nicotinic acetylcholine receptors. These findings indicate that continuous overproduction of Aβ at dendrites or axons acts locally to reduce the number and plasticity of synapses.

III. Tau and AD

Tau is a soluble microtubule-binding protein predominantly found in axons (Fig. 5). The best established functions of tau are thought to be the stabilization of microtubules and the regulation of motor-driven axonal transport (Götz et al., 2006). Tau and tangle pathology are not specific for AD, but are part of the
pathology in a number of other disorders such as Pick’s disease, progressive supranuclear palsy, corticobasal degeneration, and motor neuron diseases. As mentioned earlier, intraneuronal tangles containing hyperphosphorylated tau are a hallmark of AD pathology, and there is a strong correlation between cognitive dysfunction and tangle load and localization in AD (Thal et al., 2000).

Tau has as many as 84 putative phosphorylation sites, of which 45 are serines, 35 threonines, and 4 tyrosines. In filamentous tau extracted from AD brain, almost 50 of these residues are phosphorylated, in comparison with only nine sites identified in normal human brain (Hanger et al., 2007, 2009). Tau phosphorylation is believed to contribute to neuronal cell death by decreasing the assembly of tubulin (Amniai et al., 2009), disrupting axonal transport (Cuchillo-Ibanez et al., 2008), causing reentry of neurons into the cell cycle (Andorfer et al., 2005), and possibly leading to further abnormal tau processing, for example, truncation by proteases (Rametti et al., 2004) or inhibition of tau degradation (Litersky and Johnson, 1992). Phosphorylation of tau regulates its association with signaling proteins, proposing that tau may be involved in pathways critical for proper neuron function and survival (Götz et al., 2006; Ittner et al., 2009; Reynolds et al., 2008). Tau hyperphosphorylation in disease may be caused by disruptions in the balance of protein phosphatase and kinase activities (Billingsley and Kincaid, 1997), or

FIG. 5. Human tau isoforms. Tau is found mainly in the axonal compartment, where it binds to tubulin, and is involved in microtubule assembly and stabilization. There are six major isoforms of human tau derived by alternative mRNA splicing from a single gene on human chromosome 17. Alternative splicing of exon 10 gives rise to 3-repeat (3R) and 4-repeat (4R) forms. All six tau isoforms are expressed in adult brain, but only tau-352 is expressed in fetal brain. Alzheimer’s disease-specific epitopes include S202/S205, T212/S214, T231/S235, S396 S404, and S422.
structural alterations that result in changes in the availability of specific residues in these enzymes (Mandelkow et al., 2007). Several key tau kinases have been identified that are associated with the accumulation of phosphorylated tau in AD and related tauopathies, with cyclin-dependent kinase-5 and glycogen synthase kinase-3 thought to play especially prominent roles in the development of tauopathy (Engmann and Giese, 2009; Hanger et al., 2009; Lau et al., 2002).

Under physiological conditions, tau has also been localized to dendrites, although there are much lower levels (Ittner et al., 2010). A further compartment in which tau has been found is the somatodendritic domain; tau is localized here under pathological conditions. Upon interaction with tau, the tyrosine protein kinase Fyn is localized to the dendritic compartment, where it phosphorylates NMDA receptors and thereby mediates their interaction with postsynaptic density protein 95 (PSD95)—an interaction required for Aβ toxicity in AD and in APP transgenic mice, resulting in excitotoxicity, memory deficits, impairment of hippocampal LTP, and premature mortality (Salter and Kalia, 2004; Shipton et al., 2011). Increased levels of tau in transgenic mice result in accumulation of tau in the soma and dendrite of neurons, together with increased postsynaptic Fyn levels. This is associated with increased Aβ toxicity in double APP/tau transgenic mice, which show an early mortality compared with APP transgenic mice. Truncated tau that lacks microtubule-binding properties does not localize to dendrites, but interacts with Fyn in the soma, in a dominant negative manner. Truncated tau acts on the tau–Fyn interaction, thereby preventing Fyn from accessing dendrites, which consequently protects APP/tau transgenic mice from Aβ toxicity (Ittner et al., 2010; Roberson et al., 2007).

Inhibition of tau hyperphosphorylation and blockade of aggregation have been explored from a therapeutic angle, although from a drug development perspective these represent rather challenging avenues (Lee and Trojanowski, 2006; Schneider and Mandelkow, 2008). For example, disrupting protein–protein interactions over large surfaces is theoretically quite difficult. Reducing tau hyperphosphorylation, on the other hand, requires knowing which kinase(s) to inhibit, as well as generating a small-molecule inhibitor that is highly specific, brain penetrant, and suitable for chronic dosing from a safety standpoint in terms of mechanism-based side effects.

**IV. AD and Tau: A Double Act?**

The amyloid cascade hypothesis posits that Aβ formation is the critical step in driving AD pathogenesis, a concept derived from the identification of pathogenic mutations in patients with familial AD that are linked to Aβ formation, as well as increased Aβ levels and a higher frequency of AD in individuals with trisomy 21, who carry an additional APP allele (Bertram and Tanzi, 2005). Unlike the APP and presenilin-1 (PS-1 genes), no AD causing mutations had been identified in the
microtubule-associated protein, tau gene. However, the subsequent identification of mutations in the tau gene, that were linked to familial forms of frontotemporal dementia with parkinsonism linked to chromosome 17, demonstrated that dysfunction of tau could indeed lead to neurodegeneration and dementia (Hutton et al., 1998).

In APP transgenic mice, Aβ formation causes tau hyperphosphorylation, whereas tau transgenic mice do not show Aβ plaque pathology (Götz et al., 2004; Hussain, 2010; Zempel et al., 2010). Crossing APP transgenic mice with tau transgenic mice results in a greatly enhanced quantity and distribution of the NFT pathology, without altering Aβ plaque pathology (Hussain, 2010; Lewis et al., 2001; Terwel et al., 2008). The development and characterization of a triple-transgenic model expressing mutant APP, mutant tau, and mutant PS-1 transgenes further reinforced the relationship between Aβ deposition and NFT pathology (Oddo et al., 2003b). Plaque pathology preceded that of tangle pathology in these mice, while synaptic dysfunction and cognitive impairments preceded both Aβ plaque and NFT formation (Billings et al., 2005; Oddo et al., 2003b). Synergistic interplay between Aβ and tau may occur, for example, at the level of mitochondria, where both polypeptides compromise respiration in triple-transgenic mice at distinct points, leading to an aggravated impairment in the oxidative phosphorylation system (Rhein et al., 2009).

Intriguingly, there is evidence that tau may mediate Aβ neurotoxicity. For example, hippocampal neurons cultured from tau−/− mice are resistant to Aβ-induced injury (Rapoport et al., 2002). Furthermore, reducing endogenous tau reportedly ameliorates Aβ-induced deficits in APP transgenic mouse strains (Ittner et al., 2010; Roberson et al., 2007). As mentioned above, tau has a dendritic function in postsynaptic targeting of the Src kinase Fyn, a substrate of which is the NMDA receptor. Missorting of tau in transgenic mice disrupts postsynaptic targeting of Fyn, thereby uncoupling NMDA receptor-mediated excitotoxicity and hence mitigates Aβ toxicity. Tau deficiency prevents memory deficits and improves survival in Aβ-forming APP23 mice, a model of AD. These deficits are also fully rescued with a peptide that uncouples the Fyn-mediated interaction of NMDA receptors and PSD-95 in vivo (Ittner et al., 2010). This suggests that tau-dependent dendritic signaling is pivotal in mediating Aβ toxicity, at least in these transgenic animal models. Interestingly, tau reduction also prevents Aβ-induced defects in axonal transport of mitochondria and the nerve growth factor (NGF) receptor TrkA (Vossel et al., 2010).

V. White Matter Pathology and AD

AD traditionally has been considered a disease marked by neuronal cell loss and widespread gray matter atrophy, but degeneration of myelin in white matter fiber pathways is increasingly considered a key disease component (Bartzokis, 2009; Braak and Braak, 1996; Hua et al., 2008). White matter lesions and pathology have been extensively documented in the brains of incipient and mildly
afflicted AD patients (Brun and Englund, 1986; Dickerson and Sperling, 2008; Roth et al., 2005). More specifically, white matter aberrations have been reported in late-myelinating brain regions of presymptomatic and preclinical carriers of familial AD-associated PS-1 mutations (Ringman et al., 2007). Several studies have recorded myelin degeneration in the brains of PS-1 mutation carriers that exhibit non-AD-related symptomatic dementia, thus incriminating PS-1 mutations in white matter pathology (Dermaut et al., 2004; Marrosu et al., 2006). Moreover, triple-transgenic AD (3×Tg-AD) mice (Oddo et al., 2003a), which express the human PS-1 M146V mutation (hPS1M146V), human APP Swedish mutation (hAPPswe), and the human tau P301L mutation (htauP301L), exhibit white matter deficits in comparable brain regions at ages prior to the appearance of overt plaque and tangle pathology (Desai et al., 2009; Wirths et al., 2006). Of note, the 3×Tg-AD mouse-harbored hPS1 M146V knockin mutation can be expressed in cell types supportive of murine PS-1 promoter-driven transcription, including oligodendrocytes, whereas the hAPPswe and htauP301L mutant transgenes are expressed exclusively by neurons.

Myelin breakdown is not exclusive to PS-1 mutation carriers, as white matter alterations are also seen in the brains of individuals with late-onset AD (Firbank et al., 2007), and hAPPswe and PDAPP transgenic mice, coinciding with stages of advanced amyloid plaque pathology (Harms et al., 2006; Song et al., 2004). Thus, Aβ-related insults also impact oligodendrocyte and/or myelin integrity independent of PS-1 mutant expression. Even so, the early onset of white matter pathology in the PS-1 knockin mouse models implicates PS-1 dysfunction as a predisposing condition that can be aggravated by coincident Aβ accumulation. Oligodendrocytes expressing hPS1M146V in a transgenic mouse model exhibited increased vulnerability to Aβ peptides in vitro and enhanced white matter pathology in vivo (Pak et al., 2003). Differentiated rat oligodendrocytes are sensitive to the toxic action of micromolar levels of Aβ42 (Fig. 6). The latter observation may be of relevance to AD, as the concentration of Aβ in the CNS of elderly AD patients can exceed 15 μM (Vasilevko et al., 2007). In a new study, Desai et al. (2011) show that differentiated mouse oligodendrocyte precursor cells, when simultaneously expressing hPS1M146V and exposed to Aβ42, are impaired in their abilities to properly traffic myelin basic protein to their distal processes and elaborate myelin sheaths in vitro. Moreover, the myelination defect and myelin basic protein subcellular mislocalization triggered by hPS1M146V and Aβ42 could be effectively prevented by treatment with a glycogen synthase kinase-3β inhibitor, thereby implicating glycogen synthase kinase-3β in this pathogenic cascade. Understanding how the signaling pathways that control the complex stages of oligodendrocyte differentiation and myelin development are affected by AD-related pathogenic factors may facilitate the discovery of strategies to promote the maintenance, repair, and restoration of myelin in AD patients. Because current APP transgenic mouse models are based on the neuron-specific thy-1 promoter, it would be interesting
The majority of chronic neurodegenerative diseases, including AD, are associated with the accumulation of misfolded proteins into aggregates that contain fibrillar structures, eventually causing the progressive loss of neurons in the brain and nervous system. Most of these proteinopathies are sporadic and the cause of pathogenesis remains elusive. Heritable forms are associated with genetic defects, suggesting that the affected protein is causally related to disease formation and/or progression. However, determining whether a given pathological structure drives the disease, is a neutral bystander, or just represents an unsuccessful repair attempt remains challenging. Moreover, in an end-stage AD brain, there are many biochemical changes relative to normal brain, and numerous strategies can be rationalized by differences in gene expression or protein concentration between them. In addition, the limitation of human genetics makes it necessary to use model systems to analyze affected genes and pathways in more detail. Animal models have contributed considerably to advancing our understanding of the pathophysiological mechanisms underlying neurodegenerative disorders and, in some cases, pointed to novel strategies for drug development. The successful use of animal models in drug discovery relies on both the development of valid disease models and the availability of adequate testing paradigms for evaluating the effects of different therapeutic approaches.
The development of a mouse model of AD which recapitulates the pathological and behavioral features of this complex, chronic and progressive disease has, not surprisingly, proven to be a major challenge. Despite this, considerable progress has been made in recent years and transgenic mice depicting both Aβ plaques and NFTs have been successfully generated and characterized. These mice have proven invaluable for exploring the pathophysiology and neurobiology of this disease. In addition, they have supported drug discovery efforts, by providing what may be disease-relevant models in which to test a wealth of putative AD therapeutics.

A number of transgenic mouse models have been generated that replicate one of the key pathological features of AD, namely, the deposition of Aβ as senile plaque-like structures (Hussain, 2010). This was achieved through the overexpression of human APP transgenes-bearing mutations associated with early-onset familial forms of AD. This resulted in an age-dependent accumulation of Aβ peptides to a threshold which was sufficient to drive the aggregation and deposition of Aβ in the brains of these mice. The PDAPP mouse was the first mutant human APP transgenic mouse reported to exhibit Aβ plaque pathology (Games et al., 1995). Subsequently, a number of other APP transgenic mouse models were developed and characterized, including the Tg2576 mouse (Hsiao et al., 1996; Moechars et al., 1999; Richardson et al., 2003; Sturchler-Pierrat, 1997). In general, early plaque pathology was evident in the hippocampus and cortical brain regions of these mice between 6 and 12 months of age, comprised both diffuse and dense core plaques and was accompanied by astrocystosis, microgliosis, and dystrophic neurites. A number of the APP transgenic mice also exhibited age-dependent cognitive deficits, suggesting Aβ accumulation and deposition in the brain could induce behavioral impairments (Hsiao et al., 1996; Moechars et al., 1999; Richardson et al., 2003). Interestingly, learning and memory deficits that occurred prior to Aβ deposition in the brain were also described (Richardson et al., 2003; Van Dam et al., 2003; Westerman et al., 2002), raising the possibility that soluble oligomeric Aβ species could also induce cognitive dysfunction.

Transgenic mouse models with accelerated Aβ plaque pathology have been described. Mice expressing double mutant human APP transgenes (Chishti et al., 2001) (e.g., TgCRND8) show increased Aβ production and Aβ plaque pathology already at 3 months of age. The association of mutations in the PS-1 and PS-2 genes with increased Aβ42 production and familial forms of AD (Scheuner et al., 1996; Sherrington et al., 1995) provided a basis for the generation of APP and presenilin double transgenic mice (Borchelt et al., 1997; Holcomb et al., 1998; Richards et al., 2003). Compared to single APP transgenic mice, APP-persenilin transgenics are characterized by rapid accumulation and deposition of Aβ, with plaques being observed at a much earlier age. A transgenic mouse model containing a total of five different familial AD mutations has been
generated as well, in which Aβ plaques were evident from as early as 2 months of age (Oakley et al., 2006).

As described in the preceding section, the development of a more complete mouse model of AD subsequently led to the generation of a transgenic mouse displaying both Aβ plaque and NFT pathology in the brain. Mutant forms of human tau were subsequently used to model tauopathy in vivo. Transgenic mouse lines overexpressing mutant human tau were generated and found to exhibit an age-dependent hyperphosphorylation of tau, and NFT-like pathology, cognitive impairment, and neuronal cell loss (Allen et al., 2002; Lewis et al., 2000; Ramsden et al., 2005). Mutant tau mice have been crossed with mutant APP mice to generate bi-transgenic APP/tau mutant mice depicting both Aβ plaque and NFT pathologies in the brain (Lewis et al., 2001; Ribe´ et al., 2005). Aβ deposits were observed in the APP/tau mice at the same age as in the single mutant APP transgenic mice. However, the quantity and distribution of the NFT pathology was greatly enhanced in the mutant APP/tau mice compared to the single mutant tau transgenic mice, suggesting overexpression of Aβ or APP could accelerate downstream tau pathology. The development and characterization of a triple-transgenic model expressing mutant tau, mutant APP, and mutant PS-1 transgenes (3×Tg-AD) further reinforced the relationship between Aβ deposition and NFT pathology (Oddo et al., 2003b). These mice exhibited an age-dependent deposition of Aβ, with plaques being observed from 6 months of age. This was followed by the development of neurofibrillary tangle pathology between 12 and 15 months of age. Synaptic dysfunction and cognitive impairments were also reported in these mice (Billings et al., 2005; Oddo et al., 2003b) and interestingly these preceded both Aβ plaque and NFT formation. More recently, a triple-transgenic mouse model expressing mutant APP, PS-2, and tau was described in which tau pathology was enhanced by the accumulation of Aβ (Grueninger et al., 2010).

Significant efforts in the pharmaceutical industry have focused on the development of therapeutic agents aimed at lowering Aβ production, inhibiting Aβ deposition or facilitating Aβ clearance. These drug discovery efforts were boosted by the development of transgenic mouse models depicting Aβ pathology, as described above, as they allowed the in vivo testing of Aβ lowering agents and supported the progression of candidate compounds to the clinic (Citron, 2010; Hussain, 2010). These approaches have included secretase inhibitors, Aβ fibrillation inhibitors, antagonists for the receptor for advanced glycation end products, and immunotherapeutics utilizing both active and passive immunization against Aβ. More recently, naturally occurring autoantibodies against Aβ, which have been characterized in different experimental settings to inhibit Aβ fibrillation and toxicity (Dodel et al., 2004; Taguchi et al., 2008), were found to be reduced in patients with AD (Du et al., 2001), and to preferentially bind to Aβ oligomers (Dodel et al., 2011). Administration of autoantibodies against Aβ in TgCRND8 mice improved neurological performance (Dodel et al., 2011).
Other studies have led to the “neurotrophic deficit” hypothesis, more recently defined as “neurotrophic unbalance,” which states that loss of basal forebrain cholinergic neurons (one major neuronal population affected and progressively degenerating in AD) and altered APP processing are due to alterations in NGF trophic support (Capsoni et al., 2010; Cuello and Bruno, 2007). This hypothesis is supported by analysis of AD11 transgenic mice, where anti-NGF antibodies, neutralizing NGF versus pro-NGF, are expressed both peripherally and within the CNS from postnatal day 45, thus reducing mature NGF availability throughout adulthood (Ruberti et al., 2000). This model provided evidence correlating NGF deprivation and Aβ accumulation (Matrone et al., 2008; Nikolaev et al., 2009). Besides Aβ accumulation, AD11 mice display a comprehensive AD-like pathology including Aβ plaques, loss of basal forebrain cholinergic neurons, hyperphosphorylated tau tangles, and hippocampal-dependent memory deficits (Capsoni et al., 2011; Cattaneo et al., 2008). These mice also share with etiologically different familial AD mouse models, a common phenotype of age-dependent short- and long-term dentate gyrus plasticity deficits (Houeland et al., 2010).

In spite of numerous studies with animal models, it is difficult to reconcile that Aβ alone is able to account for all aspects of AD. For example, recent neuroimaging studies confirm earlier autopsy findings that amyloid deposits are present in cognitively normal individuals, whereas some AD patients show no amyloid deposits in positron emission tomography scans (Edison et al., 2007; Li et al., 2008). Likewise, it is possible that all of the amyloid-focused clinical trials failed because they were started too late in the disease progression, but the negative outcome is also consistent with the notion that AD can be caused by Aβ/amyloid-independent factors. The fact that vast overproduction of Aβ peptides in the mouse fails to cause frank neurodegeneration raises further questions as to whether accumulation of Aβ peptides is indeed the culprit for neurodegeneration in AD.

VII. Oxidative Stress and AD

As cellular energy machinery, mitochondria produce the largest amount of reactive oxygen species (ROS) in mammalian cells. Their numerous redox enzymes transfer single electrons to oxygen, with superoxide being the predominant species leading to the formation of other ROS (Balaban et al., 2005; Murphy, 2009). The transfer of electrons to oxygen, generating superoxide, is more likely when these redox carriers are abundantly charged with electrons and the potential energy for transfer is high, as reflected by a high mitochondrial membrane potential. ROS generation is decreased when available electrons are few and
potential energy for the transfer is low. Mitochondria also contain an extensive antioxidant defense system to detoxify the ROS generated by the above reactions: nonenzymatic components (\(\alpha\)-tocopherol, coenzyme Q10, cytochrome c, and glutathione), and enzymatic components (e.g., manganese superoxide dismutase (MnSOD, which rapidly catalyzes dismutation of superoxide to \(\text{H}_2\text{O}_2\) (Murphy, 2009)), catalase, glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase, glutathione reductase; peroxiredoxins, glutaredoxin, thioredoxin, and thioredoxin reductase). Like ROS generation, antioxidant defenses are tied to the redox and energetic state of mitochondria. In structurally and functionally intact mitochondria, a large antioxidant defense capacity balances ROS generation, and there is little net ROS production. Mitochondrial damage with decrease of antioxidant defense capacity is a prerequisite for net ROS production. Once this occurs, a vicious cycle can ensue whereby ROS can further damage mitochondria, causing more free-radical generation and loss or consumption of antioxidant capacity.

Net production of ROS is an important mechanism by which mitochondria are thought to contribute to aging. Mitochondrial insults, including oxidative damage itself, can cause an imbalance between ROS production and removal, resulting in net ROS production (Andreyev et al., 2005). The importance to aging of net mitochondrial ROS production is supported by observations that enhancing mitochondrial antioxidant defenses can increase longevity. In Drosophila, overexpression of the mitochondrial antioxidant enzymes, MnSOD (Sun et al., 2002) and methionine sulphoxide reductase, (Ruan et al., 2002) prolongs lifespan. This strategy appears to work with short-lived strains of Drosophila and has no effect in already long-lived strains. However, overexpression of catalase experimentally targeted to mitochondria increased lifespan in an already long-lived mouse strain (Schriner et al., 2005). These authors generated transgenic mice overexpressing catalase targeted to peroxisomes, nuclei, or mitochondria. The mitochondrial targeted construct provided the maximal benefit, increasing median and maximal lifespan by 20%. Hydrogen peroxide production and oxidative inactivation of aconitase were reduced in isolated cardiac mitochondria; DNA oxidation and levels of mitochondrial deletions were reduced in skeletal muscle; and cardiac pathology, arteriosclerosis, and cataract development were delayed.

In humans, the study by Lu et al. (2004) of gene expression in the brain suggests that oxidative damage has a major role in the cognitive decline that accompanies aging. Transcriptional profiling of postmortem frontal cortex samples from individuals aged from 26 to 106 revealed that after the age of 40, there was a decrease in the expression of genes involved in synaptic plasticity, vesicular transport, and mitochondrial function, followed by increased expression of stress–response, antioxidant, and DNA-repair genes. In the brain, the age-downregulated genes were characterized by a markedly increased oxidative DNA damage compared with the age-stable or age-upregulated genes.
Oxidative damage and mitochondrial dysfunction probably contribute causally to AD-related pathology (Eckert et al., 2011; Lin and Beal, 2006). Oxidative damage occurs early in the AD brain, before the onset of significant plaque pathology (Nunomura et al., 2001). Oxidative damage also precedes Aβ deposition in transgenic APP mice (Praticò et al., 2001), with upregulation of genes relating to mitochondrial metabolism and apoptosis occurring even earlier and colocalizing with the neurons undergoing oxidative damage (Reddy et al., 2004). In transgenic APP-mutant mice, energy metabolism inhibitors such as 2-deoxyglucose, 3-nitropropionic acid, and kainic acid elevated β-secretase levels and activity, as well as Aβ levels (Velliquette et al., 2005). In fetal guinea pig neurons, hydrogen peroxide treatment increased intracellular Aβ levels (Ohyagi et al., 2000). Treatment with a mitochondrial uncoupler caused cultured astrocytes to mimic amyloidogenic APP processing and intracellular Aβ accumulation as seen in Down syndrome astrocytes (Busciglio et al., 2002). AD mutant mice with decreased MnSOD expression exhibit increased levels of brain Aβ and accelerated behavioral abnormalities, including cognitive dysfunction (Esposito et al., 2006; Li et al., 2004). Conversely, overexpression of MnSOD in two different AD mouse models reduced Aβ deposition and prevented memory deficits (Dumont et al., 2009; Massaad et al., 2009). However, the last two studies did not address whether the decrease in superoxide that results in the aforementioned behavioral improvements in AD model mice is correlated with improvements in synaptic plasticity. In a just-published study, Ma and coworkers (2011) demonstrated that Aβ42-induced inhibition of LTP was reversed by a mitochondria-targeted ROS scavenger, general ROS scavengers, and by genetically overexpressing MnSOD. The reversal of synaptic plasticity deficits by the mitochondria-targeted scavengers correlated with their ability to prevent increases in mitochondrial superoxide elicited by Aβ42. The findings by Ma et al. (2011) thus suggest a causal relationship between mitochondrial ROS imbalance and Aβ-induced impairments in hippocampal synaptic plasticity.

What might be the pathways connecting oxidative stress and AD pathology? One can imagine that oxidative stress activates signaling pathways that alter APP or tau processing. For example, oxidative stress increases the expression of β-secretase through activation of c-Jun amino-terminal kinase and the stress-activated protein kinase p38 (Tamagno et al., 2005), and increases aberrant tau phosphorylation by activating glycogen synthase kinase-3 (Lovell et al., 2004). Oxidation-induced inactivation of critical molecules may also be a contributing element. The prolyl isomerase PIN1 is especially sensitive to oxidative damage (Sultana et al., 2006). PIN1 catalyzes protein conformational changes that affect both APP and tau processing. Knockout of Pin1 increases amyloidogenic APP processing and intracellular Aβ levels (Pastorino et al., 2006), and Pin1-null mice also exhibit tau hyperphosphorylation, motor and behavioral deficits, and neuronal degeneration (Liou et al., 2003).
It is well established that mitochondrial DNA (mtDNA) accumulates mutations with aging, especially large-scale deletions (Corral-Debrinski et al., 1992) and point mutations. There is some evidence that mtDNA may be involved also in the mitochondrial dysfunction seen in AD. Transfer of patient mtDNA into mtDNA-deficient cell lines results in “cybrids” which reproduce the respiratory enzyme deficiency seen in the brain and other tissues in AD, suggesting that the defect is carried at least in part by mtDNA abnormalities (Swerdlow et al., 1997). However, identifying AD-specific mtDNA mutations presents challenges. Elson et al. (2006) completely sequenced mtDNA from 145 AD patients and 128 controls but did not find any significant association with mitochondrial haplogroup or with inherited mtDNA mutations. There was also no association with acquired mtDNA mutations when a coding region was examined (Lin et al., 2002). However, in the same way that promoters appeared more sensitive to damage than coding regions in nuclear genes (Lu et al., 2004), the mtDNA control region showed an increase in acquired mutations in AD (Coskun et al., 2004). AD brains had on average a 63% increase in heteroplasmic mtDNA control-region mutations, while individuals older than 80 years had a 130% increase in mutations. These mutations preferentially altered known mtDNA regulatory elements and suppressed mitochondrial transcription and replication.

A number of proteins implicated in AD pathogenesis may, in fact, have direct physical involvement with mitochondria or mitochondrial proteins. APP has a dual endoplasmic reticulum/mitochondrial-targeting sequence. APP overexpression in cells and in transgenic mice results in the “clogging” of mitochondrial protein importation machinery, causing mitochondrial dysfunction and impaired energy metabolism (Anandatheerthavarada et al., 2003). \( \beta \) binds to a mitochondrial-matrix protein termed \( \beta \)-binding alcohol dehydrogenase (Lustbader et al., 2004). Preventing \( \beta \) from interacting with \( \beta \)-binding alcohol dehydrogenase by means of a “decoy peptide” suppresses \( \beta \)-induced apoptosis and free-radical generation in neurons (Yao et al., 2011). Likewise, overexpressing \( \beta \)-binding alcohol dehydrogenase in transgenic APP-mutant mice exaggerated neuronal oxidative stress and impaired memory. Crouch et al. (2005) and Manczak et al. (2006) have also reported that \( \beta \) interacts with mitochondria, inhibiting cytochrome oxidase activity and increasing free-radical generation. \( \beta \) inhibits \( \alpha \)-ketoglutarate dehydrogenase activity in isolated mitochondria (Casley et al., 2002), and deficiency of \( \alpha \)-ketoglutarate dehydrogenase (Gibson et al., 1988) and cytochrome oxidase activities (Parker et al., 1990) has been found in the brain and other tissues in AD. In addition, \( \beta \) interacts with the serine protease HtrA2/Omi (Park et al., 2004). Presenilin and all the other components of the \( \gamma \)-secretase complex also localize to mitochondria, where they form an active \( \gamma \)-secretase complex (Hansson et al., 2004).

Can one draw from the above observations a therapeutic approach which targets mitochondria? Previous studies indicate that the role that ROS play in
synaptic plasticity and memory is a double-edged sword. Apart from their link to synaptic pathology associated with aging and neurodegenerative diseases, ROS have a physiological role, in that their production is necessary to maintain normal synaptic plasticity. For example, LTP induction was abolished by exogenous SOD and cell-permeable manganese porphyrin compounds that mimic SOD (Klann et al., 1998). Further, the effects of ROS on LTP involve the activation of protein kinase C and mitogen-activated protein kinases, both critical signaling molecules for LTP induction (Huddleston et al., 2008; Knapp and Klann, 2002). Various types of ROS-mediated oxidative stress have been (and continue and to be) used as biomarkers of AD brain pathology, with antioxidants being utilized in AD clinical trials (Praticò, 2008), albeit with negative outcomes. While endogenous antioxidants are capable of neutralizing sufficient levels of ROS and preventing neuron injury, aging and pathological conditions like AD may well produce very high levels of ROS that overwhelm the capacity of endogenous antioxidant defenses. ROS include a variety of species produced from different sources (Balaban et al., 2005; Murphy, 2009) and appear to play different roles in synaptic plasticity, depending on their subcellular localization and the age of animals being studied (Hu et al., 2006, 2007; Kamsler and Segal, 2003; Thiels et al., 2000). With this complexity of ROS function in the nervous system and their diverse effects on synaptic function, it is probably not surprising that clinical trials with antioxidants such as vitamin E resulted in either a marginally positive effect or no effect on cognitive function in AD patients (Praticò, 2008). Findings suggesting that the specificity and subcellular targeting of antioxidants play an important role in the modulation of synaptic plasticity by ROS and thus may determine their effectiveness in treating cognitive dysfunction (Ma et al., 2011) propose that generating more specific mitochondrial antioxidants might provide an avenue to improved antioxidant therapy for the treatment of AD.

VIII. Inflammation and AD

Neuroinflammation in disorders such as AD was viewed at one time as an epiphenomenon, with inflammation occurring when damaged neurons provoke an activation response from glia. Today’s research is challenging this earlier perspective and points to a more active role of neuroinflammation in pathophysiology onset and progression. In the CNS, glial cells (microglia, astroglia, and oligodendroglia) not only serve supportive and nutritive roles for neurons but also in the healthy brain often respond to stress and insults by transiently upregulating inflammatory processes. Otherwise “normal” glial functions can sometimes result in a more severe and chronic neuroinflammatory cycle that actually
promotes or propagates neurodegenerative disease (Block et al., 2007; Hanisch and Kettenmann, 2007). The delicate balance in this homeostasis can be disturbed, resulting in disease or exacerbation of initiating factors that result in disease (Craft et al., 2005). Thus, suppression of neurotoxic products by glial activation should result in neuroprotection.

Microglia are often found near damaged tissue in AD patients, but whether the brain’s innate immune cells are helpful or harmful in the disease has been an open question. Microglia activation adjacent to amyloid deposits has been used to suggest that microglia-mediated neuroinflammatory responses mediate AD-associated neurodegeneration (El Khoury and Luster, 2008; Wyss-Coray, 2006). In particular, it has been argued that cytokines, chemokines, and neurotoxins generated by amyloid-activated microglia cause neuronal cell damage (El Khoury and Luster, 2008). Indeed, microglial Toll-like receptors-2 and -4, and their co-receptor CD14, appear to be required for amyloid stimulation of microglia (Reed-Geaghan et al., 2009, 2010). This has led to the hypothesis that anti-inflammatory therapy could be beneficial, and this idea is supported by lower incidence of AD in patients with arthritis, most of whom use nonsteroidal anti-inflammatory drugs (McGeer and McGeer, 2007). However, clinical trials in AD have been disappointing (McGeer and McGeer, 2007). It may be that nonsteroidal anti-inflammatory drugs and anti-inflammatory approaches in general work only in primary prevention of AD, not in treatment. Also, these trials may not have addressed the right molecular targets (cf., e.g., Weggen et al., 2001).

Two new studies paint a picture of microglia as a double-edged sword, playing opposing roles in AD pathogenesis: they not only eliminate Aβ aggregates via phagocytosis but also kill nearby neurons by causing inflammation and the release of neurotoxic proteases. One report makes a case for antagonizing chemokine CX3C motif receptor 1 (CX3CR1), which promotes inflammation and neuron killing by microglia (Fuhrmann et al., 2010). The other shows that an adrenergic receptor agonist stimulates a microglial mechanism for clearing Aβ deposits while suppressing inflammation (Heneka et al., 2010). The Cx3cr1 knockout rescued neuron loss, although these mice had Aβ levels that were comparable to those in wild-type controls. The authors believe that microglia do not affect the initial formation of Aβ, but rather that turning off a signaling cascade in microglia dampens the production of neurotoxic substances such as inflammatory cytokines and proteases. A recent study showing that eliminating the majority of microglia did not prevent Aβ production (Grathwohl et al., 2009) is in line with the conclusions of Fuhrmann et al. (2010). Although both functions are likely to be necessary in a healthy brain, the studies argue that an imbalance in these activities can exacerbate neurodegeneration in AD. It remains to be determined what attracts microglia toward Aβ-producing neurons in AD. Still, distinguishing and modulating beneficial and detrimental parts of the immune response in AD will be an exciting and challenging field in the coming years.
A growing number of studies show that familial AD mutations in APP and PS-1 can exert deleterious effects independent of Aβ. For example, many familial AD mutants increase neither production of Aβ42 nor the Aβ42/Aβ40 ratio (Bentahir et al., 2006; Shioi et al., 2007). Aβ peptides are normal components of human serum and cerebrospinal fluid. As it happens, in APP-based mouse models of AD, all products of APP metabolism are increased together with Aβ, and there is evidence that some of these non-Aβ products are neurotoxic (Ghosal et al., 2009; Nikolaev et al., 2009). Behavioral abnormalities of animal models overexpressing APP should be viewed with some caution, as in addition to Aβ, other APP metabolites may affect outcome and contribute to neurodegeneration (Robakis, 2011).

The ε-cleavage of transmembrane proteins (e.g., Notch 1 receptor, cadherins, APP, and EphB receptors) is mediated by γ-secretase and inhibited by PS-1 familial AD mutations (Marambaud et al., 2003). This cleavage takes place downstream from the γ-cleavage site resulting in the release of soluble cytoplasmic peptides, the intracellular C-terminal fragments (Litterst et al., 2007). These peptides travel to the nucleus where they can regulate gene expression (Gao and Pimplikar, 2001) or sequester transcription factors in the cytoplasm (Marambaud and Robakis, 2005). Familial AD mutations may thus promote neurodegeneration by altering the production of peptides with important transcriptional and signal transduction properties (Robakis, 2003).

PS-1 is essential for lysosomal proteolysis and autophagy by enabling the acidification of lysosomes required for protease activation (Lee et al., 2010). Recent evidence suggests that mutations of PS-1 and APP (or APP gene duplication), independent of Aβ, directly disrupt autophagy or alter endocytosis, which impairs neuronal function and reduces neuron survival. The lysosomal network, comprising the endocytic and autophagic pathways, mediates the processing, sorting, and turnover of proteins and other cellular constituents. Endocytosis is especially critical in neurons, as it supports such specialized functions as synaptic transmission and retrograde trophic signaling (Nixon et al., 2008). Autophagy, the principal degradative pathway for organelles and long-lived proteins, is essential for neuronal cell survival by clearing damaged, aggregated, or obsolete proteins in disease states and cellular aging (Wong and Cuervo, 2010). Deficit lysosomal proteolysis leads to the extensive “neuritic dystrophy” of AD (Suzuki and Terry, 1967) characterized by grossly swollen neurites packed with autophagic vacuoles containing Aβ and other incompletely degraded substrates (Yu et al., 2005) that are potentially neurotoxic (Yang et al., 2008). As it happens, impaired autophagy in the AD brain results in Aβ accumulation in autolysosomes (Glabe, 2001). Autophagy deficits in AD are part of a continuum of lysosomal system deficits (Jiang et al., 2010), and abnormal acceleration of neuronal cell endocytosis is
evident before cortical amyloid deposition (Cataldo et al., 2000). Genes related to endocytosis, such as Rab5, Rab7, and Rab4, are among the first to be upregulated in AD (Ginsberg et al., 2010) and are abnormally recruited to endosomes. This pattern is specific for AD among aging-related neurodegenerative diseases so far studied and is accelerated by inheritance of the \(\epsilon 4\) allele of apolipoprotein E, the major genetic risk factor for late-onset AD (Cataldo et al., 2000).

Analysis of viable presenilin conditional knockout mice, in which presenilin expression is selectively inactivated in excitatory pyramidal neurons of the postnatal forebrain, reveals that loss of presenilins affects both short- and long-term plasticity, in the absence of neurodegeneration (Saura et al., 2004). Furthermore, NMDA receptor-mediated responses are impaired and synaptic levels of NMDA receptor subunits are reduced in the absence of presenilin. Detailed genetic and electrophysiological studies (for a review, see Pimplikar et al., 2010) demonstrate that loss of presenilin function impairs LTP induction and glutamatergic neurotransmitter release by a presynaptic mechanism and raise the possibility that presynaptic mechanisms may play an important role in AD pathophysiology (Shen, 2010). Cyclin-dependent kinase-5 is a proline-directed serine/threonine kinase that has important roles in various neuronal functions including brain development, synaptogenesis, synaptic plasticity, and memory formation (Dhavan and Tsai, 2001). Evidence for a role of cyclin-dependent kinase-5 in the pathogenesis of AD has been discussed in detail recently (Crews et al., 2011; Pimplikar et al., 2010) and will not be covered further here.

X. Future Perspectives

Interventions in the amyloid pathway continue to be the focus of most drug discovery efforts for AD, and a number of programs have advanced into the clinic. While at least some of these treatments may be safe, their potential for successful outcome remains unknown. In fact, over the past several years, no fewer than 11 drugs for AD have failed in clinical trials. Because most drugs in these trials take a collective approach to targeting the same point (amyloid) in the pathological cascade, the prospects for a successful drug in the near term are not great. As Alzheimer’s is a disease with a 10- to 20-year natural history, drugs may be failing in clinical trials because they are simply tested too late in the course of AD. Imaging studies with amyloid ligands suggest that significant plaque deposition occurs already before clinical decline (Jack et al., 2009). Reducing the generation or enhancing the clearance of new A\(\beta\) monomers and oligomers, together with approaches that clear existing plaques and soluble species (Wang et al., 2011a,b) at
the same time may afford greater benefit. Conceivably, anti-amyloid therapy may be most efficacious in prevention paradigms.

Transgenic animal models of AD have proven of considerable value in elucidating the molecular events involved in amyloid-dependent neuronal injury and in designing therapeutics. Yet, to date, no rodent model of AD exists that develops the disease spontaneously. The disease must be induced by a particular treatment or by the presence of mutated genes. What is more, rarely are all aspects of disease pathology present. Indeed, one must remember that familial AD accounts for approximately 3–5% of AD cases. So although rodent models can help demonstrate whether a drug candidate is having the intended effect on the brain, results need to be interpreted within the context of these limitations.

An important challenge for future studies will be to determine the extent to which amyloid-independent mechanisms contribute to AD. Apart from genetics, environment may be an element to consider. For example, in rats stress acts cumulatively, to precipitate AD-like tau pathology and cognitive deficits (Sotiropoulos et al., 2011). The implicit assumption underlying current drug trials is that the prime causative agent of AD is amyloid, and therefore blocking amyloid accumulation will prevent AD. However, if amyloid-independent mechanisms also contribute to AD, then drug trials will yield only moderately positive results. We need to also understand why AD takes so long to manifest itself (like other neurodegenerative diseases) and why certain parts of the brain are more susceptible to AD.

The development of new diagnostic criteria that include biomarkers to diagnose early forms of AD before full-blown dementia is vital to the field (Dubois et al., 2007). The development of a new assay that can detect Aβ oligomers in the cerebrospinal fluid (Fukumoto et al., 2010), together with data from a phase II, double-blind, placebo-controlled clinical trial, suggesting that Aβ immunotherapy can attenuate cortical fibrillar Aβ load (Rinne et al., 2010) provides the first direct therapeutic evidence of amyloid burden reduction in patients with AD and is good news. It now remains to be seen if these findings translate into clinical benefit. In addition to biomarkers of disease progression, advances in imaging technology may make it possible to test drugs on people before symptoms arise. One encouraging advance is the ability to image amyloid load in the brain of cognitively normal people and track what types of changes correlate with AD (Sinha, 2011), as well as identifying biomarkers to segment the heterogeneous population (e.g., those with the apolipoprotein ε4 allele) of AD suffers. It is hoped that these biomarkers will provide a means to separate cognitively normal people from those who will go on to develop AD, thereby helping to understand why drugs are failing, or better identify drug responders. Current clinical trial design is hampered by many apparently healthy individuals who go on to develop AD, thereby confounding the data. The ability to identify asymptomatic persons predisposed to the disease would be a big plus for clinical research. Genome-wide association studies to identify Alzheimer’s susceptibility loci such as clusterin
(Harold et al., 2009; Lambert et al., 2009) may be an additional way to identify normal people who have a high risk of developing AD in the future.

It will not be surprising if future studies lead us to find that both amyloid-dependent and -independent mechanisms participate in AD pathology. In this regard, an effective disease-modifying treatment will probably come about only from a strategy that addresses both of these mechanisms.

References


AMYLOID AND ALZHEIMER’S DISEASE


