Introduction

Alzheimer's disease is the most prevalent neurodegenerative disorder.

The clinical syndrome, ranging from mild cognitive impairment to severe dementia, reflects cellular abnormalities in specific regions of the brain/circuits.

Advances in laboratory measurements and imaging of amyloid burden in brain will be of value in establishing the diagnosis of Alzheimer's disease (AD).

Some cases of familial AD (fAD) are due to mutant genes inherited as autosomal dominants; ApoE4 allele is associated with increased risk.

Multiple neurotransmitter circuits are damaged by the disease.

Neuritic plaques, one of the pathological hallmarks of the disease, are composed of swollen neurites, extracellular deposits of Aβ 40-42 peptides, (derived from β- and γ-secretase cleavages of APP) and surrounding astrocytes and microglia.

Neurofibrillary tangles (NFT) are comprised of bundles of paired helical filaments (PHF) made up of hyperphosphorylated tau.

Aspartyl proteases carry out the β- and γ-secretase cleavages of APP to generate Aβ peptides.

Transgenic strategies have been used to create models of Aβ amyloidosis.

Gene targeting approaches have identified and validated certain genes as targets for therapy.

Transgenic mouse models are being used to test a variety of novel therapies, including ways of reducing secretase activities and decreasing Aβ burden by Aβ immunotherapy.

Several of these experimental approaches are moving into clinical trials.

Alzheimer's disease (AD), manifesting as progressive memory loss and cognitive impairments [1], affects more than 4 million elderly individuals in the USA [1, 2]. This syndrome results from abnormalities associated with dysfunction and death of specific populations of neurons, particularly those in neural systems involved in memory and cognition [3]. The pathology is characterized by intracellular and extracellular protein aggregates (tau and Aβ related abnormalities) in neurofibrillary tangles (NFT) and neuritic amyloid plaques, respectively [4-6]. Genetic evidence indicates that the inheritance of mutations in several genes causes autosomal dominant familial AD (fAD), while the presence of certain alleles of ApoE4 are significant risk factors for putative sporadic disease [3, 7, 8].

Due to increased life expectancy and the postwar baby boom, the elderly are the most rapidly growing segment of our society. Thus, over the next several decades, the number of persons with AD in the USA will triple. Because of prevalence, lack of mechanism-based treatments, cost of care, and impact on individuals and caregivers, AD is one of the most challenging diseases in medicine [3, 4, 9, 10].

This review briefly discusses the clinical syndrome, laboratory tests, genetics, and pathological/biochemical features of the human illness. Subsequently, we describe selected aspects of: the biology of proteins implicated in pathogenesis of disease; the value of transgenic and gene-targeted mouse models; the identification of several therapeutic targets; and results of selected experimental treatments in the models. We demonstrate how information from...
genetics, pathology, and biochemistry has been used to create models of disease (i.e., mice expressing mutant transgenes). In parallel, we demonstrate how results of targeting genes encoding proteins implicated in disease pathways have provided insights of the roles of these proteins in the pathogenesis of AD and the potential of these proteins as therapeutic targets. The value of these targets for new treatment strategies is being tested in model systems. As safety and efficacy are assured, some of these therapeutic approaches will be increasingly used in human trials [10]. The development of effective new therapies will have a significant impact on the health and care of the elderly.

The clinical syndrome, ranging from mild cognitive impairment to severe dementia, reflects cellular abnormalities in specific regions of the brain/circuits. The disease often initially manifests as a syndrome termed mild cognitive impairment (MCI), which is usually characterized by a memory complaint and impairments on formal testing, intact general cognition, preserved daily activities and absence of overt dementia [11]. MCI is regarded by many as a transitional stage between normal aging and early AD [11–13]. The clinical manifestations of symptomatic AD include increasing difficulties with memory and with other cognitive functions (executive functions, language, attention, judgment, etc.) [1, 6, 12–14]. Some patients develop psychotic symptoms. Over time, mental functions and activities of daily living are increasingly impaired. In the late stages, these individuals become profoundly demented and usually die of intercurrent illnesses.

For a diagnosis, clinicians rely on histories, on physical, neurological and psychiatric examinations, on neuropsychological tests [1, 14], and on laboratory studies [15, 16].

Advances in laboratory measurements and imaging of amyloid burden in brain will be of value in establishing the diagnosis of Alzheimer’s disease (AD). In cases of AD, the CSF levels of Aβ peptides are often low, and CSF levels of tau may be higher than in controls [15]. Values vary between individuals and single measures may not be great diagnostic value. Over several examinations, the clinical profile, in concert with laboratory assessments, allows the clinician to make a diagnosis of possible or probable AD [17].

In cases of AD, magnetic resonance imaging (MRI) often discloses regional brain atrophy, particularly involving hippocampus and entorhinal cortex [1, 14], and rates of atrophy which correlate with changes in clinical status and may have predictive value for diagnosis. Positron emission tomography (PET) using [18F]-deoxyglucose or single photon emission computerized tomography (SPECT) commonly demonstrate decreased glucose utilization and early reduction in regional blood flow in the parietal and temporal lobes.

The most exciting recent advance in imaging relevant to AD is the use of PET with the radiolabeled Pittsburgh Compound B (PIB) which demonstrates Aβ amyloid burden in vivo. This brain penetrating 11C-labeled uncharged thioflavin derivative binds to Aβ with high affinity [16]. The PET patterns of labeled PIB are interpreted to reflect the Aβ burden in the brain [16]. In comparison with controls, subjects with AD show marked retention of label in several areas of brain that usually accumulate amyloid. This approach should eventually prove useful for enhancing accuracy of diagnosis and should allow assessment of the efficacies of new anti-amyloid therapeutics. Further details of brain imaging are found in Ch. 58.

Some cases of familial AD (fAD) are due to mutant genes inherited as autosomal dominants; ApoE4 allele is associated with increased risk. Genetic risk factors for AD include: mutations in APP (chromosome 21); mutations in presenilin (PS) 1 (chromosome 14) and PS2 (chromosome 1); and different susceptibility alleles of ApoE (chromosome 19) [3, 7, 8, 18]. Autosomal dominant mutations in APP, PS1, or PS2 usually cause disease earlier than occurs in sporadic cases. The majority of mutations in APP, PS1 and PS2 influence BACE1 and γ-secretase cleavages to increase the levels of all Aβ species or the relative amounts of toxic Aβ 42 [7, 8, 18]. Individuals with trisomy 21 or Down’s syndrome (DS) have an extra copy of APP (and other genes) in the putative obligate DS region; these individuals develop AD pathology relatively early in life. The presence of ApoE4 predisposes to later onset AD and some cases of late-onset FAD [19]. Recent research has identified other loci that confer risk [8].

Mutations of APP gene. APP, a type I transmembrane protein existing as several isoforms, is abundant in the nervous system, rich in neurons, and transported anterogradely in axons to terminals [20–22]. Its specific functions remain to be defined [7, 23, 24]. APP is cleaved by activities of BACE1 (β-site APP cleaving enzyme 1) and the γ-secretase complex, which generate the N- and C- termini of Aβ peptides, respectively [7, 10, 25–32]. The APPsw mutation, a double mutation involving codons 670 and 671, enhances many-fold the BACE1 cleavage at the N-terminus of Aβ; the result is substantial elevation in levels of all Aβ peptides. With APPsw mutations, γ-secretase cleavage is altered leading to increased secretion of Aβ 42, which is a more toxic peptide. Thus, some of the APP mutations linked to FAD can change the processing of APP and influence the biology of Aβ by increasing the production of Aβ peptides or the amounts of the more toxic Aβ 42; other mutations may promote local fibril formation.

Mutations of PS1 and PS2 genes. PS1 and PS2, two highly homologous and conserved 43- to 50-kDa multi-pass transmembrane proteins [3, 7, 33, 34], are involved in Notch1 signaling pathways critical for cell fate decisions [7, 28]. They are endoproteolytically cleaved to form an N-terminal 28 kDa fragment and a C-terminal 18 kDa fragment [33, 35], both of which (along with several other proteins described below) are critical components of the
γ-secretase complex [7, 27–32, 36]. Nearly 50% of cases of early-onset fAD are linked to approximately 90 different mutations in PS1 [3, 7, 8, 34]. A small number of PS2 mutations cause autosomal dominant fAD in several pedigrees [3, 8]. The majority of abnormalities in PS genes are single amino acid missense mutations that enhance γ-secretase activities and increase the levels of the Aβ42 peptides.

**ApoE alleles.** ApoE carries cholesterol and other lipids in the blood. In humans, three alleles exist: ApoE2, ApoE3 and ApoE4 [19]. The ApoE4 allele is most common in the general population (frequency of 0.78), whereas the allelic frequency of ApoE4 is 0.14. However, in clinic-based studies, the ApoE4 allelic frequency in patients with late-onset disease (>65 years of age) is 0.50; thus, the presence of ApoE4 increases the risk for AD [8, 19]. Significant differences exist in the abilities of ApoE isoforms to bind Aβ and these features of the individual protein are hypothesized to differentially influence aggregation, deposition and/or clearance of Aβ by different ApoE isoforms.

**Multiple neurotransmitter circuits are damaged by the disease.** The clinical signs of AD reflect the distribution of abnormalities among different populations of neurons in brain regions/systems critical for memory, learning, and cognitive performance. Circuits damaged by the disease include: the basal forebrain cholinergic system; hippocampus; entorhinal cortex; limbic cortex; and neocortex [13, 37–39]. Neurodegeneration in these regions/circuits are reflected by: NFT and neurites, related to the presence of cytoskeletal abnormalities, particularly involving conformational alterations in phospho-tau leading to PHF [5]; the presence of neuritic Aβ-containing plaques (sites of synaptic disconnection) in brain regions receiving inputs from these neurons [7, 40]; reductions in both generic and transmitter-specific synaptic markers in the target fields of these cells [40]; evidence of death of neurons in these regions [37, 40]; and local glial and inflammatory responses, particularly associated with plaques. Disruption of synaptic communication in these circuits, associated with degeneration of neurons, has profound clinical consequences [40, 41]. Abnormalities that damage the circuits involving the entorhinal cortex, medial temporal cortex, and hippocampus, contribute significantly to memory impairments. Pathology in the neocortex is reflected by deficits in higher cognitive functions, such as disturbances in language, calculation, problem solving, and judgment. Alterations in the basal forebrain cholinergic system may contribute to difficulties in memory, arousal and attention, while involvement of the limbic cortex, amygdala, thalamus, and monoaminergic systems result in behavioral and emotional disturbances [1].

Neuritic plaques, one of the pathological hallmarks of the disease, are composed of swollen neurites, extracellular deposits of Aβ 40–42 peptides, (derived from β- and γ-secretase cleavages of APP) and surrounding astrocytes and microglia. Neuritic Aβ plaques are composed of Aβ deposits of β-pleated sheet peptides surrounded by swollen neurites (nerve terminals). Aβ1–40, 42 and 11–40, 42 amyloid peptides are derived by β- and γ-secretase cleavages of APP to generate Aβ1–40, 42 and 11–40, 42 peptides [3, 7, 26, 42]. These Aβ peptides accumulate in the extracellular space of the neopil of the neocortex and hippocampus. In neurons, APP is delivered by anterograde axonal transport to terminals [20–22], from which, following β- and γ-secretase cleavages, extracellular monomeric Aβ 40 and 42, 43 peptides are released [7, 21, 36, 43]. Aβ multimers assemble into β sheets, into protofilaments, and into fibrils [44]; these aggregates are birefringent when stained with Congo red or thioflavin dyes and viewed in polarized light or fluorescence illumination, respectively. Considerable debate exists concerning the Aβ species/conformational state exhibiting the greatest toxicity, with plaques, fibrils, protofibrils, or oligomers proposed as principal offenders [44, 45]. It now is believed that multimers, sometimes termed Aβ derived diffusible ligands (ADDLs), are the principal toxic entities [41, 42, 45, 46].

In one model, APP and pro-amyloidogenic secretases are transported to terminals and thus neurons are thought to be the major source of APP that gives rise to Aβ [43]. At synapses, BACE1 cleaves APP to form amyloidogenic C-terminal derivatives [21], which are then cleaved by γ-secretase to generate Aβ 40, 42, 43 peptides. Released normally at terminals, Aβ may influence synaptic functions [28, 40], perhaps behaving as a modulator depressing activity at excitatory, glutamatergic synapses via the NMDA receptor [24]. With increasing accumulations of Aβ 42 multimers at terminals, synaptic functions, including long term potentiation (LTP, see discussion in Ch. 53), are disrupted [47]. In this scenario, neuritic amyloid plaques, a classical feature of AD, are complex structures representing sites of Aβ-mediated damage to synapses associated with degeneration of neurites and disconnection of terminals from targets. Surrounding plaques are astrocytes and microglia which produce cytokines, chemokines and other factors (including complement components) involved in inflammatory processes (see Inflammation in Ch. 33).

**Neurofibrillary tangles (NFT)** are comprised of bundles of paired helical filaments (PHF) made up of hyperphosphorylated tau. Tangles are fibrillar intracytoplasmic inclusions in cell bodies/proximal dendrites of affected neurons, while neuropil threads and neurites are predominantly swollen, filament-containing dendrites and distal axons/terminals, respectively [5]. These intracellular lesions are rich in PHF comprised of poorly soluble hyperphosphorylated isoforms of tau, a low-molecular-weight microtubule-associated protein [5]. In human brain, alternative splicing from a single gene leads to formation of six tau isoforms, consisting of three isoforms of three repeat (3-R) tau, and three isoforms of four repeat (4-R) tau, the latter derived by inclusion of exon 10 in the transcript [5, 48]. Normally, tau, synthesized in neuronal cell bodies, is transported anterograde in axons, where it acts, via repeat
regions that interact with tubulin, to stabilize tubulin polymers critical for microtubule assembly and stability [5, 48] (see also axonal transport in Ch. 28). The post-translational modification of tau, which exhibits abnormal conformations, differs somewhat in the different tauopathies: in cases of AD, the PHF are composed of six isofoms of tau; in contrast, the inclusions occurring in cases of progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are characterized by 4-R tau, while the inclusions seen in individuals with Pick's disease (PD) are enriched in 3-R tau [5] (see also Taupathies in Ch. 45).

In one hypothetical model linking Aβ and phosphorylated tau, Aβ42 damages terminals leading to synaptic disconnection which, perhaps preferentially in primates with six isoforms of tau, in turn lead to retrograde signals that ultimately trigger the activation of kinases (or the suppression of phosphatases) whose activities lead to excessive phosphorylation of tau at certain residues. Subsequently, conformational changes in the protein cause the formation of PHF. Since the cytoskeleton is essential for maintaining cell geometry and for the intracellular trafficking and transport of proteins and organelles, disturbances of the cytoskeleton can lead to alterations in axonal transport which, in turn, can compromise the functions and viability of neurons (see Chs 8, 9 and 28). Eventually, affected nerve cells die (possibly by apoptosis; apoptosis is discussed in Ch. 35) [37, 49] and extracellular tangles remain as ‘tombstones’ of the nerve cells destroyed by disease.

Aspartyl proteases carry out the β- and γ-secretase cleavages of APP to generate Aβ peptides. APP is processed by β- and γ-secretase enzymes resulting in release of the ectodomain of APP (APPs), the production of a cytosolic fragment termed APP intracellular domain (AICD), and the generation of several Aβ peptides. In the CNS, Aβ peptides are generated by sequential endoproteolytic cleavages of neuronal APP by two membrane-bound enzymes: as described below, BACE1 cleaves APP at the Aβ +1 and +11 sites to generate APP-β carboxy terminal fragments (APP-βCTFs) [26, 50]; while γ-secretase complex cleaves, via regulated intramembranous proteolysis, APP-βCTFs at several sites including Aβ 40, 42, 43 to form these peptides [10, 27, 29–32, 34, 49, 51, 52]. The γ-secretase cleavages of APP-βCTF or αCTF release the AICD, which forms a multimeric complex with Fe 65, a nuclear adaptor protein [23]. It has been suggested that the complex of Fe 65 and Aβ or Fe 65 alone (in a new conformation), enters the nucleus and binds to the histone acetyltransferase, Tip60, to influence gene transcription [23]. This signaling mechanism is analogous to that occurring in the Notch1 pathway following the S3 cleavage of NEXT to produce NICD [28, 31]. In other cells in other organs, APP can also be cleaved endoproteolytically within the Aβ sequence through alternative, nonamyloidogenic pathways involving α-secretase (TACE or TNF-alpha converting enzyme) or BACE2 [30, 53]. The α-secretase and BACE2 cleavages, which occur in non-neural tissues, preclude the formation of Aβ peptides and thus are thought to protect these organs from Aβ amyloidosis [43].

BACE1 and BACE2, encoded by genes on chromosomes 11 and 21, respectively, are transmembrane aspartyl proteases that are directly involved in the cleavages of APP [25, 26, 30, 50, 53, 54]. Analyses of cells and brains from BACE1−/− mice [26, 50] discloses that Aβ1–40/42 and Aβ11–40/42 are not secreted in these samples [26, 50]. BACE1 is demonstrable in the CNS and immunoreactivity is visualized in some synaptic regions. BACE1 preferentially cleaves APP at the +11/+1 sites of Aβ in APP [26] and this enzyme is essential for the generation of Aβ [26, 50]. Significantly, APPswe is cleaved perhaps 100-fold more efficiently at the +1 site than is wild type APP. Thus, the presence of this mutation greatly increases BACE1 cleavage and accounts for the elevation of Aβ species in the presence of this mutation. It has been reported that the expression of BACE1 is increased in certain regions of brain from some cases of sporadic AD [55, 56]. Thus, BACE1 is the principal neuronal β-secretase and is responsible for the critical penultimate pro-amyloidogenic cleavages. Although BACE1 mRNA is present in a variety of tissues (particularly the pancreas), levels of this protein are low in most non-neural tissues. In the pancreas, BACE1 mRNA is high, but the transcript is alternatively spliced to produce a smaller protein incapable of cleaving APP.

BACE2 mRNA, present in a variety of systemic organs, is very low in neural tissues, except for scattered nuclei in the hypothalamus and brainstem. BACE2 activity appears to be virtually undetectable in brain regions involved in AD. BACE2 is responsible for generation of anti-amyloidogenic cleavages at +19/+20 of Aβ [53]. Thus, BACE2, an anti-amyloidogenic enzyme, acts like α-secretase or TACE which cleave between residues 16 and 17 of the Aβ peptide [7, 57].

γ-Secretase, essential for the regulated intramembranous proteolysis of a variety of transmembrane proteins, is a multiprotein catalytic complex, which includes: PS; Nicastrin (Nct), a type I transmembrane glycoprotein [7, 27, 48, 51]; and Aph-1 and Pen-2, two multipass transmembrane proteins [7, 27–32, 34, 51, 52, 58, 59]. PS1 is isolated with γ-secretase under specific detergent soluble conditions; it is selectively crosslinked or photoaffinity-labeled by transition state inhibitors [60, 61]; substitutions of aspartate residues at D257 in TM 6 and at D385 in TM 7 have been reported to reduce secretion of Aβ and cleavage of Notch1 in vitro. PS1−/− cells show decreased levels of secretion of Aβ [27, 29, 51, 62]. Aph-1 and Pen-2 [27, 29, 59] are novel transmembrane proteins: Aph-1 has seven predicted transmembrane domains; and Pen-2 has two predicted transmembrane regions [27, 29].

The functions of these protein and their interactions with each other in the complex and in γ-secretase activity are not yet fully defined. PS1 may: act as an aspartyl protease itself; function as a cofactor critical for the activity of
γ-secretase; or play a role in trafficking of APP or proteins critical for enzyme activity to the proper compartment for γ-secretase cleavage [36, 61, 62]. In one model, Aph-1 and Nct form a pre-complex that interacts with PS; subsequently Pen-2 influences the cleavage of PS into two fragments; all proteins are critical for the activities of γ-secretase complex [51, 52].

Significantly, γ-secretase cleaves both Notch1 and APP [28], generating intracellular peptides termed NICD and AICD [23] which influence transcription [23, 28]. As described above, the AICD interacts with FE65, a cytosolic adaptor; this interaction leads to a signal which influences transcription [23, 28]. Results of targeting of PS1, Nct and Aph-1 in mice [51, 52, 63, 64], which are described below, are consistent with the concept that PS1, Nct and Aph-1 [27, 29, 30, 65] are critical components of the γ-secretase complex. The phenotypes of targeted PS1, Nct, and Aph-1 mice are the result of impaired Notch1 signaling.

Transgenic strategies have been used to create models of Aβ amyloidosis. In mice, expression of APPsw and APP17 minigenes (with or without mutant PS1) leads to an Aβ amyloidosis in the CNS [66], with the severity of pathology influenced by the nature and levels of the expressed transgene and the specific mutation. Mice expressing both mutant APP and PS1 develop accelerated disease. In these animals, levels of Aβ (particularly Aβ 42) in brain are elevated and diffuse Aβ deposits and neuritic plaques appear in the hippocampus and cortex. In transgenic mice generated at Johns Hopkins University by Dr David Borchelt [67–69], the pathology evolves in stages: levels of Aβ peptides in brain increase with age; over time, Aβ deposits appear to become increasingly abundant; swollen neurites develop in proximity to these deposits; and neuritic plaques are associated with glial responses. In these mice, aggregated tau and tangles are not detectable. The density of synaptic terminals is reduced and several neurotransmitter markers are decreased; in some settings, these abnormalities appear linked to deficiencies in synaptic transmission [47, 70]. Moreover, some lines of mice show degeneration of subsets of neurons. Although amyloid has been detected in vivo by invasive techniques [71], it has proved more difficult to detect Aβ with whole brain imaging.

The paucity of tau abnormalities in these various lines of mutant APP and PS1 mice described above may be related to differences in tau isoforms expressed in this species. Early efforts to express mutant tau transgenes in mice did not lead to striking clinical phenotypes or pathology. More recently, mice overexpressing tau show clinical signs, attributed to degeneration of motor axons [5]. When the prion or Thy1 promoters are used to drive tauP301L (a mutation linked to autosomal dominant frontotemporal dementia with parkinsonism; see Ch. 45), tangles develop in neurons of the brain and spinal cord [72]. Mice expressing APPsw/tauP301L exhibit enhanced tangle-like pathology in limbic system and olfactory cortex [73]. Moreover, when Aβ 42 fibrils are injected into specific brain regions of tauP301L mice, the number of tangles is increased in those neurons projecting to sites of Aβ injection.

A triple transgenic mouse (3xTg-AD) was created by microinjecting APPswe and tauP301L into single cells derived from monoygous PS1M146V knockin mice [74]. These mice develop age-related plaques and tangles as well as deficits in LTP which appear to antedate overt pathology [74]. However, mice bearing both mutant tau and APP (or APP/PS1) or mutant tau mice injected with Aβ are not fully faithful models of FAD because the presence of the tau mutation alone is associated with the development of tangles.

Finally, learning deficits, problems in object recognition memory, and difficulties in performing tasks to assess spatial reference and working memory, have been identified in some of the lines of mutant mice with high levels of mutant transgene expression [70, 75]. Although these mice do not fully recapitulate the complete phenotype of AD, these animals are very useful subjects for research designed to examine disease mechanisms and to test novel therapies.

Gene targeting approaches have identified and validated certain genes as targets for therapy. To begin to understand the functions of some of the proteins thought to play roles in AD, investigators have targeted in mice a variety of genes encoding these proteins, including APP, amyloid precursor like proteins (APLPs), BACE1, PS1, Nct and Aph-1.

Homoygous APPfem mice are viable and fertile, but appear to have subtle decreases in locomotor activity and forelimb grip strength. The absence of substantial phenotypes in APPfem mice is thought to be related to functional redundancy of two amyloid-precursor-like proteins, APLP1 and APLP2, homologous to APP. APLP2fem mice appear relatively normal, while APLP1fem mice exhibit a postnatal growth deficit [76]. APLP1fem mice are viable, but APP/APLP2fem mice and APLP1/APLP2fem mice do not survive the perinatal period [76]. These observations support the concept that some redundancy exists between members of this interesting family of proteins [76].

BACE1fem mice are viable and healthy, have no obvious phenotype or pathology, and can mate successfully [26, 50]. Importantly, cortical neurons cultured from BACE1fem embryos do not show cleavages at the +1 and +11 sites of Aβ, and the secretion of Aβ peptides is abolished even in the presence of elevated levels of exogenous wt mutant APP [26]. The brains of BACE1fem mice appear morphologically normal and Aβ peptides are not produced [26, 50]. These results establish that BACE1 is the neuronal β-secretase required to generate the N-termini of Aβ [26]. At present, a consensus has not been reached with regards to other substrates cleaved by BACE1 [30]. Moreover, preliminary behavioral studies of the BACE1fem mice indicate that these animals show altered performance on some tests.
of cognition and emotion. Nevertheless, BACE1 appears to be an outstanding target for development of an anti-amyloidogenic therapy.

PS1+/– mice, in contrast to BACE1−/− mice, do not survive beyond the early postnatal period and show severe developmental abnormalities of the axial skeleton, ribs and spinal ganglia; resembling a partial Notch1−/− phenotype [63, 64], these features occur because PS1 (along with Nct, Aph-1 and Pen-2) are components of the γ-secretase complex that carries out the S intramembranous cleavage of Notch1 [27–29, 48, 51, 77, 78]. Without this cleavage, NICD is not released from the plasma membrane and, therefore, the signal does not reach the nucleus to initiate transcriptional processes essential for cell fate decisions. PS2+/– mice are viable and fertile, although they develop age-associated mild pulmonary fibrosis and hemorrhage. Mice lacking PS1 and PS2 die midway through gestation showing a Notch1−/− phenotype.

Nct−/− embryos die by embryonic day 10.5 and exhibit several developmental patterning defects, including abnormal segmentation of somites; the phenotype closely resembles that seen in embryos lacking Notch1 or both PS. Importantly, secretion of Aβ peptides is abolished in Nct−/− fibroblasts, whereas it is reduced by ~50% in Nct T−/− cells [51]. The failure to generate Aβ peptides in Nct T−/− cells is accompanied by destabilization of the β-secretase complex and by accumulation of APP C-terminal fragments. Moreover, analysis of APP trafficking in Nct T−/− fibroblasts reveals a significant delay in the rate of APP re-internalization compared with that of control cells.

To determine the contributions of mammalian Aph-1 homologs in functional γ-secretase complexes, we generated Aph-1a−/− mice [52]. As compared to littermate controls, the development of Aph-1a−/− embryos was dramatically retarded by embryonic day 9.5 and exhibited patterning defects that resemble, but are not identical to those of Notch1, Nct or PS−/− embryos. Moreover, in immortalized Aph-1a−/− fibroblasts, the levels of Nct, PS fragments and Pen-2 are dramatically decreased. Consequently, deletion of Aph-1a results in significant reductions in levels of the high molecular weight γ-secretase complex and in the secretion of Aβ. Three murine Aph-1 alleles, termed Aph-1a, Aph-1b and Aph-1c, encode four distinct Aph-1 isoforms: Aph-1αL and Aph-1αS derived from differential splicing of Aph-1a, Aph-1b and Aph-1c [52]. Importantly, complementation analysis reveals that all mammalian Aph-1 isoforms are capable of restoring the levels of Nct, PS and Pen-2 in Aph-1a−/− cells. Taken together, the findings establish that Aph-1a is the major mammalian Aph-1 homolog present in PS-dependent γ-secretase complexes during embryogenesis, and support the view that mammalian Aph-1 isoforms define a set of functional γ-secretase complexes.

Transgenic mouse models are being used to test a variety of novel therapies, including ways of reducing secretase activities and decreasing Aβ burden by Aβ immunotherapy. Many experimental therapeutic efforts have focused on influencing Aβ production (by inhibiting or modulating secretase activities), the aggregation of Aβ, its clearance, and the neurotoxicity of Aβ [9, 10, 79]. Although mutant transgenic mice do not recapitulate the full phenotype of AD, they represent excellent models of Aβ amyloidosis and are highly suitable for identification of therapeutic targets, and for testing new treatments in vitro. Because it is not possible to discuss all treatment efforts in transgenic mice here, we focus on a few selected studies to illustrate experimental therapeutic strategies.

BACE1 is the principal β-secretase in neurons in vitro. BACE1-deficient neurons fail to secrete Aβ even when co-expressing the APPswe and mutant PS1 genes [25, 26, 36, 43, 50]. Significantly, BACE1−/−, APPswe, PS1ΔE9 mice do not develop the Aβ deposits and age-associated abnormalities in working memory that occur in the APPswe, PS1ΔE9 model of Aβ amyloidosis [67]. Similarly, BACE1−/− TG2576 mice appear to be rescued from age dependent memory deficits and physiological abnormalities [80]. These data indicate that BACE1 is a very attractive therapeutic target. However, BACE1−/− mice develop abnormalities in performance of tasks assessing cognition and emotion, which can be rescued if APP is overexpressed in brain. In trials, it will be important to critically examine the influences of BACE1 inhibitors on memory, cognition and emotion.

The γ-secretase complex catalyzes the final cleavage of APP, which liberates the Aβ peptide into the extracellular space (amyloid deposits) [9, 10, 27, 30–32, 51, 52]. As demonstrated by the gene targeting strategies described above, this complex is critically dependent upon the presence of PS1 and 2, Nct, Aph-1a and Pen-2. Because reductions in these components decrease levels of Aβ, γ-secretase activity is a significant target for therapy. Inhibition of γ-secretase decreases production of Aβ in cell-free and cell-based systems and in levels of Aβ mutant mice with Aβ amyloidosis. However, γ-secretase activity is also essential for Notch processing critical for lineage specification and cell growth during embryonic development. Inhibitors could influence these activities. Significantly, LY-411, 575 not only reduced production of Aβ, but it also had profound effects on T and B cell development and on the appearance of intestinal mucosa (proliferation of goblet cells, increased mucin in gut lumen and crypt necrosis). Thus, several adverse effects could occur following inhibition of this enzyme, and it will be important for investigators to be alert for these effects.

Many pharmaceutical and biotechnology companies, and some academic laboratories, are using high throughput screen and molecular modeling strategies to discover compounds that inhibit these enzyme activities [9, 10, 32, 36]. Once lead compounds are identified, medicinal chemists will modify these compounds to enhance efficacy, allow passage through the blood–brain barrier, and reduce any potential toxicities.

Aβ immunotherapy has been used in both prevention and treatment trials in mutant mice. Both Aβ immunization (with Freund’s adjuvant) and passive transfer of Aβ antibodies reduce levels of Aβ and plaque burden in
mutant APP and APP/PS1 transgenic mice [79, 81–87]. Efficacy seems to be related to antibody titer. The mechanisms of enhanced clearance are not certain, but two, not mutually exclusive, hypotheses have been suggested:

1. A small amount of Aβ antibody reaches the brain, binds to Aβ peptides, promotes the disassembly of fibrils, and, via the Fc antibody domain, encourages activated microglia to enter the affected region and remove Aβ [86]; and/or

2. Serum antibodies serve as a sink to draw the amyloid peptides from the brain into the circulation, thus changing the equilibrium of Aβ in different compartments and promoting removal of Aβ from the brain [82, 84, 85, 88].

Significantly, immunotherapy in transgenic mice is successful in partially clearing Aβ, in attenuating learning and behavioral deficits in at least two cohorts of mutant APP mice, and in reducing tau abnormalities in the triple transgenic mice [83–85].

Several of these experimental approaches are moving into clinical trials. Presently available therapies for patients include: cholinesterase inhibitors; agents that influence glutamate neurotransmission; neuroprotective and anti-inflammatory approaches; pharmacological agents useful for behavioral disturbances [1]; and statins that reduce brain amyloid burden [89]. However, these approaches do not directly influence the disease process, and new disease-modifying treatments are the major unmet need in AD. In addition to the investigations described, a variety of other treatments are being tested in model systems.

The identification of genes mutated or deleted in the inherited forms of AD has allowed investigators to create in vitro and in vivo models relevant to this illness. In this review, we have emphasized the value of transgenic and gene-targeted models and the lessons they provided for understanding mechanisms of AD, for identifying therapeutic targets, and for testing new treatments in models.

First, for illustrative purposes, we focused on studies of the progeny of BACE1−/− mice and mutant APP/PS1 transgenic mice, which provides a dramatic example of the impact of reducing activity of secretases. New models using conditional expression systems, or RNAi silencing, will allow investigators to examine the pathogenesis of diseases and to assess the degree of reversibility of the disease processes. The results of these approaches will provide us with a better understanding of the mechanisms that lead to disease and help us to design new treatments.

Second, we described the successful outcomes of Aβ immunotherapy in transgenic mice with Aβ amyloidosis. Unfortunately, in humans, although Phase 1 trials with Aβ peptide and adjuvant vaccination were not associated with any adverse events, Phase 2 trials were suspended because of severe adverse reactions (meningoencephalitis) in a subset of patients [79, 90]. The pathology in a single case, consistent with T-cell meningitis [90], was interpreted to show some clearance of Aβ deposits, yet these regions contained a relatively high density of tangles, neuropil threads and vascular amyloid deposits [90]. In some regions, Aβ immunoreactivity was associated with microglia. T-cells were conspicuous in subarachnoid space and around some vessels [90]. The trial was truncated. The events occurring in this subset of patients illustrate the challenges of extrapolating outcomes in mutant mice to human trials. Interestingly, assessment of cognitive functions in a small subset of patients (30) who received vaccination and booster immunizations, disclosed that patients who generated Aβ antibodies as measured by a new assay, had a slower decline in several functional measures over time [91]. Investigators have continued to pursue the passive immunization approach and attempting to make antibodies that do not stimulate T-cell-mediated immunologic attacks [79].

The lines of research described above have greatly enhanced our understanding of AD. We are now on the threshold of implementation of novel treatments, based on an understanding of the neurobiology and biochemistry of the illness. We anticipate that future discoveries will lead to the design of new mechanism-based therapies that can be tested in models of AD, and, eventually, these approaches can be introduced into clinical settings for the benefit of patients with this devastating disease. We are confident that parallel strategies will provide similar benefits for the treatment of other diseases of the nervous system.

ACKNOWLEDGMENTS

The authors wish to thank the many colleagues who have worked at JHMI, as well as those at other institutions for their contributions to some of the original work cited in this review and for their helpful discussions. Aspects of this work were supported by grants from the U.S. Public Health Service (AG05146, NS41438, NS45150, NS049088, AG14248) as well as the Metropolitan Life Foundation, Adler Foundation, Alzheimer’s Association, CART Foundation, and Bristol-Myers Squibb Foundation.

REFERENCES


