Annu. Rev. Pharmacol. Toxicol. 2003. 43:545–84
doi: 10.1146/annurev.pharmtox.43.100901.140248

First published online as a Review in Advance on October 4, 2002

ALZHEIMER'S DISEASE: Molecular Understanding Predicts Amyloid-Based Therapeutics

Dennis J. Selkoe[1] and Dale Schenk[2]
[1]*Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts 02115; email: dselkoe@rics.bwh.harvard.edu*
[2]*Elan Pharmaceuticals, South San Francisco, California 94080*

Key Words amyloid β-protein, secretases, protease inhibitors, immunization, monoclonal antibodies

■ **Abstract** Degenerative diseases of the brain were long considered among the most obscure and intractable of human maladies. However, recent advances in understanding their mechanisms have brought us to the verge of potential disease-modifying agents. This progress is perhaps best exemplified by the case of Alzheimer's disease. The application of molecular pathology and genetics has led to the recognition that the four genes implicated to date in familial Alzheimer's disease all chronically elevate cerebral levels of the amyloid β-protein (Aβ). Accordingly, small molecule inhibitors of the β- and γ-secretases, the proteases that generate Aβ from its precursor, are under active development, and some have shown in vivo efficacy in mouse models. An alternative approach, active or passive immunization against Aβ, has received extensive pre-clinical validation in mice, but an effective preparation free of significant side effects in humans is still awaited. Several other potential therapies are also reviewed here. If one or more of these varied approaches is ultimately proven to slow or prevent dementia, Alzheimer's disease will become a salient example of the successful application of reductionist biology to the most complex of organs, the human cerebral cortex.

INTRODUCTION

Few biomedical problems have captured the attention of the scientific and lay communities alike as has Alzheimer's disease (AD). This insidious and devastating brain degeneration that robs its victims of their most human qualities—memory, reasoning, abstraction, and language—is believed to afflict some 4 million Americans and perhaps 20–30 million people worldwide. Once classified as an obscure, "presenile" dementia that was relegated to a few short paragraphs in neurological textbooks, AD is now recognized as a major public health problem in developed nations, and knowledge of its causes and mechanisms has grown enormously in the past decade. In this review, we briefly summarize the current understanding and management of AD and then explore in detail several discrete therapeutic targets that have emerged from the ongoing elucidation of its molecular basis.

Defining Alzheimer's Disease

THE CLINICAL SYNDROME AD begins almost imperceptibly, most often with occasional, minor lapses in recalling recent events of daily life (episodic memory). Patients may fail to remember a conversation or activity, or may become confused about an item of information recently received. The syndrome referred to as mild cognitive impairment (MCI) is often a harbinger of AD and begins with pure amnestic symptoms, with little or no difficulty in other cognitive spheres (1, 2). Patients with MCI or early (mild) AD have fully preserved alertness, no significant language disturbance, and intact motor and sensory function.

During or after the first couple of years of amnestic symptoms, most patients develop additional minor problems with some aspects of general cognitive function, such as being oriented to time and place and executing complex tasks easily and correctly (executive function). As such deficits become increasingly noticeable, patients may experience disinterest in activities and hobbies, apathy, emotional lability, word-finding difficulty, spatial disorientation (e.g., getting lost), and trouble with mathematical concepts (e.g., keeping bank accounts). After a few years of progressive memory and cognitive decline, many patients begin to experience first mild and then more noticeable deficits in motor function, including stereotyped manual tasks (e.g., handiwork, writing, and drawing), balance, and walking. Over several years or even a decade or more, AD patients will gradually deteriorate into a marked dementia, with full disorientation, profound memory impairment, and global cognitive deficits. Many patients become immobile, confined to a chair or bed, and ultimately succumb to minor respiratory difficulties, such as aspiration or pneumonia.

THE NEUROPATHOLOGICAL PHENOTYPE Although a clinical diagnosis of AD based on the above signs and symptoms can be made with considerable certainty during life, confirmation still requires postmortem observation of the classical lesions in microscopic sections of hippocampus, amygdala, and the association cortices of the frontal, temporal, and parietal lobes. Although mild ($\sim$8%–15%) atrophy of the cerebral hemispheres may be observed on gross inspection of the brain, this is often hardly more than occurs with age in non-demented individuals. Light microscopy, however, reveals a myriad of neuritic ("senile") plaques and neurofibrillary tangles in the aforementioned brain areas of the AD patient. Neuritic plaques are roughly spherical, extracellular deposits of amyloid β-protein (Aβ) fibrils intimately surrounded by dystrophic axons and dendrites, activated microglia, and reactive astrocytes. Such fibrillar amyloid plaques are invariably accompanied by many "diffuse" (pre-amyloid) plaques in the same brain regions. These consist of amorphous extracellular deposits of Aβ-immunoreactive granular material that generally lack amyloid fibrils and are associated with very few or no dystrophic neurites or altered glia. In most AD cases, the number of diffuse plaques clearly exceeds that of the neuritic plaques. Diffuse plaques appear to represent the earliest light-microscopically detectable lesion in AD brains. They seem

to predate fibrillar neuritic plaques, as judged by their occurrence in cognitively normal, late middle-aged and elderly healthy individuals (3), as well as by their development in teenagers with Down's syndrome long before the latter individuals develop the neuritic plaques and neurofibrillary tangles typical of AD (4). It should be emphasized that diffuse and neuritic plaques actually exist in the cortex in a morphological continuum, rather than as two distinct types of lesions.

Aβ also accumulates in the small blood vessels of the meninges and cerebral cortex, mostly in the outer walls of arterioles and capillaries (5). The extent of this congophilic amyloid angiopathy (CAA) varies greatly among AD brains, even when parenchymal Aβ deposits are at roughly similar levels. The pathophysiological contribution of CAA to the dementing symptoms of AD, if any, remains unclear. CAA occurs not only in AD patients, but also in isolation in elderly humans lacking the clinical and neuropathological features of AD. If the amyloid deposition becomes severe enough to cause substantial hyaline necrosis of the microvessel wall, cerebral hemorrhage may ensue. However, this is a rare clinical complication in sporadic AD.

Besides the neuritic plaque, the other diagnostic lesion of AD is the neurofibrillary tangle. Tangles are non-membrane-bound masses of paired helical filaments, usually intermixed with straight filaments, found in the perinuclear cytoplasm of many limbic and cortical neuronal cell bodies. Smaller bundles of these abnormal filaments may occur in many, but not all, of the cortical dystrophic neurites found within and also separate from the neuritic plaques. Tangles are also observed in neurons of the subcortical nuclei (e.g., the cholinergic septal nuclei and nucleus basalis of Meynert) that project widely to limbic and association cortices rich in Aβ deposits.

A recurring concern in the study of AD is that Aβ plaques can be found at autopsy in individuals who had few or no cognitive symptoms during life. However, it is important to note that almost all of the plaques in aged normal brain tissue are of the diffuse type—that is, they lack associated neuritic and glial cytopatholgy—and they are accompanied by very few or no neocortical tangles. On this basis, it has been postulated that diffuse plaques are "pre-clinical" lesions not yet associated with microscopically visible injury to neurons and their processes. A rough analogy can be drawn to the fatty streaks of cholesterol in systemic blood vessels that occur in most older asymptomatic patients and are often a precursor to clinically important, mature atherosclerotic plaques.

THE NEUROTRANSMITTER PHENOTYPE The first transmitter abnormality to be documented in AD brain tissue was the loss of enzymes that synthesize and degrade acetylcholine (6–8). Accordingly, cholinergic neurons in the septum and basal forebrain were found to decline in both size and number in AD (9). However, these deficits were soon shown to be accompanied by losses of neurons using other neurotransmitters, including glutamate, GABA, somatostatin, corticotropin-releasing factor, serotonin, and several others (10–12). Thus, the neurotransmitter deficits of AD are multiple and provide no clear clue as to the process destroying

these diverse neuronal subtypes. To date, only the cholinergic deficiency has been seriously addressed therapeutically.

BASIC BIOLOGY OF AMYLOID β-PROTEIN PRECURSOR Aβ is a small hydrophobic peptide with N- and C-terminal heterogeneity that occurs in two principal lengths: Aβ40 and Aβ42. Aβ is proteolytically released from a large type 1 membrane glycoprotein of unknown function, the Aβ precursor (APP) (13), via sequential cleavages by two aspartyl proteases, referred to as the β- and γ-secretases (Figure 1) (14). The Aβ region of APP comprises the 28 residues just outside the single transmembrane domain (TMD), plus the first 12–14 residues of that buried domain. On this basis, Aβ was originally assumed to arise only under pathological circumstances, in that the second cleavage was thought to require some kind of prior membrane disruption to allow access of γ-secretase and a water molecule to

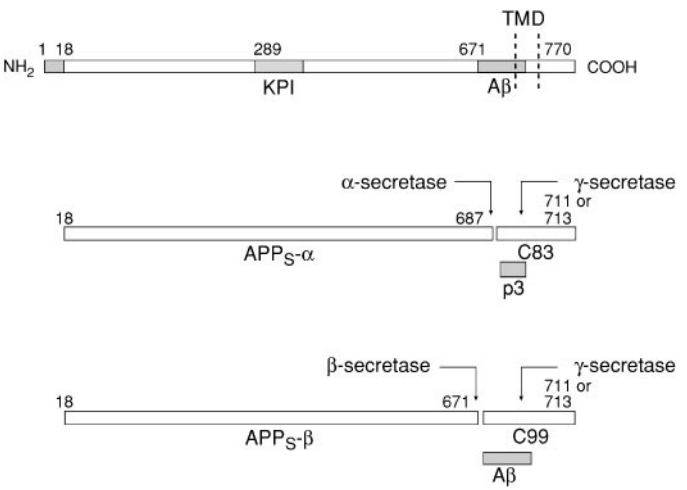

Figure 1 Schematic representation of APP processing. The top diagram represents the largest known splice variant of APP, which contains a signal peptide (residues 1–17), two alternatively spliced exons (at residue 289), and a single TMD (700-723). Constitutive α-secretase-mediated proteolytic cleavage of APP occurs after residue 687, yielding APP_s-α and the C83 fragment (*second line*) Alternatively, β-secretase-mediated cleavage occurs after residue 671, yielding APP_s-β and C99 (*third line*). γ-secretase cleavage at position 711 or 713 releases the p3 peptides from C83 and Aβ40 and Aβ42 from C99. Numbers represent amino acid positions; arrows indicate sites of secretase cleavages. Aβ: amyloid β-protein; APP: amyloid β-protein precursor; APP_s-α: soluble ectodomain of APP formed by α-secretase cleavage; APP_s-β: soluble ectodomain of APP formed by β-secretase cleavage; KPI: Kunitz type serine protease inhibitor motif; TMD: transmembrane domain. Reproduced with permission from Reference 14.

the otherwise intramembranous region. This concept was disproved in 1992, when Aβ was shown to be constitutively released from APP and secreted by mammalian cells throughout life and thus occur normally in plasma and cerebrospinal fluid (CSF) (15–17). This discovery enabled the dynamic study of Aβ production in cell culture and animal models, including examination of the effects of AD-causing genetic mutations. Moreover, high-throughput screening could now be conducted on cultured cells to identify Aβ-lowering compounds and determine their mechanism.

Most APP molecules that undergo secretory processing are cleaved by α-secretase, rather than β-secretase, near the middle of the Aβ region (18, 19). This releases the large, soluble ectodomain (APP_s-α) into the medium and allows the resultant 83-residue, membrane-retained, C-terminal fragment (C83) to be cleaved by γ-secretase, generating the small p3 peptide (Figure 1). α-secretase acts on APP molecules at the cell surface, although some processing also occurs in intracellular secretory compartments. The precise subcellular loci of the β- and γ-secretase cleavages are unclear, but likely include early, recycling endosomes (20, 21). The functional consequences of the proteolytic processing of APP remain ill-defined. The current leading hypothesis is that cleavage by α-secretase followed by γ-secretase enables the release of the APP intracellular domain (AICD) into the nucleus, where it may participate in transcriptional signaling (22–24). The APP_s-α derivative secreted as a result of this processing appears to have distinct extracellular functions. For example, those APP_s-α isoforms that contain an alternatively spliced Kunitz protease inhibitor domain function as serine protease inhibitors, including by inhibiting of Factor XIa in the coagulation cascade (25).

GENOTYPE TO PHENOTYPE CONVERSIONS IN FAMILIAL ALZHEIMER'S DISEASE As knowledge of the complex processing of APP unfolded, the question of its significance in the pathogenesis of AD came to the fore. This question was largely answered by the discovery of various genetic alterations that strongly predispose individuals to AD. The first familial AD gene to be identified was APP itself (26). Missense mutations located within and immediately flanking the Aβ sequence were found to cause autosomal dominant forms of early-onset AD and/or CAA (27). No AD-causing mutations in the large APP molecule have been found away from the Aβ region, strongly suggesting that the substitutions alter the proteolytic generation of Aβ and/or its aggregation into neurotoxic assemblies. This conclusion has been amply confirmed in cell culture assays, APP transgenic mice, and patients bearing these mutations.

The ε4 allele of the cholesterol transport protein, apolipoprotein E, was the second predisposing genetic factor to be discovered (28). Extensive genetic epidemiology has shown that inheritance of one apoε4 allele increases the likelihood of developing late-onset AD by $\sim$2–5-fold, and the inheritance of two alleles raises the risk by 4–10-fold or more (29). Interestingly, inheritance of the apoε2 allele appears to confer some protection from AD (30). The mechanism by which

apoE4 promotes the disease is unclear. Crossing mutant APP transgenic mice with mice lacking the endogenous apoE gene results in offspring with far fewer Aβ deposits (31). Expressing human apoE4 in such mice leads to heightened Aβ deposition, compared to expressing human apoE3 (32). In vitro studies also suggest that apoE4 is a less effective inhibitor of Aβ aggregation than is apoE3 (33, 34). Such experimental data fit well with neuropathological analyses in humans, which show that the inheritance of one or two apoE4 alleles significantly heightens cerebral Aβ burden, even in those who have not yet developed clinical AD (35, 36).

The two other genes clearly linked to AD are presenilins (PS) 1 and 2 (37, 38). Missense mutations in these homologous 8-TMD polypeptides cause the most aggressive form of AD known, with onset commonly occurring in the 40s and 50s and very rarely as early as the late teens. Many studies in cell culture, animal models, and patients demonstrate that presenilin missense mutations elevate the γ-secretase-mediated production of the strongly self-aggregating Aβ42 peptide, perhaps at the expense of the Aβ40 peptide (14). This mechanism is discussed in detail when we consider inhibition of γ-secretase as a therapeutic target.

The APP, PS1, and PS2 mutations cumulatively account for close to half of early-onset (<65 years) familial AD cases, with PS1 responsible for almost all of these. Inheritance of apoE4 is variously estimated to be the principal pathogenic factor in 10%–40% of all AD cases. It should be emphasized that apoε4 acts as a risk factor for AD, not a deterministic gene, so that octogenarians carrying one or even two ε4 alleles may have normal cognitive function. Several other chromosomal loci and candidate genes are under active study, but not unequivocally confirmed. When they are, the same kind of genotype-to-phenotype analysis should establish whether or not they operate by altering the economy of Aβ in the brain.

Progress in deciphering the genetic forms of AD has led to several compelling transgenic mouse models. The first models expressed human APP that contained missense mutations which cause autosomal dominant AD (39, 40). A number of distinct mouse lines ensued, carrying various mutant forms of human APP driven by different promoters and having subtly different neuropathological and biochemical phenotypes (41, 42). The cloning of PS1 led to crossed mouse lines expressing mutant human APP plus a missense mutation in human PS1, and these mice show accelerated Aβ deposition and generally more severe neuritic and glial cytopathology than APP single transgenic mice (43). Crossing mutant APP mice with those expressing human transforming growth factor-β produced a line with prominent microvascular amyloidosis (44). Particularly useful is the recent development of crossed mice expressing human mutant APP plus human mutant tau, as the progeny develop tangle-like neuronal cytopathology in addition to diffuse and neuritic plaques (45). In general, the various mouse models show progressive Aβ accumulation and deposition in the hippocampus and association cortices in anatomic patterns resembling those seen in AD and with the development of secondary neuritic, neuronal, and glial alterations.

Current Treatments are Largely Symptomatic

At the time of writing, no treatments have been clinically proven to modify the disease process in a way that significantly slows or prevents the progression of AD. However, there are several different pharmacological agents that may ameliorate or temporarily suppress certain debilitating symptoms. The most commonly used drugs that do so are reviewed here.

ACETYLCHOLINESTERASE INHIBITORS Four compounds in this class have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of AD. Of these, three are in active clinical use, whereas one [tetrahydroaminoacridine (Cognex)] has the potential to cause significant hepatotoxicity and is therefore rarely used. The other three [donepezil (Aricept), rivastigmine (Exelon), and galantamine (Reminyl)] have similar potential benefits and adverse events, including nausea, vomiting, diarrhea, and other cholinergic symptoms, and have generally been used interchangeably. In placebo-controlled studies of a few months in duration, these agents modestly improved certain cognitive test scores, although in clinical practice many patients show no objective evidence of improvement in memory or other cognitive tests. A minority of treated patients show modest improvement in certain mental status scores or no decline over a period of several months to a year. Patients who show no improvement or lose benefit over time can be switched to another inhibitor, but this does not usually result in a substantial difference in efficacy. Over time, many physicians and patients decide to discontinue acetylcholinesterase inhibitors due to lack of clear benefit. In Europe, the NMDA antagonist, memantine, is marketed for treatment of AD. This is the only non-cholinergic neurotransmitter agent currently in widespread use for symptomatic treatment.

PSYCHOTROPIC DRUGS THAT MODIFY BEHAVIORAL SYMPTOMS One of the most difficult aspects of AD for patients and caregivers is the occurrence of behavioral disturbances, including anxiety, apathy, agitation, aggressiveness, depression, serious delusions, and hallucinations (46). Although minor anxiolytics, such as benzodiazepines, can blunt some of these symptoms, their benefit is modest and often accompanied by unacceptable sedation or worsening of dementia. Non-phenothiazine antipsychotic agents in low-to-moderate doses are more widely used for this purpose. These include risperidone (Risperdal), olanzapine (Zyprexa), haloperidol (Haldol), and quetiapine (Seroquel). Occasional AD patients (~5%–10%) may display prominent symptoms of depression and therefore may benefit from low doses of selective serotonin re-uptake inhibitors, although these may also temporarily heighten the patient's cognitive deficits. Physicians sometimes prescribe stimulants, such as methylphenidate, for marked apathy, or anticonvulsants, such as devalproex sodium (Depakote) or carbamazepine (Tegretol), for agitation and anxiety. The administration of low-to-moderate doses of one, or sometimes more than one, psychotropic drug can significantly improve behavioral symptoms in AD patients. However, dose escalation should be slow,

and patients should later be gradually weaned off the agent to determine whether cognitive function is being compromised. Many AD patients experience significant behavioral aberration for relatively circumscribed periods of time; as their dementia worsens, they lose these symptoms and become more calm and mellow.

POTENTIAL NEUROPROTECTIVE STRATEGIES Although there are no available drugs clearly proven to modify the course of cognitive decline, several neuroprotective approaches have been suggested. Many clinicians recommend that patients take high doses (1000–2000 IU/day) of vitamin E. At least one placebo-controlled trial of vitamin E and selegiline (a monoamine oxidase inhibitor) has reported small but significant delays in time to both nursing home entry and death with each of these agents alone, but no additive effect of taking both. However, these effects may well represent benefits for cardiovascular and other systems, and there was no evidence of improvement in the cognitive symptoms of dementia. Although high doses of vitamin C have sometimes been recommended, no studies showing a definitive benefit in AD have appeared. Nonsteroidal anti-inflammatory drugs could potentially blunt the neurotoxic effects of peri-plaque microglial/astrocytic activation, cytokine release, and the acute phase protein response in the AD brain, but no prospective controlled trials have yet shown a benefit. Other neuroprotective strategies potentially relevant to AD include calcium channel modulators (47), free-radical scavengers (48), and metal ion chelators (49); published clinical trial results for the latter are emerging.

INHIBITION OF β-SECRETASE

Identification and Protease Biochemistry of β-Secretase

Early experiments established that a substantial portion of the APP normally secreted by human-mixed brain cultures, and some of that present in human CSF, is precisely cleaved at the amino terminus of Aβ (between residues 671 and 672) resulting in formation of the C99 fragment (Figure 1) (50). Well before the identification of the molecular structure of β-secretase, a considerable amount of information was obtained about its functional properties (Table 1) (51). This work culminated in the identification by five independent research groups in 1999 of a novel aspartic protease that exhibited the expected properties of β-secretase (52–56). Aspartyl proteases are a well-characterized class of proteases that include pepsin, renin, cathepsins D and E, and napsin A. Although each research group used a somewhat different detection approach [expression cloning (52), biochemical enzyme purification (53), and various genomic strategies (54–56)], the enzyme was identical (Figure 1), thus strengthening the conclusion that the structure identified was β-secretase.

β-secretase is a 501–amino acid type I transmembrane protein with a lumenal active site (Figure 2). It has an N-terminal signal peptide (residues 1–21), a

TABLE 1 Functional characteristics expected of β-secretase based on cell culture experiments

Present in neurons in the brain
Membrane-associated protease with lumenal active site
Present in Aβ-secreting cells
Co-localized with APP in Golgi vesicles
Overexpression increases APP_s-β, C99, and Aβ levels
Cleaves at Asp 1 of Aβ
Optimum pH ~4.5
Pepstatin-insensitive
Antisense inhibition reduces APP_s-β, C99, and Aβ expression
Cleavage preference at P1 (Leu $\gg$ Met $\gg$ Val)

Aβ: amyloid β-protein; APP: amyloid β-protein precursor; APP_s-β: soluble ectodomain of APP formed by β-secretase cleavage.

preprotein domain (residues 22–45), a catalytic domain (residues 45–459) with two conserved aspartyl protease active sites (DTGS at residues 93–96 and DSGT at residues 289–292), and a C-terminal extension with a TMD (residues 460–477) and a cytoplasmic tail (Figure 2) (51). The TMD distinguishes β-secretase from most other aspartic proteases and enables the enzyme to properly access the APP ectodomain at the β-cleavage site (57, 58). The lumenal part of the molecule includes four sites for N-glycosylation at 163, 172, 223, and 354 (52), and six cysteine residues (216, 278, 330, 380, 420, and 443) that can form up to three intramolecular disulfide bonds.

The newly identified β-secretase enzyme was initially designated variously as beta-site APP-cleaving enzyme, or BACE (52), novel aspartic protease 2 (Asp2) (54, 55), and membrane aspartic protease 2 (memapsin2) (56). However, with the identification of a close homolog of β-secretase (known as BACE2, Asp1, or memapsin1), which has a similar, but distinct, substrate profile (54, 59, 60), BACE is now more commonly referred to as BACE1.

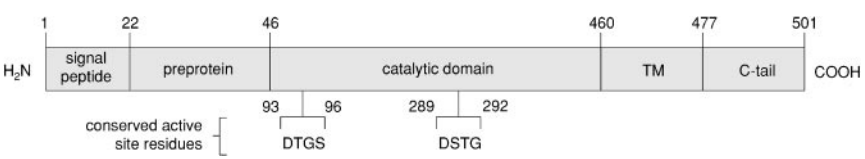

Figure 2 Schematic representation of the protein structure of human β-secretase, indicating the conserved active site residues. Numbers indicate amino acid positions. C-tail: cytoplasmic tail; TM: transmembrane domain.

Cell Biology of β-Secretase

β-secretase mRNA is widely expressed in both the peripheral tissues and brain, with the highest levels in the body found in the pancreas (52, 54, 61). In the brain, β-secretase mRNA is expressed at high levels in neurons of the hippocampus, cortex, and cerebellum (52, 61). Enzymatic activity is highest in neural tissue, and little activity occurs in peripheral tissues (52–54). Subcellular localization studies have revealed that β-secretase occurs mainly within the acidic subcellular compartments of the secretory pathway, the Golgi network and endosomes (52, 55, 58), with low amounts in the endoplasmic reticulum and lysosomes. Thus, β-secretase localizes to the same intracellular compartments at which β-cleavage of APP is believed to occur. The acidic pH of these compartments provides optimal conditions for the cleavage of APP by an aspartyl protease (52–54).

Initially expressed as an inactive pre-proprotein in the endoplasmic reticulum (57, 62), β-secretase subsequently undergoes intramolecular disulfide bond formation and N-linked glycosylation at three of its four potential N-glycosylation sites (62). On leaving the endoplasmic reticulum, the propeptide is cleaved by a furin-like endoprotease, followed by further glycosylation to produce the mature protease, which is rapidly and efficiently transported through the Golgi apparatus before being targeted to endosomes (57, 62). The cytoplasmic tail of β-secretase, and in particular its dileucine motif, appear to be important for the normal trafficking of the enzyme (62).

Substrate Specificity of β-Secretase

β-secretase cleaves APP at the N-terminus of Aβ by recognizing the VKM*DA sequence (residues 594–598). This sequence is labeled P3-P2-P1*P1′-P2′ in standard protease nomenclature, with the site of cleavage marked by *. Cleavage by β-secretase is highly sequence specific. APP containing the Swedish double mutation (KM to NL at positions 595 and 596) that is associated with early-onset AD seems to be a significantly better substrate for β-secretase than wild-type APP (63). In contrast, substitutions of other residues in the VKM*DA region, for example, valine at the P1 position, generally reduce Aβ production, sometimes markedly (64).

Sauder et al. (65) have used three-dimensional modeling to study the interactions between β-secretase and its APP substrate and to identify which residues confer specificity. This work established that the Arg296 residue of β-secretase makes a salt-bridge with the P1′ aspartic acid residue of the wild-type APP. Of additional importance are the interactions between the P2 lysine residue and Asp379 of β-secretase; the hydrophobic contacts between the P1 methionine residue and the enzyme at Leu91, Tyr132, and Ile179; and the P3 valine residue with Phe170 of β-secretase. The model also showed that the Swedish double mutation at P2-P1 interacts more favorably with Arg296 and the hydrophobic pocket of β-secretase than do residues of wild-type APP, thereby explaining the enhanced cleavage of this

mutated form of APP. Conversely, when the P1 residue is changed from methionine to valine, many of the stabilizing hydrophobic contacts between the substrate and enzyme are missing, whereas the catalytic Asp93 residue is blocked. The apparent importance of the Arg296 residue of β-secretase and the hydrophobic pocket of the active site in determining the substrate specificity of β-secretase have subsequently been confirmed by Hong et al. (66), who determined the crystal structure of the active domain of β-secretase complexed with a transition-state inhibitor.

More recently, the substrate specificity of β-secretase has been explored and compared with that of other aspartic proteases using a range of dodecameric substrates based mainly on the β-cleavage site of APP (67). The substrate recognition site of β-secretase extended over several amino acids, and β-secretase accepted a wide range of peptidic substrates. In common with other aspartic proteases, β-secretase prefers a leucine residue at position P1. However, unlike these enzymes, β-secretase accepts polar or even acidic residues at positions P1 and P2′, and prefers bulky hydrophobic residues, preferably valine, at position P3.

Strategies to Identify β-Secretase Inhibitors

β-secretase is an attractive target for a disease-modifying therapy for AD, as it initiates and is the rate-limiting step in the formation of Aβ. Now that the molecular sequence and crystal structure of the target protease have been identified, the development of specific β-secretase inhibitors is being actively pursued. The observation that mice deficient in β-secretase (BACE1 knockout mice) do not generate any Aβ (61, 68, 69) supports the validity of this target. Importantly, these mice appear to be healthy, with no obvious neurological or behavioral abnormalities, despite the complete lack of β-secretase activity in the brain (61, 68, 69).

Inhibitors of other aspartic proteases have been developed, but only those targeted against the retroviral human immunodeficiency virus protease are currently in therapeutic use (51, 70). Research groups attempting to develop β-secretase inhibitors are generally modifying peptidic molecules designed from the sequence of the APP cleavage site (53, 67, 71, 72). To date, only a few of the molecules that directly inhibit β-secretase activity at nanomolar concentrations have been described in published literature.

The first of these, StatV (Figure 3), a 14-residue transition-state analog that spans the P10 to P4′ region of Swedish mutant APP, was used as an affinity ligand by Sinha et al. when they purified and cloned β-secretase from human brain tissue (53). StatV has an IC_{50} of $\sim$30 nM. A second, smaller, and more potent compound that spans eight residues of Swedish mutant APP (P4 to P4′) is OM99-2, with an IC_{50} of $\sim$1.6 nM (Figure 3) (71). However, neither compound is entirely specific to β-secretase, as they both inhibit other aspartic proteases (67). Indeed, the IC_{50} of StatV against cathepsin D, cathepsin E, and pepsin is considerably lower than that for β-secretase (67). Furthermore, both molecules have a molecular weight that exceeds 1000 and are consequently too large for therapeutic use.

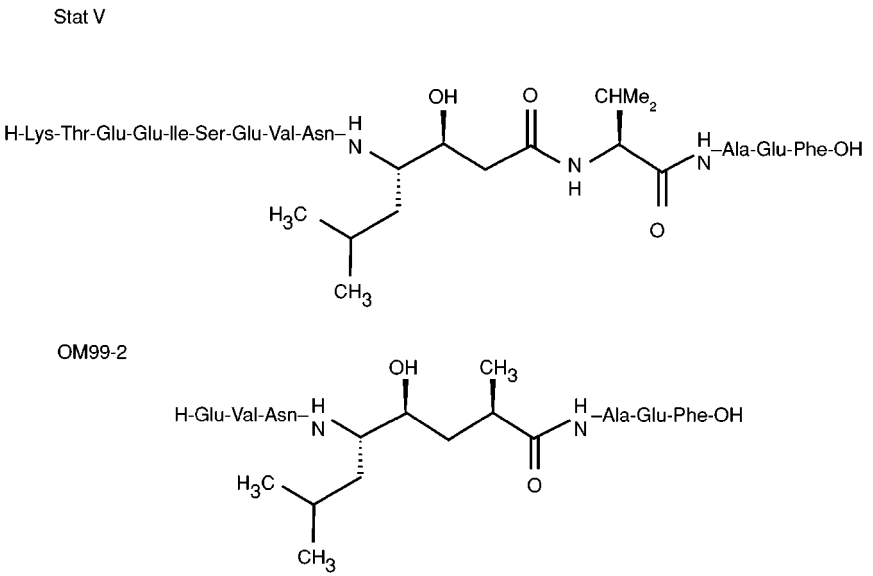

Figure 3 Structure of two direct inhibitors of β-secretase.

To facilitate the rational design of more specific, β-secretase inhibitors, Hong et al. (66) have co-crystallized OM99-2 with β-secretase with the aim of providing information on specific ligand-binding site interactions and the orientation of the inhibitor in the active domain. The group subsequently designed a series of peptidomimetic inhibitors of β-secretase using OM99-2 as a starting point. Some of these compounds were of similar potency to OM99-2, but had a much lower molecular weight (72).

INHIBITION OF γ-SECRETASE

The Concept of Intramembrane Proteolysis

When the precursor of Aβ was cloned in 1987 (13), it immediately became apparent that one of the two proteolytic scissions needed to release Aβ must occur within a hydrophobic, putatively membrane-spanning region of APP. As hydrolysis of peptide bonds requires water and as all known proteases had their active sites in aqueous compartments of the cell or extracellularly, it was assumed that at least part of the APP TMD must be transiently exposed outside of the membrane, presumably due to prior membrane injury. The only other possibility raised at the time was that the TMD of APP ended just before or at the γ-secretase cleavage site; however, this was incompatible with available information about the predicted

lengths of TMDs. The discovery of normal Aβ production in 1992 showed that neither of these explanations appeared to be correct. Rather, Aβ peptides ending at residues 40 and 42, which are clearly within the membrane-spanning region, were naturally secreted by healthy cells without evidence of membrane injury and were circulating peptides. With this discovery, it became apparent that some kind of proteolysis within the membrane could occur normally. In this sense, Aβ generation presaged the recognition of what is now appropriately referred to as regulated intramembrane proteolysis (73).

The first identification of a protease that appeared to cleave within the lipid bilayer emerged from the cloning of the site 2 protease (S2P), an unprecedented polytopic metalloprotease with its HEXXH catalytic motif interior to the membrane-cytosolic interface (74). S2P executes the second of the two cleavages of the sterol regulatory element-binding protein (SREBP), enabling the release of its N-terminal cytoplasmic domain to the nucleus, where it regulates the transcription of genes important for cholesterol homeostasis. Since this discovery, several novel and unrelated intramembrane proteases that process diverse membrane substrates have been found in organisms ranging from bacteria to man (73). A newly recognized example is the rhomboid family, responsible for releasing epidermal growth factor (75). Conserved intramembrane residues in rhomboid that are required for function (asparagine, histidine, and serine) are reminiscent of the catalytic triad typically found in serine proteases, and inhibitors of soluble serine proteases block rhomboid-mediated proteolysis. Many mechanistic details of how such molecules break amide bonds remain unknown, but progress in deciphering the identity and biochemistry of γ-secretase is helping define some of the principles of regulated intramembrane proteolysis.

Relationship of Presenilin to γ-Secretase

When presenilin was first identified (38), the mechanism by which mutations in this polytopic protein caused AD was unidentified, and early speculation about its function was wide ranging. However, it soon became clear that presenilin missense mutations alter the γ-secretase-mediated cleavage of APP in a way that elevates the ratio of Aβ42 to Aβ40 (76). Furthermore, the cloning of *sel-12*, a gene encoding the presenilin homologue in *Caenorhabditis elegans*, revealed presenilin to be critical for signaling by the Notch family of cell surface receptors (77). Deletion of the PS1 gene in mice resulted in an embryonic lethal phenotype that included severe developmental alterations in the Notch pathway (78, 79). Shortly thereafter, De Strooper et al. showed that such mice had a marked decrease in neuronal Aβ production due to a 60%–70% loss of γ-secretase activity (80), the remainder of which was later shown to be due to the residual function of PS2 (81, 82). This finding was interpreted to suggest that presenilins were critical cofactors of the unknown γ-secretase (80). An alternative explanation of these data soon emerged: that presenilin itself was γ-secretase (83). In addition to the

above findings, four principal lines of evidence led to this hypothesis. First, small amounts of APP and PS1 could be co-immunoprecipitated from the membranes of cells, and thus the two proteins could occur as complexes (84). This finding was later extended to show co-precipitation of presenilin with C83 and C99, the immediate substrates of γ-secretase (85). Second, presenilin and APP were localized to the same subcellular vesicle fractions, which mediated de novo generation of Aβ upon incubation at 37°C (86). Third, APP peptidomimetic inhibitors designed as transition-state analogs specific for an aspartyl protease blocked γ-secretase, strongly suggesting that γ-secretase was an aspartyl protease (87). Fourth, and most importantly, presenilins were observed to have two intramembrane aspartate residues predicted to lie in the middle of adjacent TMDs, flanking the hydrophobic region that undergoes endoproteolysis to create the biologically active presenilin heterodimer (83).

Based on this hypothesis, the aspartate residues in TMD 6 and TMD 7 of PS1 were independently mutated to alanine, resulting in an ~60% loss of γ-secretase-mediated Aβ generation and concomitant cellular accumulation of the C83 and C99 substrates (83). Intriguingly, mutation of either aspartate residue also abrogated the endoproteolysis of PS1. Engineering an aspartate mutation into a functional human variant of PS1 that does not undergo endoproteolysis (the ΔExon9 variant) showed that the mutation still blocked γ-secretase activity (i.e., Aβ generation), indicating that the TMD aspartates were independently required for both presenilin endoproteolysis and cleavage of C99 (83). Taken together, these findings were interpreted to suggest that presenilins were unprecedented intramembrane-cleaving aspartyl proteases activated by autoproteolysis (83, 88).

Subsequently, extensive data supporting the hypothesis that presenilins are the active site of γ-secretase has emerged. Antibodies directed against PS1 are able to precipitate in vitro γ-secretase activity from cell membranes solubilized in CHAPS and related detergents (89). Moreover, aspartyl protease transition-state analogue inhibitors of γ-secretase bind directly and specifically to both fragments of the biologically active presenilin heterodimer (90, 91), providing compelling evidence that presenilins contain the active site of γ-secretase. Deletion of both PS1 and PS2 from cells completely abrogates γ-secretase cleavage, both of APP and Notch (81, 82). Furthermore, co-expressing aspartate to alanine mutant forms of PS1 and PS2 in cells simultaneously reduces presenilin heterodimer levels to extremely low levels and essentially shuts down Aβ production, indicating that inactivation of just these residues is akin to deleting the entire protein (92). The presenilin intramembranous aspartate residues are part of signature motifs found in a bacterial family of polytopic aspartyl proteases called type-4 prepilin peptidases (93, 94). Most recently, a novel 7-TMD protein containing the two transmembrane aspartates and conserved flanking residues of presenilin has been shown to be the signal peptide peptidase (SPP), and expressing SPP alone in yeast (which have no endogenous SPP) reconstitutes proteolytic activity (95).

γ-Secretase is a Multi-Protein Complex

Despite the very substantial information implicating presenilin in γ-secretase, it has not been possible to increase substrate-cleaving activity by overexpressing PS1 and PS2 alone. This failure presumably relates to the observation that the endoproteolysis of presenilin and stabilization of the resultant heterodimers requires certain limiting cellular factor(s) (96). In other words, overexpressing presenilin by itself results in the replacement of endogenous presenilin heterodimers with their exogenous counterparts, so that little or no net increase in cleavage activity is obtained. Consequently, there has been an active search for protein cofactors that may help release this tight regulation of γ-secretase activity. The first protein found to potentially serve such a function was nicastrin (97). This large, single transmembrane glycoprotein co-precipitates with PS1 and APP, and mutations introduced into a conserved hydrophilic domain are reported to alter Aβ production (97). Presenilin heterodimers and nicastrin also co-localize in high molecular weight ($\sim$200 kDa) complexes during glycerol velocity gradient centrifugation of cellular microsomes (97, 99). Moreover, partial purification of human γ-secretase from HeLa cell microsomes yields PS1 and PS2 heterodimers and nicastrin in stoichiometric amounts, and the enriched complex cleaves the C100 fragment of APP and an equivalent Notch-based substrate in vitro (100). In accordance, conditions that prevent the co-precipitation of nicastrin and presenilin heterodimers (e.g., the presence of certain detergents) obviate γ-secretase activity (100). Although these data indicate that presenilin and nicastrin are obligatory members of the γ-secretase complex, their co-expression still does not overcome the tight cellular regulation of γ-secretase activity, and additional factors must exist in the active enzyme complex.

Evidence for two additional required components of γ-secretase has recently emerged from genetic analyses of Notch signaling in *C. elegans*. Mutational screens identified a putative 7-TMD protein, designated aph-1, mutations that result in a phenotype closely resembling loss of function in Notch (101) and pen-2, a 101-residue polypeptide with two TMDs (102). Both *aph-1* and *pen-2* showed strong genetic interaction with *sel-12*/PS and *aph-2*/nicastrin. Importantly, inactivation by RNA interference of *aph-1*, *pen-2*, and *nicastrin* in cultured *Drosophila* cells decreased γ-secretase cleavage of APP and Notch reporter substrates and reduced the levels of presenilin heterodimers (102). These results mean that all three proteins play a role in regulating presenilin, the likely catalytic component of the protease. Mutagenesis screens in nematodes have not revealed any other proteins absolutely required for Notch cleavage.

Thus, it currently appears that presenilin, nicastrin, aph-1, and pen-2 are the key components required for γ-secretase to cleave substrates. Accordingly, several experiments can now complete the story. RNA interference-mediated inactivation of each component in mammalian cells should confirm the necessity for each of them in substrate proteolysis in higher organisms. Similarly, transfection into mammalian cells, which have endogenous γ-secretase activity, could prove that

nicastrin, aph-1, and pen-2 together release the tight regulation of presenilin heterodimer levels, allowing more endoproteolysis of full-length presenilin and thus increased production of Aβ, AICD, and the Notch intracellular domain. Indeed, this has recently been accomplished (W.T. Kimberly, M. Wolfe, D.J. Selkoe, et al., unpublished data). Transfection of the four components individually and in various combinations into a cell that has no endogenous γ-secretase activity (e.g., yeast) could establish whether cleavage activity is only reconstituted when all four are present. As such experiments prove successful, the subunits of γ-secretase will have been unequivocally identified, and detailed study of the other biochemical requirements for efficient proteolysis can begin, including presenilin-associated proteins that may facilitate (or inhibit) the reaction, ionic and energy parameters, and the subcellular loci where the components assemble to yield mature enzyme.

How Does Scission by γ-Secretase Occur, and How do Presenilin Mutations Alter Cleavage Specificity?

Based on our current understanding of this very unusual protease, it appears that the two catalytic aspartates interact with a scissile bond in the TMD of a substrate within the lipid bilayer, and the protease initially engages the substrate in its α-helical conformation (103, 104). The tight α-helical conformation of the TMD presumably must be transiently relaxed to allow access of the presenilin aspartates and other residues of the active site to the scissile bond, but how this is accomplished is unclear. A water molecule is necessary for scission, and this may enter the protease via a pore-like structure formed by the eight TMDs of the presenilin heterodimer plus some or all of the TMDs of aph-1, pen-2, and/or nicastrin. The water molecules must somehow be sequestered from the substrate within the membrane until cleavage is actually required. It has been shown that deletion of TMDs 1 and 2 of PS1 abrogates its function, whereas deletion of much of the large cytoplasmic loop, which contains the binding site for the catenins, has no effect (105). Therefore, the protein-to-protein interactions needed for presenilin hydrolytic function are likely to principally involve the various TMDs and to occur within the membrane. In addition, nicastrin, the one required component of the complex with a large ectodomain, could potentially help anchor the ectodomains of the full-length substrates, prior to cleavage, and perhaps even "measure" their length in a way that allows correct cleavage by the proteases that shed the ectodomains of the substrates, such as members of the disintegrin and metalloprotease (ADAM) family. The latter speculation assumes that the α- and β-secretases transiently associate with the presenilin/γ-secretase complex to effect shedding of the substrate ectodomains, thus producing a conformational change in C83 or C99 that permits the secondary γ-secretase cleavage (106).

The two principal γ-secretase cleavage sites in APP, Aβ40 and Aβ42 are separated by two residues, i.e., they are about one half of a helical turn apart and thus on opposite faces of the substrate. It could be that the large majority of C99 and C83 substrate molecules have their Aβ40–41 bonds oriented towards the two

aspartates of particular presenilin heterodimers at the time of cleavage, whereas a small minority (~10%) have their 42–43 bonds oriented towards the aspartates of other presenilin heterodimers, thus yielding the ratio of Aß42 to Aß40 products (~1:10) documented in vivo. All of the known alterations in PS1 and 2 that cause AD are missense mutations, and most are located within the TMDs or adjacent to them. Presumably, these subtly alter the conformation of the active site and/or other critical portions of the TMDs in a way that decreases the efficiency of cleavage of C99 and C83 at the Aβ40–41 peptide bond and/or increases that at the Aβ42–43 bond. This would explain the increased ratio of Aβ42 to Aβ40 peptides that these mutations cause. Of course, even this model is a gross oversimplification because γ-secretase can cleave C99, and presumably C83 as well, at numerous different bonds between Aβ positions 35 and 43.

γ-Secretase Inhibitors Can Lower Aβ In Vitro and In Vivo and Decrease the Formation of Potentially Synaptotoxic Oligomers

Long before the identity of γ-secretase became apparent, the discovery of the normal cellular production of Aβ made the screening of compound libraries in whole cell assays possible, with the goal of detecting molecules that lowered Aβ secretion without inducing general cellular toxicity. Such screening should have uncovered both β- and γ-secretase inhibitors, but analyses of APP processing suggested that virtually all of the potential inhibitors identified acted at the level of γ-secretase. Despite the substantial difficulties of conducting screens in whole cells rather than in purified enzyme systems, the screening efforts of several companies yielded a substantial number of compounds of different structural classes that appear to inhibit the activity of γ-secretase. Extensive medicinal chemistry efforts on some of these molecules ensued. One of the resultant compounds (DAPT) has been reported to produce an acute, dose-dependent reduction of brain and plasma Aβ in vivo when administered orally to APP transgenic mice (107). A single dose of 30 mg/kg yielded an ~30% decrease in cortical Aβ40 and Aβ42 levels measured 3 h later (Figure 4). DAPT was shown to raise brain levels of C83 and C99 in vivo, confirming it as a bona fide γ-secretase inhibitor.

There is growing evidence that diffusible oligomeric assemblies of Aβ, rather than mature amyloid fibrils, may be the principal mediators of neurotoxicity in AD and models thereof (42, 108–111). In this context, certain APP-overexpressing cultured cells generate small amounts of stable Aβ oligomers that are detectable in the culture medium. Microinjection of such medium into the lateral ventricles of anesthetized rats resulted in a block of hippocampal long-term potentiation (LTP), an electrophysiological correlate of certain aspects of memory and learning (112). Moreover, pretreatment of the cultured cells with a γ-secretase inhibitor (a close analog of DAPT) partially lowered monomer production enough to markedly decrease oligomer formation, and microinjection of this medium into rat brain no longer interrupted LTP (112). These results suggest that soluble, potentially synaptotoxic oligomers of Aβ can be targeted therapeutically with γ-secretase inhibitors.

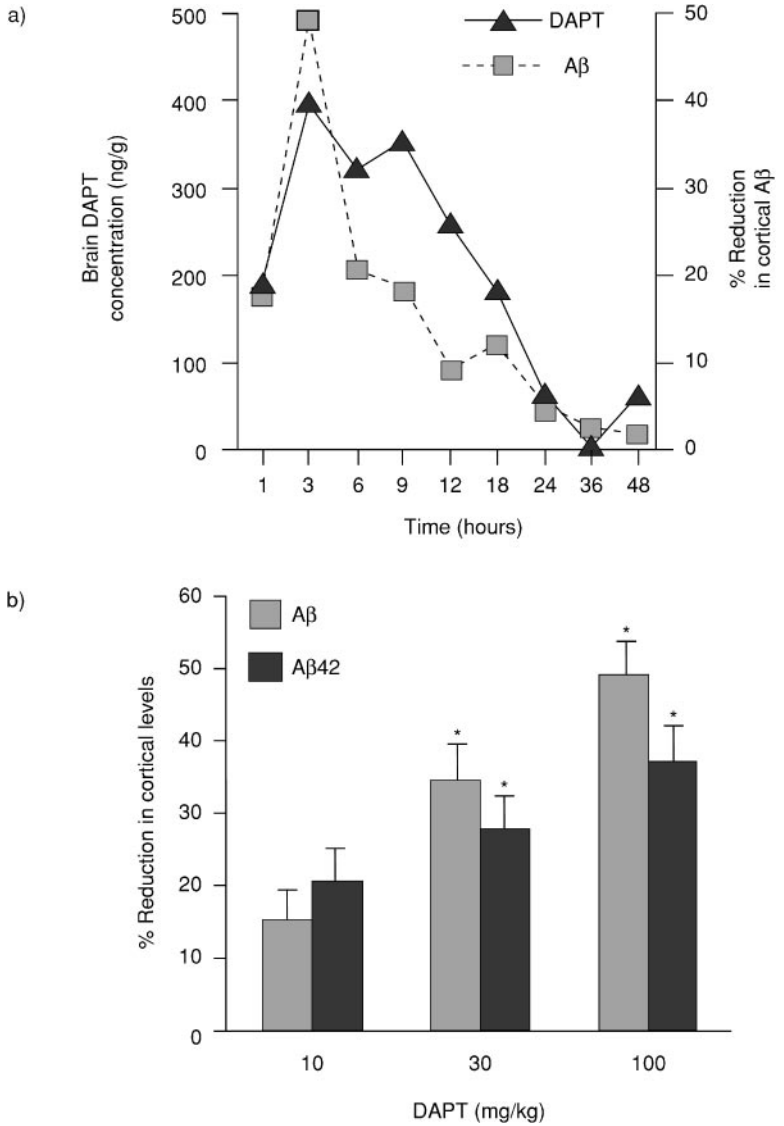

Figure 4 Effect of DAPT administration on Aβ in PDAPP mice. (*a*) Brain concentrations of DAPT and percentage reduction in cortical total Aβ levels after subcutaneous administration of DAPT (100 mg/kg). Aβ levels were significantly reduced compared with baseline from 1 to 18 h after administration, and a peak reduction of 40% occurred after 3 h. Aβ levels closely reflected DAPT concentrations. (*b*) Total cortical levels of Aβ and Aβ42 (expressed as a percentage of levels in vehicle-treated controls) 3 h after oral administration of DAPT (10, 30, or 100 mg/kg) to PDAPP mice. $^*p < 0.05$ versus vehicle-treated control. Reproduced with permission from Reference 107.

Concerns About Inhibiting the Cleavage of Notch and Other Substrates Limit the Potential Utility of Current γ-Secretase Inhibitors

The remarkably similar proteolytic processing of APP and the Notch receptors by α- and β-secretases has raised the question of whether it will be possible to reduce Aβ production by this approach without significantly interfering with the functions of Notch signaling in adults. These functions include a variety of cell fate decisions necessary for the proper differentiation of hematopoietic, immune, mucosal, and skin cells, among others. However, mutational and pharmacological studies examining Notch and presenilin suggest that presenilin-mediated cleavage can be inhibited rather substantially without significantly decreasing the amount of Notch-mediated signaling. For example, mice expressing just one allele of PS1 and no PS2 alleles show apparently normal development (113). As it is generally assumed that lowering the steady state levels of Aβ by just 30% may prove beneficial in slowing the progression of AD, this level of inhibition may leave a sufficient reserve of presenilin-mediated Notch signaling to avoid significant adverse events. However, increasing numbers of presenilin/γ-secretase substrates are emerging, and it will be necessary to determine whether chronic, partial interference with the processing of one or more of these produces adverse effects in vivo. If some of the components of the γ-secretase complex act to bind various presenilin substrates differentially and offer them to the active site, then inhibitors directed at one of these components and not the active site (the PS aspartates) may be less problematic. Of course, the use of a β-secretase inhibitor would not entail these particular risks. Resolution of the central issue of whether γ-secretase inhibitors can lower Aβ production without significantly impairing the normal functions of other substrates awaits further studies in animals and subsequent clinical trials.

An intriguing approach to the problem would be to inhibit production of only the highly amyloidogenic Aβ1–42 form of the peptide or to shift the cleavage specificity of γ-secretase away from position 42, without altering overall Aβ levels. A recent report suggests that certain members of an existing class of FDA-approved drugs, the non-steroidal anti-inflammatory drugs (NSAIDs), may do just that if administered at appropriate doses (114). The treatment of Aβ-secreting cells with the NSAIDs sulindac sulfate, ibuprofen, or indomethacin reduced Aβ42 and increased Aβ38 levels in the culture medium at doses that did not significantly decrease Aβ40 levels. This selective lowering of Aβ42 was also observed in the brains of APP transgenic mice in short-term in vivo experiments. Other NSAIDs (e.g., naproxen sodium) had no effect, indicating that the shift in Aβ production was unrelated to the inhibition of cyclo-oxygenase. This surprising result suggests that certain NSAIDs may have actions directly on γ-secretase that are independent of any anti-inflammatory effects. Chronic use in humans of some of the NSAIDs that lowered Aβ42 levels in this paradigm has been associated with a significantly lower risk of developing AD in epidemiological studies (115). Thus, this work raises

the specter of interfering selectively with Aβ42 production without otherwise perturbing γ-secretase-mediated processing of APP and other substrates. The doses of NSAIDs used to date are very high and considerable further work in animal models and prospective studies in humans is needed to determine whether chronic NSAID administration could have true therapeutic benefit.

CLEARING Aβ FROM THE BRAIN: THE IMMUNOLOGICAL APPROACH

Rationale

The immunological approach to the treatment of AD involves either stimulating the host immune system to recognize and attack Aβ or providing antibodies passively, thereby enhancing the clearance and/or preventing the deposition of Aβ plaques. The immune system can be stimulated in a number of ways, eliciting T cell–mediated and/or humoral responses. Several different types of immunotherapy are currently under investigation for AD: active immunization with synthetic intact Aβ42 or conjugated fragments thereof, and passive immunization with human anti-Aβ monoclonal antibodies (mAb). Immunization with intact Aβ42 has been evaluated in transgenic mouse models and has proceeded to clinical trials, whereas the other immunization strategies are approaching clinical development.

Active Immunization Strategies: Effects in Mouse Models

Aβ42 IMMUNIZATION The hypothesis that Aβ42 immunization could modify the disease process in AD was initially tested in young PDAPP transgenic mice carrying the V717F APP mutation (39), with treatment beginning at six weeks of age, prior to the development of any Aβ deposits (116). In the two active treatment groups, animals were injected with 100 μg of either synthetic Aβ42 or serum amyloid-P component (SAP, another protein associated with amyloid plaques) at monthly intervals for 11 months, combined with Freund's complete adjuvant for the first four doses. Control animals received adjuvant alone in phosphate-buffered saline (PBS) or no treatment. Quantitative image analysis of brains from 13-month-old mice indicated the almost complete absence of hippocampal Aβ42 plaque formation in the Aβ42 group (median plaque burden 0% of cross-sectional area versus 2.22% in adjuvant/PBS-treated controls and 2.65% in untreated mice; $p = 0.0005$). There were parallel reductions in dystrophic neurites (median 0% versus 0.28% in adjuvant-treated controls; $p = 0.0005$) and astrocytosis (median 1.55% versus >6% for all other groups; $p = 0.0017$). Histological examination of several organs, including the brain and kidneys, revealed no signs of immune-mediated complications in any treatment group. Serological analysis showed that the majority of mice immunized with Aβ42 (8 of 9) developed and maintained high serum antibody titers (>1:10,000) against Aβ42. SAP-treated mice mounted

an immune response to SAP (titers generally 1:1000–1:10,000), but exhibited an increase in plaque burden (median 5.74%), suggesting that immune responses against plaque components per se do not prevent or eliminate amyloid deposition. This particular experiment has potentially important implications regarding SAP, as it has been suggested that agents acting upon SAP may be useful in cleaving deposits in a broad range of amyloidoses (117). Subsequent experiments showed that Aβ production itself was not disrupted by the immunization. The paucity of neuritic and gliotic changes suggested that immunized mice did not develop the neurodegenerative cytopathology normally seen in the PDAPP model.

The next series of experiments were designed to establish whether the neuropathological outcome could be improved if Aβ42 immunization was initiated when there was already a substantial Aβ plaque burden (116). Immunization of PDAPP mice began at 11 months with monthly immunizations (100 μg) that continued for 4–7 months. Quantitative image analysis showed that the median cortical Aβ burden in 18-month-old immunized mice was 0.01%, compared with 4.87% for untreated age-matched controls and 0.28% for untreated 12-month-old mice. The reduction in Aβ burden was 96% after four months of treatment and $>$99% after seven months (Figure 5). Again, neuritic pathology and astrocytosis were reduced (by 55% and 34%, respectively, after seven months of immunization). The brains of 15- and 18-month-old immunized mice contained fewer diffuse and mature Aβ deposits than those of 12-month-old untreated mice, suggesting that immunization with Aβ42 had resulted in the clearance of pre-existing Aβ plaques. Analysis of different areas of the cortex indicated that immunization disrupted the normal progressive pattern of amyloidogenesis in PDAPP mice (which begins in the cingulate, frontal, and retrosplenial cortices and progresses in a lateral-ventral fashion). Immunization also prevented the usually heavy Aβ deposition in the outer molecular layer of the hippocampal dentate gyrus. Antibody titer responses to Aβ42 immunization in these experiments were similar to those described above for the younger (pre-plaque) animals.

Additional evidence supporting the concept of an immunotherapeutic approach for AD has been provided by assessments of the functional effects of Aβ42 immunization (118, 119). In experiments in a different transgenic model in which mice expressing APP (APP K670N/M671L and M146L) develop learning deficits as the amyloid burden accumulates, 5 months of immunizations with Aβ42 (100 μg/month), beginning at age 7.5 months, had no adverse effects on performance in a novel working-memory task (the radial-arm, water-maze test) (Figure 6*a*). Subsequently, at an age when the transgenic animals would be expected to exhibit cognitive deficits (15.5 months), Aβ42-immunized mice showed better cognitive performance than those who received a control immunization, and ultimately their performance became similar to that of non-transgenic mice (Figure 6*b*). Modest reductions in cortical and hippocampal Aβ40 and Aβ42 deposits were also observed (118). The observed difference in the magnitude of reductions in amyloid burden between this study and that of Schenk et al. (116) is

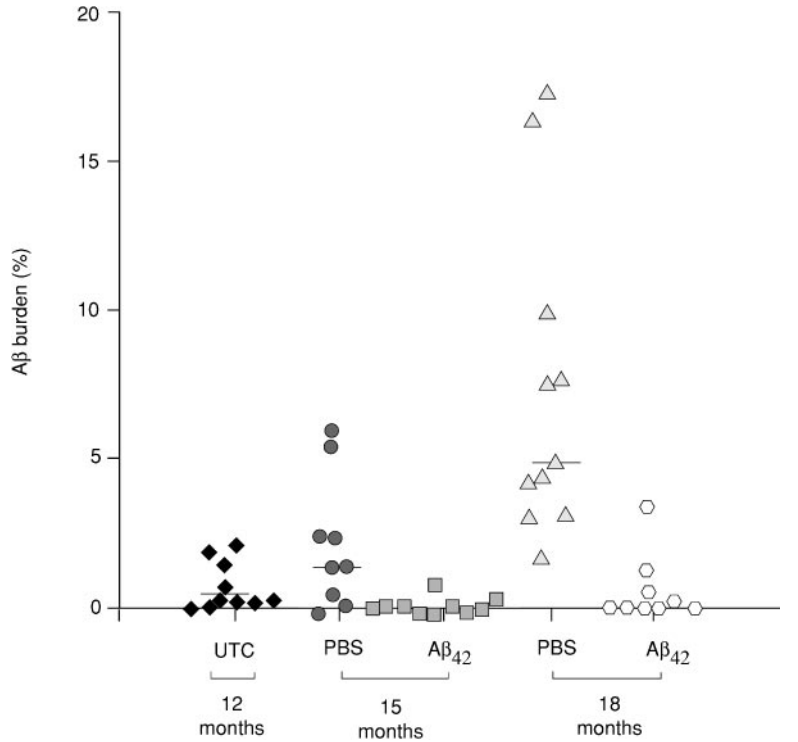

Figure 5 Quantitative image analysis of the cortical Aβ burden in older PDAPP mice (116). Treatment began at 11 months of age. Amyloid burden was significantly reduced in the Aβ group, compared with the PBS controls at both 15 and 18 months of age ($p = 0.003$ and 0.0002, respectively). The median value of the amyloid burden for each group is shown by the horizontal lines. UTC: untreated controls; PBS: phosphate-buffered saline controls; Aβ: amyloid β-protein. Reproduced with permission from Reference 116.

likely to be related to the mouse model used and the duration of the immunization period.

The behavioral consequences of Aβ42 immunization have also been explored in the TgCRND8 (APP K670N/M671L and V717F) transgenic murine model, in which the formation of Aβ plaques was accompanied by the development of spatial learning deficits by three months of age (119). Performance in the Morris water maze of 23-week-old mice immunized with Aβ42 protofibrillar assemblies in the β-pleated sheet form (100 μg/month) from 6 weeks of age was significantly better than that of control transgenic mice ($p < 0.05$), although inferior to that of non-transgenic littermates. Immunization resulted in the formation of antibodies that decorated extracellular dense-cored plaques and reduced Aβ plaque deposition,

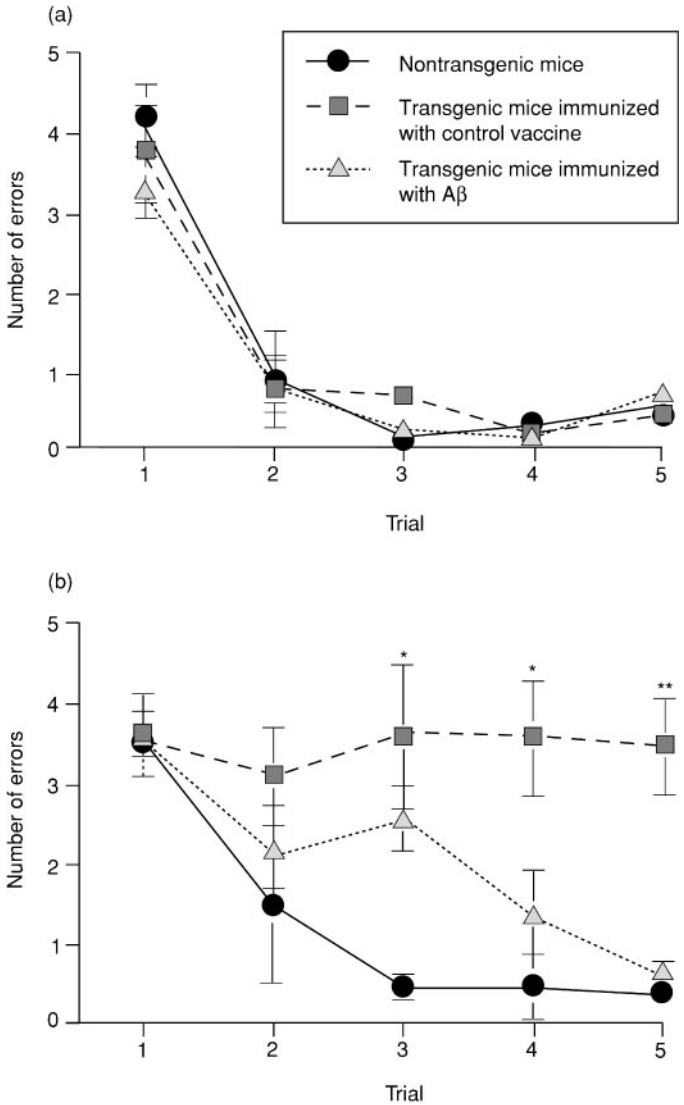

Figure 6 Effect of Aβ immunization on performance in the radial-arm water maze. (*a*) Nontransgenic mice, APP transgenic mice immunized with control vaccine, and transgenic mice immunized with Aβ were tested in the radial-arm water maze at 11.5 months of age (after 5 injections). All groups learned (*trial 4*) and remembered (*trial 5*) the platform location at this time point. (*b*) APP transgenic mice immunized with Aβ continued to show learning and memory of the platform location, whereas the transgenic mice who received the control treatment failed to show learning and memory for platform location on either trial 4 or 5 ($^{*}p < 0.05$, $^{**}p < 0.01$; control immunization group significantly different from the other two groups by LSD post-hoc analysis after MANOVA). Reproduced with permission from Reference 118.

although total brain levels of Aβ were unchanged. These results suggest that the induced antibodies were directed predominantly against Aβ in the β-sheet conformation, rather than in its more soluble form.

The nasal administration of Aβ in PDAPP mice has also been described (120). Weekly administration of Aβ40 (25 μg), beginning at age 5 months and continuing until age 12 months, was associated with a 60% reduction in plaque burden in the hippocampus, decreased neuritic dystrophy, and a reduced level of microglial and astrocytic activation. The presence of anti-Aβ antibodies was demonstrated in the serum of immunized mice, and a slight mononuclear cell infiltration in brain tissue was characterized by cytokine expression.

In summary, Aβ42 immunization results in the clearance of pre-existing Aβ deposits and also prevents the development of new Aβ plaques and associated neuropathology in transgenic murine models of AD. Furthermore, the reduction in Aβ burden is accompanied by improved cognitive performance. No adverse effects of Aβ42 immunization were observed in these models.

The underlying mechanism by which the beneficial effects of Aβ42 immunization are mediated has yet to be explained, although it is conceivable that more than one process is involved. One possibility is that the reduction in Aβ deposition is due to Fc-mediated microglial phagocytosis (116). This hypothesis is supported by the observation that a proportion of the plaques found in immunized mice were decorated with IgG and that microglia co-localized with Aβ within plaques. Furthermore, microglia/monocytes were invariably located near the remaining plaques. The effects of peripherally administered antibodies against Aβ are also consistent with this hypothesis (121).

THE IMMUNOCONJUGATE APPROACH A distinct approach to active immunization is the use of conjugated Aβ fragments from the region of the peptide that does not include the T cell epitopes (i.e., residues 16, 20, 25, and 30) and hence induces only a B cell response. The results of this immunization approach in mouse models have not yet been reported.

Passive Immunization Strategies: Effects in Mouse Models

Passive immunization using mAb raised against synthetic peptide fragments represents an alternative immunotherapeutic strategy. In addition, studies of this approach in transgenic mice have provided further insights into the possible mechanism(s) underlying the observed benefits of active immunization described above. These experiments showed that a humoral response alone, in the absence of a cellular response to Aβ, is sufficient to elicit a strongly efficacious.

In initial experiments, heterozygous PDAPP mice aged 8–10 months received weekly intravenous injections of mouse mAb, either 10D5 (Aβ1–16), 21F12 (Aβ33–42), or polyclonal immunoglobulins (Ig) to Aβ1–42. Stable serum antibody concentrations were maintained throughout the six-month study. At the study's end, mean cortical Aβ42 levels in the 10D5, 21F12, Ig, and control groups

were 6200, 13,580, 4890, and 13,800 ng/g tissue, respectively. Two further mAb, 3D6 (Aβ1−5) and 16C11 (to the tau protein), in addition to 10D5, were subsequently administered to slightly older mice (11.5–12 months). The reduction in cortical Aβ42 burden after six months of treatment with 3D6 was similar to that seen with 10D5 (86% versus 80%; both $p = 0.003$ versus controls), whereas 16C11 had no effect (Figure 7). As expected, the mice in these experiments did not exhibit a T cell proliferative response.

Examination of brain sections of mice involved in these studies revealed that 10D5 and 3D6 entered the brain and partially bound to the Aβ plaques. Subsequent experiments demonstrated that these antibodies triggered microglial cells to clear Aβ plaques through Fc receptor-mediated phagocytosis and subsequent degradation (121). However, DeMattos et al. (122, 123) have reported that a mAb

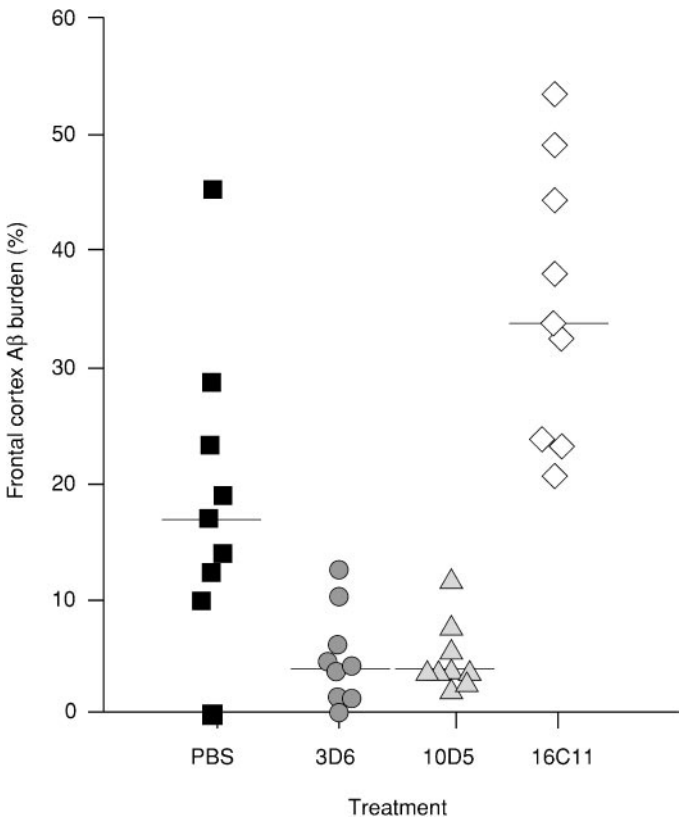

Figure 7 Effect of six months of treatment with monoclonal antibodies directed against Aβ on the Aβ burden in the frontal cortex of PDAPP mice. PBS: phosphate-buffered saline; 3D6: anti-Aβ1-5; 10D5: anti-Aβ1-16; 16C11: anti-tau. Horizontal lines indicate median values. Reproduced with permission from Reference (121).

(m266) directed against the central domain (residues 13–28) of Aβ does not bind to the Aβ plaques, yet still reduces cerebral Aβ burden. Their findings in a series of in vitro and in vivo experiments suggest that intravenous administration of m266 shifts the CNS-plasma Aβ equilibrium by acting as a peripheral sink (122, 123). They also showed that 3D6 and 10D5 [two of the mAb used by Bard et al. (121)] had a similar, although smaller, effect in their in vitro studies (122). These two mechanisms are not mutually exclusive, and both could be operative. A further potential mechanism by which Aβ immunization could work is suggested by the studies of Solomon et al. (124, 125), in which antibodies raised against the hydrophilic N-terminal region (1–28) of Aβ prevented the fibrillar aggregation of Aβ in vitro.

Moving from Mouse to Man

Following the successful outcomes of the studies of murine Aβ42 immunization in several laboratories, clinical trials were initiated. The immunogen used in these studies, AN-1792 (Elan Pharmaceuticals/Wyeth), contained synthetic Aβ42 in combination with an adjuvant (QS-21). Two Phase I studies were undertaken to evaluate the safety and tolerability of AN-1792 in patients with AD. The first was a single-dose study with three escalating Aβ42 dosage levels, but with a fixed adjuvant concentration. There were eight patients per dose level and a six-week period between escalations. The second Phase I study was a multi-dose (for both Aβ42 and QS-21 adjuvant), dose-escalation study, with a two to three month interval between each dose level.

As AN-1792 was well tolerated and a subset of patients developed an immunological response in these two studies, Phase IIa studies began in the United States and Europe. The Phase IIa program recruited 375 AD patients, and those in the active treatment group ($n = 300$) received multiple doses of the highest dose of Aβ42 combined with the lowest dose of QS-21 used in the Phase I trials. However, the program was suspended in January, 2002 after some patients in the active treatment group developed the signs and symptoms of aseptic meningo-encephalitis. All affected patients had received one to three doses of AN-1792, and their symptoms developed five days to five months after their last dose. Although the clinical development of AN-1792 has been discontinued, the study blind for the remaining patients has not been broken, and these patients will continue to be monitored for safety, CNS changes, cognitive decline, and immune function for a further year.

These reactions were unexpected, as toxicological testing in five different animal species, including primates, did not reveal any evidence of encephalitis or other adverse effects, and at the time of commencement of the Phase IIa study, none of the 64 patients receiving active treatment in the Phase I trials had developed any signs of brain inflammation. The reactions in some of the patients in the Phase IIa program do appear to be clearly linked to immunization, although the underlying mechanism has not yet been established. Ongoing tests are investigating whether the reactions in some of the patients in the Phase IIa program may be

linked to immunization, for example, through a T cell–mediated immune response to AN-1792.

Although AN-1792 has been discontinued, the development of safe and effective immunotherapies for AD is being actively pursued, based on the dramatic preclinical data. Several second-generation immunotherapeutic products with properties very different to those of AN-1792 are currently in active development.

STIMULATING α-SECRETASE CLEAVAGE OF APP TO LOWER Aβ

Neurotransmitter-Mediated Enhancement of α-Secretase Activity

Those APP molecules that undergo processing by α-secretase release p3 rather than Aβ. Although p3 has been detected in some plaques in the AD hippocampus, cortex, and cerebellum (126, 127), Aβ appears to be a far more abundant constituent of neuritic plaques. Consequently, enhancing the flux of APP molecules through the α-secretase, rather than β-secrease, pathway may be beneficial in AD. The proteolytic activities of ADAM 10 and ADAM 17 (TACE), which appear to be α-secretases for APP, can be up-regulated via stimulation of several neurotransmitter systems. The first to be recognized were the M_1 and M_3 muscarinic acetylcholine receptors acting through the phospholipase C/protein kinase C pathway (128). Agonists acting at these receptors were shown not only to increase APP_s-α levels in cultured cell medium, but also to reduce Aβ secretion, suggesting a competition for substrate between α- and β-secretases (129, 130). Moreover, systemic administration of the acetylcholinesterase inhibitor physostigmine for 10 days lowered cortical Aβ levels by ~30%–40% without altering full-length APP concentrations in guinea pigs (131). Similarly, 30%–45% and 15%–40% reductions in CSF levels of Aβ40 and Aβ42, respectively, were reported in rabbits treated for five days with one of three different M_1-selective muscarinic agonists (132). In clinical trials, two studies have reported approximately 25% reductions in CSF Aβ levels after administration of selective M_1 agonists to small numbers of AD patients (133).

Several other neurotransmitters that act through phosphatidylinositol hydrolysis and activation of protein kinases can increase APP_s-α secretion. Agonists of metabotropic glutamate receptors did so in cultured cortical astrocytes, and this was reversed by dibutryl cyclic AMP (134). Moreover, serotonin produced a three- to four-fold, dose-dependent increase in APP_s-α release in cells overexpressing 5-HT_{2A} or 5-HT_{2C} receptors (135), and stimulation of the G-protein-coupled receptors for bradykinin and vasopressin with their respective neuropeptides increased α-secretase processing of APP in PC-12 cells (136). In addition, electrical depolarization increased APP_s-α release in hippocampal and cortical slices (137).

Although such data suggest that cholinergic treatments could decrease Aβ levels while ameliorating acetylcholine deficits, there are theoretical concerns about chronic stimulation of α-secretase. As the APP ectodomain has several pharmacological activities, prolonged treatment could have adverse effects. Moreover, α-secretases have numerous substrates, making biological effects independent of those of APP_s-α likely. However, as β- and γ-secretase inhibitors are not yet available, further clinical trials of prolonged stimulation of α-secretase processing should be undertaken.

Cholesterol-Lowering Agents

Several lines of evidence lend increasing support to the concept that altering cholesterol homeostasis can change the production of Aβ in vitro and in vivo. The genetic linkage of apoE4 to both AD risk and Aβ burden first raised this issue, as the ε4 allele is associated with higher circulating cholesterol levels (138). There is also a complex body of literature suggesting that atherosclerosis may be associated with an increased risk of developing AD, although this could be explained, at least in part, by the co-occurrence in elderly patients of two independent processes: multi-infarct dementia and AD. A more direct mechanistic link between cholesterol and AD comes from the recognition that a fraction of neuronal APP is present in low-density, Triton-insoluble glycosphingolipid-cholesterol rafts (139), which appear to be one site for the conversion of APP to Aβ (140, 141). Reducing cholesterol levels in cultured hippocampal neurons by ~70% with lovastatin and methyl-β-cyclodextrin markedly inhibits Aβ formation without affecting neuronal viability or holoAPP and APP_s-α levels (142). The latter authors also obtained data suggesting that β-secretase-mediated processing of APP (not α-secretase) was affected by such treatment, as C99 levels were lowered and p3 levels were unchanged. However, another group treated non-neural and neural cells lines with either lovastatin or methyl-β-cyclodextrin and found a marked increase in APP_s-α production, including that generated by ADAM 10, with a concomitant decrease in Aβ secretion (143). In this study, cholesterol depletion in the non-neural cells inhibited internalization of surface APP, allowing more α-secretase processing. Clearly the precise mechanisms by which cholesterol-lowering agents reduce Aβ production require further study.

In vivo experiments have demonstrated that a three-week administration of very high doses of simvastatin markedly and reversibly decreased de novo brain cholesterol synthesis and lowered CSF levels of Aβ40 by ~50% and Aβ42 by ~40% in guinea pigs (144). Moreover, a similar reduction in total brain Aβ levels was observed. Treatment of double mutant PS1/APP transgenic mice for five weeks with the cholesterol-lowering drug BM15.766, which is brain-penetrant and inhibits the last step in cholesterol biosynthesis, decreased brain Aβ levels and plaque burden by ~50% (145). Brain levels of APP_s-α were increased, and those of C99 were reduced. Conversely, administration of a high fat/high cholesterol diet to this mouse line for seven weeks led to a significant 50% increase in total brain Aβ levels, which

correlated strongly with plasma and brain total cholesterol content (146). This diet increased both the number and size of Aβ deposits. The hypercholesterolemic mice showed decreased APP_s-α levels and increased C99 levels.

Three retrospective epidemiological analyses of statin usage and AD have been reported to date. One study examined records from three hospitals and found that the prevalence of a diagnosis of probable AD was approximately 60%–70% lower in patients taking lovastatin or pravastatin than in patients receiving other cardiovascular medications (147). The second study included 284 patients with dementia and 1080 matched controls (all aged >50 years) drawn from a general practice patient database in the United Kingdom (148). The adjusted relative risk of a diagnosis of dementia in the statin-treated patients was 0.29 of that in the controls ($p = 0.002$), whereas the risk in patients receiving other lipid-lowering agents was 0.96. The records did not allow a distinction between AD and other dementias. The third report was a case-control study of 492 patients with dementia, 326 of whom had clinically probable AD, and 823 controls in relation to the use of lipid-lowering agents (149). It was found that the use of statins and other lipid-lowering agents reduced the risk of AD in patients aged <80 years old (odds ratio: 0.26).

These collective data provide considerable circumstantial evidence that chronically lowering cholesterol levels, particularly with statins, may decrease cerebral Aβ concentrations, perhaps by enhancing α-secretase cleavage at the expense of the amyloidogenic processing of APP. However, no prospective randomized treatment trials of statins in AD have been reported. Until this occurs, chronic statin administration solely for the purpose of treating or preventing AD cannot be recommended.

CONCLUSIONS: THE FUTURE OF THE Aβ HYPOTHESIS AND ITS ROLE IN ALZHEIMER'S DISEASE

As with any medically driven scientific hypothesis, the ultimate value of the amyloid hypothesis is directly proportional to its clinical utility. A myriad of scientific achievements have occurred since George Glenner first identified the Aβ peptide in amyloidotic vessels in 1984 (150). Biochemical prowess combined with elegant genetics and careful neuropathological analyses have advanced the field to a point where there are numerous anti-amyloid molecules undergoing, or close to, therapeutic evaluation in AD patients.

The various treatment targets arising from research related to the amyloid hypothesis include inhibition of γ- or β-secretase activity and Aβ-related immunotherapeutic approaches. An additional approach not covered in this review involves strategies aimed at reducing the aggregation properties of the Aβ peptide. Thus far, γ-secretase inhibitors and Aβ42 peptide immunization have begun to be tested clinically, although neither approach has extended much beyond safety studies. Thus, clinical trials have not advanced to the point of actually testing the

amyloid hypothesis. It is probable, nevertheless, that modulators of γ- or β-secretase and refinements of Aβ immunotherapy (both passive antibody administration and Aβ-peptide conjugate immunization) will enter the clinic before long. In short, we currently stand on the threshold of convincingly testing the amyloid hypothesis.

Questions Remaining

Aβ NEUROTOXICITY Many questions remain regarding the relationship of Aβ to the cellular alterations underlying dementia in AD. The most notoriously difficult one to answer concerns the nature of the neurotoxic effect of the peptide itself in the brain. Both old and new work in this area has refined and clarified this issue considerably. For example, it has recently been directly shown that soluble oligomers of Aβ alter the electrophysiology of neurons in the brain at very low concentrations when microinjected in vivo, whereas monomers of the peptide do not (112). This and other related findings (108–111) have resulted in a growing consensus that it is probably small oligomers, rather than large fibrillar plaques, that principally cause the synaptotoxicity associated with the disease. Nonetheless, the complex equilibria associated with the interconversion of monomers, oligomers, protofibrils, fibrils, and very large aggregates of the Aβ peptide, together with the difficulty of measuring real-time concentrations of these various species in vivo, means that the debate about precisely which species of Aβ injures neurons and their processes in vivo will continue for some time.

γ-SECRETASE Compelling data have been reviewed here regarding the argument that presenlins mediate γ-secretase cleavage activity. In addition, a number of potent inhibitors of γ-secretase activity have been identified, and several of these have been chemically crosslinked directly to presenilins. Still unresolved, however, is exactly how many other proteins are required for full γ-secretase activity, and what their respective functions are. These are important questions, not just from the perspective of the basic science of this signaling hub, but also from the viewpoint that some compounds may be capable of selectively interacting with such proteins and thereby have an allosteric effect on γ-secretase activity. The final answer in this area will come from reconstitution studies in which various proteins are brought together in purified form and in detergents to generate γ-secretase activity, much like what has been done with β-secretase. Such an experiment could provide undeniable proof that presenilin is the authentic active site component of γ-secretase.

Also of great importance is the development of safe, orally active γ-secretase inhibitors. The observation that many general inhibitors of APP γ-secretase activity also inhibit Notch cleavage has concerned the pharmaceutical industry because Notch signaling is likely to be required for numerous normal processes in adults. The recent finding that certain NSAIDs can inhibit APP γ-secretase activity by shifting cleavage of Aβ42 to Aβ38, apparently without significantly perturbing

Notch cleavage (114), has provided encouraging results. Although the inhibitory potency of NSAIDs as a class (micromolar) does not recommend them as clinical candidates, they represent a pre-clinical proof of concept that it may be possible to design highly specific Aβ42 inhibitors.

VALIDITY OF ANIMAL MODELS The various APP and tau transgenic mouse models of AD have now been studied for several years. Nevertheless, much remains to be learned from these models. For example, it is clear from many studies that the Aβ deposits in these models are strikingly similar, though not identical, to those seen in AD. In addition, the neuritic dystrophy, gliosis, perikaryal changes, and even behavioral and electrophysiological phenotypes continue to suggest the utility of such mice for modeling the process of AD. Even neurofibrillary tangles can been seen in certain tau-APP "bigenic" mice. Though not entirely absent, neuronal loss in these animal models is subtle and not widespread. Finally, a central question remains: Will compounds that appear highly effective in APP transgenic mice, such as Aβ antibodies or γ-secretase inhibitors, prove to be useful in treating AD? Unfortunately there is little to be gained here from experience with other animal models of disease, as some have been highly predictive of human efficacy, and others not at all.

MECHANISM OF ACTION OF Aβ IMMUNOTHERAPY Immunization with Aβ and passive administration of anti-Aβ antibodies have both shown profound efficacy by many criteria in APP transgenic mice. What these results leave unanswered, however, is precisely how the antibodies are achieving their effect. Do they stimulate microglial phagocytosis of existing plaques (121), or do they achieve clearance by sequestering free Aβ in plasma, CSF, and brain extracellular fluid (122, 151). Experiments from many laboratories continue to attempt to address this question, as it may provide insight into the nature of Aβ peptide accumulation and toxicity and help direct future therapeutic approaches.

DESIGN OF CLINICAL TRIALS AIMED AT TESTING THE AMYLOID HYPOTHESIS As it is hoped that modulation or reduction of Aβ burden and associated cytopathology in AD will affect the course of the disease process, the primary outcome measures of current and future clinical trials based on the hypothesis will need to reflect this assumption. In practice, there are two main limitations of such trials. The first is that there is currently no validated method of detecting Aβ burden non-invasively in a living patient. Hence, it is not possible to know if an amyloid-lowering agent is achieving its desired immediate result. The second practical problem is the necessary length of such trials—typically a minimum of 18 months is needed to detect a sufficient change in the cognitive status of a cohort of AD patients in order to demonstrate a meaningful statistical difference. Thus, such trials require large numbers of patients and very significant time commitments to complete. Symptomatic agents, such as acetylcholinesterase inhibitors, likely have no effect on the underyling pathology but exert their pharmacological effects relatively rapidly,

thus circumventing one of the key limitations in assessing disease-modifying therapies. Ultimately, brain imaging modalities, such as functional magnetic resonance imaging and "amyloid scans," should prove useful as meaningful surrogates for testing the efficacy of anti-amyloid agents in AD.

Aβ and the Treatment of Alzheimer's Disease: The Future

Despite the formal delineation of the amyloid hypothesis well over a decade ago, we are only now beginning to test its application for treating AD. Unlike some medical hypotheses, this one appears to lend itself to many different approaches—from enzyme inhibitors to immunotherapy, to anti-aggregation compounds, to metal chelators. Each one of these approaches has its potential strengths and weaknesses. We can anticipate that meaningful treatment of this debilitating disease will involve more than a single therapeutic approach. Thus, despite the numerous approaches being actively pursued at present, still more are likely to be entertained in the future. As scientists, we eagerly await the outcome of these efforts; for patients, they cannot come soon enough.

ACKNOWLEDGMENTS

The authors thank the members of their respective research groups for many helpful discussions that contributed to the ideas presented here. DJS is a founding scientist of Athena Neurosciences, which is now a part of Elan, PLC.

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

LITERATURE CITED

1. Morris JC, Storandt M, McKeel DW, Rubin EH, Price JL, et al. 1996. Cerebral amyloid deposition and diffuse plaques in "normal" aging: evidence for presymptomatic and very mild Alzheimer's disease. *Neurology* 46:707–19
2. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. 1999. Mild cognitive impairment: clinical characterization and outcome. *Arch. Neurol.* 56:303–38
3. Dickson DW, Crystal HA, Bevona C, Honer W, Vincent I, Davies P. 1995. Correlations of synaptic and pathological markers with cognition of the elderly. *Neurobiol. Aging* 16:285–98
4. Lemere CA, Blustzjan JK, Yamaguchi H, Wisniewski T, Saido TC, Selkoe DJ. 1996. Sequence of deposition of heterogeneous amyloid beta-peptides and Apo E in Down syndrome: implications for initial events in amyloid plaque formation. *Neurobiol. Dis.* 3:16–32
5. Verbeek MM, de Waal RM, Vinters HV. 2000. *Cerebral Amyloid Angiopathy in Alzheimer's Disease and Related Disorders.* Dordrecht, The Netherlands: Kluwer. 357 pp.
6. Davies P, Maloney AJ. 1976. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* 2:1403
7. Perry EK, Gibson PH, Blessed G, Perry RH, Tomlinson BE. 1977. Neurotransmitter enzyme abnormalities in senile

dementia. Choline acetyltransferase and glutamic acid decarboxylase activities in necropsy brain tissue. *J. Neurol. Sci.* 34: 247–65
8. Bowen DM. 1977. Biochemistry of dementias. *Proc. R. Soc. Med.* 70:351–53
9. Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR. 1982. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* 215:1237–39
10. Moller HJ. 1999. Reappraising neurotransmitter-based strategies. *Eur. Neuropsychopharmacol.* 9:S53–59
11. Davies P, Anderton B, Kirsch J, Konnerth A, Nitsch R, Sheetz M. 1998. First in, last one out: the role of gabaergic transmission in generation and degeneration. *Prog. Neurobiol.* 55:651–58
12. Bennett GW, Ballard TM, Watson CD, Fone KC. 1997. Effect of neuropeptides on cognitive function. *Exp. Gerontol.* 32: 451–69
13. Kang J, Lemaire H-G, Unterbeck A, Salbaum JM, Masters CL, et al. 1987. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 325:733–36
14. Selkoe DJ. 2001. Alzheimer's disease: genes, proteins and therapies. *Physiol. Rev.* 81:742–61
15. Haass C, Schlossmacher M, Hung AY, Vigo-Pelfrey C, Mellon A, et al. 1992. Amyloid β-peptide is produced by cultured cells during normal metabolism. *Nature* 359:322–25
16. Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, et al. 1992. Isolation and quantitation of soluble Alzheimer's β-peptide from biological fluids. *Nature* 359:325–27
17. Shoji M, Golde TE, Ghiso J, Cheung TT, Estus S, et al. 1992. Production of the Alzheimer amyloid beta protein by normal proteolytic processing. *Science* 258: 126–29
18. Esch FS, Keim PS, Beattie EC, Blacher RW, Culwell AR, et al. 1990. Cleavage of amyloid beta peptide during constitutive processing of its precursor. *Science* 248:1122–24
19. Sisodia SS, Koo EH, Beyreuther K, Unterbeck A, Price DL. 1990. Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science* 248:492–95
20. Koo EH, Squazzo S. 1994. Evidence that production and release of amyloid beta-protein involves the endocytic pathway. *J. Biol. Chem.* 269:17386–89
21. Perez RG, Soriano S, Hayes JD, Ostaszewski BL, Xia WM, et al. 1999. Mutagenesis identifies new signals for β-amyloid precursor protein endotycosis, turnover and the generation of secreted fragments, including Abeta42. *J. Biol. Chem.* 274:18851–56
22. Cao X, Sudhof TC. 2001. A transcriptionally [correction of transcriptively] active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science* 293:115–20
23. Kimberly WT, Zheng JB, Guenette SY, Selkoe DJ. 2001. The intracellular domain of the beta-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a notch-like manner. *J. Biol. Chem.* 276:40288–92
24. Sastre M, Steiner H, Fuchs K, Capell A, Multhaup G, et al. 2001. Presenilin-dependent gamma-secretase processing of beta-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch. *EMBO Rep.* 2:835–41
25. Smith RP, Higuchi DA, Broze GJ. 1990. Platelet coagulation factor XIa-inhibitor, a form of Alzheimer amyloid precursor protein. *Science* 248:1126–28
26. Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, et al. 1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 349:704–6
27. Hardy J. 1997. The Alzheimer family of

diseases: many etiologies, one pathogenesis? *Proc. Natl. Acad. Sci. USA* 94:2095–97
28. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, et al. 1993. Apolipoprotein E: high-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 90:1977–81
29. Mahley RW, Rall SC Jr. 2000. Apolipoprotein E: far more than a lipid transport protein. *Annu. Rev. Genomics Hum. Genet.* 1:507–37
30. Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, et al. 1994. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer's disease. *Nat. Genet.* 7:180–84
31. Bales KR, Verina T, Dodel RC, Du Y, Altstiel L, et al. 1997. Lack of apolipoprotein E dramatically reduces amyloid β-peptide deposition. *Nat. Genet.* 17:263–64
32. Holtzman DM, Bales KR, Tenkova T, Fagan AM, Parsadanian M, et al. 2000. Apolipoprotein E isoform-dependent amyloid deposition and neuritic degeneration in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 97: 2892–97
33. Evans KC, Berger EP, Cho CG, Weisgraber KH, Lansbury PT Jr. 1995. Apolipoprotein E is a kinetic but not a thermodynamic inhibitor of amyloid formation: implications for the pathogenesis and treatment of Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 92:763–67
34. Ma J, Brewer HB Jr, Das S, Potter H. 1994. Amyloid-associated proteins alpha1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments. *Nature* 372: 92–94
35. Polvikoski T, Sulkava R, Haltia M, Kainulainen K, Vuorio A, et al. 1995. Apolipoprotein E, dementia, and cortical deposition of β-amyloid protein. *N. Engl. J. Med.* 333:1242–47
36. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, et al. 1993. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 90:9649–53
37. Levy-Lahad E, Wijsman EM, Nemens E, Anderson L, Goddard AB, et al. 1995. A familial Alzheimer's disease locus on chromosome 1. *Science* 269:970–73
38. Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, et al. 1995. Cloning of a novel gene bearing missense mutations in early onset familial Alzheimer disease. *Nature* 375:754–60
39. Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, et al. 1995. Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. *Nature* 373: 523–27
40. Hsiao K, Chapman P, Nilsen S, Ekman C, Harigaya Y, et al. 1996. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* 274: 99–102
41. Borchelt DR, Thinakaran G, Echman CB, Lee MK, Davenport F, et al. 1996. Familial Alzheimer's disease-linked presenilin 1 variants elevate A beta1-42/1-40 ratio in vitro and in vivo. *Neuron* 17:1005–13
42. Hsia AY, Masliah E, McConlogue L, Yu GQ, Tatsuno G, et al. 1999. Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc. Natl. Acad. Sci. USA* 96:3228–33
43. Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, et al. 1998. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat. Med.* 4:97–100
44. Wyss-Coray T, Masliah E, Mallory M, McConologue L, Johnson-Wood K, et al. 1997. Amyloidogenic role of cytokine TGF-beta-1 in transgenic mice and in Alzheimer's disease. *Nature* 389:603–6

45. Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, et al. 2001. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* 293:1487–91
46. Mayeux R, Sano M. 1999. Treatment of Alzheimer's disease. *N. Engl. J. Med.* 341:1670–79
47. Mattson MP, Cheng B, Davis D, Bryant K, Lieberburg I, Rydel RE. 1992. β-Amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. *J. Neurosci.* 12:379–89
48. Butterfield DA, Drake J, Pocernich C, Castegna A. 2001. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid β-peptide. *Trends Mol. Med.* 7:548–54
49. Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, et al. 2001. Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 30:665–76
50. Seubert P, Oltersdorf, Lee MG, Barbour R, Blomquist C, et al. 1993. Secretion of beta-amyloid precursor protein cleaved at the amino terminus of the beta-amyloid peptide. *Nature* 361:260–63
51. Howlett DR, Simmons DL, Dingwall C, Christie G. 2000. In search of an enzyme: the beta-secretase of Alzheimer's disease is an aspartic proteinase. *Trends Neurosci.* 23:565–70
52. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, et al. 1999. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 286:735–41
53. Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, et al. 1999. Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature* 402:537–40
54. Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, et al. 1999. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. *Nature* 402:533–37
55. Hussain I, Powell DJ, Howlett DR, Tew DG, Meek TD, et al. 1999. Identification of a novel aspartic protease (Asp2) as beta-secretase. *Mol. Cell. Neurosci.* 14: 419–27
56. Lin X, Koelsch G, Wu S, Downs D, Dashti A, Tang J. 2000. Human aspartic protease memapsin 2 cleaves the beta-secretase site of beta-amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* 97:1456–60
57. Capell A, Steiner H, Willem M, Kaiser H, Meyer C, et al. 2000. Maturation and pro-peptide cleavage of beta-secretase. *J. Biol. Chem.* 275:30849–54
58. Yan R, Han P, Miao H, Greengard P, Xu H. 2001. The transmembrane domain of the Alzheimer's beta-secretase (BACE1) determines its late Golgi localization and access to beta-amyloid precursor protein (APP) substrate. *J. Biol. Chem.* 276:36788–96
59. Hussain I, Powell DJ, Howlett DR, Chapman GA, Gilmour L, et al. 2000. ASP1 (BACE2) cleaves the amyloid precursor protein at the beta-secretase site. *Mol. Cell. Neurosci.* 16:609–19
60. Farzan M, Schnitzler CE, Vasilieva N, Leung D, Choe H. 2000. BACE2, a beta-secretase homolog, cleaves at the beta site and within the amyloid-beta region of the amyloid-beta precursor protein. *Proc. Natl. Acad. Sci. USA* 97:9712–17
61. Roberds SL, Anderson J, Basi G, Bienkowski MJ, Branstetter DG, et al. 2001. BACE knockout mice are healthy despite lacking primary-secretase activity in the brain: implications for Alzheimer's disease therapeutics. *Hum. Mol. Genet.* 10:1317–24
62. Huse JT, Pijak DS, Leslie GJ, Lee VM, Doms RW. 2000. Maturation and endosomal targeting of beta-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase. *J. Biol. Chem.* 275:33729–37

63. Citron M, Oltersodrf T, Haass C, McConlogue L, Hung AY, et al. 1992. Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. *Nature* 360:672–74
64. Citron M, Teplow DB, Selkoe DJ. 1995. Generation of amyloid beta protein from its precursor is sequence specific. *Neuron* 14:661–70
65. Sauder JM, Arthur JW, Dunbrack RL Jr. 2000. Modeling of substrate specificity of the Alzheimer's disease amyloid precursor protein beta-secretase. *J. Mol. Biol.* 300:241–48
66. Hong L, Koelsch G, Lin X, Wu S, Terzyan S, et al. 2000. Structure of the protease domain of memapsin 2 (beta-secretase) complexed with inhibitor. *Science* 290:150–53
67. Gruninger-Leitch F, Schlatter D, Kung E, Nelbock P, Dobeli H. 2002. Substrate and inhibitor profile of BACE (beta-secretase) and comparison with other mammalian aspartic proteases. *J. Biol. Chem.* 277:4687–93
68. Luo Y, Bolon B, Kahn S, Bennett BD, Babu-Khan S, Denis P, et al. 2001. Mice deficient in BACE1, the Alzheimer's β-secretase, have normal phenotype and abolished β-amyloid generation. *Nat. Neurosci.* 4:231–32
69. Cai HB, Wang YS, McCarthy D, Wen HJ, Borchelt DR, et al. 2001. BACE1 is the major beta-secretase for generation of A beta peptides by neurons. *Nat. Neurosci.* 4:233–34
70. Phimister B. 2000. Four companies announce discovery of beta-secretase gene. *Nat. Biotechnol.* 18:16
71. Ghosh AK, Shin D, Downs D, Koelsch G, Lin X, et al. 2000. Design of potent inhibitors of human brain memapsin 2 (beta-secretase). *J. Am. Chem. Soc.* 123:3522–23
72. Ghosh AK, Bilcer G, Harwood C, Kawahama R, Shin D, et al. 2001. Structure-based design: potent inhibitors of human brain memapsin 2 (beta-secretase). *J. Med. Chem.* 44:2865–68
73. Brown MS, Ye J, Rawson RB, Goldstein JL. 2000. Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. *Cell* 100:391–98
74. Rawson RB, Zelenski NG, Nijhawan D, Ye J, Sakai J, et al. 1997. Complementation cloning of S2P, a gene encoding a putative metalloprotease required for intramembrane cleavage of SREBPs. *Mol. Cell* 1:47–57
75. Urban S, Lee JR, Freeman M. 2001. Drosophila rhomboid-1 defines a family of putative intramembrane serine proteases. *Cell* 107:173–82
76. Scheuner D, Eckman C, Jensen M, Song X, Citron M, et al. 1996. Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat. Med.* 2:864–70
77. Levitan D, Greenwald I. 1995. Facilitation of *lin-12*-mediated signalling by *sel-12*, a *Caenorhabditis elegans S182* Alzheimer's disease gene. *Nature* 377: 351–54
78. Shen J, Bronson RT, Chen DF, Xia WM, Selkoe DJ, Tonegawa S. 1997. Skeletal and CNS defects in presenilin-1 deficient mice. *Cell* 89:629–39
79. Wong PC, Zhen H, Chen H, Becher MW, Sirinathsinghji DJS, et al. 1997. Presenilin 1 is required for Notch 1 and D111 expression in the paraxial mesoderm. *Nature* 397:288–92
80. De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Gundula G, et al. 1998. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* 391:387–90
81. Herreman A, Serneels L, Annaert W, Collen D, Schoonjans L, De Strooper B. 2000. Total inactivation of gamma-secretase activity in presenilin-deficient embryonic stem cells. *Nat. Cell Biol.* 2:461–62
82. Zhang Z, Nadeau P, Song W, Donoviel D, Yuan M, et al. 2000. Presenilins are

required for gamma-secretase cleavage of beta-APP and transmembrane cleavage of Notch-1. *Nat. Cell Biol.* 2:463–65
83. Wolfe MS, Xia W, Ostaszewski BL, Diehl, TS, Kimberly WT, Selkoe, DJ. 1999. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature* 398:513-517
84. Xia WM, Zhang JM, Perez R, Koo EH, Selkoe DJ. 1997. Interaction between amyloid precursor protein and presenilins in mammalian cells: implications for the pathogenesis of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 94:8208–13
85. Xia WM, Ray WJ, Ostaszewski BL, Rahmati T, Kimberly WT, et al. 2000. Presenilin complexes with the C-terminal fragments of amyloid precursor protein at the sites of amyloid β-protein generation. *Proc. Natl. Acad. Sci. USA* 97:9299–304
86. Xia W, Zhang JM, Ostaszewski BL, Kimberly WT, Seubert P, et al. 1998. Presenilin 1 regulates the processing of beta-amyloid precursor protein C-terminal fragments and the generation of amyloid β-protein in ER and Golgi. *Biochemistry* 37:16465–71
87. Wolfe MS, Citron M, Diehl TS, Xia WM, Donkor IO, Selkoe DJ. 1998. A substrate-based difluoro ketone selectively inhibits Alzheimer's gamma-secretase activity. *J. Med. Chem.* 41:6–9
88. Wolfe MS, Xia WM, Moore CL, Leatherwood DD, Ostaszewski BL, et al. 1999. Peptidomimetic probes and molecular modeling suggest Alzheimer's γ-secretase is an intramembrane-cleaving aspartyl protease. *Biochemistry* 38:4720–27
89. Li YM, Lai MT, Xu M, Huang Q, DiMuzio-Mower J, et al. 2000. Presenilin 1 is linked with gamma-secretase activity in the detergent solubilized state. *Proc. Natl. Acad. Sci. USA* 97:6138–43
90. Esler WP, Kimberly WT, Ostaszewski BL, Diehl TS, Moore CL, et al. 2000. Transition-state analogue inhibitors of γ-secretase bind directly to presenilin-1. *Nat. Cell Biol.* 2:428–34
91. Li YM, Xu M, Lai MT, Huang Q, Castro JL, et al. 2000. Photoactivated γ-secretase inhibitors directed to the active site covalently label presenilin 1. *Nature* 405:689–94
92. Kimberly WT, Xia WM, Rahmati R, Wolfe MS, Selkoe DJ. 2000. The transmembrane aspartates in presenilin 1 and 2 are obligatory for γ-secretase activity and amyloid β-protein generation. *J. Biol. Chem.* 275:3173–78
93. LaPointe CF, Taylor RK. 2000. The type 4 prepilin peptidases comprise a novel family of aspartic acid proteases. *J. Biol. Chem.* 275:1502–10
94. Steiner H, Kostka M, Romig H, Basset G, Pesold B, et al. 2000. Glycine 384 is required for presenilin-1 function and is conserved in bacterial polytopic aspartyl proteases. *Nat. Cell Biol.* 2:848–51
95. Weihofen A, Binns K, Lemberg MK, Ashman K, Martoglio B. 2002. Identification of signal peptide peptidase, a presenilin-type aspartic protease. *Science* 296:2215–18
96. Thinakaran G, Borchelt DR, Lee MK, Slunt HH, Spitzer L, et al. 1996. Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. *Neuron* 17:181–90
97. Yu G, Nishimura M, Arawaka S, Levitan D, Zhang L, et al. 2000. Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing. *Nature* 407:48–54
98. Deleted in proof
99. Yu G, Chen F, Levesque G, Nishimura M, Zhang DM, et al. 1998. The presenilin 1 protein is a component of a high molecular weight intracellular complex that contains beta-catenin. *J. Biol. Chem.* 273:16470–75
100. Esler WP, Kimberly WT, Ostaszewski BL, Ye W, Diehl TS, et al. 2002. Activity dependent isolation of the presenilin-gamma secretase complex reveals nicastrin and a

gamma substrate. *Proc. Natl. Acad. Sci. USA* 99:2720–25
101. Goutte C, Tsunozaki M, Hale VA, Priess JR. 2002. APH-1 is a multipass membrane protein essential for the Notch signaling pathway in Caenorhabditis elegans embryos. *Proc. Natl. Acad. Sci. USA* 99:775–79
102. Francis R, McGrath G, Zhang J, Ruddy DA, Sym M, et al. 2002. *aph*-1 and *pen*-2 are required for Notch pathway signaling, gamma secretase cleavage of βAPP and presenilin protein accumulation. *Dev. Cell.* 3:85–97
103. Lichtenthaler SF, Wang R, Grimm H, Uljon SN, Masters CL, Beyreuther K. 1999. Mechanism of the cleavage specificity of Alzheimer's disease gamma-secretase identified by phenylalanine-scanning mutagenesis of the transmembrane domain of the amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* 96:3053–58
104. Lichtenthaler SF, Beher D, Grimm HS, Wang R, Shearman MS, et al. 2002. The intramembrane cleavage site of the amyloid precursor protein depends on the length of its transmembrane domain. *Proc. Natl. Acad. Sci. USA* 99:1365–70
105. Saura CA, Tomita T, Soriano S, Takahashi M, Leem JY, et al. 2000. The nonconserved hydrophilic loop domain of presenilin (PS) is not required for PS endoproteolysis or enhanced A beta 42 production mediated by familial early onset Alzheimer's disease-linked PS variants. *J. Biol. Chem.* 275:17136–42
106. Struhl G, Adachi A. 2000. Requirements for presenilin-dependent cleavage of notch and other transmembrane proteins. *Mol. Cell* 6:625–36
107. Dovey HF, John V, Anderson JP, Chen LZ, de Saint Andrieu P, et al. 2001. Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain. *J. Neurochem.* 76:173–81
108. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, et al. 1999. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am. J. Pathol.* 155:853–62
109. McLean CA, Cherny RA, Fraser FW, Smith MJ, Beyreuther K, et al. 1999. Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann. Neurol.* 46:860–66
110. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, et al. 1998. Diffusible, nonfibrillar ligands derived from A beta1-42 are potent central nervous system neurotoxins. *Proc. Natl. Acad. Sci. USA* 95:6448–53
111. Hartley DM, Walsh DM, Ye CP, Diehl T, Vasquez S, et al. 1999. Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *J. Neurosci.* 19:8876–84
112. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, et al. 2002. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416:535–39
113. Annaert WG, Levesque L, Craessaerts K, Dierinck I, Snellings G, et al. 1999. Presenilin 1 controls γ-secretase processing of amyloid precursor protein in pre-Golgi compartments of hippocampal neurons. *J. Cell Biol.* 147:277–94
114. Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, et al. 2001. A subset of NSAIDs lower amyloidogenic A beta42 independently of cyclooxygenase activity. *Nature* 414:212–26
115. in t'Veld BA, Ruitenberg A, Hofman A, Launer LJ, van Duijn CM, et al. 2001. Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. *N. Engl. J. Med.* 345:1515–21
116. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, et al. 1999. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400:173–77

117. Pepys MB, Herbetrt J, Hutchinson WL, Tennent GA, Lachmann HJ, et al. 2002. Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. *Nature* 417:254–59
118. Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, et al. 2000. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408:982–85
119. Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, et al. 2000. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 408:979–82
120. Weiner HL, Lemere CA, Maron R, Spooner ET, Grenfell TJ, et al. 2000. Nasal administration of amyloid-beta peptide decreases cerebral amyloid burden in a mouse model of Alzheimer's disease. *Ann. Neurol.* 48:553–55
121. Bard F, Cannon C, Barbour R, Burke RL, Games D, et al. 2000. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat. Med.* 6:916–19
122. DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. 2001. Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* 98:8850–55
123. DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM. 2002. Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science* 295:2264–67
124. Solomon B, Koppel R, Hanan E, Katzav T. 1996. Monoclonal antibodies inhibit in vitro fibrillar aggregation of the Alzheimer β-amyloid peptide. *Proc. Natl. Acad. Sci. USA* 93:452–55
125. Solomon B, Koppel R, Frankel D, Hanan-Aharon E. 1997. Disaggregation of Alzheimer beta-amyloid by site-directed mAb. *Proc. Natl. Acad. Sci. USA* 94: 4109–12
126. Higgins LS, Murphy GM Jr, Forno LS, Catalano R, Cordell B. 1996. P3 beta-amyloid peptide has a unique and potentially pathogenic immunohistochemical profile in Alzheimer's disease brain. *Am. J. Pathol.* 149:585–96
127. Lalowski M, Golabek A, Lemere CA, Selkoe DJ, Wisniewski HM, et al. 1996. The "nonamyloidogenic" p3 fragment (amyloid beta17-42) is a major constituent of Down's syndrome cerebellar preamyloid. *J. Biol. Chem.* 271:33623–31
128. Nitsch RM, Slack BE, Wurtman RJ, Growdon JH. 1992. Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science* 258:304–7
129. Buxbaum JD, Koo EH, Greengard P. 1993. Protein phosphorylation inhibits production of Alzheimer amyloid β/A4 peptide. *Proc. Natl. Acad. Sci. USA* 90: 9195–98
130. Hung AY, Haass C, Nitsch RM, Qiu WQ, Citron M, et al. 1993. Activation of protein kinase C inhibits cellular production of the amyloid β-protein. *J. Biol. Chem.* 268:22959–62
131. Beach TG, Kuo YM, Schwab C, Walker DG, Roher AE. 2001. Reduction of cortical amyloid beta levels in guinea pig brain after systemic administration of physostigmine. *Neurosci. Lett.* 310:21–24
132. Beach TG, Walker DG, Potter PE, Sue LI, Fisher A. 2001. Reduction of cerebrospinal fluid amyloid beta after systemic administration of M1 muscarinic agonists. *Brain Res.* 905:220–23
133. Nitsch RM, Deng M, Tennis M, Schoenfeld D, Growdon JH. 2000. The selective muscarinic M1 agonist AF102B decreases levels of total A beta in cerebrospinal fluid of patients with Alzheimer's disease. *Ann. Neurol.* 48:913–18
134. Lee RK, Wurtman RJ. 1997. Metabotropic glutamate receptors increase

amyloid precursor protein processing in astrocytes: inhibition by cyclic AMP. *J. Neurochem.* 68:1830–35
135. Nitsch RM, Deng M, Growdon JH, Wurtman RJ. 1996. Serotonin 5-HT2a and 5-HT2c receptors stimulate amyloid precursor protein ectodomain secretion. *J. Biol. Chem.* 271:4188–94
136. Nitsch RM, Kim C, Growdon JH. 1998. Vasopressin and bradykinin regulate secretory processing of the amyloid protein precursor of Alzheimer's disease. *Neurochem. Res.* 23:807–14
137. Farber SA, Nitsch RM, Schulz JG, Wurtman RJ. 1995. Regulated secretion of beta-amyloid precursor protein in rat brain. *J. Neurosci.* 15:7442–51
138. Sing CF, Davignon J. 1985. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. *Am. J. Hum. Genet.* 37:268–85
139. Simons K, Ikonen E. 1997. Functional rafts in cell membranes. *Nature* 387:569–72
140. Lee SJ, Liyanage U, Bickel PE, Xia WM, Lansbury PT, Kosik KS. 1998. A detergent-insoluble membrane compartment contains Aβ in vivo. *Nat. Med.* 4:730–34
141. Yanagisawa K, Odaka A, Suzuki N, Ihara Y. 1995. GM1 ganglioside-bound amyloid beta-protein (A beta): a possible form of preamyloid in Alzheimer's disease. *Nat. Med.* 1:1062–66
142. Simons M, Keller P, De Strooper B, Beyreuther K, Dotti CG, Simons K. 1998. Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc. Natl. Acad. Sci. USA* 95:6460–64
143. Kojro E, Gimpl G, Lammich S, Marz W, Fahrenholz F. 2001. Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha-secretase ADAM 10. *Proc. Natl. Acad. Sci. USA* 98:5815–20
144. Fassbender K, Simons M, Bergmann C, Stroick M, Lutjohann D, et al. 2001. Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides A beta 42 and A beta 40 in vitro and in vivo. *Proc. Natl. Acad. Sci. USA* 98: 5856–61
145. Refolo LM, Pappolla MA, LaFrancois J, Malester B, Schmidt SD, et al. 2001. A cholesterol-lowering drug reduces beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis.* 8:890–99
146. Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, et al. 2000. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol. Dis.* 7: 321–31
147. Wolozin B, Kellman W, Ruosseau P, Celesia GG, Siegel G. 2000. Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. *Arch. Neurol.* 57:1439–43
148. Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA. 2000. Statins and the risk of dementia. *Lancet* 356:1627–31
149. Rockwood K, Kirkland S, Hogan DB, MacKnight C, Merry H, et al. 2002. Use of lipid-lowering agents, indication bias, and the risk of dementia in community-dwelling elderly people. *Arch. Neurol.* 59:223–27
150. Glenner GG, Wong CW. 1984. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* 120:885–90
151. Dodart JC, Bales KR, Gannon K-S, Greene SJ, DeMattos RB, Mathis C, et al. 2002. Immunization reverses memory deficits without reducing brain Aβ burden in Alzheimer's disease model. *Nat. Neurosci.* 5:452–57