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Chapter VIII

DEVELOPMENT IN DIAGNOSTIC AND THERAPEUTIC STRATEGIES FOR ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease for which, at present, there is no cure. Accurate diagnosis of AD is difficult because the symptoms overlap with those manifested in other forms of dementia. Acetylcholinesterase inhibitors and memantine, drugs that currently are approved by the Food and Drug Administration (FDA) for AD, treat symptoms but not the underlying cause of the disease. The neuropathology of AD begins with compromised synaptic transmission and impaired plasticity, predominantly in the entorhinal cortex and the hippocampus. These neuropathological processes may begin years or even decades before manifestation of the first symptoms. Thus, by the time the first signs of disease become apparent, the underlying neuropathology may be already fairly advanced. Therefore, development of disease-modifying therapeutic strategies and accurate diagnostic measures for AD must focus on the events that initiate the disease at the earliest stages. Substantial evidence indicates that the primary cause of AD is an age-related imbalance between production and clearance of amyloid β-protein (Aβ). Prevention of Aβ production, enhancement of Aβ clearance, and inhibition of Aβ assembly into neurotoxic aggregates, thus are

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principal focuses of current therapeutic approaches. Present diagnosis of AD is based on clinical, neurophysiologic and neuroimaging assessments. These approaches are relatively sensitive and specific at the middle or late stages of AD but early diagnosis is challenging. With the advent of new drugs now in clinical trials, there is an urgent need for early diagnosis, not only for prescribing the correct treatment and validating the efficiency of the drugs, but also for understanding the underlying disease process.

ABBREVIATIONS

AD,	Alzheimer's disease;
ADAM,	A disintegrin and metalloprotein;
ADDLs,	Aβ-derived diffusible ligands;
APLP,	APP-like protein;
APP,	amyloid β-protein precursor;
Aβ,	amyloid β-protein;
BACE-1,	β-site APP-cleaving enzyme 1;
BBB,	blood–brain barrier;
CDR,	clinical dementia rating;
CMRgl,	cerebral metabolic rate for glucose;
CNS,	central nervous system;
COX,	cyclooxygenase;
CSF,	cerebrospinal fluid;
chGly,	cyclohexylglycine;
dsRNA,	double-stranded RNA;
ECE,	endothelin-converting enzyme;
EGCG,	epigallocatechin-3-gallate;
ELISA,	enzyme-linked immunosorbent assay;
ErbB4,	erythroblastic leukemia viral oncogene homolog-4;
ERK,	extracellular signal-regulated kinase;
FAD,	familial AD;
FDA,	Food and Drug Administration;
FDG,	^{18}F-labeled fluoro-2-deoxy-D-glucose ;
^{18}F-FDDNP,	2-(1-{6-[2-[^{18}F]fluoroethylmethylamino]-2-naphthyl}ethylidene) malononitrile;
GAG,	glycosaminoglycan;
IAPP,	islet amyloid polypeptide;
IDE,	insulin degrading enzyme;
ISF,	interstitial fluid;
LRP,	low-density lipoprotein receptor-related protein;
MAO,	monoamine oxidase;
MCI,	mild cognitive impairment;
MCIa,	amnestic MCI;
mLeu,	*N*-methyl leucine;
MMSE,	mini-mental state examination;

MoCA,	Montreal cognitive assessment test;
MRI,	magnetic resonance imaging;
mRNA,	messenger RNA;
MTA,	medial temporal lobe atrophy;
NEP,	neprilysin;
NFT,	neurofibrillary tangles;
NGF,	nerve growth factor;
NMDA,	*N*-methyl-D-aspartate;
NSAID,	non-steroidal anti-inflammatory drug;
PET,	positron emission tomography;
PIB,	Pittsburgh compound B;
PKC,	protein kinase C;
RAGE,	receptor for advanced glycation-end products;
RNAi,	RNA interference;
ROS,	reactive oxygen species;
siRNA,	small interfering RNA;
SPECT,	single-photon emission computed tomography;
SREBP,	sterol regulatory element-binding protein;
TACE,	tumor necrosis factor α converting enzyme;
^{99}Tc-ECD,	^{99}Tc-ethylcysteinate dimer;
^{99}Tc-HMPAO,	^{99}Tc-hexamethylpropyleneamine oxime;
tPA,	tissue-type plasminogen activator;
TrkA,	tyrosine kinase receptor A;
uPA,	urokinase-type plasminogen activator;
VBM,	voxel-based morphometry.

INTRODUCTION

Alzheimer's disease (AD) initially manifests as episodic memory lapses and difficulty with daily tasks, and then gradually causes decline of mental faculties, dementia, and finally death (Selkoe 2001a; Cummings 2004). AD is a disease of old age, typically affecting people in the seventh or eighth decade of life with incidence numbers rising steeply after age 65. Currently, AD has no cure. Based on the year 2000 census in the United States, the prevalence of AD has been estimated at 3.9–4.5 million and predicted to triple by 2050 due to the rapid aging of the population, if no cure is found (Hebert et al. 2003; Grant 2004). A recent report by the Alzheimer Association has suggested that in 2007, the prevalence of AD in the US has exceeded 5 million and may increase up to 16 million by the middle of the century (AlzheimerAssociation 2007a). The life span of patients with AD is 8 years on average and may extend up to 20 years from the onset of symptoms (AlzheimerAssociation 2007b). During the years of illness, the patients, their families, and their caretakers suffer grave emotional and financial duress. Current cost estimates of care for patients with AD in the US are over $148 billion a year (AlzheimerAssociation 2007a). Numbers in other

countries, including developing countries, also are highly alarming (Beeri et al. 2002; Leung et al. 2003; Zencir et al. 2005).

The neuropathology of AD begins with compromised synaptic transmission and plasticity, predominantly in the entorhinal cortex and the hippocampus (Monien et al. 2006; Scheff et al. 2006). As the disease progresses, other brain regions become affected and increasing injury leads to dysfunction and eventually death of susceptible neurons. At late stages of AD, massive degeneration of affected brain areas leads to severe atrophy. Upon postmortem examination of AD brain, two hallmark lesions are observed—extracellular amyloid plaques and intracellular neurofibrillary tangles (NFT) (Alzheimer 1906; Alzheimer 1907), both contributing to the neurodegenerative process. Dead or dying neurons and an active inflammatory process including dystrophic astrocytes, and activated microglia typically surround the amyloid plaques (Heneka and O'Banion 2007). These pathological findings represent advanced stages of AD pathology. However, the events that set off the disease process may occur 10–15 years before manifestation of the first symptoms (Kawas et al. 2003). Thus, to develop efficient therapy for AD and to accurately diagnose AD early enough for the treatment to be as effective as possible, the events that initiate the disease at the earliest stages need to be targeted.

Most researchers agree that the primary cause of AD is an age-related imbalance between production and clearance of the amyloid β-protein (Aβ) or of particular forms of Aβ (Hardy and Selkoe 2002). Aβ is a small protein that is produced as part of normal metabolism (Haass et al. 1992) and has an unknown function. A plausible explanation for the fact that young people do not develop AD is that this imbalance is subtle and develops very slowly with aging. When the rate of production exceeds that of clearance, Aβ accumulates to above-optimal concentrations, leading to its self-association into neurotoxic assemblies. The association process is complex, likely proceeds via multiple pathways, and involves substantial conformational changes in Aβ. The final products of this process are Aβ polymers in which monomers are associated with each other non-covalently. The polymers, which are the form of Aβ found in amyloid plaques, are rich in β-sheet conformation and have a fibrillar morphology (Serpell 2000).

The Amyloid Cascade Hypothesis (Hardy and Higgins 1992) stipulates that the harmful action of Aβ causes the brain inflammation that is a part of the pathology of AD, and leads to hyperphosphorylation of protein tau, a component of the microtubule structure. Upon hyperphosphorylation, tau aggregates into paired helical filaments that comprise the NFT. Aggregation of hyperphosphorylated tau is not unique to AD but exists in, and may be the cause of, other neurodegenerative diseases termed “tauopathies” (Churcher et al. 2006; Iwatsubo 2006). In AD, evidence has indicated that hyperphosphorylation of tau and formation of NFT are downstream events that follow Aβ-induced insults to neurons (Lemere et al. 1996; Morishima-Kawashima and Hara 2002; Roberson et al. 2007).

Despite the fact that Aβ fibrils are the main proteinaceous components of the amyloid plaques, substantial evidence suggests that the actual disease-causing forms of Aβ are pre-fibrillar, oligomeric assemblies (Kirkitadze et al. 2002; Klein et al. 2004; Walsh and Selkoe 2004; Glabe 2006; Terry 2006; Haass and Selkoe 2007). Aβ fibrils may contribute to the neuropathology at later stages of the disease but are thought to be the end product of the self-association process rather than the predominant pathogenic form. Aβ oligomers vary in size

and structure. Despite this structural variability, the majority of the oligomeric species tested have been found to be neurotoxic. Neurotoxic Aβ oligomers may be as small as dimers and trimers (Walsh et al. 2002) and as large as protofibrils (Hartley et al. 1999; Walsh et al. 1999), which may comprise hundreds of monomers and are the latest precursor known on the pathway to fibril formation. Several types of oligomers that share a spherical morphology, but may vary in size from a few nanometers to several dozen nanometers, have been shown to be neurotoxic (Lambert et al. 1998; Hoshi et al. 2003; Barghorn et al. 2005; Fradinger et al. 2005). Direct comparison of activity has shown that spherical Aβ oligomers are 10-100 times more neurotoxic than Aβ fibrils (Dahlgren et al. 2002). Aβ oligomers have been found in brain and cerebrospinal fluid (CSF) from patients with AD in concentrations considerably higher than in age-matched healthy individuals (Pitschke et al. 1998; Gong et al. 2003; Kayed et al. 2003; Georganopoulou et al. 2005). Studies of animal models of AD have shown that decline in memory and learning ability are observed before the formation of amyloid plaques (Hsia et al. 1999; Mucke et al. 2000; Jacobsen et al. 2006) and that Aβ oligomer concentration correlates with the degree of cognitive decline (Lesné et al. 2006), strongly suggesting that oligomers are the primary neurotoxins *in vivo*.

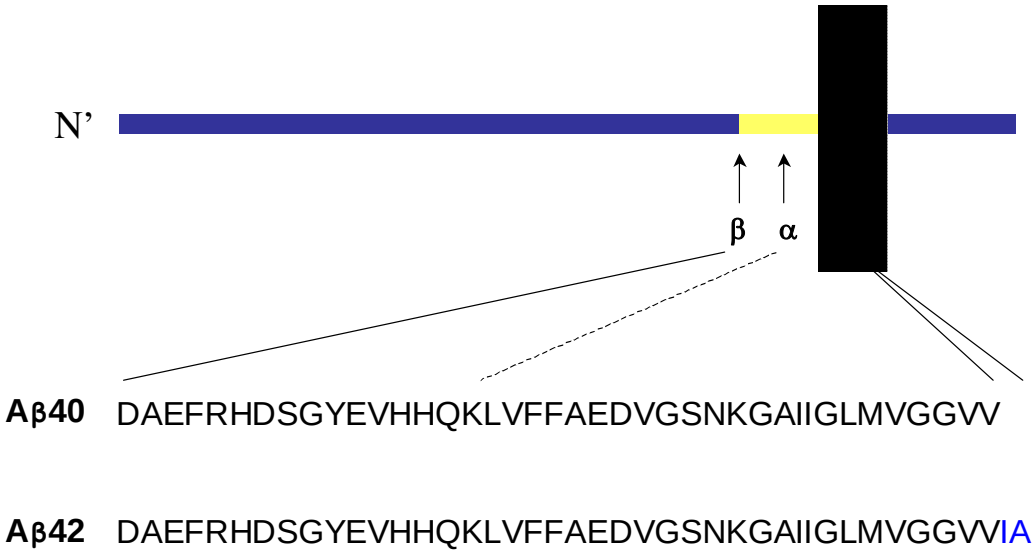

Figure 1. Proteolytic processing of APP by α-, β- and γ-secretases. Consecutive cleavage by β- and γ-secretases produces the amyloidogenic fragments Aβ40 and Aβ42, whereas cleavage by α-secretase precludes production of Aβ.

Aβ is produced from the amyloid β-protein precursor (APP), a large, type I membrane protein, through sequential proteolytic cleavage by β-secretase and γ-secretase, respectively (Nunan and Small 2000). Several forms of Aβ are produced, major among them are those comprising 40 (Aβ40) or 42 (Aβ42) amino acid residues, which differ only by the absence or presence of the two C-terminal residues, Ile[41] and Ala[42] (Figure 1). Despite this small difference in sequence, Aβ40 and Aβ42 display distinct behaviors clinically, biologically and biochemically. Aβ40 and Aβ42 exist *in vivo* at a concentration ratio of ~10:1, respectively (Wang et al. 1996). Aβ42 is deposited first during the development of AD (Suzuki et al.

1994) and is more neurotoxic than Aβ40 (Younkin 1995; El-Agnaf et al. 2000; Zhang et al. 2002). Aβ42 is the predominant component in parenchymal plaques whereas Aβ40 is the main component in vascular deposits (Roher et al. 1993; Gravina et al. 1995; Suo et al. 1998). An increase in the Aβ42/Aβ40 ratio is associated with early-onset familial AD (FAD) (Suzuki et al., 1994; Scheuner et al. 1996; De Jonghe et al. 2001), whereas treatments that decrease this ratio reduce the risk for AD (Weggen et al. 2001). Transgenic mice expressing high levels of Aβ40 in the absence of human APP do not develop overt amyloid pathology whereas mice expressing Aβ42 develop amyloid plaques and cerebral amyloid angiopathy (McGowan et al. 2005). In a drosophila model, each Aβ form expressed individually causes learning deficits, but expression of Aβ42 leads to extensive neurodegeneration, whereas expression of Aβ40 does not lead to the formation of amyloid deposits or neurodegeneration (Iijima et al. 2004). Aβ42 oligomers have been found to be substantially more neurotoxic than those of Aβ40 *in vitro* (Dahlgren et al., 2002; Fradinger et al., 2005). Aβ40 and Aβ42 oligomerize through distinct pathways (Bitan et al. 2003a; Bitan et al. 2003b) and give rise to distinct oligomers (Bitan et al., 2003a; Hoshi et al., 2003; Hepler et al. 2006). These assembly characteristics are thought to be related to formation of quasi-stable structures at the C-terminus of Aβ42 but not of Aβ40 (Urbanc et al. 2004; Lazo et al. 2005; Murakami et al. 2005; Krafft et al. 2006; Sgourakis et al. 2007). Thus, Aβ42 oligomers are believed to be the chief culprit in AD and are a major target for development of therapeutics for AD.

This chapter outlines the current status of therapy and the diagnosis in AD and discusses new, mechanism-based approaches pursued to develop disease-modifying agents that will treat the causes rather than the symptoms of AD, and to improve accuracy and sensitivity of AD diagnosis.

APPROVED THERAPY FOR AD

Cholinesterase Inhibitors

Current therapy for AD largely is restricted to symptomatic treatment. Early observations of a deficiency in cholingeric neurotransmission in AD have led to development of cholinesterase inhibitors as the first approved treatment (Bartus et al. 1982). Four of the five drugs approved in the USA for treatment of AD—Donepezil (Aricepts™, Eisai Co, Woodcliff Lake, NJ); Galantamine (Razadyne ER™, Ortho-McNeil Neurologics, Titusville, NJ); Rivastigmine (Exelon™, Novartis, Basel, Switzerland); and Tacrine (Cognex™, First Horizon Pharmaceutical, Roswell, GA), are acetylcholinesterase inhibitors, designed to compensate for the loss of cholinergic neurons by preventing the breakdown of the neurotransmitter acetylcholine by the enzyme acetylcholinesterase (Lleo et al. 2004). Tacrine, which was the first approved drug, causes considerable adverse side effects including nausea, diarrhea, urinary incontinence and liver toxicity and therefore today is prescribed rarely. The Cochrane dementia group has published three reviews on the evidence for the efficacy and safety of donepezil (Birks et al. 2000b), rivastigmine (Birks et al. 2000a), and galantamine (Olin and Schneider 2002). Although these drugs alleviate the symptoms of AD, they do so for a limited period and to a small extent. They do not resolve the underlying cause of the

disease but treat one downstream process. TV3326 (Ladostigil™, Yissum Technology Transfer Company of the Hebrew University of Jerusalem, Israel), a second-generation cholinesterase inhibitor is under phase I/IIA clinical studies for the treatment of AD (Youdim and Bakhle 2006). TV3326 combines the neuroprotective effects of the anti-Parkinson's disease drug rasagiline, a selective monoamine oxidase (MAO)-B inhibitor, with the cholinesterase inhibitory activity of rivastigmine in a single molecule. It also exerts antidepressant activity by inhibition of MAO-A (Youdim 2006). Thus, TV3326 may be effective as a potential treatment for AD, Lewy body disease, and Parkinson's disease with dementia (Youdim et al. 2006).

NMDA Receptor Modulation

Another approach for symptomatic treatment of AD is attenuating glutamatergic excitotoxicity. Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS). Under certain pathologic conditions, glutamate is involved in excitotoxicity—excessive activation of *N*-methyl-D-aspartate (NMDA) receptors with consequent intracellular accumulation of Ca^{2+}, leading to a cascade of events that results in neuronal death (Michaelis 1998). NMDA receptors recently have been reported to mediate synapse loss and oxidative stress caused by Aβ oligomers (De Felice et al. 2007; Shankar et al. 2007). The fifth drug approved by the FDA and commonly prescribed to patients with AD is the non-competitive NMDA receptor antagonist memantine (Axura™ and Akatinol™, Merz, Frankfurt, Germany; Namenda™, Forest laboratories, New York, NY; Ebixa®™, Lundbeck, Copenhagen, Denmark). Memantine was shown to delay cognitive deterioration in patients with moderate to severe AD (Reisberg et al. 2003). Compared with donepezil monotherapy, combination therapy with donepezil and memantine resulted in modest cognitive improvement (Tariot et al. 2004). Despite a possible neuroprotective mode of action of memantine (Wenk et al. 1997; Miguel-Hidalgo et al. 2002), studies have shown that the drug relieves the symptoms of AD (Tariot et al., 2004) but there is no evidence supporting a disease-modifying role.

EXPERIMENTAL THERAPY

Approaches that interfere with the fundamental causative pathological processes in AD are a primary focus of current drug-development programs (Aisen 2005; Jacobsen et al. 2005; Golde 2006; Kennedy et al. 2007). Gradual accumulation of Aβ, particularly Aβ42, initiates a complex multistep cascade that includes disruption of synaptic transmission, oxidative stress, decline in neurotransmitter level, formation of NFT, gliosis, inflammatory processes, apoptosis, and neuronal death (Hardy and Selkoe 2002). Various stages of the amyloid cascade are being explored as targets for therapeutic intervention (Hardy and Selkoe 2002; Gandy et al. 2003; Golde 2003; Selkoe and Schenk 2003; Selkoe 2005; Moreira et al. 2006). Leading approaches include inhibition of Aβ production, enhancement of Aβ clearance, and inhibition of Aβ assembly.

Inhibition of Aβ Production

Inhibiting Aβ production by targeting the secretases that cleave APP is an obvious therapeutic approach for AD. To generate Aβ, β-secretase first cleaves APP to produce a soluble N-terminal ectodomain, sAPPβ, and a membrane-anchored C-terminal stub. The latter then is cleaved within the membrane by γ-secretase to produce Aβ and a C-terminal fragment, which complexes with other proteins, relocates to the nucleus, and acts as a transcription regulator. Cleavage of APP within the Aβ domain by α-secretase is an alternative processing pathway that precludes Aβ formation (Figure 1) (Selkoe 2000). Strategies to reduce Aβ production, either through inhibition of β- or γ-secretases, or through activation of α-secretase, therefore are potential approaches for treatment and/or prevention of AD.

β-Secretase Inhibition

The membrane-anchored aspartic protease β-site APP-cleaving enzyme 1 (BACE-1) is the predominant β-secretase (Vassar 2004). Some β-secretase activity also has been assigned to the cysteine protease, cathepsin B (Hook et al. 2005). BACE-1 knockout mice are viable, show no major pathological abnormalities, and do not produce Aβ (Cai et al. 2001; Luo et al. 2001; Dominguez et al. 2005). The mice display subtle deficits in explorative activities and spatial learning and memory, suggesting that BACE-1 is important for the normal function of the brain (Laird et al. 2005). BACE-1 cleaves a variety of substrates in addition to APP, including the APP-like proteins APLP1 and APLP2, sialyltransferase, P-selectin glycoprotein ligand-1, low-density lipoprotein receptor-related protein (LRP) and the β-subunit of voltage-gated sodium channels (Hussain 2004; von Arnim et al. 2005; Wong et al. 2005). Therefore, the current approach in therapeutic inhibition of BACE-1 is to inhibit the enzyme only partially. Under partial inhibition conditions, other BACE-1 substrates may not be affected to levels that would cause damage, but Aβ production may be reduced to non-pathogenic levels.

Hopes for developing β-secretase inhibitors initially had been boosted by obtaining a high-resolution crystal structure of the BACE-1 protease domain complexed with a peptide inhibitor (Hong et al. 2000). However, the structure revealed that the active site of BACE-1 was unusually large and polar, characteristics that make obtaining potent small-molecule inhibitors difficult. Most of the inhibitors discovered initially were relatively large peptides, which did not cross cell membranes or the blood-brain barrier (BBB) and were metabolically unstable (Citron 2004; Cumming et al. 2004; Hussain 2004). Obtaining crystal structures of BACE-1 with smaller inhibitors (Patel et al. 2004) has given new insights into the way ligands bind to the active site and consequently has aided the development of more potent and selective cell-permeable compounds (Coburn et al. 2004; Stachel et al. 2004; Ghosh et al. 2005; John 2006; Stachel et al. 2006).

The first BACE-1 inhibitor shown to be effective *in vivo*, OM00-3, was a large peptide-based inhibitor that upon intraperitoneal injection into the brains of Tg2576 mice, which overexpress human APP containing the Swedish double mutation K670N and M671L, reduced soluble Aβ concentration (Chang et al. 2004). Subsequent progress in the development of BACE-1 inhibitors resulted in generation of smaller peptidomimetics (Kimura et al. 2005). Intrahippocampal injection of the peptidomimetic KMI-429 decreased

the soluble pool of brain Aβ in both wild-type mice and Tg2576 mice (Asai et al. 2006). Recently, pharmacological inhibition of BACE-1 that leads to lowering of brain Aβ, has been achieved through oral administration of a non-peptidic compound, GSK188909 (GlaxoSmithKline, Essex, UK), in TASTPM mice, which overexpresses human APP containing the FAD-linked Swedish mutation and the presenilin-1 M146V mutation resulting in over production of Aβ (Hussain et al. 2007). These findings support the initiation of clinical trials using BACE-1 inhibitors.

γ-Secretase Inhibition

γ-Secretase is a membrane-bound aspartyl protease complex composed of presenilin-1 or presenilin-2, nicastrin, Aph1, and Pen2 (Chen et al. 2006; Wolfe 2006; Lundkvist and Näslund 2007). The complex composition of γ-secretase makes obtaining high-resolution structures of γ-secretase difficult. Potential safety concerns have been raised regarding the use of γ-secretase inhibitors because γ-secretase is known to cleave multiple substrates, including Notch, erythroblastic leukemia viral oncogene homolog-4 (ErbB4), and sterol regulatory element binding protein (SREBP) in addition to APP (De Strooper et al. 1999; Kimberly and Wolfe 2003; Wong et al. 2004; Fre et al. 2005; Yoon and Gaiano 2005; Barten et al. 2006). Despite the difficulty in obtaining a high-resolution structure of γ-secretase, several potent inhibitors have been discovered, both by academic laboratories and by pharmaceutical companies (Wolfe et al. 1998; Churcher and Beher 2005; Churcher et al., 2006; Tomita and Iwatsubo 2006; Asberom et al. 2007a; Asberom et al. 2007b; Best et al. 2007; Pissarnitski et al. 2007). A phase II clinical trial of one such compound, LY450139 (Eli Lilly, Indianapolis, IN) has been reported recently (Siemers et al. 2005; Siemers et al. 2006). Patients receiving LY450139 (30 mg daily for one week followed by 40 mg daily for five weeks) had reduced Aβ40 concentration levels in the plasma (average maximum reduction 38%) but no significant changes in the Aβ40 or Aβ42 concentration levels in the CSF, possibly because the dose used was not high enough (Siemers et al., 2006).

Modulation of γ-secretase to reduce specifically the generation of Aβ42 may be a safer approach than simple inhibition because under modulation conditions, γ-secretase cleavage of essential substrates is affected minimally. A number of modulators including non-steroidal anti-inflammatory drugs (NSAIDs) and NSAID-like molecules have been shown to increase the production of shorter Aβ species such as Aβ38, and decrease the production of Aβ42 without perturbing Notch signaling at therapeutic doses (Weggen et al., 2001; Takahashi et al. 2003; Czirr and Weggen 2006). A potential caveat of this approach is that NSAIDs also act as non-selective inhibitors of cyclooxygenases. Cyclooxygenase (COX)-1 protects the stomach lining from harsh acids and digestive chemicals and helps maintain kidney function. Therefore, the clinical merit of using NSAIDs as Aβ42-lowering drugs is limited by potentially significant gastrointestinal and renal toxicity. R-flurbiprofen (Flurizan™, Myriad Pharmaceuticals Inc., Salt Lake City, UT), the R-enantiomer of the NSAID S-flurbiprofen, which modulates γ-secretase activity with little or no COX-inhibitory activity (Eriksen et al. 2003; Geerts 2007) is now in phase III clinical trials for mild to moderate AD (Czirr and Weggen 2006). The drug was well tolerated in a phase II trial in patients with mild to moderate AD and showed retardation of cognitive decline in a subgroup of patients with mild

dementia, suggesting that γ-secretase modulation is a viable disease-modifying approach (Wilcock et al. 2005).

α-Secretase Stimulation

α-Secretase cleaves APP within the Aβ sequence to produce a large N-terminal fragment, sAPPα and a small membrane-bound stub, CTFα, thereby precluding the formation of Aβ (Figure 1). sAPPα, may play a role in regulating neuronal excitability, plasticity, and survival, and has been reported to attenuate the neurotoxic effects of Aβ (Mattson et al. 1993; Furukawa and Mattson 1998; Small 1998; Kojro and Fahrenholz 2005). Several metalloprotease disintegrins, ADAM-9, ADAM-10, and TACE, have been identified as major α-secretases (Buxbaum et al. 1998; Lammich et al. 1999; Fahrenholz and Postina 2006). Neuronal overexpression of ADAM-10 in transgenic mice overexpressing human APP containing the FAD-linked London mutation, V717I, increases secretion of sAPPα, decreases Aβ production, delays plaque formation, and alleviates cognitive deficits (Postina et al. 2004). Protein kinase C (PKC) modulates the activity of α-secretase (Gandy and Greengard 1994a; Buxbaum et al., 1998) and therefore represents a possible target for drug development. Stimulation of α-secretase in Chinese hamster ovary (CHO) cells expressing human APP through activation of PKC by treatment with phorbol esters inhibits cellular production of Aβ (Zhu et al. 2001). Several groups have reported activation of α-secretase cleavage by muscarinic agonists, serotonin, glutamate, and estrogens (Vardy et al. 2005). Most of these compounds work by activating PKC-mediated signaling pathways, which alter the trafficking and subcellular distribution of APP and α-secretase (Hung et al. 1993; Gandy and Greengard 1994b; Koo 1997). Because PKC participates in many cellular processes, selective activation of α-secretase is challenging. This approach is still in development and has not yet advanced enough to support clinical trials.

Statins

Epidemiological studies have shown a link between using statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) to reduce blood cholesterol concentration and a decreased occurrence of AD (Wolozin et al. 2000). Cholesterol decreases α-secretase activity, and increases β- and γ-secretase activities (Golde and Eckman 2001). Thus cholesterol-lowering drugs may produce a net decrease in Aβ by increasing the α-secretase cleavage of APP and decreasing the β- and γ-secretase cleavage. However, two large trials using simvastatin (Zocor) (2002) and pravastatin (Shepherd et al. 2002) failed to find significant effects on the cognitive function of patients with mild to moderate AD. A pilot, proof-of-concept trial of atrovastatin (Lipitor) administered at 80 mg/day in 63 patients with mild to moderate AD showed some improvement in the cognitive functions (Sparks et al. 2006a; Sparks et al. 2006b) suggesting that larger trials with atrovastatin may be beneficial.

Enhancement of Aβ Clearance

Immunotherapy

Studies demonstrating that monoclonal antibodies could inhibit Aβ aggregation and Aβ-induced neurotoxicity *in vitro*, and that immunization with Aβ-derived antigens reduced plaque burden *in vivo* (Solomon et al. 1997; Solomon 2004), prompted major research efforts targeting Aβ clearance from the brain by the immune system. Exploration of Aβ immunotherapy in the PDAPP transgenic mouse model—a model that overexpresses the FAD-causing Indiana mutant form of human APP, V717F, has shown that either induction of humoral immune response against fibrillar Aβ42 or passive administration of anti-Aβ monoclonal antibodies (mAbs) can prevent amyloid plaque formation in young animals (Schenk et al. 1999) and reduce plaque burden with improvement in cognitive function in older mice (Morgan et al. 2000). Several hypotheses have been proposed for the mechanism of plaque clearance by Aβ immunotherapy: 1) Direct binding of antibodies to Aβ resulting in solubilization of Aβ fibrils or neutralization of Aβ oligomers (Solomon et al., 1997; Frenkel et al. 1999; Klyubin et al. 2005); 2) Fc-receptor-mediated phagocytosis of Aβ by microglial cells in the brain (Schenk et al., 1999; Wilcock et al. 2004a); and 3) "peripheral sink" action in which anti-Aβ antibodies bind Aβ in the plasma causing an efflux of Aβ from the brain by altering the equilibrium across the BBB (DeMattos et al. 2001; Matsuoka et al. 2003).

Despite initial encouraging results in animal models, a phase II clinical trial of active immunization therapy using aggregated Aβ42 with QS21 as an adjuvant (AN-1792; Elan Corporation, San Francisco, CA) was halted because approximately 6% of the patients participating in the trial developed severe meningoencephalitis (Schenk 2002). Postmortem study of brains from patients who had participated in the trial indicated that plaque densities were reduced in many areas of the neocortex, suggesting that the induced immune response was effective in clearing Aβ deposits (Nicoll et al. 2003; Ferrer et al. 2004; Masliah et al. 2005; Nicoll et al. 2006). Although a response to Aβ immunization could not be correlated with changes in cognitive and functional measures, in follow-up studies, a subgroup of patients who developed high levels of Aβ42-specific antibodies showed improvement in cognitive and functional tests compared to the control groups (Hock et al. 2003; Fox et al. 2005; Gilman et al. 2005). However, this correlation was lost when the sample size of evaluated patients was increased (Gilman et al., 2005). These data raise concerns that in contrast to data obtained with transgenic mice, clearance of brain deposits of Aβ in humans may not be a reliable proxy for cognitive improvement. Studies in a non-transgenic canine model, in which aged beagles have been actively immunized with fibrillar or oligomeric Aβ, find that although immunotherapy produces strong clearance of Aβ deposits in the brain of the animals (Pop et al. 2006), there is no significant improvement in their memory performance (Head et al. 2006). A relevant observation is that in transgenic mice, unlike humans or dogs, accumulation and deposition of Aβ in the brain does not cause neurodegeneration. Thus, removal of Aβ from the brains of the mice may be sufficient for reconstruction of synaptic networks. However, in the case of patients with AD, where massive neurodegeneration had been taking place for years, removal of Aβ deposits likely would be insufficient for resoration of cognitive abilities. It may be necessary to start immunizing humans prior to initial deposition of Aβ and/or to combine immunization with

other therapeutic means, such as antioxidants and behavior enrichment to improve the function of existing neurons and cortical circuits.

Although the exact cause of the meningoencephalitis that occurred in the AN-1792 trial is unknown, it has been suggested that patients may have developed an autoimmune T-cell response to the immunogen because the adjuvant used, QS21, was a proinflammatory Th1 response-inducing adjuvant (Cribbs et al. 2003). In order to reduce the risk of a T-cell-mediated immune response, new Aβ peptide immunogens and adjuvants currently are tested (Agadjanyan et al. 2005; Ghochikyan et al. 2006; Seabrook et al. 2007). In humans, T-cells were shown to recognize an epitope in the region Aβ(16-33), whereas the majority of antibodies generated in mice, monkeys, and humans recognize epitopes in the N-terminal region Aβ(1-16) (Lemere et al. 2006). Vaccination with N-terminal Aβ fragments may lead to a predominantly humoral response, thereby avoiding the T-cell-associated reactions believed to have caused meningoencephalitis in the AN-1792 trial. Current research efforts aim at development of Th2-inducing adjuvants that promote humoral responses and limit Th1-type cellular immunogenicity (Schenk et al. 2005).

Another approach for anti-Aβ immunotherapy without generating a cellular immune response is passive immunization. Administration of exogenous anti-Aβ antibodies has been shown to mimic many of the effects of active immunization (Bard et al. 2000; DeMattos et al., 2001; Wilcock et al. 2004b; Hartman et al. 2005). In addition to avoiding the T-cell response, patient serum antibody titers can be monitored and controlled more precisely with passive immunization. Another advantage of passive immunization is that it offers the ability to choose antibodies that recognize and bind specific forms of Aβ. Such antibodies can be modified to control binding specificity, binding affinity, and BBB permeability, and if adverse reactions to the treatment develop, the treatment can be discontinued and the antibody can be cleared rapidly (Brendza and Holtzman 2006). Currently, Elan Corporation in collaboration with Wyeth Pharmaceuticals (Madison, NJ) is performing a phase II passive immunization clinical trial with a humanized monoclonal antibody, AAB-001, and a phase I clinical trial with an active Aβ immuno-conjugate, ACC-001 (Dasilva et al. 2006; Kennedy et al., 2007). Though passive immunization offers several advantages over active immunization, if better control can be gained over the parameters of actively immunizing humans to Aβ, active immunization is a preferred long-term solution. Active immunization leads to a continuous production of antibodies, alleviating the need for continuous treatment and precluding the peaks and troughs associated with infusion of antibodies, and thus, may be less invasive and more cost-effective than passive immunization (Maier et al. 2005; Brendza and Holtzman 2006).

Enhancement of Aβ Degradation

Studies of proteases that cleave Aβ and participate in Aβ clearance have revealed that the proteases neprilysin (NEP), insulin-degrading enzyme (IDE), endothelin-converting enzymes 1 and 2 (ECE-1, ECE-2), and plasmin, each may play a role in degradation of Aβ in the brain. Consequently, selective activation of these proteases has become a target for anti-amyloid therapy (Tanzi et al. 2004; Eckman and Eckman 2005; Wang et al. 2006).

NEP, a membrane-bound, extracellular metalloendopeptidase, has been identified as a major extracellular Aβ degrading enzyme in the brain (Iwata et al. 2000). NEP levels are

reduced in normal human brains and transgenic mouse models of AD as a result of aging (Iwata et al. 2002; Apelt et al. 2003; Caccamo et al. 2005; Iwata et al. 2005). NEP levels are particularly low in vulnerable brain areas and correlate inversely with Aβ-related pathology in AD patients (Akiyama et al. 2001; Fukami et al. 2002). Intracerebral injection of a viral construct that leads to NEP expression in PDAPP mice reduced cortical amyloid deposits by ~50% (Marr et al. 2003; Marr et al. 2004). Overexpression of NEP also protects hippocampal neurons from Aβ toxicity *in vitro* (El-Amouri et al. 2007). These results suggest that overexpression of NEP by gene therapy approaches in vulnerable areas of AD brain may protect the neurons from the toxic effects of Aβ. Recent observations that the neurohormone somatostatin modulates brain Aβ levels through regulation of NEP activity suggests that increasing the brain concentration of somatostatin or using somatostatin receptor agonists is a potential therapeutic strategy for AD (Saito et al. 2005).

IDE is a large zinc-binding metalloprotease that cleaves multiple short polypeptides with little sequence specificity. Endogenous levels of both Aβ40 and Aβ42 are elevated in the brains of IDE-knockout mice (Farris et al. 2003; Miller et al. 2003a) and in primary cultured neurons derived from IDE-deficient mice (Farris et al., 2003). Overexpression of IDE or NEP reduces accumulation of Aβ in the brain of TgCRND8 transgenic mice, which expresses human APP bearing both the Swedish and Indiana FAD-linked mutations (Leissring et al. 2003; Marr et al., 2003; Iwata et al. 2004). IDE and NEP each have a number of substrates other than Aβ. Therefore, additional safety studies are needed to determine whether enhancing IDE/NEP activity may produce unwanted effects.

ECE is a membrane-bound zinc metallopeptidase homologous to NEP. Both Aβ40 and Aβ42 concentration levels are significantly higher in mice deficient of ECE-1 and the closely related ECE-2, when compared with age-matched wild-type littermate controls (Eckman et al. 2003), suggesting that ECE activity might be an important factor involved in A*β* clearance *in vivo*. Recently, Choi et al. have demonstrated that neuronal overexpression of the ε isozyme of PKC, PKCε increases ECE activity and reduces amyloid plaque pathology in transgenic mice expressing human APP bearing the Indiana mutation (Choi et al. 2006). Additional studies are needed to determine if ECE activation is a viable therapeutic strategy for AD.

Plasmin is a serine protease that plays an important role in the blood clotting system and degrades several proteins in the plasma, including fibrin clots (Henkin et al. 1991). A*β* can activate, and be degraded by, the plasmin system. Aggregated A*β* binds to and stimulates tissue-type plasminogen activator (tPA) (Kingston et al. 1995; Wnendt et al. 1997) and induces expression of tPA and urokinase-type plasminogen activator (uPA) both *in vitro* and *in vivo* (Tucker et al. 2000). Both tPA and uPA cleave plasminogen to generate active plasmin, which in turn degrades fibrin aggregates (Henkin et al., 1991). Several lines of evidence implicate the plasmin system in AD (Van Nostrand and Porter 1999; Tucker et al., 2000; Selkoe 2001b). Brain tissues from AD patients have low plasmin concentration levels and plasmin activity is reduced in serum of AD patients compared to healthy individuals (Ledesma et al. 2000; Dotti et al. 2004). Aβ injected into the hippocampus of plasmin-deficient mice is removed more slowly than in wild-type mice, providing additional evidence for the role of this enzyme in Aβ clearance (Melchor et al. 2003). However, unlike mice deficient in NEP, IDE, or ECE-1 and ECE-2, mice deficient in plasmin (plasminogen knockout mice) do not have elevated levels of endogenous Aβ (Tucker et al. 2004)

suggesting that plasmin does not contribute to the regulation of steady-state Aβ levels under non-pathogenic conditions, but may play a more important role in Aβ clearance after aggregation is initiated (Eckman and Eckman 2005).

The activity of the Aβ-degrading enzymes that help regulating accumulation of Aβ in the brain decreases with aging. Upregulation of these enzymes can reduce Aβ accumulation significantly. Regulation of each of the Aβ-degrading enzymes is complex and their brain concentrations depend on multiple factors. Therefore, more pre-clinical studies are required to assess safety and effectiveness issues before these approaches can be used in human studies.

Promoting Receptor-Mediated Aβ Efflux from the Brain

Increased Aβ concentration in brain interstitial fluid (ISF) is one source for formation of neurovascular and cerebral neurotoxic Aβ assemblies (Ghiso and Frangione 2002). Accumulation of Aβ in the ISF might be a direct consequence of deficient efflux (Zlokovic et al. 2000) or increased influx of circulating Aβ across the BBB (Zlokovic 2004). Aβ influx and efflux across the BBB are mediated by the receptor for advanced glycation-end products (RAGE) and low-density lipoprotein receptor-related protein (LRP), respectively (Deane et al. 2003; Deane et al. 2004a; Deane et al. 2004b; Donahue et al. 2006; Deane and Zlokovic 2007). Significant upregulation of RAGE at the BBB is observed in AD patients and in transgenic mouse models of AD, including PDAPP and Tg2576 mice. Activation of RAGE by its interaction with Aβ may take place at an early stage of AD (Deane et al., 2003) and prevention of this interaction, therefore, is a potential strategy for early intervention (Arancio et al. 2004; Lue et al. 2005; Geroldi et al. 2006; Deane and Zlokovic 2007). Peripheral administration of a soluble form of RAGE reduces Aβ concentration in the brain of Tg2576 and PDAPP mice either by preventing influx of Aβ by RAGE-Aβ interaction or via the egress of Aβ from the CNS through a peripheral sink mechanism (Deane et al., 2003). TTP488 (TransTech Pharma, Inc., High point, NC), an orally bioavailable RAGE inhibitor currently is in phase II clinical trials (TransTechPharma 2005; ClinicalTrials.gov 2006).

LRP is linked to AD genetically (Kang et al. 2000) and may influence APP processing and metabolism (Herz and Strickland 2001). LRP also affects neuronal Aβ uptake through complexing of Aβ with the LRP ligands such as α2-macroglobulin (Du et al. 1997), apolipoprotein J (Matsubara et al. 1995), apolipoprotein E (Yang et al. 1997), transthyretin (Schwarzman et al. 1994), and albumin (Biere et al. 1996). LRP plays an opposing role to that of RAGE by transporting brain-derived Aβ out of the brain and into the blood (Shibata et al. 2000; Herz and Marschang 2003; Wang et al. 2003). Thus, enhancing LRP-mediated Aβ efflux may be a viable pharmacological approach for decreasing Aβ in the brain and facilitating Aβ clearance (Deane and Zlokovic 2007). The functional role of LRP in Aβ clearance is under active investigation but is not ready for clinical trials.

Inhibition of Aβ Assembly

Aβ toxicity is closely related to its self-assembly (Lansbury 1997; Teplow 1998; De Felice and Ferreira 2002). Because the physiological role of Aβ is unknown, inhibiting Aβ

production or enhancing its clearance may lead to adverse side effects. In contrast, Aβ self-assembly is purely a pathogenic process. Therefore, preventing Aβ assembly may be an advantageous therapeutic approach for AD.

Small-Molecule Inhibitors

Interaction of Aβ with glycosaminoglycans (GAG) occurs at a particular binding site on Aβ, Aβ(13-16), and promotes Aβ assembly (McLaurin et al. 1999). Thus, this interaction may be viewed as a ligand–receptor system, in which GAG act as agonists, which upon binding to their specific binding site on the receptor, Aβ, promote a biological function—self assembly. If this is true, then putative antagonists may bind to the same binding site, inhibiting the interaction of GAG with Aβ and thereby preventing Aβ assembly. This rationale has led to the discovery of Tramiprosate (3-amino-1-propanesulfonic acid; Alzhemed^{TM}, Neurochem Inc., Quebec, Canada), a GAG-mimetic, which binds preferentially to soluble Aβ at the GAG-binding site and maintains Aβ in a random coil/α-helix-rich conformation. The compound reduces both soluble and fibrillar Aβ concentrations in the TgCRND8 transgenic mouse model (Gervais et al. 2006). A phase II clinical study with Alzhemed^{TM} showed a ~33% decrease of CSF Aβ42 concentration after 3 months of treatment in patients with mild AD and a ~14% decrease of Aβ42 in patients with moderate AD (Aisen et al. 2006). Alzhemed^{TM} currently is in phase III clinical studies (Neurochem 2007). Aisen et al. have hypothesized that the greater reduction of Aβ concentration in patients with mild AD suggests that the drug may be more effective during the early stages of AD, before extensive Aβ assembly into fibrils and plaques occurs (Aisen et al., 2006). Disappointingly, however, no significant differences were found in cognitive and clinical assessments between the Alzhemed^{TM} and placebo groups after three months of treatment. These results, together with findings form the AN-1792 immunotherapy trial discussed above, increase the concerns that removal of Aβ deposits from the brain may not yield the expected improvement in cognitive abilities for patients with AD.

Metal Chelators

A body of evidence suggests that Aβ deposition in AD may be influenced by endogenous transition metal ions (Bush 2003). Copper and zinc ions propagate the formation of toxic Aβ aggregates through interaction with specific amino-acid residues, such as histidine and tyrosine, in Aβ (Atwood et al. 2004). Although Aβ–metal interactions may participate in normal metal-ion homeostasis, age-dependent increase in Cu^{2+}, Zn^{2+} and Fe^{3+} concentrations, leading to accelerated Aβ aggregation may be an etiologic neurochemical event in the generation and progression of AD (Bush et al. 1994; Atwood et al. 1998). In fact, zinc and copper ions are directly coordinated with Aβ in AD-affected brain (Opazo et al. 2002; Dong et al. 2003).

A therapeutic approach has been devised based on the observations that Cu/Zn chelators can dissolve Aβ deposits in AD-affected postmortem brain tissue (Cherny et al. 1999). Bush et al. have identified chelators that are capable of interfering selectively with pathologically relevant metal–protein interaction without depleting the metal necessary for normal physiologic function (Cherny et al. 2001; Bush 2003; Ritchie et al. 2003; Opazo et al. 2006). Among these tested chelators, clioquinol (PBT-1, Prana Biotechnology Ltd., Parkville,

Australia) showed promising effects in phase I and II clinical studies (Ritchie et al., 2003). However, due to a toxic impurity in the drug, phase III studies were not carried out (PranaBiotechnology 2005). A new drug, PBT-2 (Prana Biotechnology Ltd.), which inhibits Aβ aggregation by a similar mechanism is under phase II clinical studies (AlzForum 2007).

Polyphenols

Epidemiological studies have shown that moderate red wine intake reduces the risk of developing AD (Lindsay et al. 2002; Truelsen et al. 2002; Luchsinger et al. 2004). Resveratrol, a polyphenol abundant in grapes and red wine, attenuates Aβ-induced cytotoxicity (Jang and Surh 2003; Savaskan et al. 2003). Anti-amyloidogenic effects of several polyphenols, such as tannic acid (TA), myreticin, curcumin, and rosemarinic acid, on Aβ also have been investigated (Hamaguchi et al. 2006). TA dose-dependently inhibits the formation of Aβ fibrils from freshly prepared Aβ solutions and destabilizes preformed Aβ fibrils *in vitro* (Ono et al. 2004). Curcumin, a polyphenolic diketone from turmeric, inhibits the formation of Aβ oligomers and fibrils and reduces amyloid plaques *in vivo* (Yang et al. 2005). Tg2576 mice treated with curcumin show reduced oxidative damage and amyloid pathology when compared to untreated mice (Lim et al. 2001). Binding of curcumin with copper and iron ions is suggested as one of the possible mechanisms of its protective action in AD (Baum and Ng 2004). The polyphenol epigallocatechin-3-gallate (EGCG), an ingredient extracted from green tea, is a potent anti-inflammatory agent and antioxidant (Ahmed et al. 2002; Lee et al. 2003). EGCG protects cultured hippocampal neurons exposed to Aβ-induced oxidative stress (Choi et al. 2001). These beneficial effects suggest that polyphenols are a promising class of compounds for general neuroprotection and anti-amyloid activity.

Inositol Derivatives

Derivatives of phosphatidylinositol and inositol (cyclohexanehexol) sterioisomers, have been studied as potential inhibitors of Aβ assembly (McLaurin et al. 1998; McLaurin et al. 2000) based on the observation that phosphatidylinositol lipids facilitate Aβ oligomerization and fibrillation (McLaurin and Chakrabartty 1996; McLaurin and Chakrabartty 1997). Inositol stereoisomers inhibit Aβ fibrillogenesis, accelerate disassembly of preformed fibrils, stabilize Aβ in nontoxic, β-structured spherical micellar structures, and protect primary cultured neurons against Aβ oligomer-induced toxicity (McLaurin et al., 1998; McLaurin et al., 2000). This activity is dependent on the stereochemistry of the inositols. *Scyllo*-inositol and *epi*-inositol are more effective than the *myo*-inositol when given orally to TgCRND8 mice in ameliorating several AD-like phenotypes, including impaired cognition, altered synaptic physiology, cerebral Aβ pathology, and accelerated mortality (McLaurin et al. 2006). Alimentary administration of *scyllo*-inositol (AZD 103, Transition Therapeutics Inc., Toronto, Canada) to rats injected with soluble Aβ oligomers prevents reference memory errors and inhibition of long-term potentiation induced by soluble Aβ oligomers (Townsend et al. 2006). AZD 103 currently is in a phase I clinical trial (TransitionTherapeuticsInc. 2006).

β-Sheet Breaker Peptides

β-Sheet breaker peptides are short, synthetic peptides capable of binding Aβ and destabilizing amyloidogenic Aβ conformers, thereby precluding the formation of β-sheet rich amyloid (Soto et al. 1996; Tjernberg et al. 1996; Nakagami et al. 2002). β-Sheet breaker peptides typically are derived from amino acid sequences in the central hydrophobic core of Aβ, but have a low propensity to adopt β-sheet conformation themselves and therefore inhibit the transition of full-length Aβ to a β-sheet rich conformation. The five-residue peptide LPFFD (iAβ5) blocks the formation of amyloid fibrils in a rat model of amyloidosis in which freshly prepared Aβ42 is injected directly into the amygdala (Soto et al. 1998). A derivative of iAβ5, iAβ5p (acetyl-LPFFD-amide), modified for protection against proteolytic degradation by exopeptidases and to increase transport through the blood–brain barrier (Poduslo et al. 1999), was found to reduce amyloid load and cerebral damage in double-transgenic mice overexpressing human APP containing the London mutation and human presenilin-1 bearing the FAD-causing mutation A246E (Permanne et al. 2002). Another study on the neuroprotective effect of chronic intraperitoneal administration of iAβ5p in rats, in which behavioral deficits were induced by intrahippocampal injection of Aβ-fibrils, reports that after the injection, the animals showed partial reduction of the amyloid deposits formed and decreased astrocytic response around the injection site compared to animals treated with saline. In addition, the iAβ5p-treated animals showed a significant improvement in spatial learning (Chacon et al. 2004). These results suggest that β-sheet breaker peptides may be useful for treatment of AD. Gordon et al. have developed inhibitors for Aβ40 self-assembly from peptides that are homologous to the central core domain of Aβ (Aβ16-20), but contain *N*-methyl amino acids at alternate positions (Gordon et al. 2001; Gordon et al. 2002). When these inhibitor peptides are arrayed in an extended β-strand conformation, the alternating position of *N*-methyl amino groups gives the peptides two distinct faces, one exhibiting a normal pattern of peptide backbone hydrogen bonds allowing the binding to Aβ and the other face having limited hydrogen-bonding capabilities due to the replacement of the amide protons by *N*-methyl groups, thereby preventing further assembly. Kokkoni et al. have optimized the structure of *N*-methylated peptide inhibitors of Aβ aggregation derived from the region Aβ(16–20) by varying the peptide length, *N*-methylation sites, acetylation and amidation of the N- and C-termini, side-chain identity, and chirality, via five peptide/peptidomimetic libraries (Kokkoni et al. 2006). The peptide D-[(chGly)-(Tyr)-(chGly)-(chGly)-(mLeu)]-NH_2 (where chGly = cyclohexyl glycine and mLeu = *N*-methyl leucine) was the most active inhibitor of Aβ aggregation (Kokkoni et al., 2006).

A conformationally constrained islet amyloid polypeptide (IAPP) mimic, IAPP-GI, recently has been reported as one of the most potent inhibitors of Aβ40 self-assembly (Yan et al. 2007). IAPP-GI is generated by *N*-methylation of two amide bonds on the same side of a β-strand in the amyloid core sequence IAPP(22–27) (NFGAIL sequence) of full-length IAPP (Yan et al. 2006). This sequence is analogous to the sequence NKGAII in positions 28–32 of Aβ. IAPP is a 37-residue polypeptide that acts as a neuroendocrine regulator of glucose homeostasis. Self-assembly and aggregation of IAPP into fibrillar amyloid causes type II diabetes (T2D). IAPP-GI is the only known peptide-derived compound that binds with high affinity to both IAPP (Yan et al., 2006) and Aβ40 (Yan et al., 2007) and blocks and reverses cytotoxic self-assembly of both polypeptides. Because clinical studies suggest that persons

suffering from T2D might be at risk of AD, and vice versa (Janson et al. 2004; Nicolls 2004), IAPP-GI could be a highly potent lead compound for designing novel therapeutic compounds targeting both AD and T2D.

Penke and co-workers designed β-sheet breaker peptides derived from sequences in the C-terminal region of Aβ (Hetenyi et al. 2002). The peptide RIIGLa inhibited aggregation and neurotoxicity of Aβ42 in cultured neuroblastoma cells (Fülöp et al. 2004). Based on β-sheet packing in fibril structures suggesting that Met^{35} packs against Gly^{33} in the C-terminus of Aβ40 and against Gly^{37} in the C-terminus of Aβ42, Sato et al. designed peptides for interference with β-sheet packing with the general sequence Gly-x-Phe-x-Gly-x-Phe, where x represents any amino acid. The peptides were found to inhibit Aβ fibril maturation and Aβ42-induced toxicity in rat cortical neurons (Sato et al. 2006). Recently, we found that peptides derived from the C-terminus of Aβ42 inhibited the neurotoxicity of Aβ42 oligomers and rescued Aβ42-induced inhibition of mini excitatory postsynaptic currents in primary hippocampal neurons (Fradinger et al., manuscript in preparation). These data suggest that peptide inhibitors derived from the sequence of Aβ or of related amyloidogenic peptides provide useful lead compounds for development of Aβ assembly inhibitors as potential drugs for AD.

Alternative Disease Modification Strategies

RNA Interference for Treatment of FAD

RNA interference (RNAi) is the process of mRNA degradation induced by hydbridization with a short sequence of complementary RNA, thereby forming double-stranded RNA (dsRNA) (Agrawal et al. 2003). The RNAi pathway is thought to be an ancient mechanism for protecting cells against viruses and rogue genetic elements that use dsRNA in their life cycles. By activating a sequence-specific RNA degradation process, RNAi post-transcriptionally inhibits protein expression. Early-onset FAD is caused by mutations in the genes that encode APP or presenilins (Goate et al. 1991; Mullan et al. 1992; Scheuner et al., 1996; Selkoe 2001a; Higgins and Jacobsen 2003; Rocchi et al. 2003). The majority of these mutations cause increased concentrations of Aβ or increased Aβ42/Aβ40 ratio (Suzuki et al., 1994; Scheuner et al., 1996; De Jonghe et al., 2001). The hereditary pattern is autosomal dominant, thus patients with FAD-causing mutations also have one normal allele of the mutant protein. Selective silencing of the disease-causing mutant allele by RNAi, while leaving the normal allele fully expressed, is a novel approach that has been explored in recent years as a therapeutic strategy for dominantly inherited diseases (Ding et al. 2003; Miller et al. 2003b; Miller et al. 2004). This approach recently was applied successfully to silencing APP-encoding alleles bearing the London and Swedish mutations *in vitro* (Feng et al. 2006) and the Swedish mutation *in vivo* (Rodriguez-Lebron et al. 2006), suggesting that RNAi may be a useful strategy for treatment of FAD.

Nerve growth Factor Therapy

Nerve growth factor (NGF), a member of the neurotrophin gene family maintains the survival of cholinergic neurons of the basal forebrain nociceptive dorsal root ganglion

neurons, and certain third-order sympathetic neurons (Huang and Reichardt 2001). The loss of basal forebrain cholinergic neurons, which release the majority of acetylcholine in the cerebral cortex and hippocampus (Mesulam and Geula 1988) enhancing synaptic efficacy and modulating active cortical circuits (Kilgard and Merzenich 1998; Conner et al. 2003), is at least partially responsible for the cognitive decline observed in AD patients (Perry et al. 1978; Bartus et al., 1982). NGF promotes survival of those neurons by activating its high-affinity tyrosine-kinase receptor TrkA. Downstream of TrkA, the small G-protein p21ras plays a pivotal role in controlling neuronal survival and differentiation (Hock et al. 1998; Hock et al. 2000a; Hock et al. 2000b). Therefore, treating AD patients with NGF to improve cholinergic function may ameliorate disease symptoms. NGF has been shown to improve memory functions in animal models of AD (Hagg et al. 1989; Fischer et al. 1991; Koliatsos et al. 1991; Frick et al. 1997). However, safe delivery of NGF to the brain is challenging because NGF does not cross the BBB due to its size and polarity. Gene delivery has made it possible to deliver sufficient amount of NGF specifically to sites of degenerating neurons, thereby circumventing the need to cross the BBB and avoiding adverse effects such as weight loss and pain observed during ventricular administration to the brain (Tuszynski 2002). A phase I trial of *ex vivo* NGF gene delivery using CERE-110 (Ceregene Inc., San Diego, CA), a preparation that carries the gene encoding NGF encased in a harmless viral coating that protects the gene and facilitates its delivery to brain cells, improved the rate of cognitive decline in 6 out of 8 patients with mild AD (Tuszynski et al. 2005).

Cerebrolysin is a low molecular weight peptide preparation produced by proteolytic breakdown of purified porcine brain proteins, which mimics the effects of NGF (Francis-Turner and Valouskova 1996). Intravenous infusion of Cerebrolysin, (Cere, Ebewe pharmaceuticals, Unterach, Austria) has shown promising results in several clinical trials (Ruther et al. 2000; Ruether et al. 2001; Alvarez et al. 2006). In a recent trial with Cere, a 10 ml dose per day for 24 weeks was found optimal for treatment of patients with mild to moderate AD and a 60 ml dose per day was suggested to be useful for patients with more severe stages of AD (Alvarez et al., 2006).

Antioxidants

One of the mechanisms by which Aβ causes neurotoxicity is generation of free radicals and reactive oxygen species (ROS) (Behl et al. 1994). By a vicious cycle, oxidative stress enhances the activity of β-secretase resulting in increased production and accumulation of Aβ (Tamagno et al. 2002), which increases mitochondrial dysfunction and oxidative stress (Casley et al. 2002). In fact, aging-related oxidative stress has been hypothesized to be the initial cause for increased Aβ production that leads to sporadic AD (Barger 2004; Lee et al. 2004; Lee et al. 2006). Hence, attenuation of oxidative stress by antioxidants has been explored as a potentially useful means of therapeutic intervention (Hajieva and Behl 2006). Several epidemiologic studies provide evidence supporting the concept that vitamin E and vitamin C may delay the onset of AD (Zandi et al. 2004). A retrospective review comparing patients treated with a donepezil together with vitamin E indicated that the combination treatment lowered the rate of cognitive decline significantly (Klatte et al. 2003). However, a clinical study that compared the effects of vitamin E to that of donepezil and placebo has concluded that vitamin E showed no beneficial effects in patients with mild cognitive

impairment (MCI) (Petersen et al. 2005). Thus, the value of treating patients with AD with vitamin E remains unclear.

DIAGNOSIS OF AD

Current Clinical Diagnosis

Cognitive Assessment

In clinical practice, AD usually is diagnosed by application of cognitive assessment tools that detect symptoms, such as memory loss, disorientation, and problems with routine tasks. Two common diagnostic tools for AD are the Clinical Dementia Rating (CDR) (Morris 1993) and Mini Mental State Examination (MMSE) (Folstein et al. 1975). CDR scores for staging cognitive impairment range from 0 – no impairment, through 0.5 – very mild, 1 – mild, 2 – moderate, and 3 – severe dementia (Morris 1993). In the MMSE, a score of 20–24 out of a maximum of 30 points suggests a 'mild dementia', whereas lower scores indicate more advanced stages of cognitive decline (Folstein et al., 1975). The MMSE detects AD with a sensitivity of ~ 80% (Kalbe et al. 2004). The CDR and the MMSE tests are highly important in AD research because they are used to define patient groups and to monitor the disease progress in studies of diagnostic tools and medical treatments.

Recently, the importance of early diagnosis of AD for success of therapeutic intervention has been given increased recognition (Cummings 2004; Nestor et al. 2004; Monien et al., 2006). The symptoms observed in early AD often are described as MCI, an imprecise, yet clinically useful, term for distinguishing between normal cognitive aging and the beginning of dementia (Petersen 2004). The diagnostic criteria for MCI include the following key features: 1) the person is neither cognitively normal nor demented; 2) evidence of cognitive decline over time is observed or reported by the patient and/or informant; 3) general intellectual functions and activities of daily living are preserved; and 4) according to the CDR, individuals are diagnosed with 'questionable dementia' (score = 0.5) (Petersen et al. 2001). However, this definition of MCI was reported to be too vague to allow accurate distinction of MCI from normal, age-related cognitive decline on one hand, and from mild AD on the other hand (Winblad et al. 2004). Petersen notes that accurate division of subjects into healthy individuals, patients with MCI, and patients with AD based on cognitive assessment alone is impossible because the ranges of cognitive abilities observed for those three groups overlap (Petersen 2004). Two additional problems complicate the diagnosis of MCI based on cognitive assessment alone. First, the symptoms classified as MCI may precede not only AD, but also various other types of dementia, such as dementia associated with Parkinson's disease, Pick's disease, vascular dementia, and Lewy body dementia. In addition, certain medications, such as anticholinergic (Ancelin et al. 2006) and sedative (Sjogren et al. 2005) drugs, and conditions such as depression, cerebral injuries, and alcohol abuse may cause MCI symptoms. Under these transient circumstances, MCI may be non-progressive and/or reversible. Second, the classification of the patient is subjective, depending on the judgment of the clinician and is hindered by the heterogeneity of character

and etiology among patients. The daily disposition of patients plays an important role in the result of psychometric tests, as do their levels of education, intelligence, and motivation.

Despite these difficulties with early detection of AD, cognitive tests are essential frontline tools for the assessment of cognitive (dys)function in individuals once they become present in clinical settings. Two main research approaches currently attempt to overcome the problems associated with the diagnosis of MCI by cognitive assessment. One approach to better characterize the heterogeneous nature of the symptomatic patterns of MCI and define tighter criteria for AD-specific MCI has led to the definition of four subtypes of MCI. Of these subtypes, amnestic MCI (MCIa), a variant characterized by memory loss as the predominant symptom, has been proposed as an important risk factor for AD (Petersen 2005). A different approach follows the design of novel cognitive assessment tools for detection of subtle differences between MCI and normal, age-related cognitive decline. An ideal cognitive test should be quick to administer, easy to score, well tolerated and accepted by patients, and should be relatively independent of culture, language, and education (Shulman 2000). To be effective not only for MCI and for AD but also for other forms of dementia, tests also should cover a broad range of cognitive domains. Longitudinal studies of individuals with MCIa have identified cognitive domains affected by the development of dementia, particularly episodic memory (story recall, word list learning), and semantic memory (recall of names). Attention processing, visuospatial skills, and mental speed also are affected (Nestor et al., 2004). Based on these findings, a variety of screening tools with moderate to high sensitivity and specificity capable of discerning patients with MCI from cognitively intact individuals have been developed (Flicker et al. 1991; Tierney et al. 1996; Devanand et al. 1997; Albert et al. 2001; Amieva et al. 2004). Two recent examples are the Montreal Cognitive Assessment test (MoCA) (Nasreddine et al. 2005) and the DemTect (Kalbe et al., 2004). Both psychometric tools showed high sensitivity in distinguishing MCI (90% and 80%, respectively) and AD (both 100%) from age-matched controls, in contrast to the lower sensitivity of the widely used MMSE for the detection of MCI (69% and 18% determined by Kalbe et al. (Kalbe et al., 2004) and Nasreddine et al. (Nasreddine et al., 2005), respectively). The specificity for the identification of healthy individuals in the control groups was determined to be 87% (MoCA), 92% (DemTect) and 100% (MMSE).

To enhance the predictive power of currently available cognitive assessment tools, tests may be optimized in the following ways: First, the results of psychological assessments may be improved by test repetition, following the cognitive impairment of an individual in intervals of three to six months. It has been shown that monitoring of incipient dementia progression to determine a specific rate of cognitive decline has a greater accuracy in diagnosis of MCI than a single cognitive assessment (Kurz et al. 2004). Second, knowledge of the time course in which brain regions are affected by AD and their participation in specific cognitive tasks, such as semantic memory and attention processing, may lead to a schedule that describes the loss of cognitive abilities during the progression of AD. For the establishment of such a time course, future development of tests that monitor the condition of specific cognitive domains or functions is needed. With the characteristic change of several cognitive parameters, the diagnosis of AD-specific MCI using three cognitive assessments over a period of six months is predicted to be improved substantially.

Neuroimaging

Quantitative high-resolution brain imaging techniques enable minimally invasive examination of alterations in brain anatomy and function, facilitating identification of affected regions in early stages of AD. Structural evaluation of brain damage allows differentiating among certain etiologies of cognitive decline, thereby separating patients with emerging AD from other subjects with MCI, e.g., those with brain tumors, subdural haematoma, or normal pressure hydrocephalus. In addition, neuroimaging provides diagnostic information complementary to psychometric tests, thereby improving the prediction of AD progression (Winblad et al., 2004).

Currently, standard clinical diagnostic procedures for AD include brain examination with magnetic resonance imaging (MRI), or less frequently, single-photon emission-computed tomography (SPECT). Structural imaging with MRI to find markers for AD progression has been focused on the medial temporal lobe. The medial temporal lobe consists of the hippocampal region (CA fields, dentate gyrus, and subicular complex) and the adjacent perirhinal, entorhinal, and parahippocampal cortices. These structures are essential for declarative memory (episodic and semantic memory) and are the areas primarily affected during memory impairment (Squire et al. 2004). Interestingly, NFT density in the medial temporal lobe correlates with performance in memory tests supporting the notion that NFT are a pathological substrate that causes memory loss in AD (Guillozet et al. 2003). MRI studies have confirmed that atrophy of the medial temporal lobe and volumetric decrease of the hippocampus and entorhinal cortex are sensitive markers for AD (Du et al. 2001; Scheltens et al. 2002; Du et al. 2003; Jack et al. 2004; Jack et al. 2005). However, AD progression varies widely among patients and a standardized scale, correlating specific levels of brain atrophy with stages of memory impairment applicable to all patients may not be attainable. It has been shown that the rate of atrophy in both the hippocampus and entorhinal cortex provide greater accuracy than a single-point assessment for the detection of MCI. Comparative longitudinal surveys suggest that using the rate of medial temporal lobe atrophy (MTA) may help distinguishing between healthy individuals and patients with MCI or mild AD, devising standards for prediction of the future course of dementia (Chong et al. 2006). Conversion of healthy persons to patients with MCI correlates well with reduction of hippocampal volume (Du et al., 2001), entorhinal cortex volume (Killiany et al. 2000; Killiany et al. 2002) or both (Dickerson et al. 2001; Jack et al., 2004), and MCI patients who convert to AD have greater rates of MTA than those who are cognitively stable (Jack et al. 1999; Dickerson et al., 2001; Du et al., 2001; Visser et al. 2002; deToledo-Morrell et al. 2004; Jack et al., 2004; Devanand et al. 2007). These data support the use of rates of volume loss observed in hippocampus and entorhinal cortex as surrogate markers for disease progression alongside with psychometric screening tools.

Despite encouraging results, the time-consuming analysis of MRI recordings, which includes determination of regions of interest and subsequent calculation of volumetric measures is an obstacle in clinical routine (Nestor et al., 2004). An interesting alternative to fully computerized processing of three-dimensional brain images is the ‘visual rating scale assessment’ of MTA (Scheltens et al. 1992). This method is quick, easily performed by untrained personnel, and is a more accurate evaluation system compared to semi-automatic region-of-interest analysis of hippocampus and entorhinal cortex (Wahlund et al. 2000;

Bresciani et al. 2005). MTA rates measured by visual assessment differentiate effectively patients with MCI from normal individuals and patients with AD, and therefore, yield good predictive accuracy for the conversion of MCI to AD (80–90%) (Visser et al., 2002; Rusinek et al. 2003; Schott et al. 2003; Korf et al. 2004). Voxel-based morphometry (VBM, voxel = volumetric pixel) is an automatic method for computational neuroanatomical evaluation of MRI data (Chetelat et al. 2002). VBM involves a voxel-wise comparison of the local concentration of gray matter between two groups of subjects (Ashburner and Friston 2000). The first VBM study of MCI patients showed marked gray matter loss predominantly affecting the hippocampal region and cingulate gyri, and extending into the temporal neocortex (Chetelat et al., 2002). Discussion of other methods of MRI evaluation is beyond the scope of the chapter. Further details can be found in a comprehensive review by Nestor et al. (Nestor et al., 2004).

As an alternative to MRI, SPECT may be used to support the diagnosis of AD based on neuropsychological tests. Detection of single γ-photons emitted from molecules containing the radioactive isotope ^{99}Tc, e.g., ^{99}Tc-hexamethylpropyleneamine oxime (^{99}Tc-HMPAO) and ^{99}Tc-ethylcysteinate dimer (^{99}Tc-ECD) (van Dyck et al. 1996) enables measurements of cerebral blood flow. SPECT is used to confirm the clinical diagnosis of AD by measuring bilateral parieto-temporal perfusion deficits (Vlasenko et al. 1997), enhancing the sensitivity of the diagnosis by cognitive assessment from ~ 75% to ~ 90% (Read et al. 1995). The progression of AD has been tracked upon serial SPECT examinations by showing a progressive reduction of cerebral blood flow in the left hippocampus, parahippocampus, and cerebral association cortex (Kogure et al. 2000). The value of SPECT in the diagnosis of questionable or early dementia cases recently has been studied. Decreased cerebral blood flow in individuals with MCI who subsequently converted to AD was most prominent in cingulate gyri (Johnson et al. 1998; Kogure et al., 2000; Huang et al. 2002; Borroni et al. 2006), the temporo-parietal lobes (Encinas et al. 2003; Hirao et al. 2005; Borroni et al., 2006), and the precunei (Kogure et al., 2000; Hirao et al., 2005; Borroni et al., 2006). Compared with data from stable MCI patients, these markers could predict the conversion of MCI to AD with a sensitivity of 70–80% at least 2 years before the patients were clinically diagnosed with AD (Kogure et al., 2000).

RECENT PROGRESS IN DIAGNOSIS OF EARLY AD

In recent years, development and fine-tuning of diagnostic techniques, which are essential for detection of AD at an early stage, and standardized monitoring of disease progress during clinical trials have advanced at a rapid pace. To diagnose early AD reliably, the techniques used should be reproducible, highly sensitive, and specific for AD relative to other forms of dementia. Ideally, they also would be inexpensive, easy to perform, and non-invasive. Recent promising advances in the fields of neuroimaging and biomarker assays that have not yet become part of routine clinical practice are presented here.

Brain Glucose Metabolism as an Imaging Marker for AD

Whereas structural imaging methods detect structural changes of the brain during the progress of AD, functional imaging techniques are directed towards understanding the molecular level of pathophysiological processes underlying the disease. A common method for imaging of resting cerebral activity is detection of changes in regional glucose metabolism by positron-emission tomography (PET) using ^{18}F-labeled fluoro-2-deoxy-D-glucose (FDG) as a tracer. The radioactive decay of the isotope results in emission of a positron. Subsequent annihilation on contact with an electron yields two γ-photons emitted with equal energy in opposite directions enabling highly sensitive detection (Hoffman and Phelps 1986). FDG PET studies reveal characteristic and progressive reduction in regional measurements of cerebral metabolic rate for glucose (CMRgl) in patients with AD (Minoshima et al. 1995; Moriearty et al. 1999; Silverman et al. 2001) and MCI (Berent et al. 1999; Arnaiz et al. 2001; Chetelat et al. 2003; Drzezga et al. 2003). In patients with AD, CMRgl reduction in the posterior cingulate, parietal, temporal, and prefrontal cortices are correlated with dementia severity (Minoshima et al., 1995) and progression (Alexander et al. 2002). In a retrospective study of patients with mild to moderate dementia, the pattern of hypometabolism offered a sensitivity and specificity of 94% and 73%, respectively, for the prediction of subsequent clinical decline and the histopathological diagnosis of AD (Silverman et al., 2001). In patients diagnosed with MCIa (Arnaiz et al., 2001; Drzezga et al., 2003), isolated memory impairment (Berent et al., 1999), or non-amnestic MCI (Chetelat et al., 2003), regional CMRgl reduction helped distinguish subsequent AD converters from non-converters. Some overlap between the groups was found (Berent et al., 1999; Arnaiz et al., 2001; Chetelat et al., 2003; Drzezga et al., 2003). In a longitudinal study of MCIa patients, the one-year rate of decline for CMRgl was greater in subsequent AD converters than in non-converters (Drzezga et al., 2003). These studies indicate that discrimination of various stages during progression of AD by neuroimaging and cautious prediction of progression to AD within the heterogeneous group of patients with MCI subjects may be feasible.

Molecular Probes as Imaging Markers for AD

Another promising area of brain imaging research focuses on developing tracer compounds that bind to abnormal brain deposits implicated in AD. Initial data indicate that one such tracer, Pittsburgh compound B (PIB), binds to amyloid plaques and reveals their presence and number in a PET scan (Klunk et al. 2004; Mathis et al. 2005). PIB is a thioflavin derivative that is selective for Aβ-containing plaques at the concentrations used for imaging studies. A number of clinical PIB PET studies have begun recently in order to accrue longitudinal data for the diagnostic value of PIB. Another imaging probe allowing visualizing brain pathology in living AD patients with PET is 2-(1-{6-[2-[^{18}F]fluoroethylmethylamino]-2-naphthyl}ethylidene)malononitrile (^{18}F-FDDNP). ^{18}F-FDDNP PET was used to discern AD patients and cognitively normal persons with high accuracy by labelling senile plaques and NFT in brain tissue (Shoghi-Jadid et al. 2002). More recently, ^{18}F-FDDNP PET was shown to track disease progression from MCIa to AD efficiently (Small et al. 2006). In the latter study,

which included 25 patients with AD, 28 patients with MCI, and 30 normal individuals, the PET imaging showed a clear correlation between cognitive deterioration and FDDNP concentration in the temporal, parietal and frontal brain regions, where the abnormal protein deposits typically accumulate early in patients with MCI and advancing in those with AD. ^{18}F-FDDNP PET yielded excellent diagnostic accuracy with sensitivity values of 95 and 98% for the distinction between MCI patients and normal individuals, and between MCI patients and AD patients, respectively, and predicted disease progression and brain pathology precisely (Small et al., 2006).

Metabolic Markers for AD

Metabolic biomarkers for AD may be assessed in different body fluids, including CSF, blood, and urine (Thal et al. 2006). Thus far, the most encouraging results were obtained in studies of CSF biomarkers. The CSF is in direct contact with the brain and thus reflects biochemical changes due to pathological processes. Analyses of various biomolecules in the CSF by lumbar puncture was routine in neurological practice to diagnose infectious, inflammatory, and degenerative conditions of the CNS, such as meningitis, Guillain-Barré syndrome, and multiple sclerosis (Andreasen and Blennow 2005). With the advent of less invasive diagnostic modalities, currently this procedure is performed mostly in research settings.

Leading candidate biomarkers for early AD are the proteins that reflect key features of AD pathology, namely Aβ, tau, and hyperphosphorylated tau (p-tau). The clinical diagnosis of AD was shown to correlate with increased CSF levels of tau and p-tau, and with decreased levels of Aβ42 (Andreasen and Blennow 2005). The mean sensitivities of the assays are 85% for Aβ42, and 80% for tau and p-tau for the differentiation between patients with AD and non-demented individuals, whereas the overall specificity is ~ 90% (Blennow 2004b). The ratio of Aβ42/Aβ40 has a higher diagnostic accuracy for differentiating patients with AD from normal individuals than Aβ42 alone, with a sensitivity of 94% (Lewczuk et al. 2004).

To distinguish patients with MCI from normal individuals, low Aβ42, and high tau and p-tau CSF concentration levels are equally sensitive compared to the differentiation between healthy persons and AD patients at later stages of the disease (Andreasen et al. 1999; Arai et al. 2000; Andreasen et al. 2001; Gottfries et al. 2001; Lautenschlager et al. 2001; Maruyama et al. 2001; Riemenschneider et al. 2002; Andreasen et al. 2003). The prognostic value of CSF biomarkers for the conversion of patients with MCI to AD has been evaluated in a longitudinal study, showing that decreased levels of Aβ42 and increased levels of tau discriminate patients with MCI that progress to AD from those that do not progress with sensitivities (and specificities) of 59% (100%) and 83% (90%), respectively (Hampel et al. 2004). The data suggest that CSF markers are indicative of pathology very early in the disease process in AD, and may be of clinical value to differentiate MCI cases with incipient AD, which will progress to full-fledged AD, from incidents of non-progressive or reversible MCI.

Despite the high accuracy of the biomarker assays for distinction of MCI cases from healthy individuals, the data must be interpreted with caution. Current quantitative analyses

of CSF or serum biomarkers for AD diagnosis use enzyme-linked immunosorbent assay (ELISA). The technical limitations and confounding factors of this technique have been discussed in detail by Andreasen and Blennow (Blennow 2004a; Andreasen and Blennow 2005). In addition, changes in levels of Aβ, tau, and p-tau are not necessarily specific for AD and do not allow differentiating AD accurately from other forms of dementia, such as Lewy body dementia or vascular dementia (Andreasen and Blennow 2005). Because the current average sensitivity for the discrimination between patients with AD and healthy individuals using CSF biomarker detection is ~ 85%, these assays do not offer a considerable increase in predictive value over existing algorithms comprising neuropsychological and imaging modalities (Andreasen and Blennow 2005).

New approaches in the field of AD biomarkers target the detection of soluble assemblies of Aβ. In contrast to the less toxic amyloid plaques and Aβ monomers, soluble Aβ oligomers play a key role in the early pathogenesis of AD. Aβ oligomers are believed to be the primary neurotoxins causing synaptic impairment and cognitive deterioration in early AD, several years before plaques are formed and brain atrophy is observed (Lue et al. 1999; McLean et al. 1999; Wang et al. 1999; Gong et al., 2003). Two novel approaches that do not rely on ELISA target the monitoring of soluble Aβ42 oligomers. Recently, a compound derived from a library of benzofurans was shown to bind selectively to Aβ oligomers but not to Aβ fibrils. The specificity of this compound may be used to detect and quantify soluble Aβ oligomers in CSF samples as a surrogate marker for the early detection of AD (Tan-Hehir et al. 2006). Another technique for specific detection of soluble Aβ assemblies that may offer increased sensitivity and specificity over ELISA used a 'bio-barcode' assay, in which Aβ-derived diffusible ligands (ADDLs) were detected with femtomolar sensitivity in CSF of patients with AD but not in age-matched healthy individuals (Georganopoulou et al., 2005). The bio-barcode assay is based on ADDL recognition with conformation-specific antibodies linked to oligonucleotide-modified nanoparticles. Several hundred copies of the bio-barcode DNA are released from the nanoparticle and amplified to provide a highly sensitive signal for antigen identification (Fradinger and Bitan 2005).

To avoid a lumbar puncture, detection of plasma levels of AD-related biomarkers has been studied. Unfortunately, the results of these studies have been disappointing. Plasma concentrations of Aβ42 do not correlate with those in CSF (Mehta et al. 2001). Longitudinal studies did not show consistent changes in plasma Aβ over time in patients with AD (Mayeux et al. 2003), and cross-sectional differences between patients with AD and healthy individuals that would allow plasma Aβ concentrations to be used as a diagnostic measure have not been identified (Blasko et al. 2006). Secondary pathophysiologic and metabolic alterations in AD, including those related to inflammation (interleukins), cholesterol metabolism (cholesterol, apolipoprotein E, and homocysteine), and oxidative stress (antioxidants and lipid peroxides) also have been studied as potential biomarkers (Thal et al., 2006). Although serum and plasma levels of these biomarkers are altered in AD relative to non-demented, age-matched individuals, they do not have sufficient discriminatory power to allow reliable diagnosis (Irizarry 2004).

Two promising alternatives for fast and non-invasive tests for AD currently are under development. One approach is based on the finding that Aβ aggregates can form deposits in the eye lens of patients with AD (Goldstein et al. 2003). Accumulation of Aβ causes

scattering of a weak laser beam directed into the lens allowing detection of protein deposits. In laboratory experiments using transgenic mouse models of AD, this approach distinguished transgenic mice from healthy mice with a sensitivity of 100% (Moncaster et al. 2006). More research is needed to determine the applicability of this test in patients with AD and to correlate between the amount of protein deposit found in the eye and the level of dementia. Another test that may be applied at very early stages of AD is a skin test based on detection of AD-specific abnormalities related to an inflammatory response. Khan et al. have shown that the level of phosphorylation of extracellular signal-regulated kinases ERK1 and ERK2 in fibroblasts is significantly lower in patients with AD than in healthy individuals. This difference has been used to distinguish between the two groups with excellent accuracy in a cohort of 60 patients (Khan and Alkon 2006). Further work is directed at reproduction of the results in trials with greater numbers of patients to verify the sensitivity and reproducibility of these observations.

CONCLUSION

Currently, there are no disease-modifying therapies approved for AD. A number of promising targets and therapeutic strategies for the treatment of AD are under active investigation. At present, it is too early to determine if one strategy, and which strategy, will work best. Quite likely, a combination of treatments tailored for individual patients will be the most beneficial route. Inhibition of Aβ production, assembly, and toxicity has become a primary focus for contemporary drug development programs. Recent developments include identification and clinical assessment of β- and γ-secretase inhibitors and modulators, inhibitors of Aβ oligomerization, and safer active and passive anti-Aβ immunization strategies. NGF gene therapy and RNA interference for FAD are alternative promising approaches. These therapies are likely to have the best efficacy in the early or even preclinical phases of the disease, before cognitive deficits have become apparent because at these stages the main pathological process is synapse loss but not neuronal loss. If the pathologic molecular processes causing synapse impairment can be inhibited, the brain likely will have sufficient plasticity to repair injured neurons and regenerate affected synapses. At later stages of AD, when substantial neuronal loss has occurred, regeneration may be more difficult or impossible. Development of tools for early diagnosis of AD at preclinical stages is invaluable for evaluating the outcome of clinical trials and for successful application of these therapies.

Currently, unequivocal diagnosis of AD is not possible in living patients. It is still equally difficult to discriminate with certainty patients with MCI from healthy individuals and from patients with AD. Despite several confounding factors, cognitive assessment of patients is the most common tool in clinical routine. Imaging techniques, such as MRI and SPECT help support the initial diagnosis and to rule out other sources of cognitive decline. A combination of the rate of cognitive deterioration and the rate of MTA has superior diagnostic/prognostic value compared to a single assessment. Periodic repetition of examination is recommended. An important goal of current research in brain imaging and biomarker detection is to shift the limit of detection in the development of AD to an earlier

stage when the future patient is still presymptomatic. New developments in brain imaging target visualization of Aβ lesions rather than brain atrophy using tracer compounds such as PIB and FDDNP. However, Aβ deposition occurs relatively late in the pathogenesis of AD and is preceded by soluble Aβ assemblies. Targeting the detection of non-fibrillar Aβ assemblies will be highly valuable for distinguishing effectively normal elderly people from those with MCIa at presymptomatic stages. These developments and future non-invasive diagnostic methods possibly using detection of Aβ aggregates in the eye or AD-specific inflammatory markers in the skin hold promise for fast and reliable early diagnosis of AD allowing initiation of immediate therapeutic interventions that would prevent and/or reverse the disease.

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