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Alzheimer's Disease and Inflammation

Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by accumulation of extracellular deposits of beta-amyloid in brain regions that are important for memory and cognition (e.g. the hippocampus and cerebral cortex). Beta-amyloid (Aβ) is comprised of 40 and 42 amino-acid peptides (Aβ40 and Aβ42), generated by proteolytic processing of a widely expressed cell surface protein called amyloid precursor protein (APP). Aβ, in particular Aβ42, is prone to concentration-dependent oligomerization and aggregation. Rising levels of Aβ in the cerebrospinal fluid gradually lead to formation of small oligomers followed by growth into protofibrils and fibrils, which can associate with other peptides and proteins to form highly insoluble neuritic plaques (also called senile plaques). The buildup of Aβ aggregates in the AD brain is followed by formation of intracellular neurofibrillary tangles and activation of local inflammatory reactions. These brain changes ultimately lead to a widespread loss of synapses, neuronal degeneration, and neurotransmitter deficits.

The sporadic form of AD usually begins after the age of 60 years, and is the most common cause of dementia in the elderly. Its prevalence rises steadily with age, affecting less than 3% of persons between ages 60 and 70, up to 12% of those between 70 and 80, and more than 40% of those over 85. Besides age, the greatest risk factor for AD is inheritance of one or two ε4 alleles of the single human apolipoprotein E gene (chromosome 19). The ε4 genetic variation increases the likelihood of developing AD 3-8 fold and lowers the age of onset by 5-10 years, as compared with ε3 homozygotes. The familial form of AD, which accounts for less than 10% of AD cases, typically begins during the fifth decade of life. It is caused by inherited mutations in the genes for APP (chromosome 21), presenilin (PS)-1 (chromosome 14), and PS-2 (chromosome 1). Warning signs of AD are subtle and include mild forgetfulness and difficulty in identifying familiar smells. The actual development of AD is marked by progressive decline in memory and language function, personality changes, and finally dementia. Death usually occurs as a result of minor respiratory complications in the middle of the night. In the year 2000, there were an estimated 4.5 million people in the U.S. with AD, and this number is predicted to triple by 2050, if no therapy intervenes.

APP and Generation of Aβ Plaques

Amyloid precursor protein (APP) occurs in 3 major isoforms containing 770, 751, or 695 amino-acid residues, arising from alternative splicing of a single gene. The main difference between these isoforms is the presence or absence of a 51-residue Kunitz protease inhibitor (KPI) domain. Interestingly, APP-695 which lacks the KPI domain is expressed exclusively in neurons and at higher levels than the KPI-containing forms (APP-751 and APP-770). The latter are widely expressed in non-neuronal cells throughout the body, including astrocytes, microglia, and other brain cells. The APP gene is highly conserved in evolution, and nearly identical in mammalian species. It encodes a single transmembrane polypeptide whose primary structure contains a 17-residue signal peptide for secretion, a large extracellular N-terminal domain, a 24-residue transmembrane domain, and a cytoplasmic C-terminal tail of 47 amino-acid residues (Figure 1). The Aβ sequence, marked in red in figure 1, lies partially outside the cell membrane (amino acids 1-28 of Aβ) and partially within the membrane (amino acids 29-40 and

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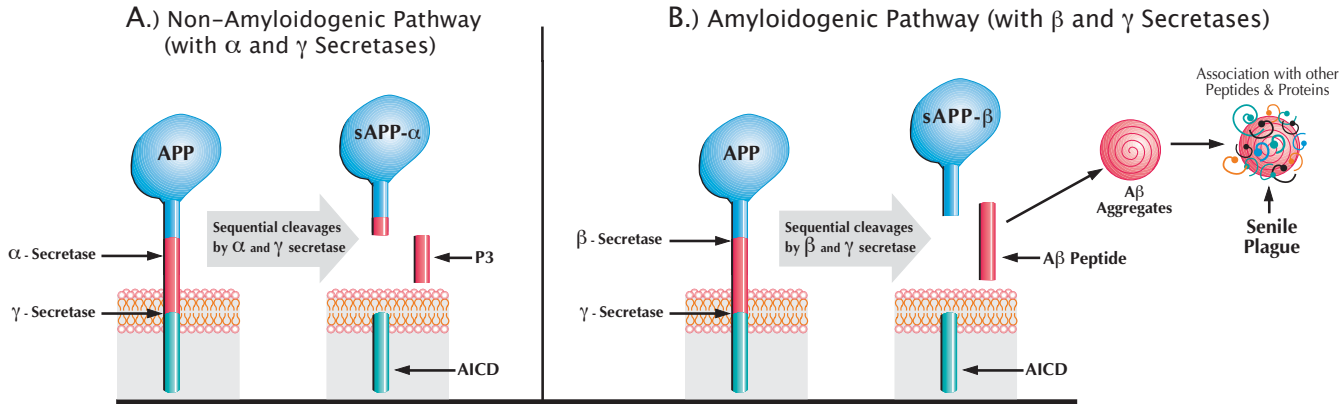
Figure 1: Proteolytic Processing of APP



29-42 of Aβ40 and Aβ42, respectively). During normal metabolism, APP, whose half-life is less than an hour, can be processed by three proteolytic activities, designated α–, β–, and γ-secretase (Table I).

Cleavage of APP by α-secretase, which splits the peptide bond between residues 16 and 17 of the Aβ sequence, precludes Aβ formation and is referred to as the non-amyloidogenic pathway. It leads to release of a large soluble extracellular fragment (sAPP-α) and retention of an 83-residue membrane-bound fragment (C83). Alternatively, APP can be cleaved by β-secretase, which cuts at the N-terminus of Aβ, thus generating a slightly smaller extracellular fragment (sAPP-β) and a 99-residue membrane-bound fragment (C99). Subsequent to their formation, C83 and C99 undergo conformational change and become targets for the presenilin (PS1/PS2)-dependent γ-secretase, which cleaves them within their plasma membrane domain. This unusual proteolysis is heterogeneous and yields peptides with slightly different lengths. It generates 24 and 26 amino-acid peptides (P3) from C83, and Aβ40 and Aβ42 from C99. The cleavage of either C83 or C99 by γ-secretase also yields an amyloid intracellular domain (AICD) of 57-59 amino-acid residues, which moves to the nucleus where it may modulate transcription of target genes. Although neither the physiological role of APP nor that of its proteolytically-derived fragments has been demonstrated directly, several lines of evidence suggest that at least one of the γ-secretase-derived fragments may play an important neuroprotective role. For example, mice whose cerebral γ-secretase activity was eliminated postnatally by combined genetic ablation of both PS1 and PS2 genes developed age-dependent cognitive deficits and neurodegeneration. As expected, amyloid plaques, the pathological hallmark of AD, were absent in the brain of these animals (1).

The steady-state levels of Aβ in the cerebrospinal fluid and plasma of healthy individuals are 3-8 nM and less than 0.5 nM, respectively. Normally, the Aβ pool contains predominantly Aβ40, which is much less prone to aggregation than Aβ42. In familial AD, the Aβ42/Aβ40 ratio is considerably higher than normal as a result of mutated forms of APP, PS1, and PS2, which share a common feature of modulating γ-secretase cleavage to increase production of the highly amyloidogenic Aβ42 peptide. Overexpression of normal APP due to duplication of chromosome 21 (Down's syndrome) results in increased secretion of Aβ from birth and invariably leads to premature AD, usually during the fourth decade of life. An interesting exception is a rare case of a Down's syndrome patient whose chromosome 21 was diploid for the APP gene, and who lived to the age of 78 years with no signs of dementia (2).

Aβ-induced Inflammation

Inflammation is a bodily response to irritation or injury, aimed at eliminating both foreign and endogenously-derived contaminating agents. The pivotal cells involved in this activity are blood polymorphonuclear leukocytes (PMNLs) and monocyte-derived tissue macrophages. These cells, dubbed "the professional phagocytes", are capable of ingesting and degrading particulate matter. Their phagocytic activity is often accompanied by release and/or leakage of oxidants (e.g. nitric oxide, hydrogen peroxide, and hydroxyl radicals), which are implicated in a variety of tissue damage mechanisms. The recognition signal for ingestion may be either particle-surface determinants, or certain serum proteins, called opsonins, which bind to the particle and render it ingestible. Circulating PMNLs are highly specialized to engulf and kill invading microorganisms; however they often die shortly thereafter, leaving puss behind. Macrophage-mediated responses, on the other hand, are typically more durable and less harsh than PMNL responses, and can accomplish degradation and recycling of accumulated debris. Normally, the immediate and early response to tissue injury is acute and involves massive infiltration with PMNL (mainly neutrophils). The cardinal signs of this reaction are redness and swelling with heat and pain ("rubor et tumor cum calore et dolore"). The PMNL-mediated response is usually phased out within hours, giving way to repair processes to heal and reconstitute the sites of injury. If clearance of irritants is incomplete, inflammation can persist indefinitely, primarily as a low-grade macrophage-mediated response. The collateral damage caused by this type of inflammation usually accumulates slowly, sometimes asymptomatically for years, and if unabated, can lead to severe tissue deterioration. Accelerated tissue damage results from chronic inflammation that has the potential to erupt episodically, as in Gout and Crohn's disease. In recent years it has become generally accepted that low-grade chronic inflammation is a silent killer, underlying severe human diseases including atherosclerosis, colorectal cancer, and Alzheimer's disease.

Although the precise relationship between inflammation and the pathogenesis of AD remains unclear, there is compelling evidence that neuroinflammation is critical for driving the disease process. Numerous epidemiological and retrospective studies have demonstrated that long term use of nonsteroidal anti-inflammatory drugs (NSAIDs) significantly reduces the risk for developing AD (3). During the past 15 years, a wide range of inflammatory mediators have been found to be upregulated in the AD brain, including complement components, acute phage reactants, oxidative stress enzymes, and inflammatory cytokines. The presence of these mediators in the AD brain is associated with abundance of activated microglia and astrocytes in regions with high Aβ-plaque burden. Microglial cells, which constitute 10-15% of the cellular population in the brain, have a monocytic origin and appear to possess a macrophage-like-phagocytic capacity. The accumulation of activated monocyte-derived cells around insoluble deposits of phagocytosis-resistant material is a common phenomenon in peripheral amyloidogenesis. Such a response has been defined as inflammatory over a century ago. However, unlike inflammatory responses in other tissues and organs, neuroinflammation is exclusively mediated by resident brain cells. The blood-brain barrier (BBB), which separates the central nervous system (CNS) from the blood, prevents not only harmful agents such as microbes and viruses but also circulating leukocytes and antibodies from entering into the brain. Consequently, the CNS can sustain only low-grade chronic inflammation, which is primarily mediated by activated microglia. Resting microglial cells play an important neurotrophic

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Table 1: Compiled Data on α, β, and γ Secretases

α-secretase has not been identified as any single proteinase, but three members of the ADAM (α **d**isintegrin **a**nd **m**etalloproteinase) family, ADAM-9, ADAM-10, and ADAM-17 (TACE) are candidate α-secretases. Up-regulation of α-secretase activity reduces Aβ formation, and is being tested as a possible therapeutic treatment for AD.

β-secretase also known as BACE1 (β-site **A**PP **c**leaving **e**nzyme) is a unique member of the pepsin family of aspartyl proteinases. It is a type I transmembrane protein whose absence in BACE knockout mice has no adverse consequences, but dramatically reduces levels of Aβ. Currently, BACE1 is a major target for development of inhibitors to treat AD.

γ-secretase is a membrane protein complex comprised of presenilin homodimer (PS1 or PS2), nicastrin, Aph1 (Aph1a or Aph1b) and Pen2. Such minimal complex is sufficient for γ-secretase activity, although other components may exist. γ-Secretase is predominantly located in the endoplasmic reticulum and the cis-Golgi, and is capable of cleaving intra-membrane peptide bonds in a variety of additional proteins, including Notch, E-cadherin, and ErbB4.

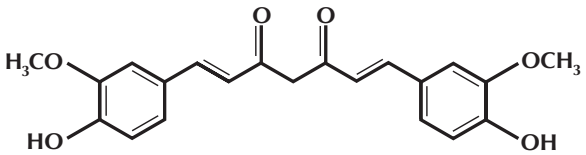
role, regulate various metabolic processes, and help to maintain homeostasis in the CNS. Of particular relevance to the pathogenesis of AD is the role of microglial-derived apoE, which is commonly associated with Aβ deposits. ApoE is a 34 kDa protein of 299 amino-acid residues, occurring as three major isoforms that differ at two residues: apoE2 (Cys^{112}, Cys^{158}), apoE3 (Cys^{112}, Arg^{158}), and apoE4 (Arg^{112}, Arg^{158}). ApoE is a key component of most lipoproteins (e.g. HDL and LDL), and plays an essential role in the redistribution of cholesterol and other lipids throughout the body. In the brain, apoE appears to inhibit Aβ-induced neurotoxicity and inflammation, and to promote clearance of amyloid deposits (4–7). ApoE is capable of solubilizing hydrophobic compounds by incorporating them into its core lipid-binding domain and delivering them to target cells via apoE receptors. However, apoE, especially the E4 isoform, is susceptible to proteolytic cleavage by a chymotrypsin-like protease, which removes a 27-residue C-terminal domain and generates a truncated apoE protein (apoE-272) with an exposed lipid-binding domain (8). When encountering hydrophobic Aβ assemblies, ApoE-272 is not only unable to solubilize them, but sticks to them and enhances their aggregation into highly insoluble plaques. Transgenic mice expressing high levels of apoE4-272 died at 2-4 months of age, and their brain cortex and hippocampus displayed AD-like alterations (8).

Aggregated forms of Aβ peptides induce transformation of resting microglia and astrocytes into activated cells, both in-vitro and in-vivo (9). These activated cells can express a variety of inflammatory mediators including chemokines, cytokines, complement and other acute-phase proteins. Interestingly, recent studies have demonstrated that under stressful conditions, neurons can produce a number of these factors including complement, cyclooxygenase-2 (COX-2), and inflammatory cytokines. In the vicinity of amyloid plaques, activated astrocytes undergo both hyperplasia and hypertrophy, and become large, star-shaped cells. These astrocytes form halos around the plaques, thus insulating them from nearby neurons and providing neuronal protection from toxic effects of Aβ deposits. In contrast to the peripheral positioning of astrocytes, activated microglia assume a more central location and deeply interdigitate the plaques. Accumulating evidence suggest that the initial attempt of these cells to phagocytose Aβ plaques involves limited activation of the complement pathway and generation of relatively non-neurotoxic opsonizing proteins such as the C1q and C3b complement components (10). Failure in this attempt appears to trigger further activation of the complement cascade, gradually leading to generation of more neurotoxic complement fragments and ultimately results in formation of the highly neurotoxic C5b-9 complex, also known as MAC ("membrane attack complex"). The presence of MAC and other complement activation proteins in the AD brain have been demonstrated by immunochemical staining procedures, indicating the significance of complement inflammation in the pathogenesis of AD (10). The perpetuation of neuroinflammation, which is accompanied by altered ionic homeostasis, free radical formation, oxidative damage, and neuritic dystrophy, may be the primary cause of neuronal death in AD. Once neuronal injury or death occurs, either due to Aβ neurotoxicity or as a result of Aβ-induced inflammation, the resulting debris may overtime create a self-amplifying cycle of neurodegenerative events.

Treating and Preventing AD

To date, there are no available therapeutic interventions that halt or reverse AD. As noted earlier, chronically taken NSAIDs appear to delay the onset of AD symptoms, probably by limiting neuronal damage caused by Aβ-induced inflammation. However, excessive use of NSAIDs targeting cyclooxygenase (COX) can cause gastrointestinal, liver, and renal toxicity. Certain NSAIDs including ibuprofen can confer protection against AD by modulating γ-secretase cleavage to lower Aβ42 production, a mechanism that does not require inhibition of COX activity (11). Derivatives of NSAIDS, which lack COX inhibitory activity but can still lower Aβ42

Figure 2: Molecular Structure of Curcumin



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production, are presently being tested in patients with AD. There is evidence that maintaining low LDL levels by various cholesterol-lowering regiments decreases the risk for developing cardiovascular disease and AD. Taken together, these findings suggest that agents such as statins, which possess both cholesterol-lowering capacity and anti-inflammatory properties, could be useful anti-AD agents. The statins are FDA-approved drugs for LDL-lowering therapy, and their therapeutic usefulness in AD is currently being tested in clinical trials.

The notion that plaque formation in AD is enhanced by oxidative damage is supported by studies indicating that antioxidants can be useful inhibitors of AD. For example, high daily doses of both vitamins E and C, which possess antioxidant properties, have been reported to considerably reduce the risk of AD in elderly people (12). Another antioxidant that has been shown to possess therapeutic potential against AD is curcumin, a yellow phenolic compound found in the spice tumeric, which is a key ingredient in American mustard and Indian curry.

Curcumin has long been known for its potent anti-inflammatory and antioxidant activities and its widespread consumption in India is believed to be a major reason for the unusually low incidence of AD in this country, with just 1% of those 65 and older contracting the disease. In recent years, curcumin has become a subject of intense investigation because of its protective effects against human malignancies, which have been demonstrated by a number of studies. The therapeutic potential of curcumin as an anti-AD agent has recently been tested both in-vitro and in a transgenic mouse model for AD (APPsw mice) (13, 14). At concentrations of around 1 μM, curcumin was found to effectively prevent oligomerization and aggregation of Aβ peptides as well as disassemble preformed Aβ fibrils (14). When fed to aged APPsw mice with advanced amyloid levels, curcumin crossed the blood-brain barrier, bound to Aβ, and significantly reduced amyloid levels and plaque burden (13, 14). These findings suggest that the anti-inflammatory activity of curcumin stems, at least in part, from its ability to facilitate amyloid clearance. They also provide strong rationale for curcumin use in the treatment and prevention of AD, and should encourage further search for potential anti-AD agents that could be even more effective than curcumin in disaggregating Aβ fibrils.

References

(1) C.A. Saura, et al. *Neuron*, Vol. 42, 23-36 (2004)
(2) D.M. Walsh and D.J. Selkoe, *Neuron*, Vol. 44, 183-193 (2004)
(3) P.L. McGeer, et al, *Neurlogy*, Vol. 47, 425-432 (1996)
(4) A.M. Manelli, et al. *J. Molecular Neuroscience*, Vol. 23, 235-246 (2004)
(5) J. Jordan, et al. *J. Neurosci.*, Vol. 18, 195-204 (1998)
(6) J.R. Lynch, et al. *J.Neuroimmunol.*, Vol. 114, 107-113 (2001)
(7) D.S. Yang, et al. *Neuroscience*, Vol. 192, 1217-1226 (1999)
(8) F.M. Harris, et al. *PNAS (USA)*, Vol. 100, 10966-10971 (2003)
(9) R. Strohemer and J. Rogers, *J. of Alzheimer's Disease*, Vol. 3, 131-157 (2001)
(10) D.A. Loeffler, *J. of Neuroinflammation*, Vol. 1, 18-29 (2004)
(11) S.Weggen, et al. *Nature*, Vol. 414, 212-216 (2001).
(12) P.P. Zandi, et al. *Arch. Neurol.*, Vol. 62, 82-88 (2004)
(13) G.P. Lim, et al. *J. Neurosci.*, Vol. 21, 8370-8377 (2001)
(14) F.Yang, et al. *J. Biol. Chem.*, Vol. 280, 5892-5901 (2005)