Chapter 8

Beta Amyloid Peptides: Extracellular and Intracellular Mechanisms of Clearance in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease and the most common form of dementia, characterized by the overproduction and accumulation of different amyloid-β peptide peptides (Aβ) within different areas in the brain conducting to memory loss and dementia. The Aβ cascade hypothesis of AD was originally proposed by Selkoe in 1991 by the theory that accumulation of Aβ42 is the initial trigger for neurodegeneration. The Aβ cascade hypothesis assumes that changes in the production or accumulation of Aβ are responsible for AD pathology. Different Aβ clearance mechanisms are also affected by AD pathology. Studies from the past years have revealed that the blocking of Aβ production is not effective for reducing the brain Aβ levels. However, the relevance of Aβ clearance in AD, especially in late-onset sporadic AD (LOAD), has been heightened, and the study of the Aβ clearance mechanisms has elucidated new possible therapeutic targets. This chapter summarizes recent data underlying the idea of the reduced Aβ clearance and subsequent Aβ spread in AD. We discuss the Aβ clearance mechanisms altered in AD, and the Aβ clearance through autophagy in more detail, a more recent mechanism proposed, and the new strategies to eliminate Aβ42 inducing autophagy.

Keywords: β-amyloid peptide, alzheimer´s disease, clearance, autophagy, neurodegeneration

1. Introduction

The removal of accumulated amyloid peptide in the brain is carried out by several mechanisms. The clearance of Aβ by nonenzymatic pathway is performed as follows: the total flux of the interstitial fluid (ISF) into the cerebrospinal fluid (CSF) accompanied by the ISF drainage pathway, phagocytosis by microglial or astrocytic cells, and a mechanism named peripheral sink (transport through the blood vessel walls into the circulation); the last one could be regulated by different receptors.

The enzymatic pathway uses the proteolytic machinery in the brain in order to clean the Aβ excess and includes the participation of multiple Aβ-degrading enzymes (ADE) implicated in the clearance of the different Aβ peptides, which include neprilysin, insulin-degrading enzyme, matrix metalloproteinase-9, glutamate carboxypeptidase II, the mitochondrial human preprotease, and others.

Finally, the last Aβ clearance mechanism involved in the Aβ degradation is related with the proteasome and Aβ degradation by autophagy. The proteasome is important for the degradation of worn out and misfolded proteins. Decreased proteasome activity has been implicated in Alzheimer's disease (AD) and proteasome inhibition induces autophagy. Autophagy is a catabolic process involved in the degradation of aberrant organelles and macromolecules, into double membrane vesicles, and delivers it to lysosomes for degradation and the eventual recycling of the resulting macromolecules, and more recently, autophagy has been related with Aβ clearance, but it is still unknown whether autophagy is beneficial or deleterious to AD neurons as the autophagosome has been suggested as a site of amyloid-β (Aβ) generation. In addition, there is little information about the autophagic processes on neurons or microglia involved in the degradation of amyloid peptides.

A series of studies on the Aβ clearance mechanism provide new insight into the pathogenesis of AD at the molecular level and suggest a new target for the development of novel therapeutics. There are a lot of publications dealing with signaling pathways of Aβ synthesis and related enzymes but the identification of molecules responsible for Aβ clearance pathways and their mechanistic links to AD is still a matter of debate. The recent results have shown that Aβ-degrading enzymes have played an important role in reducing AD pathology in both cell and animal models. However, the induction of intracellular clearance of Aβ42 by autophagy is becoming an important proposed mechanism to improve the degradation of Aβ peptides.

2. Methods

2.1. Literature review

This chapter reviews the current available information about beta amyloid clearance mechanisms. We performed a systematic review and exploratory analysis of articles in order to identify, select, and synthesize all high-quality research evidence and arguments relevant to

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the mechanisms of clearance of beta amyloid peptides form CNS in Alzheimer´s disease. By high-quality evidence we mean the whole of peer-reviewed articles in indexed journals to guarantee the quality and reliability of data in this chapter. Specifically, a manual literature search was carried out using the Medline, NCBI, and Embase databases. The following text words and MeSH headings were used in this search: "beta amyloid 1-42", "beta amyloid 3-42", and "beta amyloid 11-42", "clearance", "degradation", "enzymatic clearance", "microglial activation", "autophagy", and combinations of these terms, and the search was extended for papers referenced by other papers, papers and authors known by reputation, and papers from personal databases.

3. Alzheimer´s disease: a brief description and pathological markers (Aβ1-42, 3-42, 11-42)

Alzheimer's disease (AD) is the most common cause of dementia in the elderly population. It is characterized by a progressive atrophy in several brain areas such as the entorhinal cortex, hippocampus, corpus callosum, and also areas outside the limbic system [1]. This process is irreversible and results in memory loss, inability to learn, performing calculation, unbalanced perception of space, and depression. AD is classified in three stages: mild, moderate, and severe.

AD commences with signs of mild cognitive impairment characterized by memory loss, poor judgment, mood swings, repetitive questions, and difficulty in doing mathematical calculations. The symptoms of moderate AD include the inability to learn new things, difficulty to recognized people, hallucinations, delusions, paranoia, and impulsive behavior. Finally, severe AD patients are dependent and bedridden [2].

Pathological hallmarks of AD include the presence of neurofibrillary tangles, senile plaques, neuronal death, synapsis loss, astrogliosis in the enthorinal cortex, hippocampus, amygdala, and frontal, temporal, parietal, and occipital cortex [3].

Neurofibrillary tangles are intracellular deposits of paired helical filaments formed by hyperphosphorylated tau protein. On the other hand, senil plaques are present as diffuse plaques composed of amorphous extracellular deposits of amyloid β (Aβ) that lacks neurites and neuritic plaques composed of extracellular deposits of insoluble Aβ surrounded by dystrophic neurites, reactive astrocytes, and activated microglia [3, 4].

The etiology of AD is not yet fully understood, but genetic and environmental factors are involved in the disease pathogenesis and progression. Early-onset, so-called familial AD occurs in 1% of cases that are linked to autosomal dominant mutation in amyloid β precursor protein (APP), presenillin (PSEN) 1, and PSEN2. The rest of AD cases are sporadic, the late-onset form (so-called "sporadic AD"). There are also genetic risk factors associated with late-onset AD, for example, the presence of the ε4 allele of the gene for apo-lipoprotein E, which has been shown to increase the probability of the development of AD, whereas the presence of an ε2 allele appears to protect against the disease [5, 6]. Nongenetic risk factors include

cerebrovascular changes (hemorrhagic or ischemic), hypertension, type 2 diabetes, and metabolic syndrome [4].

Aβ is generated from the amyloid precursor protein, an ~105 kDa single-pass transmembrane glycoprotein found at presynaptic and postsynaptic terminals in the brain. APP gene in human is located on chromosome 21 and alternative splicing of APP transcript generates 8 isoform, of which the 695, 751, and 770 amino acids forms are the most common [7]. APP695 is predominantly expressed in neurons, especially during neuronal differentiation whereas APP751 and APP770 are more ubiquitous, although during brain injury their expression increases in astrocytes and microglia [8]. APP plays an important role in neuronal functions such as synapse formation, neuronal migration, neurite outgrowth, synaptic plasticity, synaptic transmission and learning, and memory [9]. APP is synthetized in endoplasmic reticulum and is modified in the Golgi apparatus. The ectodomain contains part of the Aβ sequence, which extends into the transmembrane domain.

Proteolytic processing of APP includes two different pathways (**Figure 1**): (1) nonamyloidogenic processing and (2) amyloidogenic processing. The first is the cleavage by α-secretase within the Aβ domain releasing a soluble α-secretase-released N-terminal of APP (sAPPα) and generating a truncated APP CTF (αCTF or C83). The latter is subsequently intramembrane cut by γ-secretase, which liberates a truncated Aβ peptide called p3 and generates the APP intracellular domain (AICD). This process stops the production of β-amyloid peptide and prevents its deposition in plaques. On the other hand, APP can be cleaved by β-secretase at the beginning of Aβ sequence liberating a soluble sAPPβ and generating a membrane-associated *C*-terminal fragment (βCTF or C99) whose subsequent cleavage by γ-secretase activity results in the generation of Aβ peptides ranging in length from 38 to 42 residues, where Aβ1-42 is the most neurotoxic form [10–13]. The resulting peptides are liberated into extracellular fluids such as cerebrospinal fluid (CSF), plasma, or interstitial fluid [14].

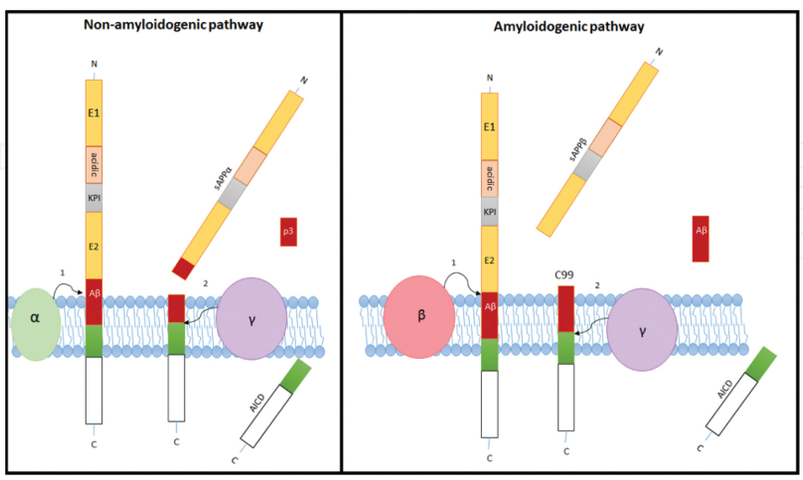

Figure 1. APP schematic structure and processing by secretases.

Mutation in APP molecule has differential effect depending on the location of the mutated residue. Amino acid substitution flanking the Aβ region close to β-secretase cleavage site like

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Swedish mutation modulates the rate of enzymatic processing of APP maintaining the ratio Aβ-42/Aβ-40. In contrast, mutation occurring in close proximity to γ-secretase cleavage site (such as the so-called Austrian, Iranian, French, German, London, and Florida mutations) is associated with increasing production of Aβ-42 and lower levels of Aβ-40 [15]. Mutations in the mid region of Aβ domain affect the primary sequence of Aβ peptide resulting in enhanced aggregation propensity. Some of these intraAβ mutations can lead to mixed amyloid pathologies: marked cerebral angiopathy and marked amyloid plaque formation [11]. The α-secretase activity is mediated by a series of membrane-bound proteases, which are member of the ADAM (a disintegrin and metalloprotease) family. In neuron, the principal constitutive α-secretase activity is exerted by ADAM10. The processing of APP by α-secretase is postulated to be protective in the context of AD because the enzymes cleave within the Aβ sequence, thereby preventing the production of Aβ [16].

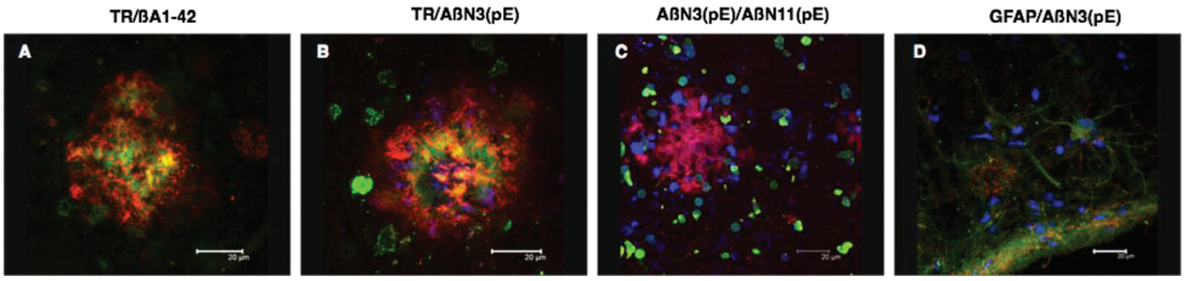

Figure 2. Amyloid plaques of 1-42, AβN3(pE), and AβN11(pE) present in human brain. (A) Merge (yellow) between βA1-42 plaque (green) and the fibrillar βA1-42 marker TR (red). (B) Merge (yellow) between AβN3(pE) plaque (green) and TR (red). (C) Merge between AβN3(pE) (green), AβN11(pE) (red), and the nuclear marker DAPI (blue). (D) Merge between AβN3(pE) (red) and the glial cell marker GFAP (green) showing a glial cell surrounding AβN3(pE) aggregates. These amyloid aggregates were observed in 50 mm thick brain tissue sections of temporal cortex from AD patients. Scale bar represents 20 μm; A–D.

The major neuronal β-secretase, termed BACE-1, is a transmembrane 501 amino acid aspartyl protease. After synthesis, BACE-1 is transported to the cell surface via the endoplasmic reticulum and Golgi. APP and BACE-1 are both endocytosed where the APP cleavage occurs as the optimum pH of BACE is 3.5–4.4. Mutations of BACE-1 have not been identified in familial AD cases, but the activity of BACE is increased in both familial and sporadic AD [17]. The γ-secretase activity is executed by a high molecular weight, membrane-embedded protein complex consisting of PSEN, nicastrin, anterior pharynx defective (APH1), and presenilin enhancer (PEN2), although PSEN seems to provide the active core γ-secretase complex functioning as an aspartyl protease [18]. In mammals, two homologous, PSEN1 and PSEN2, are found whose mutation alters the biochemical character of the γ-secretase complex and its interaction with APP substrate to skew the transmembrane cleavage toward longer more aggregation-prone forms of Aβ, increasing the ratio Aβ-42/Aβ-40, which is associated to early onset of AD [19]. The proteolytic APP processing preferentially generates Aβ1-40/1-42; however, there is a great diversity of Aβ peptides depending on γ-secretase and shorter peptides resulting from γ-secretase activity upon C99. In addition, a significant proportion of Aβ consist of N-terminal truncated/modified species, which increase the Aβ propensity to form aggregates [14, 20] with the most prominent forms identified starting at position 3 or 11 and

possessing N-terminal pyroglutamic acid (pyroE), generated by glutamic acid [21]. The N-terminal truncated Aβ3-42/Aβ3 is generated by the zinc-metalloprotease neutral endopeptidase or neprilysin (NeP)-40 cleaving Aβ between Arg-2 and Glu-3. On the other hand, BACE-1 is also capable of cleaving between Tyr-10 and Glu-11, leading to the release of Aβ11-42/Aβ11-40 peptides [22]. Then, the GluN-terminal undergoes N-terminal pyroglutamate (pGlu) modification catalyzed by glutaminylcyclase (**Figure 2**) [23].

4. Aβ structure

The native conformation of Aβ is an unfolded protein. Aβ forms amyloid fibrils by folding from the native random-coil-rich state to α-helical-rich intermediate, and finally to a β-sheet-rich amyloid monomer that self-assembles into the fibrils [24].

Another protein closely related to AD pathology is the tau protein. This is encoded in mapt gene, located on chromosome 17q21. Several tau isoforms are generated by alternative splicing, creating high and low isoforms. The human central nervous system expresses six low molecular weight isoform ranging from 352 to 441 amino acids [25].

Tau is a neuronal cytosolic protein whose function is to promote microtubules polymerization and stabilization. In addition, Tau has importance in maintaining an appropriate morphology of neurons and it appear to modulate axonal transport [26, 27].

Domains of Tau are defined on the basis of their microtubule interaction and their amino acid character. The C-terminal domain (assembly domain) binds microtubules while the N-terminal domain projects away from microtubule (projection domain). The overall amino acid tau composition is hydrophilic, consistent with its unfolded character; however, the N-terminal is predominantly acidic and the C-terminal roughly neutral, which is important for microtubule interaction. The middle region is a proline-rich domain which is targets of many proline-directed kinases and binding sites for proteins with SH3 domains [28]. Tau is highly regulated and is subject to multiple post-translational modifications. Phosphorylation is the most common tau post-translational modification described resulted from the equilibrium between the amount and activity of protein kinases and phosphatases.

Hyperphosphorylation of tau is not only associated with the disease, but is also employed by the neuron to downregulate its activity transiently and reversibly where required, for example during development, anesthesia, and hypothermia. It is the nonreversible nature of the abnormal hyperphosphorylation of tau in AD and other tauopathies that results in an involuntary slowing down of neuronal activity and a consequent chronic progressive neurodegeneration [29]. Increased tau phosphorylation decreased its affinity for microtubules resulting in an abnormal increase in the levels of the free (unbound) Tau fraction; next small nonfibrillary tau deposits (normally referred to as "pretangles") are formed followed by structural rearrangement involving the formation of the characteristic pleated β-sheet structures, which finally form the neurofibrillary tangles by self-assemble [26].

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5. Clearance mechanisms

5.1. Alzheimer's disease clearance: is microglia involved?

Since Alois Alzheimer described the disease in 1907 several therapeutic options have been developed. The therapeutic treatments available today treat the symptoms without targeting the cause of the disease [30] and as a consequence the disease follows it's natural course [31]. Cholinesterase inhibitors and memantine are FDA-approved therapies against the cognitive symptoms for AD [32]. These drugs favor short-term cognitive benefits, so even though patients are receiving the ideal treatment, they will return to their baseline cognitive decline [33].

Since the amyloid cascade was described research for a disease modifying therapy is being aimed toward the study of Aβ. β-Secretase inhibitors have been tested in an attempt to reduce the production of Aβ. It was demonstrated that β-secretase inhibitors reduced plasma and CSF levels of Aβ but concerns have emerged about potential side effects with chronic administration [34].

One of the most recent strategies, known for its ability to reduce the accumulation of Aβ and promote a cognitive benefit in preclinical trials, is the immunotherapy. The immunotherapeutic approach can be classified as either active or passive. Passive immunotherapy refers to the administration of anti-Aβ antibodies, bypassing the patient need to mount an immunological response toward Aβ. Active immunotherapy involves the administration of full length Aβ or peptide fragments conjugated to a carrier protein, with a T-cell epitope and with an adjuvant in order to stimulate the patient own immune response. The basis of both immunotherapeutic approaches relies on the recognition of Aβ aggregates by specific anti-Aβ antibodies [35].

Extensive studies of active and passive immunization with Aβ showed promising benefits. Schenk et al. were the first to report the beneficial effects of Aβ immunotherapy in a preclinical study with active immunization in PDAPP mouse. The immunization with Aβ reduced levels of cerebral amyloid and produced high serum antibody titers. A year later Morgan et al. reported that Aβ immunization improved behavioral performance in learning and memory tasks [36]. Passive immunization studies showed that antibodies were able to enter the central nervous system, 0.01% of the peripherally administered antibodies, bind plaques, and induce clearance of preexisting amyloid lesions. Passive immunization of PDAPP mice led to reduce plaque burden, increase blood circulating Aβ, and improve cognitive performance [37]. Immunotherapy via active or passive immunization against Aβ peptides has shown to be very successful in reducing Aβ aggregates in AD animal models [38–41]. The immunotherapeutic approach was translated to clinical trials by ELAN/Wyeth in 2000. After a few immunized subjects, the trial was stopped due to the development of meningoencephalitis in 6% of immunized AD patients [42]. The postmortem analysis of participants, who died from causes not related to the immunization, showed patchy clearance of amyloid plaques in the brain [43]. These areas of clearance were accompanied by Aβ immunoreactive microglia cells, supporting

the hypothesis that Aβ-specific antibodies may lead to the phagocytosis of Aβ by microglia cells [39].

The involvement of microglia in clearance of Aβ aggregates after immunotherapy has been demonstrated through several studies. After a single injection of anti-Aβ antibody to APP mice, the antibodies were found associated not only with amyloid deposits but also with microglia surrounding the plaques [44]. Wilcock et al. reported that 24 and 72 hours after the injection of anti-Aβ antibodies to Tg2576 APP mice there was a reduction in fibrillar amyloid deposits and showed an increase in microglial activation, evaluated by CD45, a protein tyrosine phosphatase commonly used as a marker for microglia activation, and MHC-II staining. Intracraneal injections of anti-Aβ antibodies to APP mice demonstrated that the increase in CD45 expression of microglia is evident after the clearance of diffuse deposits and is parallel with the clearance of fibrillar deposits [36]. The temporal association of fibrillar amyloid loss with microglia activation suggests some causal role for microglial activation in the process [45]. In 2004 Wilcock reported that 1 month after the administration of anti-Aβ antibodies to APP transgenic mice an increase in CD45 expression on microglia surrounding amyloid deposits in both the hippocampus and frontal cortex. After 2 months of treatment there was an additional increase in CD45 not only in microglia surrounding amyloid plaques but also in microglia associated with soluble aggregate [46, 47]. This microglial activation also takes form of an increased transcript level of proinflammatorycyctokines and iNOS [47].

The role of microglia in the clearance of amyloid deposits after the administration of anti-Aβ antibodies was analyzed *in vivo* through the generation of the CX3CR1-GFP protein. CX3XR1 is a gene specifically expressed in microglia in the CNS [48]. After administration of an anti-Aβ antibody that recognizes both aggregated and soluble Aβ, PDAPP mice contained more levels of CX3CR1-GFP positive cells and these cells had twice as many protruding processes from their cell bodies. These changes were detected surrounding amyloid plaques and amyloid deposits associated with blood vessels. These changes were also seen with the microglia marker Iba-1 and with CD45 staining [49]. When Fab fragments of the antibody were injected there was no effect on the number of microglia CX3CR1-GFP positive cells or on microglia morphology, suggesting that the Fc is required to elicit the microglial changes observed in the mouse treated with the full-length antibody [49].

One of the mechanisms for plaques clearance is by anti-Aβ immunotherapy through FcγR-mediated phagocytosis of plaques by microglia [50]. After administration of anti-Aβ antibodies to APP mice there was an increase in FcRII and FcRIII on microglia. The microglia expressing the FcR were associated with amyloid plaques and with diffuse aggregates [46]. When examined in *an ex vivo* assay with sections of PDAPP or AD brain tissue, antibodies against Aβ-activated microglia cells clear amyloid plaques through FcR-mediated phagocytosis and subsequent peptide degradation [39]. Anti-Aβ antibodies with binding affinity for FcγR increased the Aβ oligomer induced p38MAPK activity in microglia [51]. The p38MAPK pathway is responsible for the upregulation of proinflamatory cytokines in microglia, such as TNF-α and IL-1β [52].

After the role of microglia in anti-Aβ antibodies amyloid clearance was proposed, and the effect of microglia inhibition was assessed. The anti-Mac-1-saporin immunotoxin was used to

kill activated microglia in APP mice. The elimination of activated microglia reduced Aβ clearance by anti-Aβ antibodies, although appreciable clearance was still present [53]. This suggests that microglia, dependent and independent mechanisms, are likely involved in the clearance of amyloid aggregates following immunotherapy [54]. Additional mechanisms for clearance of amyloid peptides are possible *in vivo*. If Fab2 fragments, which fail to activate microglia, are injected in transgenic mice, the clearance of Aβ deposits is blocked, but the clearance of diffuse aggregates is unaffected [46]. These data suggest that the clearance of diffuse aggregates may proceed by the catalytic dissolution mechanism proposed by Solomon [50], who postulated that direct interaction of antibodies with Aβ may lead to the disruption of aggregates [55]. This process does not depend on FcR activation. Even though there are several non-Fc-dependent mechanisms for the removal of Aβ aggregates, previous studies demonstrate, through the analysis of microglial cells and frozen tissue sections, that the Fc-mediated mechanism is dominant in the removal of amyloid deposits [39].

Even though the involvement of microglia is important for the removal of amyloid deposits, the activation of microglia by anti Aβ antibodies is accompanied with microhemorrhages and edema. It has been proposed that microglia activation by antibodies induces damage to the vasculature and to the neurons [50]. The prevention of microhemorrhages is achieved when antibodies with reduced affinity for FcγR are used. This was shown by using Crenezumab, a humanized antibody with lower affinity for all FcγR; this antibody promotes a reduction in microglial activation, limiting the release of inflammatory cytokines to avoid side effects, such as vasogenic edema. In the phase I of the safety trial, no vasogenic edema or microhemorrhage were found (Clinicaltrials.gov). The current immunotherapeutic approaches for AD are directed to the design of an optimized antibody that could separate the phagocytic and inflammatory response, promoting an efficient clearance of Aβ aggregates the induction of the detrimental proinflammatory citokyne release [56].

6. Intracellular mechanisms

6.1. βA peptides degradation by autophagy

Cellular homeostasis largely depends on the proteostasis network. Under normal conditions, this network senses and rectifies disturbances in the proteome to restore homeostasis in cells. The main players in proteostasis maintenance are chaperones and two proteolitic systems: the ubiquitin-proteasome and the autophagy system.

Although there are some differences in this proteolitic systems, substrates of the ubiquitin-proteasomes pathway are predominantly short-live proteins and misfolded or damaged proteins. Meanwhile, the autophagy substrates are long-live proteins and multiple proteins organized into oligomeric complex or aggregates that cannot be degraded by others systems [53].

In this sense, macroautophagy (hereafter referred to as autophagy) has been characterized as a catabolic process that engulfs aberrant organelles, misfolded proteins, and protein aggregates

into double membrane vesicles (named autophagosomes) and delivers it to lysosomas [54]. The correct function of this catabolic process is very important because it is the only known mechanism that eukaryotic cells possess to degrade protein aggregates and the only one by which entire organelles such as mitochondria and peroxisomes are recycled. Several studies proposed that autophagy helps to relieve the proteotoxic stress of misfolded proteins by degrading toxic oligomers in the cytosol [55].

Posmitotic cells like neurons are highly dependent on autophagy. Mainly because once neurons mature and become postmitotic, they lose their ability to dilute insults by cell division. Thus, neuronal survival heavily depends on housekeeping processes to maintain cellular quality control [56]. In this regard, the loss of autophagy particularly in neurons causes the accumulation of ubiquitin-positive inclusion bodies and triggers a process of neurodegeneration [57]. Evidence points that dysfunction in the autophagy processes is part of Alzheimer's disease pathogenesis [58] and the clearance of autophagic vacuoles and lysosomal degradation of Aβ could prevent the intracellular accumulation.

6.2. Autophagy

Nowadays it is recognized that autophagy has a fundamental role in homeostasis through the degradation of components that would be toxics for the cell.

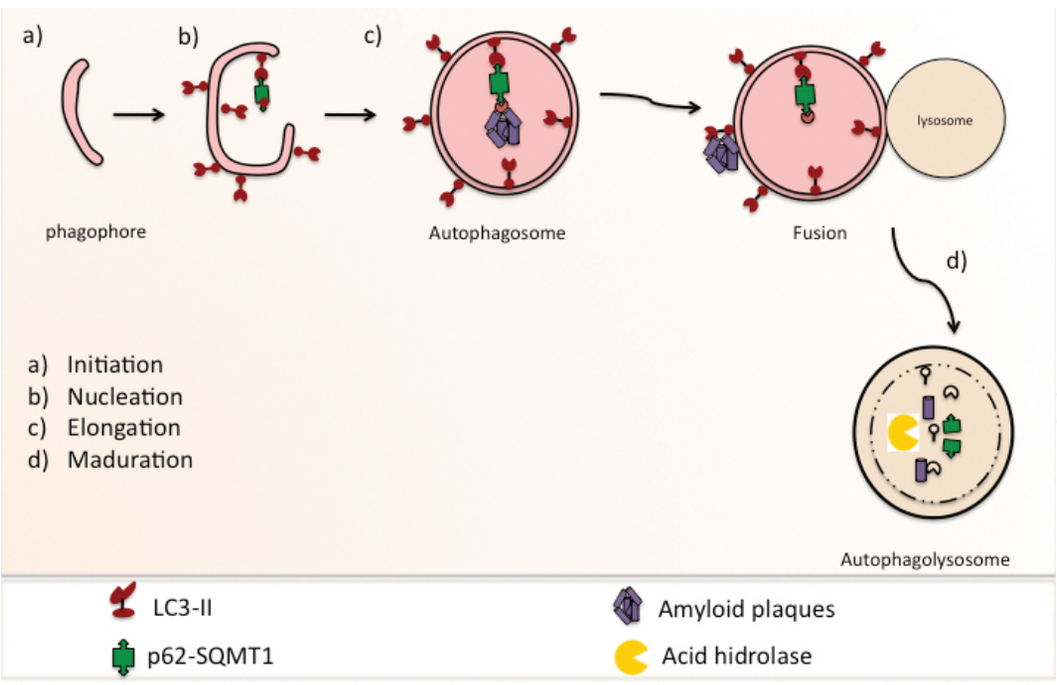

Figure 3. Autophagy elimination of Aβ plaques. Autophagy is the only known mechanisms that eukaryotic cells possess to degrade Aβ aggregates.

Autophagy is a complex process that requires a series of coordinated steps. In the first place, the formation of a vesicle isolation called phagophore is described. After the phagophore

formation, it elongates around the cytoplasmatic components selected for degradation. The recognition of the components that will be degraded and the closing of the vesicle are dependent on the lipidated form of LC3 protein (microtubule-associated protein light chain 3). The lipidated form of LC3 is associated with the outer and inner membranes of the autophagosome and has become a reliable method for monitoring autophagy and autophagy-related processes [59].

Finally, the late stage of autophagy or maturation depends on the successful fusion of autophagosome with lysosome. This fusion allows contact of autophagosome cargo with lysosomal hydrolases and consequently the degradation of the components that in some cases are recycled.

These steps are fundamentaly important for the autophagic flux (defined as the continuous series of events since the cargo is engulf until it is degraded). Any event that alters the autophagic flux also alters the degradation process and consequently leads to the accumulation of autophagosomes (**Figure 3**).

6.3. Autophagy and Alzheimer's disease

Autophagic vacuoles are uncommon in neurons of the healthy brain because this process is constitutive active in neurons and the efficient clearance of autophagosomes keeps their presence low [60]. Moreover, in AD there is an accumulation of autophagic vesicles preferentially in dystrophic neuritis [61]. This evidence suggests that some of the later steps in the autophagic process is altered; this idea is supported by the observation that the lysosomal hidrolases are increased and abnormally distributed in AD brain indicating the defective maturation of autophagosomes [62]. Additionally, it was observed that acidification of lysosomes causes an autophagosome accumulation without altering the induction [62].

6.4. Why autophagy is altered in AD?

It is not clear, but it has been demonstrated, that a large number of autophagic vacuoles are observed in dystrophic neuritis before extracellular Aβ deposition in neurons of AD patients and transgenic mouse models [63, 64]. This suggests that autophagy dysfunction leads to the accumulation of Aβ, avoiding proper degradation.

Inductors of autophagy as trehalosa (a natural disaccharide that block glucose transporters) could rescue the AD-like phenotype in APP/PS1 transgenic mice. In this sense, trehalosa treatment significantly improves the performance of memory and learning tasks. In accordance with behavioral test, Aβ deposits were significantly reduced in hippocampus [65].

In addition, the induction of autophagy by rapamicyn (an mTORC1 complex inhibitor) in PD/APP transgenic mice improves the cognitive performance through the degradation of extracellular Aβ depositions.

In this model, autophagy induction was higher in the hippocampus of transgenic mice compared with nontransgenic mice [66]. This suggests that autophagy dysfunctions could be reversed through the pharmacological stimulation and these inductions have beneficial effects

by promoting Aβ degradation. Different studies have been performed finding that rapamycin reduces the accumulation of Aβ levels and fibrillar aggregates approximately 40–50% in 3XAD-Tg mice and APP transgenic mice [67].

Morphological evidence shows that APP and Aβ peptide are colocalized with LC3-positive autophagosomes and autophagy induction shows a greater colocalization of Aβ in autophagic vacuoles, suggesting a more active degradation [68]. However, the mechanism by which autophagy can degrade extracellular amyloid plaque content is unknown. But autophagic process of microglia (the resident macrophages in the brain) seems to play an important role.

The degradation of extracellular amyloid content through microglia involves at the first step phagocytosis; once Aβ peptide is in the cytosol it is exposed to be recognized by LC3-II via optineurin (an adaptor protein). LC3-II/OPTN recognition allows Aβ degradation via the autophagic–lysosomal system [69].

The exact pathology of AD is still unknown, but it is widely believed that the deposition of Aβ is one of the main causes leading to the degeneration and death of neurons. So, finding alternatives that avoid Aβ accumulation or enhance its degradation could be a strong therapeutic target. In this sense, autophagy seems to be the first line of defense to face accumulation but it is not clear how autophagy dysfunctions are related to Aβ aggregation or if Aβ overproduction directly induces autophagy defects.

6.5. Differential autophagy activation by monomers and oligomers

Some observations have demonstrated that a large number of autophagic vacuoles are observed in dystrophic neuritis before extracellular Aβ depositions in the neurons of AD patients and murine models of AD [70], but recently it has been demonstrated that Aβ monomers and oligomers differentially modulate autophagy in neurons. In a different way, monomers stimulate autophagy increasing autophagosome rates and the elevation of LC3-II protein levels, but at the same time monomers impaired lysosomal pathway affecting the autophagy flux. These events resulted in autophagosome accumulation. On the other hand, Aβ oligomers cause a less pronounced increase in LC3-II protein levels and does not affect the autophagy flux [58], suggesting that defects in autophagy could be the result of an increase in amyloid monomers.

Enhancing autophagic clearance of toxic protein aggregates through rapamycin or trehalosa ameliorates protein aggregation, neuron survival, and this is reflected in the improvement of cognitive skills. However, converging evidence suggests that improvement in autophagic flux through stimulation is a promising therapeutic intervention, and this field is still developing.

In other words, there are barely some studies showing the degradation by autophagy of the N-truncated beta amyloid peptide [71–74]. Recently, it has been determined that pE3-Aβ is strongly reduced in the TgCRND8 mice fed with a normal diet supplemented with the antioxidant Oleurupein (OLE) and that such a decrease likely reflects the parallel reduction of QC expression. In addition, their model of (Aβ) peptide deposition displayed strongly improved performance in behavioral and cognitive tests, reduced inflammatory response, and recovered dysfunctions of transgene-induced long-term potentiation (LTP) in the CA1

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hippocampal area. These effects were induced, at least in part, by a strong activation of autophagy. All these results suggest new perspectives for AD not only at the prevention but also at the therapeutic level [75–77].

7. β-Amyloid peptides altered clearance in loss vision in AD

Until now, we have been discussing the altered clearance mechanisms of beta amyloid peptides in AD. However, these peptides are involved in other pathological mechanisms during the development and progression of AD. Recently, the βA42 peptide has been involved in vision loss and its presence in the retina has been proposed as an early diagnostic marker for AD [78–80]. The presence of multiple βA42 reservoirs in the eye, especially in the retina environment, also induces different pathologies that lead to deficient vision as blindness [81, 82]. Pathologies such as age-related macular degeneration (AMD) and cataracts may contribute to the local inflammatory events involved in the formation of local deposits of lipids and beta amyloid peptide called drusens, atrophy of retinal pigment epithelium, lens degeneration, and photoreceptor cell death [82, 83]. In this section of the chapter, we will discuss the most recent findings emerging from the altered mechanisms of beta amyloid clearance in the eye.

As mentioned above, one of the principal degradation mechanism of amyloid beta peptides is the enzymatic pathway. In the brain, the principal βA-degrading enzymes are neprilysin, endothelin-converting enzyme (ECE), insulin-degrading enzyme (IDE), angiotensin-converting enzyme (ACE), and in mitochondria the enzyme is called hPreP [84]. However, in the eye the presence of the Neprilysin specifically in the lens [82] has been described. In other words, it has been reported that autophagy could be involved in the degradation of beta amyloid peptides through the internalization in clathrin-positive endosomes. However, these mechanisms have not been totally elucidated and current research is being performed in order to probe it in the eye [85, 86]. Finally, as mentioned above, antibeta amyloid antibody therapy is a currently applicable treatment. In the eye, this therapy was designed to target the C-terminal fragment of βA42 in a mouse model of AMD resulting in a protective effect. All this mechanisms of Alzheimer pathophysiology can contribute to vision degeneration, suggesting further that therapeutics targeting Aβ proteases or induced autophagy may be applicable to avoid vision loss.

8. Conclusions

As discussed in this chapter, there are some βA clearance mechanisms that are altered in Alzheimer´s disease. A large number of evidence exists about the βA1-42 clearance mechanism. However, despite of this, there is a lack of evidence related to the βA3-42 and βA11-42 degradation mechanisms. The principal βA variants detected in the human brain are A1-40 and A1-42; however, a significant proportion of AD brain A also consists of N-terminal-truncated species and the latest hypothesis pointing that they are seeding species. For this reason, N-terminal peptides represent highly desirable and abundant therapeutic targets.

We have an urgent need to perform immunotherapy strategies directed against N-truncated/pyroglutamate-modified βA peptides and consider them for vaccine development for AD. These kinds of analysis may provide promising diagnostic and therapeutic tools, targeting all pathological amyloid species involved in AD in the future.

In other words, autophagy is a hot topic in the recent years, enhancing autophagic clearance of toxic protein aggregates through rapamycin or trehalosa ameliorates protein aggregation and neuron survival, and this is reflected in the improvement of cognitive skills. However, converging evidence suggests that the improvement in the autophagic flux through stimulation is a promising therapeutic intervention that is still developing. Importantly, the combination of different strategies targeting simultaneously different pathological pathways, "systems therapeutics", might be more appropriate for a multifactorial disease like AD. For example, we should try to design an anti-βA, anti-tau, anti-inflammatory, anti-oxidative stress, and autophagy enhancing strategy in preclinical trials with a hope to translate them to human research.

Interestingly, there is no information about the presence of the N-truncated species of beta amyloid peptides in the eye. The big question is: Why? Is not there relevance? Or only has not been studied? More research in this topic is urgently needed in order to improve the quality of life of patients with AD.

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