REVIEW ARTICLE

Amyloid and Alzheimer's Disease: Inside and Out

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ABSTRACT: Alzheimer's disease (AD) is poised to become the most serious healthcare issue of our generation. The leading theory of AD pathophysiology is the Amyloid Cascade Hypothesis, and clinical trials are now proceeding based on this hypothesis. Here, we review the original evidence for the Amyloid Hypothesis, which was originally focused on the extracellular deposition of beta amyloid peptides (Aβ) in large fibrillar aggregates, as well as how this theory has been extended in recent years to focus on highly toxic small soluble amyloid oligomers. We will also examine emerging evidence that Aβ may actually begin to accumulate intracellularly in lysosomes, and the role for intracellular Aβ and lysosomal dysfunction may play in AD pathophysiology. Finally, we will review the clinical implications of these findings.

RÉSUMÉ: Tout sur l'amyloïde et la maladie d'Alzheimer. La maladie d'Alzheimer (MA) est sur le point de devenir le problème le plus important du secteur des soins de santé de notre génération. La théorie qui prévaut concernant la physiopathologie de la MA est l'hypothèse de la cascade amyloïde. Des essais cliniques fondés sur cette théorie sont actuellement en cours. Nous revoyons les données originales qui y ont donné lieu. Elle est centrée sur la déposition extracellulaire du peptide bêta-amyloïde (βA) sous forme de gros amas fibrillaires. Cette théorie a été élargie au cours des dernières années aux petits oligomères amyloïdes solubles qui sont extrêmement toxiques. Nous examinons les données de plus en plus nombreuses à l'effet que la βA pourrait commencer à s'accumuler dans les lysosomes intracellulaires et comment la βA intracellulaire et la dysfonction lysosomiale joueraient un rôle dans la physiopathologie de la MA. Enfin, nous revoyons les implications cliniques de ces observations.

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Alzheimer's disease (AD) is the most common neurodegenerative illness. The main risk factor for AD is age. AD affects about 1% of individuals at age 65, with this rate doubling every five years, so that 30% of 80-year-olds are affected[1,2]. The Alzheimer's Society of Canada estimates that there are currently 500,000 Canadians suffering with Alzheimer's disease and this number is expected to rise to more than one million by 2038[3]. These numbers are mirrored in the United States and the rest of the world where there are 5.1 million and 35 million affected respectively, and these numbers are expected to grow to 15 million and 115 million by 2050[4-6]. Alzheimer's disease is expensive, with the cost of caring for Canadians with Alzheimer's disease estimated at $15 billion/year now and projected to rise to a staggering $158 billion per year by 2038[3]. Alzheimer's disease already costs the American economy more than US$150 billion/year[5]. These estimates suggest that AD is poised to become a major challenge for our health care system and our society.

Alzheimer's disease is characterized by many neuropathological changes including neurofibrillary tangles, and loss of synapses and neurons, but it is amyloid plaques that distinguish Alzheimer's disease from other neurodegenerative diseases[7]. Although Alois Alzheimer first described this disease more than 100 years ago, it was only in the 1980's that β-amyloid peptide (Aβ) was identified as the major component of amyloid plaques. This led to the Amyloid Cascade Hypothesis, which is the current leading model of pathophysiology in Alzheimer's disease. In its initial form, the Amyloid Hypothesis posited that amyloid deposition in large macromolecular fibrils was the initiating factor for AD, with numerous other pathological changes occurring secondarily[8,9]. With anti-amyloid therapies in phase 3 clinical trials, it is timely to review the Amyloid Hypothesis as it was originally proposed and the new directions that it is taking. The role of amyloid in intracellular compartments will also be reviewed.

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Aβ is produced by the cleavage of a large transmembrane protein call the Amyloid Precursor Protein (APP) (Figure 1). First, APP is cleaved at a β-site by an aspartyl proteinase referred to as BACE (beta-site APP-cleaving enzyme)[10-15]. Subsequently, APP is cleaved again at a variable "γ" cleavage site by an enzyme referred to as the γ-secretase (described below) to release peptides ranging from 38-43 amino acids. The γ-cleavage regulates the amount of Aβ produced, as well as the relative amount of the more toxic 42 amino acid form of Aβ. Amyloid precursor protein may also be cleaved at an α position within the Aβ sequence, by an enzyme (or family of enzymes) referred to as α-secretases, which prevents the production of Aβ[16-18]. β-cleavage is the preferred pathway in neurons[19]. Once produced, individual amyloid peptides (Aβ42 in particular) have a high propensity to form aggregates that begin as small assemblies of dimers and trimers, followed by 'oligomers' and protofibrils, and the large insoluble fibrils that are seen in amyloid plaques[20,21].

The Amyloid Hypothesis

The literature of AD encompasses numerous patho-physiological mechanisms (reviewed in[22]) that often seem to be dueling for the status of "most important". Indeed the tissue loss seen in gross pathology of AD (Figure 2) is likely the result of many processes, and not merely the result of deposition of amyloid plaques (Figure 3). However, the origins of amyloid hypothesis are not rooted only in pathology, but also in genetics.

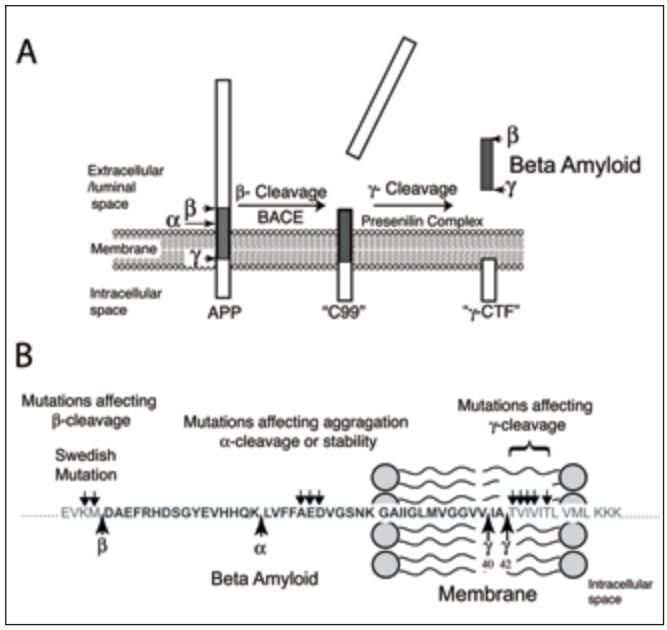

Figure 1: *Schematic of beta amyloid production. A. APP is a large transmembrane protein that is cleaved first at a beta site by BACE, and then at a gamma site by the gamma secretase to release Aβ. B. The sequence of APP spanning the Aβ and transmembrane regions of APP showing the sites of the α-, β- and γ-secretase cleavage sites. Examples of some of the sites of AD-causing mutations are indicated.*

Chromosome 21

The genetic view of Alzheimer's disease begins with the longstanding observation that patients with Down's syndrome (Trisomy 21) invariably develop neuropathological features indistinguishable from Alzheimer's disease in early adulthood[23,24]. This suggested a simple gene-dosage effect caused by an extra copy of a critical gene on chromosome 21. The purification and sequencing of the components in vascular amyloid and amyloid plaques in 1984 lead to the discovery of Aβ[25,26] and to the subsequent cloning of the APP gene on chromosome 21[27,28]. The cloning of APP (and subsequent Familial Alzheimer's Disease (FAD) genes below) has allowed their study in cultured cells and the generation of mouse models. In the case of APP, this led to the surprising finding that Aβ production was not a rare pathological event but rather a normal process. Aβ is produced by many cell types and is normally present in plasma and cerebrospinal fluid (CSF)[29-31]. Synaptic activity regulates the amount of Aβ secreted into CSF[32,33] in mice. In humans, Aβ is also rapidly secreted and cleared from the CSF, presumably governed by similar mechanisms[34].

The next major insight came from families with autosomal dominant Alzheimer's disease (Familial AD- FAD) that occurred well before the age of 65. In some of these families, Alzheimer's

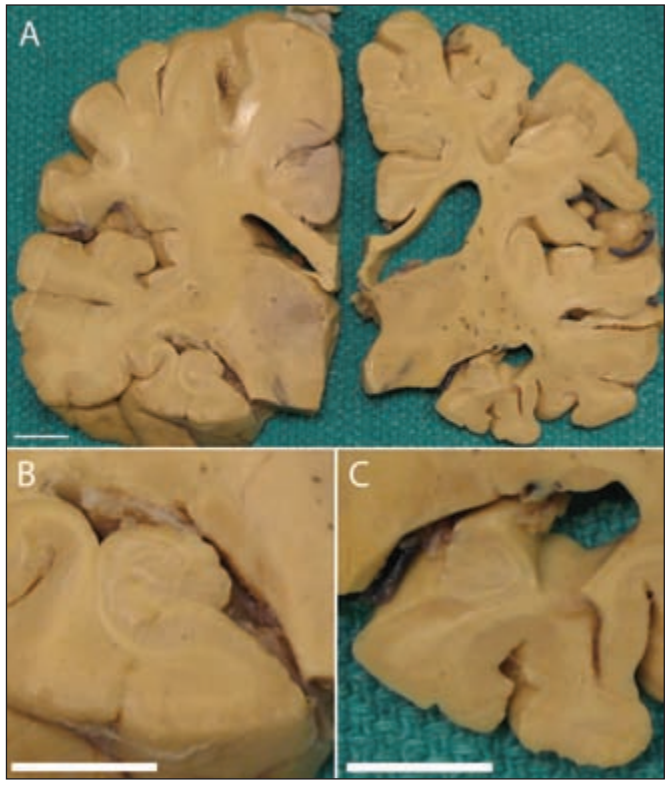

Figure 2: *Alzheimer's disease gross pathology. Panel A shows comparable coronal sections of a normal brain on the (left) compared with the brain with a neuropathological diagnosis of AD (right). Inset are close up images of the hippocampus showing a normal brain (B) and demonstrating the marked atrophy/tissue loss in Alzheimer's disease (C). Scale bar 1 cm.*

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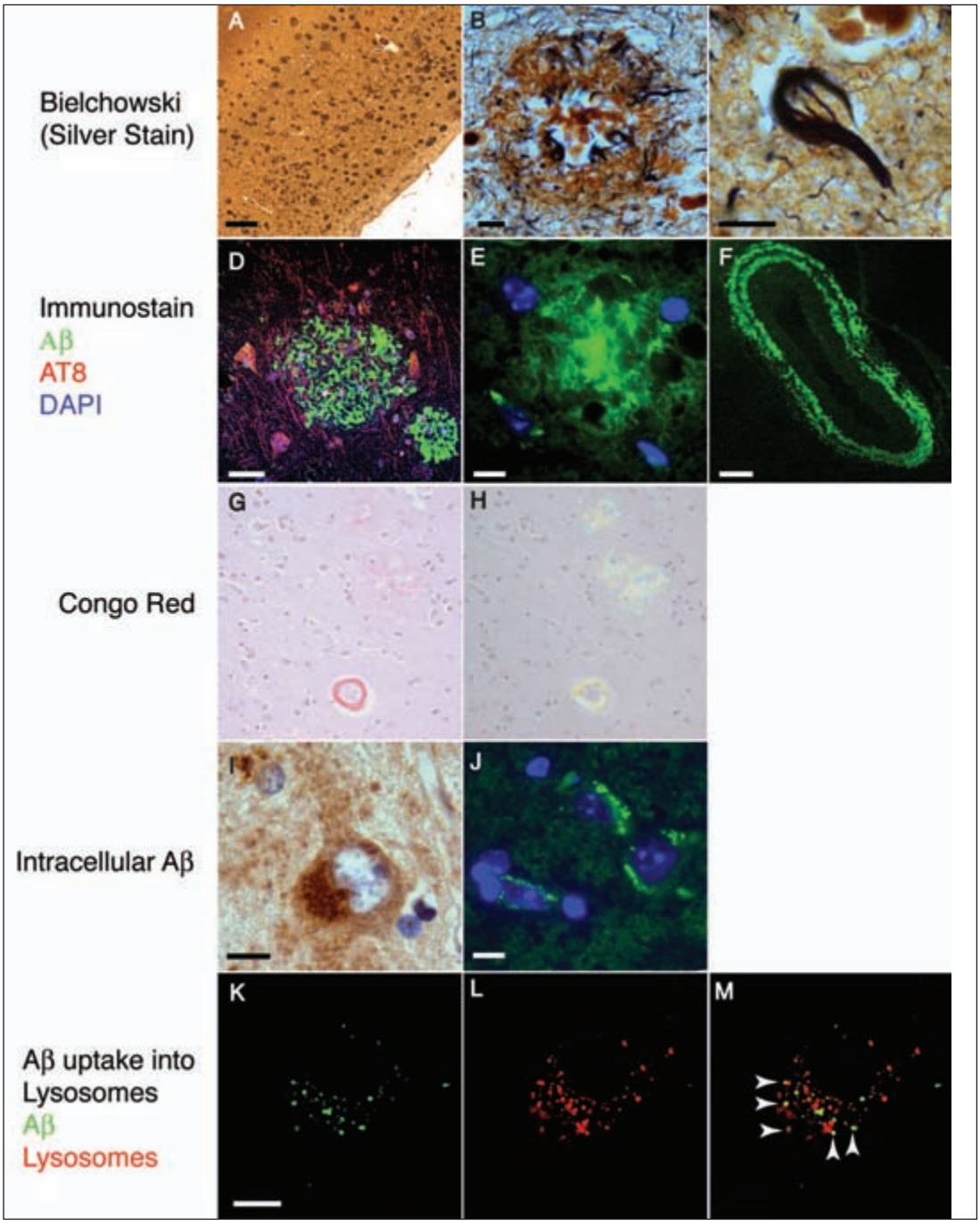

Figure 3: *Extracellular and Intracellular Pathology in Alzheimer's disease. A-C) Human brain from an AD patient stained with Modified Bielschowsky silver stain. A low power view of human temporal cortex (A) (scale bar 200 μm). High power views of the same case, showing an amyloid plaque (B) with amyloid appearing brown and dystrophic neuritis and tangles appearing black (Scale bar = 10μm) and (C) a neurofibrillary tangle (Scale bar = 10μm). D- F) Immunohistochemistry with formic acid pretreatment (antigen retrieval) shows an (D) amyloid plaque stained with antibodies to Aβ42 (green) and an antibody to AT8 (abnormally phosphorylated tau) in red. Nuclei are in blue. (Scale Bar = 30 μm). (E) An amyloid plaque immunostained with an antibody against Aβ42 (green) in the brain of a transgenic APP-Swe PS1-Δ exon 9 mouse (Scale bar 10 μm). F) Vascular amyloid in a human stained with an antibody to Aβ42 (green). (Scale bar 150 μm). G-H Classical Congo red stain of vascular and plaque amyloid showing red staining under while light G (G), but apple-green birefringence under polarized light (H; 400X). I-J Intracellular amyloid is seen using heat treatment (Retriever 2100) to immunostain intracellular granules of Aβ42 inclusions in humans (I, brown) (Scale Bar 10mm). and transgenic mice Aβ42 (J; green) and nuclei (blue) (Scale Bar 10μm). K-L. Aβ42 is taken up into lysosomes. Neuronal SN56 cells allowed to take up 250nM HiLyte Fluor 488 labeled Aβ42 for 24 hours. Aβ42 is green (K) and lysosomes were marked by transfected LAMP1 tagged with mRFP (L; red). Merged image (M) shows colocalized pixels in yellow marked with arrows (Scale Bar 10 μm)*

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disease mapped to the APP gene. To date, 32 mutations (in 86 families) in APP have been described (http://www.molgen.ua.ac.be/ADMutations). These mutations generally fall into three classes. One type of mutation, the Swedish mutation, is located adjacent to the β-cleavage site of APP where it increases the rate of β-cleavage up to 10-fold, resulting in increased production of all amyloid species[35]. Another group of mutations near the γ-site of APP alters the specificity of the γ-secretase cleavage by increasing the relative amount of the more toxic Aβ42 produced (e.g. the London mutation)[36-38]. In addition, point mutations within the Aβ sequence itself that appear to decrease α-cleavage, increase the stability of Aβ or increase its propensity to aggregate (e.g. the Arctic, Dutch and the Iowa mutations[39-41]). FAD can also occur due to increased APP expression due to promoter mutations[42] or by the simple gene duplication of APP[43-45]. Thus, from the point of view of genetic defects on chromosome 21, anything that increases the amount of Aβ or Aβ42 is tightly associated with AD.

Not just chromosome 21

Even early on, it was apparent that many FAD families mapped outside chromosome 21. A FAD-linked locus was mapped to chromosome 14 by Peter St. George Hyslop's group at the University of Toronto[46]. This led to the identification of mutations in the protein presenilin-1 (PS1) and a second homologous gene on chromosome 1 dubbed PS2[47,48]. These patients often have additional features such as seizures, myoclonus, long tract signs, ataxia and psychiatric symptoms[49]. With much subsequent work, presenilin was found to be the catalytic protein in a large enzyme complex called the γ-secretase, which is composed of at least 4 proteins (presenilin, nicastrin, mAph1 and PEN2[50-55]) Recombinant PS1 is able to catalyze γ-secretase cleavage on its own[56], and mutations at either of two critical aspartate residues in PS-1 and PS-2 within the catalytic site abolishes its enzymatic activity[57]. PS also contains the binding site of pharmacological γ-secretase inhibitors[58,59]. Currently, mutations in PS1 account for the largest identified group of FAD, with 177 mutations described in PS1 (392 families) and 14 mutations described in PS2 (23 families) (http://www.molgen.ua.ac.be/ADMutations)[60].

While the exact mechanism(s) by which FAD PS mutations alter γ-secretase function remain to be elucidated, they are believed to cause AD by increasing the relative amount of Aβ42 to Aβ40[56,61,62], even when they reduce the total amount of Aβ produced[63]. The average age of onset in human families correlates with the Aβ42/40 ratio[64]. It is interesting to note that the identification of PS was based solely on genetics with no preconceived notion of their biochemical functions.

Late Onset AD

The genetics of Late Onset Alzheimer's disease has proven more difficult. To date, the best-characterized locus is the Apoliporotein (ApoE) gene. In humans, ApoE has three alleles namely e2, e3, and e4 which differ by only a few amino acids. Individuals with 1 e4 allele are at a 2-3 fold increased risk for Alzheimer's disease and having 2 e4 increases the risk about 12 fold[65]. Some studies suggest that the ApoE2 allele is protective against AD[66]. The mode of action of ApoE also supports the importance of amyloid, as the e4 allele appears to increase the rate of Aβ fibril formation *in-vitro*[67,68] and to increase the observed amount of Aβ deposition in mice and humans[69,70]. More recent evidence suggests that ApoE isoform also controls Aβ clearance from the CSF of transgenic mice[71].

It is estimated that 60-80% of Alzheimer's disease risk may be heritable[72] and many groups are searching for the missing genetic risk factors. In the last few years, new micro-array based techniques of screening referred to as Genome Wide Association Studies (GWAS) have substantially increased the number of candidate genes[73-76]. The AlzGene database (http://www.alzgene.org/TopResults.asp) currently lists the top ten genes likely to be risk factors for AD. Unfortunately these putative genes (assuming that they prove to be correct) each accounts for only very small risk of AD (Odds Ratio of ~1.25) meaning that they are much less powerful than ApoE[73-77].

Challenges To The Original Amyloid Hypothesis?

Despite the importance of Aβ, there were a number of important drawbacks to the original Amyloid Hypothesis. The most serious of these were that amyloid plaque load does not correlate well with cognitive function or disease progression in humans[78,79] or mice[80]. In fact, neurofibrillary tangles (NFT's) correlated better with cognitive impairment, leading many to suspect they might be the critical causative agent in AD[78,79].

Tangles: the fall and the return

Neurofibrillary tangles (NFTs) are made up of intracellular aggregates of paired helical filaments of the protein tau, which have become hyperphosphorylated[78,79] (Figure 3). Although NFT's correlate well with AD onset and progression[81], to date, no tau mutations have been found in AD families. Instead, tau mutations cause phenotypically distinct diseases including frontotemporal dementia, which do not display plaques, suggesting that tau pathology in AD is downstream of Aβ[79,82]. This idea also is supported by pathological studies in Down syndrome and transgenic mice that demonstrate that amyloid plaques precede the appearance of NFT's[83,84]. Furthermore, treatments that increase Aβ oligomers in mouse models lead to increased tau pathology[85-88]. Conversely, immunotherapy to deplete Aβ clears amyloid plaques and reduces early tau aggregates, although later stage tau aggregates are not reversible[87]. Other experiments suggest that tau does have a role in AD as reducing tau levels or disrupting tau's interactions with signaling proteins reduces pathology and improves memory[89-91]. Therefore, tau is likely still important in AD, but its effects are downstream from Aβ.

Amyloid: Are Plaques a distraction?

Although amyloid plaques might be the most striking aspect of AD pathology (Figure 3), the best pathological correlate of cognitive impairment is loss of synapses[92-95]. Synaptic loss correlates not with insoluble amyloid fibrils in plaques but with levels of soluble amyloid species in the brain[96,97]. Aβ are now recognized to aggregate into a wide variety of soluble structures, from simple dimers and trimers to large soluble oligomers sometimes referred to as Amyloid Derived Diffusible Ligands (ADDLs) and Aβ56*[95,98-100]. These soluble aggregates are orders of magnitude more toxic to neurons and synapses than the

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insoluble amyloid fibrils in plaques[20,21]. In animal models, soluble Aβ impairs learning performance and decreases the number of synapses[100-102]. These changes are reversible through clearing or antibody chelation of Aβ[103-105]. Furthermore, oligomeric Aβ binds directly to synapes containing glutamate neurotransmitter receptors (including N-methyl D-Aspartic Acid (NMDA) receptors) causing a rapid decrease in receptors, reducing signaling, disrupting the structure of the synapse[99,103,106,107] and depleting synaptic vesicles[108]. Aβ oligomers have also been proposed to impair LTP by binding to the prion protein (PrP)[109-111] although this is currently controversial[105,112]. It is these small soluble amyloid oligomers that are now the main focus of the Amyloid Hypothesis.

Intracellular Amyloid. The Other Half?

While the discovery of soluble amyloid aggregates may explain the acute effects of amyloid toxicity, it might not explain more chronic changes of AD including alterations of neuronal structure, neuronal loss, or the origin of plaques. One possible inroad to understanding these changes comes from the study of Aβ aggregation inside living cells.

The endosomal/ lysosomal system comprises a series of intracellular compartments that are responsible for taking up extracellular material and proteins from the cell surface. Internalized material is transported to early endosomes, where they are sorted, and then either recycled to the cell surface or transported to late endosomes/ multivesicular bodies and then to lysosomes (See Figure 4). A parallel system called macro-autophagy (referred to hereafter as autophagy) provides a parallel pathway for the degradation of long-lived intracellular proteins and organelles such as mitochondria. Autophagy begins with double-layered sheets of membrane arising from the endoplasmic reticulum engulfing regions of cytoplasm to form double walled autophagic vesicles. These vesicles may then fuse with endosomes to acquire hydrolytic (digestive) enzymes, and eventually fuse with lysosomes[113,114].

Lysosomes are highly acidic (pH 4.5) compartments containing > 80 hydrolytic (digestive) enzymes.[115-118]. Lysosomes are recognized clinically because of more than 40 Lysosomal Storage Diseases (LSDs), which are usually caused by the absence of a critical catabolic (digestive) enzyme resulting in a buildup of undigested material in lysosomes. When they involve the central nervous system, these diseases lead to dementia and death[119]. Although lysosomes are traditionally thought of as simply a waste disposal/digestive system, they are now also recognized as a secretory compartment in a wide variety of cell types including thyroid hormone, pulmonary surfactant, albumin, cytotoxic compounds from lymphocytes and neurtrophils[120-122]. Lysosomes are also able to fuse with the cell membrane in order to repair damage to the cell surface[123].

The endosomal/ lysosomal system plays a role in Aβ production. This was best demonstrated in experiments in which APP is labeled on the cell surface and followed as it is internalized, cleaved into Aβ, and then secreted or retained intracellularly[124-128]. Moreover, increasing the rate of internalization of APP increases Aβ generation, and blocking internalization reduces Aβ levels[32,125,129-137]. Autophagosomes have also been demonstrated to contain APP, β- and γ-secretases and to produce Aβ[138, 139]. Our own work has demonstrated that APP and γ-secretase activity are highly enriched in lysosomes[140,141]. We have also found that APP undergoes unexpectedly rapid direct transport to the lysosome from the cell surface[142] and from internal compartments and these pathways may play a role in Aβ production (unpublished observations)[143].

Although the spontaneous intracellular accumulation of Aβ42 has long been recognized in cultured neuronal cells[144-148], the histological detection of intracellular Aβ in tissue has only been recognized relatively recently. This is likely because the standard techniques used to immunostain amyloid plaques rely on concentrated formic acid; formic acid improves appearance of plaques, but can wash away intracellular deposits[149-151]. Reliable detection of intracellular Aβ therefore requires optimizal tissue preparation techniques (antigen retrieval) along with careful selection of highly specific, high affinity antibodies[152] (reviewed in[153]). Although it still has detractors[154], the concept of intracellular Aβ accumulation is now widely accepted[153,155-157]. Figure 3 shows examples of extracellular and intracellular Aβ.

Intracellular accumulation of Aβ42 in the endosomal/ lysosomal system has been observed in transgenic Alzheimer's disease mice either before or accompanying cognitive impairment, but well before the appearance of amyloid plaques[158-166]. Intracellular Aβ42 has been shown in human

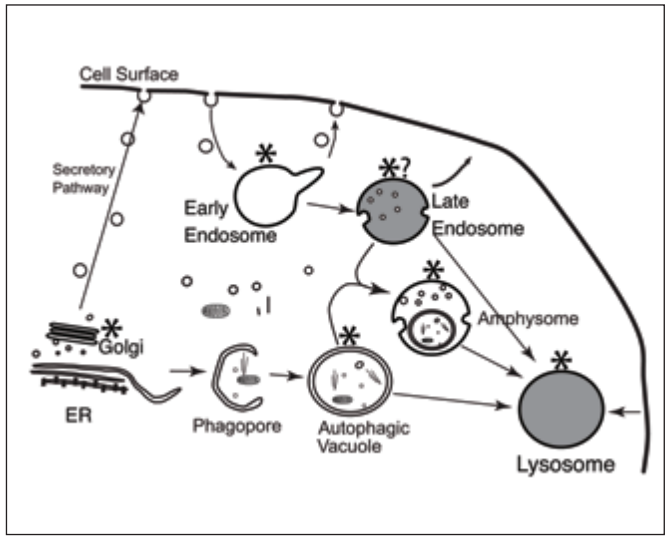

Figure 4: *Overview of the endosomal/lysosomal and autophagy systems. Proteins are synthesized in the ER and transit to the Golgi, where they are glycosylated and exported. Cell surface proteins, transmembrane proteins, and extracellular material are endocytosed to early endosomes (EE). From there they may recycle to the cell surface or transit to the late endosome (LE) and to the lysosome. APP can also be transported directly from the cell surface to the lysosome. Macroautophagy begins with membrane extending out of the ER, which becomes a Phagopore that engulfs cytoplasm and organelles into double membrane bound Autophagic Vacuoles. Autophagic Vacuoles can fuse with endosomes to form Amphysomes or directly with lysosomes. Lysosomes that contain residual indigestible autofluorescent material remain as lipofuscin granules. Compartments implicated in Aβ production are marked * (it is not known if Aβ is made in the late endosome and this compartment is labeled *?). Compartments implicated in Aβ accumulation are shaded.*

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neuropathological material in both Alzheimer's disease and Down's Syndrome patients, where it also appears to fill neuronal lysosomal compartments before the appearance of plaques[44,149,151,167-170]. Recent studies using Laser Capture Microdissection to collect groups of neurons from AD brains and directly assay their Aβ content have confirmed that the histological appearance of increased Aβ in fact reflects true intracellular amyloid accumulation in human brain[171,172].

The intracellular accumulation of Aβ may play a role in AD pathology. This is because Aβ fibrils found in plaques are preferentially nucleated by both lysosomal gangliosides (complex lipids and carbohydrates) and the lysosomal pH of 4.5[173-177]. Furthermore, *in-vitro* experiments demonstrate that Aβ fibril formation directly disrupts lipid membranes[175,178]. These effects come together in experiments that show Aβ taken up from the media can nucleate further Aβ aggregation[155,179-182]. From there, it can directly disrupt the structures of neurons and synapses[183] or cause lysosomal rupture leading to cell death[184,185]. Cell death is likely due to the release of digestive enzymes into the cytoplasm, or their secondary activation of programmed cell death pathways[143,186].

The propensity for Aβ to aggregate in the acidic environment of the lysosome suggests that extracellular amyloid plaques may begin as amyloid 'seeds' in the lysosome. This idea is supported by evidence that cell death begins only after intracellular Aβ42 is detected[187] and that the amount of intracellular Aβ42 decrease as plaques appear[164,188]. In addition, the presence of many active lysosomal enzymes in plaques suggests that lysosomal contents directly contributed to plaques[189-192]. Furthermore, remnants of neuronal cell bodies are often seen in the center of mature 'dense core' plaques suggesting that these cells were lysed to form the beginnings of the plaque[167,193]. Recent experiments using confocal microscopy in live mouse brains have demonstrated that plaques can appear rapidly over 24 hours[194], confirming that plaques can appear acutely.

A different route to pathology in AD?

Abnormalities in the endosomal/lysosomal system have been recognized in Alzheimer's disease pathological material since the early 1990's. Even before the onset of clinical disease, there is a marked increase in the number and size of lysosomes in brain regions most vulnerable to Alzheimer's disease. As Alzheimer's disease advances, lysosomes multiply and appear to fill neurons[115,191,195-197]. Using electron microscopy, it is now recognized that many of these compartments are autophagic vacuoles that are also undergoing massive upregulation[138,198]. In fact, the dystrophic neurites characteristic of AD are actually neuronal processes filled predominantly with autophagic vacuoles[199]. Autophagy in neurons is highly efficient and autophagosomes are not normally observed, and their appearance suggests a pathological failure of this system[200].

The accumulation of lysosomes and autophagosomes in AD is strikingly similar to the pathology seen in Lysosomal Storage Diseases (LSDs)[199]. These diseases are also accompanied by a prominent failure of autophagy[119,199], and the failure of autophagy can cause neurodegeneration on its own[201] and may represent a mode of cell death[119]. Conversely, the failure of the lysosomal system in a number of LSD's can also lead to elevated levels of Aβ and tau; these include Niemann-Pick Type C[202], Tay Sach's and Sandhoff's disease[203] and mucopolysaccharidosis[204]. These effects can be partly replicated experimentally; inhibiting lysosomal proteolysis causes buildup of autophagic vacuoles in processes very similar to AD[205], and overloading cells with gangliosides inhibits APP degradation and increases Aβ production[203,206].

Recently, PSs have emerged as a potentially unifying factor in these pathologies. PS1 is important for clearance of proteins from endosomes[207], for clearance of proteins by autophagy[199,208,209] and for trafficking through the endosomal lysosomal system[210]. In a recent twist, PS mutations have been shown to directly impair lysosomal function by preventing lysosomal acidification[211] suggesting that FAD might be in effect a Lysosomal Storage Disease. Thus, PS mutations can lead to cell death by two different pathophysiological processes. On the one hand they are responsible for producing toxic Aβ species, and on the other hand they block autophagy, impairing the cell's ability to clear Aβ (and perhaps tau).

Defects in autophagy and intracellular Aβ clearance may represent a therapeutic target. This was shown dramatically in recent experiments in which knocking-out an endogenous lysosomal protease inhibitor (cystatin B) increases the clearance of intracellular Aβ, reduces the number of autophagic vesicles, decreases plaques, and prevents the development of cognitive changes[212]. A number of 'lysosomal modulatory' drugs are under study, that increase the levels of lysosomal enzymes and appear to reduce Alzheimer's pathology in a tissue slice model of AD[213,214].

Taken together, these data suggest that in addition to the acute extracellular effects of Aβ oligomers, Aβ also accumulates intracellularly as either a cause or a consequence of lysosomal dysfunction, which results in neurons exhibiting neuropathological changes reminiscent of classic LSD. These intracellular changes can cause pathology independently from the acute synaptotoxic effects of extracellular Aβ oligomers. Amyloid plaque formation might be secondary to neuronal lysis and release of intracellular Aβ.

Conclusions: Where To From Here?

Although the Amyloid Hypothesis has gone through a number of twists and turns, amyloid still appears to be an important causal factor in AD. However, the true proof of this model will ultimately rest on whether amyloid-reducing therapies can treat Alzheimer's disease. A large number of compounds are currently in clinical trials. Generally anti-amyloid treatments fall into several classes, including compounds that inhibit Aβ production (by inhibiting secretase enzymes) and compounds which bind Aβ to impair aggregation, neutralize toxicity or increase clearance[215,216]. These include antibody-based strategies of active immunization and passive immunization with monoclonal or polyclonal antibodies [87,217-222], or small molecules such as Scyloinositol[223]. Although these strategies are effective in treating mice, there have been a number of high profile failures in clinical trials, including Tarenflurbil and LY451039 (secretase modulators/ inhibitors) and Alzmed (tramiprosate; an aggregation inhibitor)[224]. In addition, we know from a discontinued Aβ vaccination trial in humans, that clearance of Aβ plaques alone is not sufficient to improve symptoms[225,226].

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Some have taken these failures to mean that there is a problem with the Amyloid hypothesis itself. Before coming to that conclusion, we must first look at the limitations of these trials. Currently, AD is a clinical diagnosis, made only after the appearance of cognitive impairment. However, AD has a long presyptomatic phase suggested to be years to decades[227,228], during which neuropathological changes and subtle cognitive and imaging changes can be observed[229-232]. While the transition from presymptomatic disease to AD is difficult to identify clinically, it is important to be able to do so, because neuronal loss begins at the earliest clinical changes of AD (transition from a Clinical Dementia Rating scale score (CDR) of 0 (asymptomatic) to a CDR of 0.5 (very mild dementia)[233,234]. Numerous biomarkers are under study to try to detect or predict this transition to AD. These include CSF protein levels (tau and Aβ), volumetric MRI to quantitate atrophy, and nuclear medicine-based scans of brain glucose uptake and amyloid load (e.g. Pittsburgh compound or PiB scans). Although these are promising, they are still considered research tools[227,235-238].

From animal studies, we know that even modest reductions in Aβ production can dramatically reduce amyloid deposits and improve memory[165,223], but only early pathology may be reversible[87]. In humans, a number of other factors will likely complicate clinical applications. For example, amyloid found in human brains is much more insoluble than amyloid produced in mice,[239] suggesting that it will be more difficult to clear. In addition, the γ-secretase also processes a large number of other important regulatory proteins (most notably the Notch receptor) and inactivation is toxic in many tissues and lethal to embryos[240-242]. A safe γ-secretase inhibitor will need to selectively block Aβ production without impairing its other functions. It must also be pointed out that the failed trials were first generation agents which had significant technical limitations and were advanced to phase 3 trials without being successful in phase 2 trials[224].

More concerning, however, is that transgenic mice used to develop these treatments generally do not exhibit the high levels of neuronal loss seen in AD. In fact, it has been argued that transgenic mice only model early AD, while the trials were looking at established AD[224]. This fundamental mismatch between the mouse models and the human patients might therefore be the largest obstacle for the therapeutic application of the Amyloid Hypothesis.

It may be that that anti-amyloid agents will only be effective if they are used before irreversible damage has occurred, or in other words before symptoms appear. Therapeutic trials may not work without better diagnostics to allow identification of patients with presymptomatic or very early AD,[224]. With the oncoming tidal wave of patients, the cost of failure to treat or cure Alzheimer's disease will be staggering. If we are to affect the course of AD, safer and more effective amyloid lowering treatments will need to be coupled with better, earlier, presymptomatic diagnosis.

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