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REVIEW

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Repercussions of microglial efferocytosis on neurodegeneration in Alzheimer's Disease (AD): a double-edged sword and perplexing factor warranting scrutiny in AD research

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Abstract

Background Alzheimer's disease (AD) is a neurodegenerative disease characterized by the accumulation of amyloid beta (Aβ) and tau aggregates within the neuronal milieu. To prevent their neurotoxicity, these pathological aggregates will be cleared from the neuronal environment by extracellular, intracellular, and excretory mechanisms. As these compensatory mechanisms become overwhelmed, these left-behind aggregates will instigate neuronal loss via varied downstream signaling events. As a result, neurons undergo cell death through apoptosis and necrosis leading to the accumulation of cellular debris. Timely clearance of this cellular debris is critical, otherwise it can further potentiate neuronal loss by perpetuating pro-inflammatory environment.

Results Microglial cells migrate and engulf these dead neurons by a process known as canonical efferocytosis. On the other hand, normal living neurons will be cleared by microglial cells through extracellular exposure of phosphatidyl serine (PS) under the pathological influence of Aβ and tau through non-canonical efferocytosis. Canonical efferocytosis should be predominant with the absence of the non-canonical efferocytosis during the physiological conditions. Upregulation of cytokines, and chemokines in AD creates a fertile ground for the amplification of non-canonical efferocytosis in parallel to canonical efferocytosis. The preponderance of the non-canonical over canonical pathways leads to exuberant clearance of stressed and normal living neurons along with dead neurons, thereby leading to exacerbated neuronal loss, brain tissue thinning and severe cognitive disturbances in AD.

Conclusions Research efforts should be directed to understanding the factors that fine-tune the balance between these clearance processes. Novel therapeutic strategies that reinforce canonical efferocytosis will be beneficial by improving tissue repair, healing, and regeneration in AD.

Keywords Microglia, Efferocytosis, Neuronal death, Alzheimer disease and amyloid beta, Necrosis, Apoptosis, Phagocytosis, Canonical, Non-canonical

Background

Microglial cells are the most important glial cells in the central nervous system. They are responsible for numerous physiological functions including neurogenesis, preservation of neuronal homeostasis, rehabilitation following neuronal injury and scavenging unwanted material accumulating in the brain tissues [1]. Microglial cells function as primary phagocytes during physiological and pathological conditions [2]. In this regard, they tend to

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migrate to the site of injury and ingest dead microbes, dying neurons, degenerating synapses and unwanted protein aggregates [2]. This physiological function of ingesting dead apoptotic and necrotic neuronal cells is officially defined as canonical efferocytosis [3]. Canonical efferocytosis occurs in a stepwise fashion through the involvement of specific chemotactic signals, bridging molecules and receptors [4, 5]. A recent report revealed that, migration of centrosomes into microglial invagination is the preliminary step for microglial pseudopodia formation, phagosome maturation and polarized vesicular trafficking necessary for engulfment of apoptotic cells [6].

Microglial TLR4 (Toll-Like Receptor-4) and Axl receptor (TAM family tyrosine kinase receptor) upregulation and their contribution to the pathogenesis of AD has been well documented [7, 8]. As microglial receptors are involved in initiating the contact for engulfing the dead neurons, any presence of inflammatory mediators might upregulate or stimulate the expression of these receptors, thus accelerating the process of canonical efferocytosis. Lipopolysaccharide (LPS) from neuroinflammation, and high mobility group box protein 1 (HMGB-1) secreted from the necrotic dying neurons along with Aβ are known to activate microglial TLR4 receptors [9–11]. This leads to the activation of downstream P2Y2 [purinergic receptor 2Y2]-Axl pathway-mediated removal of dying neurons in AD [9]. It is important to understand that, the momentum of the microglial efferocytosis should match the pace at which the dead apoptotic neurons accumulate near the site of the active disease process. If not, there is the risk of accumulation of this toxic neuronal cellular debris resulting in deleterious consequences in AD. A recent study showed that, perivascular cells secreted phosphoprotein-1 (SPP-1) is essentially indispensable for overlooking the microglial engulfment of neuronal synapses along with boosted the expression of phagocytic markers (C1q [complement 1q], Grn [progranulin] and Ctsb [cathepsin-b]) under the presence of amyloid beta (Aβ) oligomers[12]. In instances where microglial efferocytosis becomes subpar, then astrocytes step in and execute pathological elimination of excitatory and inhibitory synapses adjoining the pathological plaques in a C1q-dependent manner [13]. That being the case, complement-1q (C1q) deletion afforded better clinical outcomes by attenuating the pathological synaptic elimination in the mouse AD disease models [13].

Along with AD, microglial efferocytosis also seems to play an important role in modulating the disease pathogenesis for facilitating neuroprotection and recovering neurological function in the stroke models. STAT6 [signal transducer and activator of transcription 6]/Arg1 [arginine-1] signaling was found to be critical in regulating the microglial phenotype, removal of dead/dying neurons, neuroinflammation, infarct size and clinical outcomes in the mouse models of ischemic stroke as well as stroke patients [14]. Furthermore, in a study by "Zhang", and colleagues sigma-1 receptor (Sig-1R) was demonstrated to be responsible for regulating dead cell removal, neuroinflammation, and neurological deficits via boosting RAC1 [Rho family GTPase protein] signaling-dependent actin polymerization, a pivotal step for the engulfment of apoptotic neurons in the transient middle artery occlusion (tMCAO)-induced stroke animal models[15].

M2 macrophage phenotype is primarily implicated in the removal of dead and apoptotic cells following pathological insults, a critical process also associated with the resolution of inflammation [16]. Pro-resolving mediators such as resolvin D1, resolvin E1, maresin1, protectin D1, lipoxin A4, Myc-Nick, and LTB4 (leukotrienes B4) are implicated in the transformation of M1 to M2 microglial phenotype and thereby facilitate the removal of apoptotic cellular debris during neuroinflammatory and neurodegenerative conditions [16–20]. Canonical efferocytosis (Fig. 1) is essential for maintaining homeostasis, normal brain functional connectivity and neurodevelopment by synaptic pruning [21, 22]. Taken together, this process is helpful in the efficient clearing of dead cells accumulated during the disease process in the physiological and pathological conditions. Nevertheless, uncontrolled, and excessive occurrence of this phenomenon is detrimental as it can potentially hasten neuronal loss, particularly in AD. Accordingly, in a few studies arresting microglial efferocytosis has prevented neuronal death and yielded favorable outcomes in AD disease models [23, 24].

In contrast, the non-canonical efferocytosis (Fig. 1) involves the same mechanism but involves the removal of normal and healthy living neurons instead of dead cells. In AD-model of P301 tau mice, tau-exposed neurons were cleared by adjoining microglia due to PS exposure on their plasma membrane [23]. Elevation of tau, LPS and Aβ in AD resulted in the removal of normal living neurons because they instigated extracellular PS exposure by various mechanisms including increase in oxidative stress, nitric oxide synthase (NOS), nitric oxide (NO) nitrate a(NO_3-), and Milk factor globulin-E8 (MFG-E8) [23–25].

In ideal conditions, canonical efferocytosis should be functional, whereas non-canonical efferocytosis should be switched off for healing and tissue regeneration to proceed following neuronal insults, injury, and infections. Any clinical scenario which alters this balance between canonical and non-canonical efferocytosis tends to have disastrous consequences by amplifying the removal of dead as well as normal/viable neuronal cells (Fig. 1). This will expedite neuronal loss and accelerate the course of

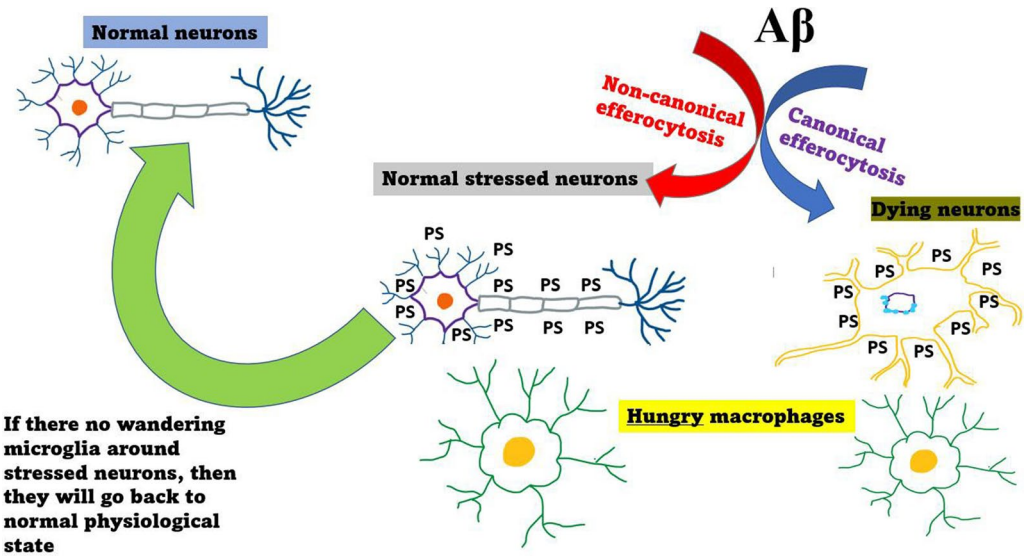

Fig. 1 Happening of canonical and non-canonical efferocytosis in the neuronal niche during AD pathogenesis: under the influence of Aβ, neurons undergo cell death by apoptosis leading to exposure of PS extracellularly thereby attracting microglia for clearance through canonical efferocytosis. Even normal living neurons which are stressed under the toxic clout of Aβ will expose PS thus becoming a meal for wandering hungry macrophages by non-canonical efferocytosis. If there are no surrounding macrophages near these stressed normal neurons, then can potentially revert into normal physiological state. Taken together, preponderance of non-canonical efferocytosis in AD can be a counterplot as it instigates riddance of normal stressed neurons over and above already dying neurons by just around the corner macrophages. This could potentially have clinical implications as it would scuttle the progression of neurodegeneration and onset of cognitive impairment in AD

disease progression in neurological disorders such as AD, Parkinson's disease and stroke. This will set the stage for the emergence of excessive and widespread brain tissue loss and atrophy, a factor that is directly correlated with the inception of severe cognitive disturbances in neurodegenerative diseases like AD [26, 27].

Therefore, comprehension, of these two vital processes is necessary to understand their influence upon the pathogenesis of neurodegeneration in AD. In this review, we discussed the following sections including neurotoxic effects of amyloid and tau, microglial mediated, innate immune responses, heaping of dead cellular debris, types of the microglial efferocytosis, evidence of their simultaneous occurrences in AD, conclusion, limitations, and future research warranted. We particularly enumerated the processes of canonical and non-canonical efferocytosis in a great detail with a thorough discussion of their pathways involved, study models enumerated, mediators—and receptors involved, and the consequence of their derailment on the pathological process of AD. This review is an effort to highlight the omnipresence of these two clearance pathways and underscore their importance on the neurodegeneration in AD. Kickstarting appropriate research studies to explore them will unveil new molecular targets that might be useful in tweaking these processes so that the efficient purging of accumulated dead neuronal cells ensues without unconditioned removal of normal/living neurons. This will ultimately pave the way for better tissue healing, regeneration, lesser cognitive decline as well as optimal clinical outcomes in AD.

Methods

We performed a PubMed search of relevant articles that describe microglial innate responses in response to the active pathological process of AD. Specifically, we focused on microglial efferocytosis (canonical and non-canonical), its mechanism, pathways involved, the models studied and its effect on the clearance of dead neurons that pile up during the disease process of AD. We explored the current literature that enumerated these two processes and underscored their relevance in the context of AD disease pathogenesis. We also highlighted the repercussions of the imperfect regulation of these two processes on the disease progression in AD.

Discussion

Amyloid plaques and neurofibrillary tangles are the hallmark pathological signatures that were originally revealed by Alos Alzheimer in 1907 [28, 29]. As Aβ and tau aggregates start accumulating within the brain tissues in the preclinical stage of AD, both intracellular and extracellular mechanisms are potentially required for clearing each of these pathological processes [30–32].

Intracellular mechanisms that tend to clear these aggregates include the ubiquitin–proteosome pathway, autophagy–lysosome pathway, endosome–lysosome, endosome–lysosome pathway, and proteases [31]. Extracellular mechanisms that were hypothesized for purging these pathological aggregates include proteases and microglial phagocytosis [30, 31]. Apart from these, excretory drainage pathways such as perivascular drainage, glymphatic clearance and CSF (cerebrospinal fluid) absorbance clearance were also implicated to purge these aggregates from the brain interstitial fluid into the systemic circulation [30, 31, 33].

As these clearance mechanisms tend to be exhausted, these uncleared pathological aggregates are inclined to accumulate and exert their toxic influence on the neighboring neurons. Under the influence of Aβ, some of the mechanisms leading to the cell death include loss of mitochondrial membrane potential, increased mitophagy, JNK activation (C-jun terminal kinase), Bcl-w down-regulation (B-cell lymphoma-w), ER (endoplasmic reticulum) stress, lysosomal leakage, synaptic degradation, and oxidative stress [34–39].

Aβ oligomer accumulation is associated with microtubule disassembly, inhibition of microtubule transport, impaired long-term potentiation and ectopic cell cycle reentry of neurons, all of which in combination can result in neuronal loss [40]. Anatomically, tau is principally expressed in the axons and very little can be traced to the dendrites or neuronal soma. Under the pathological influence of Aβ, tau is redistributed to the somatic dendrites [41]. This anatomical redistribution of tau incites signaling events including enhanced NMDA [N-methyl-D aspartate] receptor activity, calcium excitotoxicity, tau phosphorylation and microtubule destruction in the dendrites [42]. This tau-dependent microtubule disarray will impair the physiological delivery of presynaptic components to the axon terminals and postsynaptic components to the dendritic terminals [43]. Taken together, Aβ and tau work in tandem and instigate these changes which form the underlying pathological basis for synaptic dysfunction, neuronal death, brain tissue changes and cognitive abnormalities in AD [40].

Due to compensatory mechanisms launched by innate immune defenses, brain tissues can withstand these pathologies for some time as is evidenced by the presence of pathology many years ahead of symptom onset [44–47]. However, they are eventually exhausted and neuronal loss results in cholinergic dysfunction followed by emanation of the hippocampal atrophy and cognitive symptoms [48–51]. As neuronal cells undergo apoptosis, microglial cells come to the rescue and mount compensatory defenses to arrest or reverse these processes. They accomplish this by various mechanisms including phagocytosis of Aβ aggregates, dysfunctional synapses, and dead/dying neurons, oxidative stress and pro-inflammatory cytokine secretion [52–54]. Furthermore, one of the important mechanisms acting to counteract the effects of Aβ and tau pathologies is microglial cell activation which occurs when these aggregates are ingested by phagocytosis via scavenger receptor (SR) which would elicit an inflammatory response (IL-1; interleukin-1) and could contribute to AD disease pathology [55, 56]. Microglial cells migrate, phagocytose, and degrade Aβ aggregates through fluid phase micropinocytosis, protease-mediated degradation, and SRs [57–59]. According to a recent study by Joshi, and colleagues, as the microglial phagocytic function and micropinocytosis function of extracellular Aβ is overwhelmed, these Aβ oligomers will be transferred to the micro-vesicles (MVs) where they are anchored to the PrPc (cellular prion protein) or GM1 gangliosides on the external surface [60–63]. Subsequently, these Aβ were solubilized by the lipid content of these MVs and thus become more neurotoxic [60]. It has been speculated that more toxic forms of Aβ resurface secondary to the uncanny and eccentric lipid composition of these micro-vesicles, a turning point that eventually instigates neuronal demise and progressive neurodegeneration in AD [60]. Some of the MVs plasma membrane lipids such as cholesterol, sphingomyelin, ceramide, and lipid rafts components are implicated in this transpiration [60, 64]. Microglial cells eventually release these MVs loaded with solubilized Aβ into the extracellular environment, which sooner or later reaches the neighboring neurons to exert their neurotoxicity [60]

Future studies should be focused on understanding the lipid profile of these released microglial micro-vesicles so that the underlying basis for the generation of neurotoxic Aβ aggregates are clearly ascertained.

Coincidentally, after engulfing these extracellular Aβ aggregates, microglial cells are persistently activated via CD36 [receptor for thrombospondin; scavenger receptor], Fc receptors [ITAM associated receptor family], TLRs and RAGE (receptors for advanced glycosylation end products) which has counterproductive effects by increasing the Aβ production and attenuating their removal from the neuronal tissues [58, 65–67]. Activated microglia in AD disease models are broadly classified into M1 (classically activated) and M2 (anti-inflammatory phenotype) [58]. M1 phenotype is mainly responsible for the secretion of pro-inflammatory cytokines (IL-1β [interleukin-1 beta], TNF-α [tumor necrosis factor alpha], STAT3 [signal transducer and activator of transcription 3], IL-6 [interleukin-6], IL-12 [interleukin-12], IL-23 [interleukin-23] and ROS [reactive oxygen species]) while the M2 phenotype is mainly incriminated with the release of anti-inflammatory cytokines (IL-10

[interleukin-10], IL-4 [interleukin-4], IL-13 [interleukin-13], and TGF-β [transforming growth factor beta]) [58]. Unfortunately, M1 microglia secreted pro-inflammatory cytokines IL-1β, IL-6, IL-18 [interleukin-18], and TNF-α further exacerbate the ongoing disease pathogenesis by increasing the Aβ synthesis, decreasing the Aβ clearance and increasing the tau hyperphosphorylation [68–71]. However, M2 microglia secreted anti-inflammatory cytokines such as TGF-β and IL-4 were deemed to be protective by facilitating the Aβ removal, reducing the brain parenchymal plaque burden and downgrading the amyloid-induced neuroinflammation [58, 72–74].

Furthermore, in AD disease models, there is concurrent activation of both M1 and M2 microglial phenotypes in the early stages but as the disease progresses the microglial populations around parenchymal plaque accumulations tend to be predominantly M1 phenotype, which becomes a powerful drive for advancing the Aβ induced neuronal demise in AD [58]. Due to the lack of any checkpoint to reverse this microglial-induced toxicity, neuroinflammation, and neurotoxicity, the disease process marches forward unabated. Ultimately this leads to excessive neuronal death, brain tissue thinning, progressive neurodegeneration and cognitive disturbances in AD.

Cytokines secreted by the microglia can have harmful effects on the neurons and associated synapses [75–77]. Furthermore, Aβ-stimulated microglial cells can cause neuronal toxicity via stimulation of TNF-alpha receptors as well as NMDA receptors [78]. Upon microglial ingestion of these Aβ aggregates, acidification of lysosomes (PH < 5) will facilitate their degradation [79]. Moreover, as Aβ aggregates are engulfed by microglia, they can induce collateral damage via the upregulation of ROS, NOS, and peroxy-nitrate (HNO_4.) thereby resulting in oxidative stress [80]. This Aβ-induced microglial oxidative stress can result in neuronal loss in addition to their direct neurotoxicity.

Aβ aggregates exert direct toxic effects on the microglial cells and an indirect toxic effect on the neurons through microglia cells [81]. In a study by Lorenzi and colleagues, microglial cells treated with high molecular weight Aβ 42 oligomers for 24-48 h demonstrated a significant decrease in the cell viability along with increased secretion of pro-inflammatory cytokines IL-1 β and TNF-alpha [81].

Eventually, microglial cells will be overwhelmed by excessive piling of Aβ aggregates and thus there is a possibility that their engulfing capacity is saturated. Alternatively, it was shown that microglial phagocytic capacity for Aβ was impaired in the mice with AD disease pathology as compared to age-matched controls which was partially restored by decreasing the amyloid burden by Aβ vaccination [82]. These findings underscore the probability that the presence of Aβ will cause impairment of the microglial phagocytic capacity and facilitate their aggregation and subsequent neurotoxicity.

This scenario leads to the piling up of these toxic aggregates within the vicinity of neurons leading to the kick-starting of apoptotic or necrotic signaling pathways within them. Additionally, micro-vesicles secreted by microglial cells were shown to interact and convert insoluble extracellular Aβ aggregates into soluble neurotoxic forms due to lipid content in their membranes [60, 83]. Moreover, micro-vesicles isolated from CSF of AD patients should be neurotoxic to cultured neuronal cell lines [60]

Neuronal death can occur through apoptosis or necroptosis contributing to the accumulation of extracellular debris in the brain [84–87]. As time progresses, aggregation of cellular debris adds to the pathological effects of Aβ aggregates [87–89]. Necrotic dead and dying cells release toxic mediators that can augment the ongoing neuroinflammation mediated by microglial cells [90]. In the knee osteoarthritis, defective efferocytosis leads to lysis of necrotic cells and release of danger signals including DAMPs [damage associated molecular patterns] (DNA [deoxy ribonuclear acid], uric acid, Hsp-90 [heat shock protein-90]), HMGB-1, receptor for advanced glycation end products and alarmins (S100A8 and S100 A9) within the synovial joint [91, 92]. This leads to the activation of innate immune system, overproduction of pro-inflammatory cytokines, enhanced cartilage destruction and inflammation in the joint [91, 92]. Moreover, danger signals such as HMGB-1 were recently shown to directly inhibit the microglial phagocytic capacity which might delay the clearance of these toxic Aβ aggregates resulting in excessive neuronal demise and rapid disease progression in AD [93]. In a similar manner, the defective removal of apoptotic and necrotic neurons can incur disastrous consequences due to the release of danger signals and activation of pro-inflammatory pathways in the neuronal milieu in AD. To prevent this from happening, these dead neurons should be cleared by microglial efferocytosis which facilitates repair, healing, and tissue regeneration in AD.

It is defined as the ingestion and digestion of dead accumulated cells by primary professional phagocytes. The pathway usually basically involves two primary phases namely the ingestion phase and digestion phase [94]. The ingestion phase is further subdivided into five sub-phases including recruitment, recognition, tethering, signaling, and engulfment [95]. The pathway of canonical efferocytosis can be briefly summarized in four steps beginning with dead apoptotic cells mounting flashing signals (eat-me signals) as well as releasing chemical

messengers (find-me markers) to signal the neighboring microglial cells in step I (Fig. 2) [95]. In step II, microglial cells migrate and physically couple with these dead cells utilizing eat-me receptors [95]. Post interaction, there will be the engagement of CRKII [CT10 regulator of kinase]-DOCK180 [CED-5/180 kDa protein downstream of chicken tumor virus no. 10]-ELMO [CED-12/engulfment and migration] complex followed by stimulation of RAC1-mediated signaling pathway leading to the reshuffling of the actin cytoskeleton (Step III) (Fig. 2) [95]. This will result in the invagination of the microglial membrane and subsequent engulfment of dead apoptotic neurons. Following the successful ingestion, processing and degradation of dead cells transpires in the phagolysosome (step IV) through a unique pathway known as light chain-3-associated phagocytosis (LAP) (Fig. 2) [95, 96]. In this step, maturing efferosome containing dead cells is sequentially fused with early endosome, late endosome, and lysosome for consequent processing [97]. Regular canonical autophagy is characterized by double-membrane structures and delayed formation of autophagosomes, whereas LAP encompasses single membrane structures and quicker assembly of autophagosomes as short as 10 min as compared to hours in the former [98, 99]. Translocation of LC3 and beclin1 to phagosome was a nascent step that formed the infrastructural foundation for the ensuing phagolysosome formation, brisk acidification, and annihilation of ingested dead cell [98]. LAP is also functionally distinct from regular canonical autophagy as the former is primarily implicated in shrinking pro-inflammatory signals, amplifying anti-inflammatory cytokines, and abolishment of auto-antigen presentation following successful digestion of dead cells [100, 101]. As long as LAP operates, canonical efferocytosis will accomplish immunologically silent depopulation of dead apoptotic neuronal cells from the extracellular space, a factor critical in preventing future arousal of autoimmune processes within the brain tissues [100]. Cholesterol, protein, DNA, proteins, and amino acid arginine released from dead apoptotic cells will be processed in an immaculate manner so that ensuing negative microglial metabolism, inflammation and death are averted. Specifically, the processing of arginine into putrescine and Dbl [GTP-exchange factor] is of foremost significance as continuous activation of actin regulating RAC1 ensures that microglial will be forearmed for subsequent rounds of efferocytosis [102]. Lipid-derived metabolites are responsible for the activation of nuclear receptors (PPAR [peroxisome proliferator-activated receptors] and LXR [liver X receptors]) which triggers the expression of cholesterol efflux transporters (ABCA1 [ATP binding cassette transporter subfamily A1] and ABCG1 [ATP binding cassette transporter subfamily AG]) and downregulated inflammatory gene expression (iNOS [inducible nitric oxide synthase] and IL-6 [103]. Over and above that, this stimulation of nuclear receptors effectuates the heightened proliferation of efferocytosis machinery including phagocytic receptors (MerTK [Mer tyrosine-protein kinase], CD36 [scavenging receptor] and Axl and bridging molecules (Gas6 [growth arrest-specific 6], MFG-E8 andC1q, which ensures incessant and unhampered furtherance of efferocytosis will transpire for safe and immunologically silent elimination of the accumulated cellular debris from the neuronal niche with evolving active disease process [100].

On top of this, the successful purging of dead neuronal cells by efferocytosis paves the way for mounting an anti-inflammatory response by diminution pro-inflammatory cytokines (TNF-α, IL-1 and IL-12) as well as by an upsurge of anti-inflammatory cytokines IL-10 and TGFβ (Fig. 2) [103–105]. Microglial efferocytosis entails the participation of numerous mediators including cytokines, chemokines, find-me signals, eat-me signals, bridging molecules, and receptors [4, 106–111]. In canonical efferocytosis microglial cells are capable of recognizing and ingesting dead neuronal and microglial debris [112–116]. Briefly, in canonical efferocytosis dying neuronal cells secrete find-me signals or chemotactic factors such as sphingosine-1-phosphate (SIP), chemokine-like fractalkine (CX3CL1), lyso-phosphatidylcholine (Lyso-PC), ATP [adenosine triphosphate] and UTP [uridine triphosphate] whose main function is to attract the neighboring microglial cells towards them. Microglial sense these signals and migrate towards these dying neurons. Upon reaching their neurons, they utilize microglial receptors and bridging molecules (protein-S [vitamin K-dependent plasma clotting factor] and Gas-6) to bind to the apoptotic neurons with the help of eat-me signals such as PS expressed extracellularly [117, 118]. Engagement of dead/apoptotic neuronal cells lead to their engulfment via downstream activation of PI3K [phosphoinositide-3 kinase]/RAC1-mediated signaling pathways and actin polymerization [119, 120]. Sustaining this process of canonical efferocytosis via continual non-inflammatory microglial (M2) proliferation is critical for the apoptotic cell clearance, disease regression, tissue healing as well as resolution of inflammation [114].

Microglial receptors that are implicated in this physiological process include (P2Y6 [purinergic receptor P2Y6], S1PR [sphingosine1-phosphate receptor], G2A [G protein-coupled receptor 132], CX3CR1 [C-X3-C chemokine receptor] for the find-me phase as well as BAI-1 [brain angiogenesis inhibitor-1], integrin β1 [integrin beta-1], CD300b [cluster of differentiation 300b], TIM1 and 4 [T cell immunoglobulin mucin domain-1], stabilin 1and2, RAGE [receptor for advanced glycosylation end products] and MERTK in eat-me phase [90].

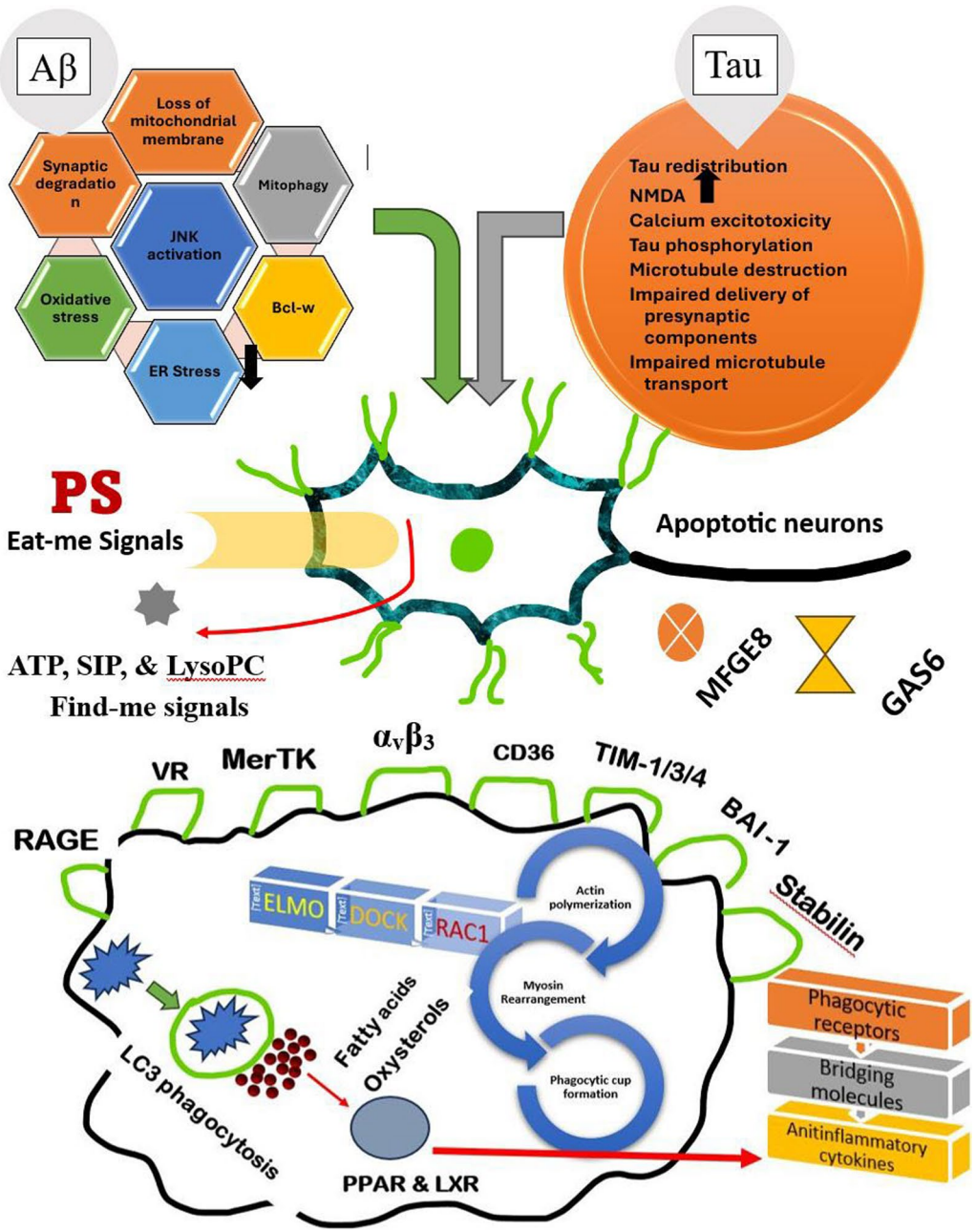

Fig. 2 Microglial canonical efferocytosis during AD neurodegeneration. Alzheimer's disease is characterized by accumulation of amyloid beta and tau aggregates in the neuronal vicinity. These toxic aggregates will provoke neuronal death by various cellular downstream signaling events including oxidative stress, ER stress, mitochondrial damage, microtubule dysfunction and synaptic degradation. Neuronal cells undergo apoptotic cell death as a result and these cellular debris accumulates in the neuronal milieu. This cellular debris need to be removed in a timely manner otherwise they progress to undergo necrotic type of cell death, a crucial turning point that leads to disastrous consequences including heightened neuroinflammation, pacing disease progression and autoimmunity. To avert this from happening, microglial cells perform an important physiological function namely efferocytosis, which literally means engulfing and processing the neuronal debris. Apoptotic neurons expose eat-me signals and release find-me signals that fulfill the function of attracting microglial cells towards them. As these microglial cells approach these dead neurons, they are recognized by eat-me signals and utilizing the recognition machinery (bridging molecules and receptors), they engulf, process and degrade them in an immunological silent manner (LC3 phagocytosis). This transpires through involvement of ELMO-DOCK-RAC1 induced actin polymerization for phagocytic cup formation. An important byproduct of this purging process is secretion of anti-inflammatory cytokines due to stimulation of nuclear receptors such as PPAR-gamma and LXR

Excessive activation of these microglial receptors can have potential implications in the pathogenesis of various neurological diseases including AD. For example, the activation of STAT1 [signal transducer and activator of transcription-1]/Arg1 in the brain resident microglia resulted in modulation of the microglial phenotype, enhanced resolution of inflammation and better clinical outcomes in stroke model [14, 112]. In a similar manner, the sigma-1 receptor (Sig-1 R) knock-out mice that exhibited disablement of the microglial phagocytic activity, excess brain damage and severe neurological deficits, thus emphasizing the involvement of this receptor in the efferocytosis signaling events [15]. Accordingly, administration of Sig-1 R expressed bone marrow macrophages to these knock-out mice resulted in the restoration of the microglial engulfment of dead neurons, attenuated infarction, reduced neuroinflammation and enhanced clinical recovery following ischemic middle cerebral artery occlusion [15]. Likewise, the CX3CR1 receptor is a microglial receptor involved in a cortical III-layer neuronal loss in AD [121]. By knocking out this CX3CR1 receptor, Fuhrmann, and colleagues were able to show that neuronal loss is reduced, and microglial–neuronal communication is restored, a culmination that revived the neuronal homeostasis in AD disease models [121].

Demonstrating the signature of canonical efferocytosis might be helpful in comprehending the genes involved as well as gauging the extent of occurrence of this physiological process in the neuronal tissues during various disease stages of AD.

In this regard, it was unearthed that there were approximately 80 efferocytosis-specific genes which were significantly upregulated in the hepatocellular carcinoma (HCC) cohorts as compared to controls [122, 123]. In atherosclerosis, some of the genes associated with phagocytic receptors MerTK [, Lrp1 [LDL receptor protein], Tim-1and4 [T cell immunoglobulin mucin domain-1 and4], SRB-1 [scavenger receptor B-1] and G2A [G *protein*-coupled receptor 2A]); bridging molecules (Gas-6, Fas [type-II transmembrane protein], Fas ligand [type-II transmembrane protein ligand] and C1q); find-me signals (CX3CL1); eat-me signals (calreticulin [CRT]); and don't eat-me signals (CD47) were studied to ascertain their role in revamping the pathogenesis of atherosclerosis[124]. In atherosclerosis, studies which employed knock-out mouse models of Mertk, Lrp1, Tim1and4, C1q, Fas ligand, and calreticulin displayed increased plaque size, elastic lamina disruption, aneurysm formation, TUNEL [terminal deoxynucleotidyl transferase dUTP nick-end labeling]-positive cells in the necrotic core and plaque vulnerability [106, 124–128]. In tandem with these findings, few studies were performed which demonstrate that markers of the microglial efferocytosis were significant in influencing the pathogenesis of neurodegeneration in AD. Tyro3 (TAM receptor subfamily of tyrosine kinase receptor 3) is a receptor present on microglial membrane and moderates the interaction, ingestion, and destruction of apoptotic cells [129, 130]. According to Zheng, and Colleagues, Tyro3 overexpression in 293APPswe cells were instrumental in decreasing the Aβ production as well as curtailing the activity of BACE1 (β-site amyloid precursor protein cleaving enzyme) enzyme. Over and above that, incorporating Tyro3 knockdown into the AD transgenic mouse model instigated the tweaking of pathogenic mechanisms so that there was a heightened assembly of Aβ aggregates in the hippocampal tissues of the mice brain along with increased density of plaque-associated astrocyte congregations [131]. TGF-β1 which was previously implicated in the microglial activation was shown to increase the expression of MFG-E8 in microglia [132, 133]. Apoptotic cells also release TGF-β1 which subsequently facilitates their clearance through canonical microglial efferocytosis [133]. The critical interaction that is essential for the apoptotic cell clearance involves interlinkage between MFG-E8 and oxidized PS-expressed apoptotic cells [134]. Decreased production or the absence of MFG-E8 is detrimental as mouse knock-out models (MFG-E8−/− mice) developed clinical sequelae including splenomegaly and glomerulonephritis secondary to generation of autoantibodies [135].

Non-canonical efferocytosis is a pathological process while canonical efferocytosis is physiological process, with both processes set in motion in varying proportions during the onset and progression of various neurological diseases. Fundamentally, the delicate balance and the preponderance of either of these processes is primarily contingent upon the presence or the absence of neurotoxins as well as cytokine and chemokine profiles of the neuronal milieu. The fundamental and main difference between these two processes is that the former removes normal viable neurons, whereas later encompasses the clearance of dead apoptotic cells that accumulate during the course of neurodegeneration in AD.

The process of the non-canonical efferocytosis begins with exposure to the most common find-me signal namely PS on the outer leaflet of the plasma membrane of viable neuronal cells, in contrast to the canonical efferocytosis which starts with the demise of neurons under the toxic influence of LPS, Aβ and tau aggregates (Fig. 3) [25, 136, 137]. It has been speculated that, healthy neuronal cells upregulate find-me signals (PS) and downgrade don't-eat-me signals particularly when exposed to these stressful and toxic signals [112]. The underlying mechanism for this occurrence is still obscure and is a matter of investigation, although few researchers speculated a plausible hypothesis in this regard. LPS and Aβ

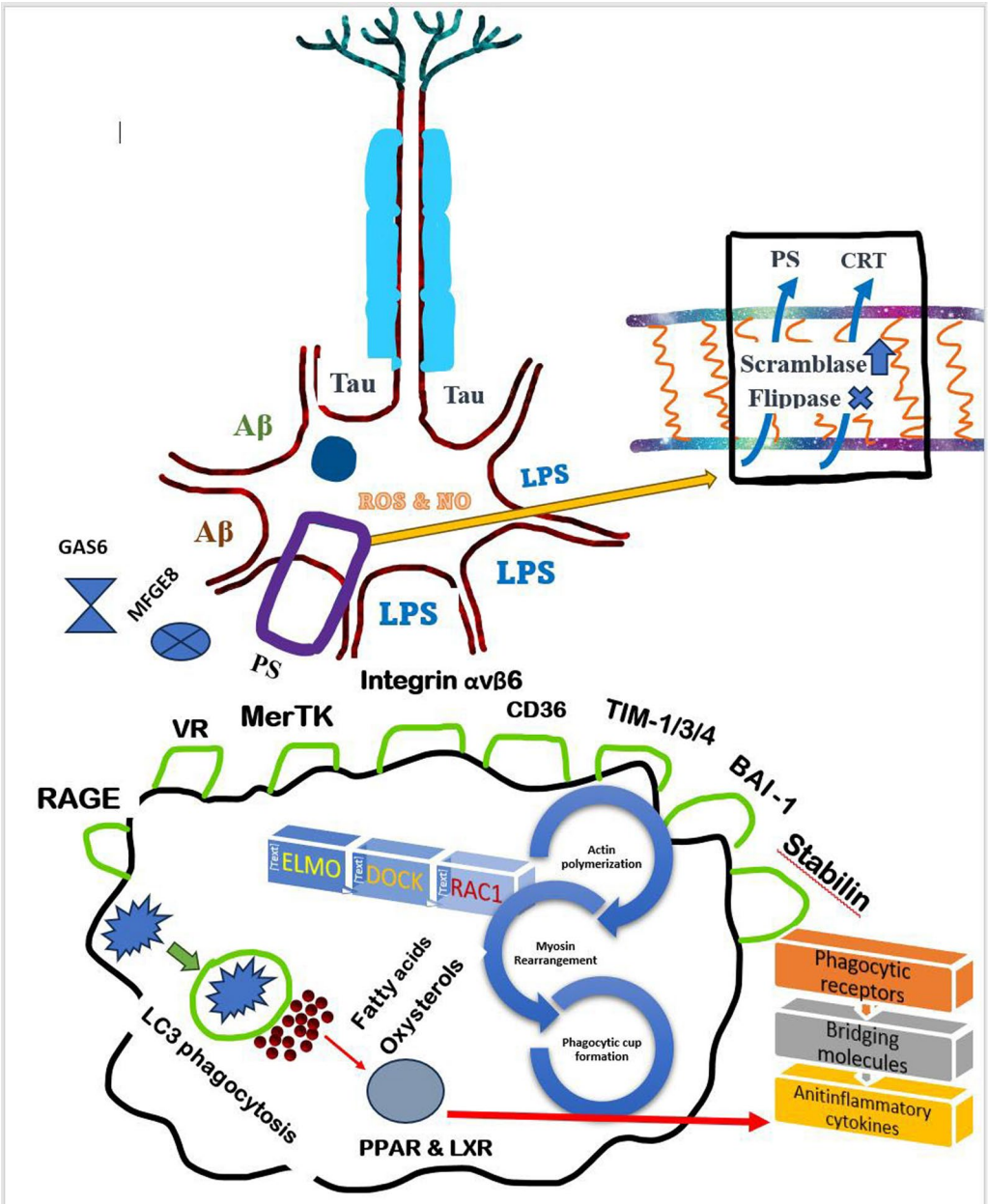

Fig. 3 Microglial non-canonical efferocytosis during AD neurodegeneration. It is quite evident that, the presence of lethal concentrations of Amyloid beta and tau is potent to provoke neuronal cell death. However, some of the normal and viable neurons at the site of active disease will be laid bare to the sub-lethal concentrations of these neurotoxicants. Although sub-lethal exposure is not neurotoxic, it incites neuronal alterations that can indirectly enkindles their removal by wandering activated macrophages. These sub-therapeutic concentrations will foment the production of ROS, nitric oxide and peroxy-nitrate with the neurons. Building up of these reactive oxidative species within the neurons will stir up the exposure of eat-me signals PS and CRT on their plasma membrane through inactivation of flippase and activation of scramblase enzymes. These PS-exposed stressed but normal and viable neurons can be recognized, engulfed [ELMO-DOCK-RAC1 induced actin polymerization] and processed [LC3 phagocytosis] by activated wandering microglial cells utilizing bridging molecules and recognition receptors

accumulation during neuroinflammation will initiate the process by acting on the microglial cells and cause upregulation of peroxy-nitrite via stimulation of iNOS and PHOX (Phagocytic NADPH [nicotinamide adenine dinucleotide phosphate] oxidase) enzymes (Fig. 3) [138–141]. This peroxy-nitrite will act on the neighboring viable neurons thereby instigating the outside exposure of the PS, providing a glaring flashing signal for microglial identification. This step is followed by the migration of neighboring macrophages towards the normal and viable neuronal

cells expressing PS extracellularly [95, 142]. Microglial cells will in turn, secrete (MFG E8), a bridging molecule which interlinks PS-expressed neurons with microglial vitronectin (VR) or $\alpha_v\beta_{3/5}$ integrin receptor [95, 138]. This interaction becomes the springboard for the consequent generation of ELMO–DOCK180 complex which causes ensuing RAC1-mediated actin cytoskeletal rearrangement and F-actin remodeling (Fig. 3) [95]. These steps become the underlying foundational basis for the invagination and formation of the phagocytic cup for successful engulfment of the PS-exposed viable neurons. After ingestion, they will be boarded in single membrane vesicles, fused with phagolysosomes and degraded through a unique process called LC3-associated phagocytosis to ensure dead clearance proceeds in an immunological silent manner [100]. That being said, it is quite obvious that the non-canonical efferocytosis follows the same steps as canonical efferocytosis apart from the initial step encompassing exteriorization of the find-me signal for sparking the transmigration of the microglial cells towards stressed but viable neurons[112]. Interestingly, using blocking agents to either MFG-E8 or PS or phagocytic receptor might be beneficial as these stressed might revert back to their normal state by internalization of the PS and thus evading their ability to be recognized by hungry macrophages [138]. Over and above that, stressed but viable neurons will unsheathe their expressed PS from their plasma membrane whenever they escape from the influence of these toxic stimulants (Aβ and tau), which might come into existence as their concentration scales down secondary to their degradation by cellular proteases or microglial ingestion. That being said, this unconventional phenomenon is a reversible process as compared to canonical efferocytosis which is a physiological protective endeavor.

Therefore, inhibition of this inadvertent pathway might be beneficial as it can potentially limit the advancement of inflammatory neurodegeneration, which is widely prevalent in AD disease models [24].

MFG-E8 present in the brain is responsible for linking PS-exposed apoptotic neuronal cells with the neighboring microglia and thus is primarily involved in instigating the signaling pathways necessary for the enforcement of efferocytosis [143]. Studies indicate that levels of MFG-E8 are significantly altered in AD, thus increasing the chances of hampered clearance of dead apoptotic cells from the neuronal extracellular space [143]. In the LPS-induced neuroinflammatory models, MFG-8 was shown to facilitate microglial phagocytosis of viable neurons [136]. These findings thus underline the existence of non-apoptotic neuronal loss during neuroinflammation and this can be blocked by the administration of inhibitors of the PS/MFG E8/VR pathway of the non-canonical microglial efferocytosis [136]. Along with that, MFG-E8 was implicated in modulating microglial M1 /M2 phenotype and astrocyte A1/A2 phenotype via NF-kB [nuclear factor kappa B] and P13k–Akt [phosphoinositide-3 kinase–protein kinase B] pathways in AD disease models [144, 145]. Moreover, MFG-E8 was revealed to hamper the Aβ supervised microglial production of cathelicidin-related antimicrobial peptide (CRAMP), a protein primarily incriminated in launching innate immune responses of chronic inflammation in AD [146]. As neuroinflammation favors the exteriorization of the PS on the viable neurons and potentiates the non-canonical efferocytosis, any credible efforts to prevent this from happening will have better clinical outcomes in AD disease models. By transfiguring the cytotoxic glial phenotype as well as by encumbering the generation of pro-inflammatory factors, MFG-E8 might be a potential therapeutic strategy in AD as it has the potential to alleviate the neuroinflammation-mediated non-canonical efferocytosis. Microglial inflammation and neuronal demise demonstrated in the LPS-treated mice was lessened by 50% when the non-canonical efferocytosis pathway was blocked using PS-blocking antibodies, Annexin V, mutant MFG-E8 unable to bind VR, or VR antagonist [136]. Likewise, even MFGE8 knock-out mice experienced less neuronal loss upon challenged with LPS, thus reinforcing this speculative hypothesis of its manifestation during neuroinflammation [136].

On the contrary, the disarray of cytokine/chemokines and their respective receptor signals can bring forth a situation where microglial cells seem to feed on normal living neurons and microglial cells by an uncommon pathway known as non-canonical efferocytosis (Fig. 1) or phagoptosis [24, 112]. Reports suggest that canonical efferocytosis is an essential phenomenon required for the physiological development of hippocampus in the newborns [112, 147–151]. Along these lines, microglia can regulate the number of neuro-progenitor cells for facilitating the development of the cortex and cerebellum via canonical efferocytosis during the early phases of brain maturation [152, 153]. During neuronal development, microglial clearance of neuro-progenitor cells from the cortex and cerebellum was known to be bolstered by the presence of LPS and reactive oxygen species. Plausibly, the presence of these toxic stimulants might exacerbate the PS exposure on these neuro-progenitor cells thus transfiguring them into a more palatable meal for microglial engulfment and elimination [152, 154].

In the setting of neuroinflammation, stressful but living neurons are inclined to upregulate PS extracellularly which makes them more susceptible to being ingested by neighboring inflamed microglia through MFG-E8

receptor [24, 155]. Accordingly, MFG-E8 knock-out mice demonstrated reduced neuronal loss via attenuated macrophage-mediated removal [136]. Previous studies revealed that microglial cells tend to be actively ingesting viable neurons under the virulent influence of LPS, tau and Aβ aggregates [23, 25, 136, 137]. Microglial cells treated with neurotoxicants such as LPS, Aβ and tau respond by an increase in sialidase activity and desialylation of their surface plasma membrane [156]. Accordingly, induction of desialylation of neuron–microglial cultures resulted in increased microglial phagocytic activity and ensured the removal of normal living neurons through activation of phagocytic receptor CR3 (complement receptor 3) [156]. It is important to understand that, sialic acid present in the neuronal glycocalyx preserves neuronal integrity by inhibiting complement C1 binding and CR3-mediated microglial removal [157, 158]. Accordingly, loss of these protective glycans from the neuronal surface makes them liable to expedited microglial removal through CR3 receptor [157, 158]. Likewise, analogous potential mechanisms speculated through which desialylation activates microglial phagocytosis can range from the exacerbated binding of galactin-3, less trans-activation of Siglec-11 [sialic acid-binding immunoglobulin-like lectins-11] and Siglec-E [sialic acid-binding immunoglobulin-like lectins-E] to declined cis-activation of Siglec-2 [sialic acid-binding immunoglobulin-like lectins-2] and Siglec-3 [sialic acid-binding immunoglobulin-like lectins-3] [156].

In a study by Neher, and colleagues, primary rat neuronal and microglial cultures treated with LPS demonstrated increased neuronal loss secondary to phagocytosis of dead/dying neurons within 3 days [24]. LPS stimulation of microglia resulted in their proliferation, enhanced phagocytic activity and increased secretion of peroxy-nitrate [24]. Microglial secreted peroxy-nitrate acted on the neighboring neurons and triggered the exposure of eat-me signal PS (Fig. 3) [24]. Mechanistically, nitric oxide interaction with normal living neurons leads to extracellular PS exposure due to membrane lipid peroxidation and blockage of aminophospholipid translocase [159] (Fig. 3).

This exposure of the PS extracellularly on the neurons is the key step and a harbinger for their exacerbated elimination by microglial engulfment secondary to loss of plasma membrane phospholipid symmetry (Fig. 3) [24, 160, 161]. Necessary measures are taken to decrease this PS exposure might be beneficial in reducing their removal by macrophages [24]. This study provides an underlying basis for the presumption that inflammatory mediators such as LPS can trigger microglial-mediated removal of otherwise normal neurons by exposing PS extracellularly on their outer membranes. In addition to PS exposure, alternative mechanisms were also implicated in the removal of living neurons with the presence of LPS. In a recent study by Hide, and colleagues LPS stimulation resulted in the upregulation of phagocytic receptor $P2Y_2$ as well as death cell sensing receptor Axl on microglial cells both are which might be responsible for the removal of the PS-exposed normal living neurons in AD [9].

Aβ activation of microglia will bring out stimulation of NADPH [] oxidase, NOS production and [NO_4^-] generation (Fig. 3) [162–166]. The buildup of this NO_4^-around the neurons will stimulate the exposure of the PS extracellularly in neurons due to stimulation of scramblase and concomitant weakening of flippase or translocase [25, 166]. Aβ enhancing the phagocytic activity of surrounding microglia in addition to precipitating the PS exposure on the neuronal cells [25]. In a study by Neniskyte, and colleagues, the administration of blocking agents to PS exposure (Annexin V or antibody to PS) and microglial phagocytosis (cytochalasin-D or cyclo RGDfV) was sufficient to attenuate neuronal loss secondary to Aβ toxicity [25]. Aβ induced loss of phospholipid asymmetry along with extracellular PS exposure in the neuronal synapses due to lipid peroxidation-induced covalent modification of cysteine residues in the flippase enzyme or amino-phospholipid translocase [167]. Accordingly, pretreatment of modulators of phospholipid asymmetry [tricyclodecan-9-xanthogenate (D609) and ferulic acid ethyl ester (FAEE)] offered a protective effect against Aβ-induced neuronal loss in AD disease models [167]. Furthermore, pathological accumulation of tau causes PTEN (phosphatase and tensin homolog) activation, which underlies the extracellular exposure of the PS on the neurons and synapses for their subsequent microglial removal [137]. In P301S-tau mice, living neurons with tau inclusions expose PS due to the production of ROS [23]. In a previous study, PS-exposed neurons induce microglial secretion of MFG-E8 and nitric oxide for potentiating their removal in P301S-tau mice [23]. In microglial–neuronal co-cultures, the addition of extracellular tau was responsible for outside exposure of the PS on living neurons as well as stimulating the phagocytic activity of the microglial cells, both of which operate synchronously to instigate successful removal of living neurons without undergoing necrosis or apoptosis [168].

Taken together, all these studies support the hypothesis that Aβ, tau and LPS-effect microglial engulfing capacity as well as reduce their ability to discern between normal neurons and dead or dying neurons [25, 169]. Desensitized microglia can become a liability in AD as they can actively ingest normal neurons in addition to dead or dying neurons. Presumably, aggregation of Aβ clusters in the brain tissues can potentially exacerbate

the microglial-induced neuronal shrinkage by shifting the balance towards the non-canonical efferocytosis. The preponderance of the non-canonical efferocytosis is deleterious as there are higher chances of elimination of normal living neurons as well as dead neurons. This will lead to disproportionate brain atrophy and increase the risk of developing severe cognitive abnormalities in AD [170, 171]. Taken together, we hypothesize that dysregulation of efferocytosis is a primary pathology during all clinical stages of AD. This pathology can be exacerbated by factors beyond Aβ aggregates that contribute to the overactivation of efferocytosis and excessive clearance of normal neurons and microglial cells.

It is important to understand that the neurotoxic effect of Aβ is primarily dependent upon its concentration, with higher concentrations directly provoking neuronal death, whereas low concentrations might indirectly affect neuronal viability via stimulating microglial activity [34, 138, 172]. Higher concentrations of Aβ were known to incite neuronal death by a multitude of mechanisms ranging from excitotoxicity, mitochondrial toxicity, synaptic alteration, dysfunction of calcium homeostasis, ER stress, DNA damage, tau hyperphosphorylation to oxidative stress [173, 174]. Apoptotic type of cell death has been reported as a primary mode of neuronal loss in AD [175]. As neurons undergo apoptotic cell death, there should be mechanisms set in motion for prompt clearance of accumulated cellular debris from the extracellular space to prevent ensuing adverse consequences. So, apoptotic cells exteriorize PS and secrete find-me signals which serve as a forewarning as well as a glaring signal to the neighboring microglial cells in purging them immediately. This will commence the onset of necessary signaling pathways for kick-starting canonical efferocytosis in the neuronal environment.

However, the scenario will be quite different if the concentration of Aβ aggregates is sub-therapeutic and thus does not have the full ability to exert a direct neurotoxic effect [138]. During these instances, Aβ will trigger the activation of alternative signaling pathways by acting on the neighboring microglial cells and neurons, which will eventually execute clearance of normal but stressed neurons via non-canonical efferocytosis. Recently it was proven that, low nanomolar concentrations of Aβ instigated microglial-mediated neuronal loss from the cultures and hippocampal slices by acting through calcium-activated potassium channel and nitric oxide upregulation [172]. At the same time, Aβ acting on the neurons might potentiate extracellular exposure of the PS due to inactivation of flippase enzyme on the plasma membrane (transmembrane enzyme amino-phospholipid translocase) [176]. Therefore, due to a combination of effects of neurons and microglial cells, sub-toxic concentrations of Aβ might lay the foundation for the commencement of non-canonical efferocytosis in the neuronal milieu.

Regarding tau, its effects can be understood based on its location and concentration in the neuronal milieu. First, when intracellular tau concentration reaches super-threshold levels within the neurons, then neuronal death and canonical efferocytosis will ensue [177]. In a second setting, if the neuronal concentration of tau is sub-threshold and neuronal death is not a possibility, then neuronal stress, exposure of the PS, and tagging of stressed but viable neurons will ensue [23, 168]. In third scenario, excess intracellular tau might leak into the extracellular space and stimulate neighboring microglial cells thus making them vigilant and sharp-eyed for watching out for depopulating PS tagged viable neurons by non-canonical efferocytosis [23, 168]. Lastly, excess extracellular tau might enter the neuronal cells, incite PS exposure, and ultimately facilitate their removal by already hungry stimulated macrophages in their vicinity [23, 168]. Taken together, these scenarios come into play in various permutations and combinations when there is an appearance of Aβ and tau accumulation in the neuronal milieu.

A recent study reported that LPS-induced neuronal death secondary to neuroinflammation as well as increased amassment of amyloid ($A\beta_{1-42}$) in the cortex and hippocampus which directly translated to memory impairment and cognitive disturbances in AD [140, 178, 179]. Neuronal demise under the influence of LPS engenders the release of find-me and exposure of eat-me signals, which precipitates the migration of the microglial cells for favoring their clearance through canonical efferocytosis. However, when LPS concentrations are sub-optimal, microglial stimulation and discharge of nitrite radicals will be immediate aftereffects thus kick-starting the process of non-canonical efferocytosis.

Taken together, the process of neurodegeneration in AD is a complex phenomenon and is particularly driven by the relative concentration of cytokines, chemokines, and other toxic mediators. In all probability, the concentration of toxicants tends to fluctuate depending upon the stage of the disease process and the robustness of immune defenses to ward the toxic stimulus from the neuronal environment. Depending upon the sturdiness of the host defenses, the concentration of the toxic stimulus might vary from sub-optimal to above-threshold levels. Moreover, we can also speculate that there might be some vacillation in the concentration of different toxic mediators (LPS, Aβ and tau) at any stage of the disease process. Regardless, the immune defenses might not be completely efficacious in purging all the toxic stimulants that accumulate at the same point, thus exposing

the neurons and microglia to different concentrations of toxic mediators. This sets the precedence for pushing forward the hypothesis that gives rise to a framework where the deployment of both canonical and canonical efferocytosis at the same point at any given stage of AD is a strong possibility. The ratio of canonical to non-canonical efferocytosis is also of paramount significance and a crucial decisive factor that influences neuronal loss and brain tissue thinning in AD models. Considering these arguments, we propose necessary research investigations in this regard to shed light on these submerged pathological set of circumstances so that appropriate therapeutic strategies can be crafted for shrinking neuronal loss and brain tissue damage which forms the underlying basis for cognitive disturbances in AD.

Conclusions

The main purpose of this review is to underscore the importance of this physiological pathway (microglial efferocytosis) that plays a key role in tweaking the disease pathogenesis of AD. There are two forms of the microglial efferocytosis, a canonical pathway which is involved in the removal of dead neurons accumulating in the neuronal niche and the non-canonical pathway implicated in the removal of stressed but viable neurons displaying PS extracellularly on their plasma membrane.

M2 microglial cells are the primary phagocytes that tend to migrate, make initial contact, engulf dead, dying, and necrotic neurons during the physiological conditions as well as pathological scenarios like AD through canonical efferocytosis, a process with the added benefits of suppressing the associated neuroinflammation and facilitating tissue healing endeavors in AD. This canonical process of the microglial efferocytosis entails the engagement of bridging molecules, receptors, and signaling pathways, which act synchronously in effectuating actin polymerization-driven phagocytic cup formation for dead neuronal engulfment [94, 96, 180, 181]. As a part of an innate immune response, initiation, and full-fledged deployment of canonical efferocytosis will be beneficial not only in clearing the dead/dying cells accumulated during the course of the disease process, but also in the resolution of inflammation, tissue repair, regeneration, and homeostasis[96]. By RNA sequencing analysis of the human microglia, and neurons of the cerebral cortex, it was revealed that the microglial expression of phagocytosis receptors (CX3CR1, P2Y6, P2Y12 [purinergic receptor 2Y12], stabilin 1, SIRPα [signal regulatory protein α], TREM2 [triggering receptor expressed on myeloid cells 2] MerTK, and CD11b [integrin or adhesion receptors]) is required for the microglial-mediated clearance of the PS-exposed neurons [112, 182, 183]. In physiological conditions and during exposure to acute stressors (LPS), microglial cells will counterpoise to heighten their efferocytosis efficiency for having an equivalence between efferocytosis and neuronal apoptosis, mostly by incrementing their recognition receptor machinery and surveillance abilities [184]. However, during chronic pathological conditions like epilepsy, hyperactivity of the hippocampal network, altered ATP micro-gradients, piling of pro-inflammatory cytokines and attenuated receptor machinery sparks off uncoupling between microglial efferocytosis and neuronal apoptosis, thereby engendering the accumulation of dead apoptotic cells in the neuronal milieu[184]. The significance of the canonical pathway of the microglial efferocytosis in the pathogenesis of AD had recently gained significance and warrants further investigation [185, 186].

On the flip side, neurotoxicants such as LPS, tau and Aβ upregulated in AD can facilitate the removal of normal living neuronal cells by the non-canonical efferocytosis [23–25, 187]. As living neurons are exposed to sub-lethal concentrations of these above-mentioned toxicants, the release of nitric oxide with subsequent lipid peroxidation triggers the exteriorization of the PS due to incapacitated flippase as well as activation of plasma membrane scramblase enzyme [159, 162, 188]. These PS-exposed neurons are cleared by the neighboring wandering microglial cells through this idiosyncratic and unusual non-canonical pathway [23, 161]

Conceivably, it is plausible that both canonical, and non-canonical efferocytosis can be happening at the same time in varying proportions during the inception and progression of neurodegeneration in AD. It is plausible that, concurrent playing out of these two processes at the neuronal–synapse interface presents a predicament due to which aggravated neuronal loss can be a culminating effect in AD. As a matter of fact, the co-existence of both canonical and non-canonical efferocytosis is detrimental as it leads to the vigorous removal of dead as well as living neurons from the brain tissues. This will cause excessive brain tissue thinning and severe cognitive disturbances with an associated increase in morbidity and mortality in AD. Alternatively, overpowering non-canonical efferocytosis is also deleterious as the removal of normal living neurons takes precedence over clearing dead and dying neurons. In this regard, identifying the relative proportion of these processes and their relative impact on the disease progression during the preclinical and clinical stages of AD would be worthwhile. It is also feasible to understand whether the effects of LPS, tau and Aβ are synergistic in inducing neurotoxicity as well as triggering stress-related PS exposure, thus opening the doors for the concurrent existence of these two divergent pathways [168].

Limitations

Microglial efferocytosis is a budding research topic with researchers still trying to ascertain its prominence in the CNS, particularly its enactment and precision in the physiological and pathological conditions still being nebulous. Currently, microglial efferocytosis is predominantly executed by microglial cells with secondary contribution from astrocytes and non-microglial mononuclear cells [189]. A recent study by Konishi, and colleagues revealed that in the mice with microglial ablation, astrocytes expressing phagocytic machinery (Axl and MerTK) come to the rescue to scavenge the dead neuronal debris [189]. Currently, it is not known whether the meticulousness of astrocyte efferocytosis matches that of the microglial purging process, thus knock-out models of the microglial efferocytosis should be interpreted with caution until we compute the soundness of the compensatory mechanisms. That being said, to study the full effects of efferocytosis derailment on the neurodegenerative process in AD, we should eliminate the all the cells contributing to this purging process including microglial cells, astrocytes and non-microglial mononuclear cells, which thus might be a challenging process [189].

Studies indicate that boosting canonical efferocytosis is beneficial by clearing the accumulated dead neurons in the neuronal niche during AD pathogenesis [190]. This requires dead neurons to proficiently display eat-me signals (PS) to facilitate their effortless clearance by the incoming microglial cells. With LPS or Aβ piling up during the active disease stage, even neighboring viable neurons displaying calreticulin can be engulfed and cleared by activated microglial cells through CRT–LRP pathway [191]. Moreover, as microglial cells ingest and clear the Aβ aggregates, they might inherently develop encumbrances and weaknesses in their physiological functions including efferocytosis. Due to these transpirations, microglial will lose their decoding ability to pertinently distinguish between normal/viable and apoptotic under the toxic spell of LPS/ Aβ [190]. This collateral damage might magnify the neuronal loss and neuronal degeneration in AD. Thus, interventions that heighten the microglial efferocytosis in AD might not be alone sufficient to lessen neurodegenerative process in AD.

Downgrading the non-canonical pathway is essential for restraining unwanted removal of viable neurons from the neuronal niche and subsequent brain thinning in AD. ROS and nitric oxide generated by oxidative stress under the hegemony of LPS or Aβ are partially implicated for eat-me signal exposure in normal/viable cells, an unfolding that incites their impending removal through PS–MFG-E8–VR pathway [24, 191]. Neutralizing this unconventional neuronal removal process is indirectly dependent upon the near complete elimination of ROS and NO from the neuronal environment, an endeavor that is a little strenuous to accomplish.

Despite these transgressions, it would be worthwhile to delve into these processes and as a result we propose future research studies to get to the bottom of these clearance processes which might have a profound impact on the disease pathogenesis in AD. Therapeutic interventions that promote efferocytosis in the neuronal niche can at least be applied as an adjunctive therapy to currently FDA-approved anti-amyloid medications to lessen the pile of neuronal cellular debris [192, 193]. This can become a turning point for setting in motion disease healing processes promptly as soon as neuronal damage materializes for furthering optimal disease outcomes and decreasing clinical symptom profile in AD.

Future research

Previous studies established that cytokines, chemokines, and other toxin factors spawned during AD pathogenesis disrupts the delicate balance between canonical and non-canonical efferocytosis. Under the influence of these toxic factors, the balance might be disproportionately swayed towards the non-canonical efferocytosis, a process which should be relatively absent or minimal under physiological conditions. The preponderance of this process is counterproductive as it can foment hastened removal of stressed viable neurons along with bringing the tissue healing mechanisms to a standstill. As the consequences of the preponderance of this non-canonical efferocytosis can be deleterious, further research is warranted in delineating the protective strategies that might curb or lessen the activation of this pathological process so that neuronal loss and brain thinning can be scaled down. So, kick-starting research efforts to expose the necessary factors that govern the fine balance between canonical and non-canonical efferocytosis in AD is very much a future necessity.

It has been speculated that LPS-stimulated microglia overproduce peroxy-nitrate which acts on the neighboring stressed neurons to exteriorize PS, which becomes a first step before the dawning of non-canonical efferocytosis [138]. With that being said, it would be worthwhile to explore the possible mechanisms by which peroxynitrate-induced wreckage of neuronal plasma membrane transpires, a vulnerability that sparks off exposure of the PS consequently for the materialization of non-canonical efferocytosis.

Furthermore, PS/MFG-E8/VR pathway has been primarily implicated in the phagocytosis of stressed neurons under the influence of LPS [25, 138]. Ramifications of blocking this phagocytosis pathway in the neurons stimulated with tau, and Aβ should be investigated in the future. Protein Annexin A1 (ANXA1) and MFG-E8

which are released from the microglial cells bound to play a crucial role in clearing the neurons during inflammatory and non-inflammatory conditions [136, 169]. Previous reports suggest their expression is increased in AD and they serve as an eat-me signal and bridging factor for clearing the PS-expressed apoptotic neurons [136, 169]. Knockout models of these bridging molecules will need to be included in the research studies to assess their efficacy in attenuating the loss of normal and stressed neurons in AD [136, 169].

With regard to canonical efferocytosis, it is imperative to delve into the influence of LPS, tau and Aβ on the efferocytosis machinery including microglial surface receptors, bridging molecules and metabolism for comprehending the integrity of these processes during the dawning of neurodegeneration in AD. Furthermore, knock-out models of the microglial efferocytosis machinery will succinctly identify the significance of these processes in purging the accumulated dead neuronal cells and their lasting impact of untimely removal.

Given that the efficient removal of dead cells from the extracellular space requires efficient functioning of canonical pathways and switching of non-canonical pathways, research in this regard tends to highlight the key signaling factors involved in these concurrent processes operating during neurodegeneration inAD. This might serve as a framework for developing novel interventions for reducing the unnecessary neuronal loss and onset of cognitive disturbances in AD.

We anticipate that, full execution of above-mentioned research studies will become an initial-stepping stone by shedding light upon effect of toxic (LPS, Aβ & tau) aggrgates on microglial clearance processes in AD. This will yield valuable preliminary data that will help in comprehending the the status quo of microglial purging and subsequent heaping of neuronal debris during neurodegeneration in AD.

Abbreviations

TLR4	Toll-like receptor-4
Axl	TAM family of receptor tyrosine kinase receptors
LPS	Lipopolysaccharide
HMGB1	High mobility group box1
Aβ	Amyloid beta
SPP1	Phosphoprotein1
C1qa	Complement 1qa
AD	Alzheimer's disease
STAT6	Signal transducer and activator of transcription 6
Arg1	Arginine1
Sig-1R	Sigma-1 receptor
tMCAO	Transient middle cerebral artery occlusion
LTB4	Leukotrienes B4
NOS	Nitric oxide synthase
NO	Nitrate
MFG-E8	Milk factor globulin E8
JNK	C-Jun terminal kinases
ER	Endoplasmic reticulum
Bcl-w	B-cell lymphoma w
NMDA	N-Methyl-D aspartate
SR	Scavenger receptor
MV	Microvesicles
PrPC	Cellular prior protein
GM1	Monosialotetrahexosylganglioside
RAGE	Receptors for advanced glycosylation end products
M1	Classically activated phenotype
M2	Anti-inflammatory phenotype
IL-1β	Interleukin-1 beta
TNF-α	Tumor necrosis factor alpha
STAT3	Signal transducer and activator of transcription 3
IL-6	Interleukin-6
IL-12	Interleukin-12
IL-23	Interleukin-23
ROS	Reactive oxygen species
IL-10	Interleukin-10
IL-4	Interleukin-4
IL-13	Interleukin-13
TGF-β	Transforming growth factor beta
DAMP	Damage associated molecular patterns
DNA	Deoxy nucleic acid
HSP-90	Heat shock protein 90
S100A8	Alarmin
S100A9	Alarmin
LAP	LC3 associated phagocytosis
CRKII	10 (CT10) regulator of kinase (Crk)II
DOCK180	CED-5/180 kDa protein downstream of chicken tumor virus no. 10
ELMO	CED-12/engulfment and migration
RAC1	Rho family GTPase protein
GTP	Guanosine-5′-triphosphate
Dbl	MRNA encoding the GTP-exchange factor
PPAR	Peroxisome proliferator-activated receptors
LXR	Liver X receptors
iNOS	Inducible nitric oxide synthase
CX3CL1	Chemokine-like fractalkine
Lyso-PC	Lyso-phosphatidyl choline
ATP	Adenosine triphosphate
UTP	Uridine triphosphate
PS	Phosphatidyl serine
Gas-6	Gamma-carboxyglutamic acid (Gla) domain-containing protein
STAT1	Signal transducer and activator of transcription 1
Tyro3 receptor	TAM family of tyrosine kinase receptors
BACE1	β-Site amyloid precursor protein cleaving enzyme
PHOX	Phagocytic NADPH oxidase
VR	Vitronectin receptor
CRAMP	Cathelicidin-related antimicrobial peptide
PTEN	Phosphatase and tensin homolog
ANXA1	Protein Annexin1
RAGE	Receptor for advanced glycation end products
MERTK	MER proto-oncogene, tyrosine kinase
LRP1	Low density lipoprotein receptor
SRB1	Scavenger receptor, class B type 1
P2Y6	Purinergic receptor P2Y6
P2Y12	Purinergic receptor P2Y12
S1PR	Sphingosine1-phosphate receptor
G2A	G protein-coupled receptor 132
BAI-1	Brain specific angiogenesis inhibitor 1
CD300b	Cluster of differentiation 300 b
ER	Endoplasmic reticulum
HCC	Hepatocellular carcinoma
TUNEL	Deoxynucleotidyl transferase-mediated dUTP nick-end labeling
CX3CR1	C-X3-C chemokine receptor
TIM1and4	T cell immunoglobulin mucin domain-1and4
CRT	Calreticulin
TREM2	Triggering receptor expressed on myeloid cells 2
SIRPα	Signal regulatory protein α
P2Y12	Purinergic receptor 2Y12
CD11b	Integrin or adhesion receptors

NADPH	Nicotinamide adenine dinucleotide phosphate
Siglec-11	Sialic acid-binding immunoglobulin-like lectins-11
Siglec-E	Sialic acid-binding immunoglobulin-like lectins-E
Siglec-2	Sialic acid-binding immunoglobulin-like lectins-2
Siglec-3	Sialic acid-binding immunoglobulin-like lectins-2
NF-kB	Nuclear factor kappa B
P13k–Akt	Phosphoinositide-3 kinase–protein kinase B
TGF-β1	Transforming growth factor beta
Tim1and4	T cell immunoglobulin mucin domain-1and4
Fas	Type-II transmembrane protein
Fas ligand	Type-II transmembrane protein ligand
PI3K	Phosphoinositide-3 kinase
ABCA1	ATP binding cassette transporter sub-family A1
ABCG1	ATP binding cassette transporter sub-family AG
CSF	Cerebrospinal fluid
CD36	Receptor for thrombospondin; scavenger receptor
Fc receptors	ITAM associated receptor family
Grn	Progranulin
Ctsb	Cathepsin-b

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