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REVIEW PAPER

The Role of Lipid Bodies in the Microglial Aging Process and Related Diseases

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Abstract Lipid bodies (LBs) have long been considered to be organelles merely for the storage of neutral lipids. However, recent studies have shown the significance of LBs in signal transduction, especially in glial cells, including microglia. Microglial cells are the resident mononuclear phagocytes in the central nervous system and have a close relationship with the aging process and neurodegenerative diseases. Evidence suggests that LBs accumulate and are remodeled during the aging process and the development of neuroinflammatory conditions. However, the mechanisms underlying the formation of LBs under these conditions and the mechanism by which LB remodeling influences the progression of neurodegeneration remain to be clarified. In this review, we have summarized the findings from recent studies with the aim of further elucidating these issues.

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Abbreviations

ADRP	Adipose differentiation-related protein
AD	Alzheimer's disease
CNS	Central nervous system
JNK	c-Jun-N-terminal kinase
DAG	Diacylglycerols
DHA	Docosahaxaenoic acid
ER	Endoplasmic reticulum
HSN-1	Hereditary sensory neuropathy type 1
LBs	Lipid bodies
LPS	Lipopolysaccharide
LXRs	Liver X receptors
OGA	O-GlcNAcase
SPT	Palmitoyltransferase
SNAP23	Synaptosome-associated protein 23
CPT2	Palmitoyltransferase 2
PD	Parkinson's disease
PS	Phosphatidyl serine
SREBP	Sterol regulatory element binding protein
TEM	Transmission electric microscopy
TAG	Triacylglycerols

Introduction

Lipid bodies (LBs), also referred to lipid droplets (LD), lipid particles and liposomes, are dynamic lipid-rich organelles of various sizes and compositions that contain neutral lipids, such as triacylglycerols (TAG), diacylglycerols (DAG) or steryl esters [1]. The LB surface is composed of a monolayer of amphipathic phospholipids, glycolipids and/or sterols [2] and is also decorated with various proteins, such as PAT family proteins (perilipin, adipose differentiation-related protein (ADRP), tail-interacting protein of 47 kDa (TIP-47) and caveolins [3]. Although the functions

of these proteins on LBs were still not fully understood, the identities and functions of various proteins have been revealed gradually by proteomics analyses. These surface proteins play vital roles in the formation, maintenance, metabolism and degradation of LBs under the influence of various stimuli and physiological conditions. LBs were first thought to serve as a reservoir of lipids and to play a vital role in lipid metabolism. However, research suggests that LBs are closely related to atherosclerosis, metabolic disorders [4], cancer [5] and especially, aging and aging-related diseases. Evidence suggests that LB accumulation in the microglia during the aging process is associated with remodeling and altered interactions with other organelles [6]. Thus, we hypothesized that LBs play important roles in brain aging, especially in some neurodegenerative diseases.

Microglial cells are the resident macrophages and immune cells in the central nervous system (CNS) and play an important role in the aging process [7–9], neuroinflammation and as well as neuron circuit integrity and plasticity [10]. Most popular models of the aging process and other studies suggest that LBs originate in the endoplasmic reticulum (ER) [11]. ER stress and mitochondrial defects are characteristic of brain aging and result in the microglial cell activation. Another key consequence of mitochondrial dysfunction and reactive oxygen species (ROS) generation is the accumulation of LBs [12]. Activated microglia secretes proinflammatory cytokines, nitric oxide (NO) and ROS [13, 14]. These inflammatory conditions induce LBs to undergo a variety of changes via specific signaling pathways. All these reports imply that LBs play a pivotal role in brain aging.

Dysregulated microglia play a vital role in aging and related neurodegenerative conditions, such as Alzheimer's, Parkinson's, and Huntington's diseases as well as in schizophrenia [15]. The disruption of cholesterol metabolism and inflammation is closely linked to the initiation and progression of AD, which is modulated by liver X receptors (LXRs). APP/PS1 transgenic mice lacking either of the *Lxrα* or *Lxrβ* genes exhibit an increasing amyloid plaque load [16]. The disruption of LXRα or LXRβ also leads to excessive LB accumulation in mouse brain [17] We have summarized these studies in this review and proposed three potential mechanisms of LB formation. We also discuss reduction in LB numbers and remodeling as a therapeutic strategy in neurodegenerative diseases.

LB Formation in Microglia

LBs exist in different cell types in various numbers and sizes, potentially reflecting lipid storage capacity. It is widely accepted that LBs originate in the ER [18–20]. LBs could be formed under two distinct but not mutually

exclusive environmental conditions. First, LBs accumulate in cells in response to exogenous lipid availability [21]. Studies have shown that free fatty acids (FAs) and cholesterol from serum lipoproteins induce the formation of LBs, probably to meet the needs of the cell in terms of energy generation and biosynthesis of steroid hormones [22, 23]. Second, LB biogenesis is induced by a variety of cellular stress conditions, including starvation [24], inflammation and oxidative stress [21], the latter being closely associated with the aging process in microglia. Based on this emerging evidence, we propose three potential molecular mechanisms to account for the formation of LBs; these mechanisms are summarized in Fig. 1.

The first two potential mechanisms are based on the observation that LB formation in the microglia is induced by ROS, generated either in the microglial cell itself or in neurons in the vicinity. This discovery was the result of intense investigations and continues to be a focus of research [25]. Although the relationship between ROS and LB formation has been known for some time, this association in the context of microglia has only recently been discovered. Lee et al. showed that ROS induced by hyperoxia/hypoxia resulted in loss of mitochondrial DNA (mtDNA) and also enhanced LB formation. This group also showed that LB formation is greatly influenced by heme oxygenase (HO)-1, an inducible stress protein [21]. Subsequently, Khatchadourian et al. reported that ROS produced by lipopolysaccharide (LPS)-stimulated microglia induce LB formation via the JNK and p38 MAPK stress signaling pathways, in which LD-associated protein perilipin-2 (also known as adipose differentiation-related protein, ADRP) and cytosolic phospholipase A_2 (cPLA$_2$-α) play vital roles [25]. The mechanism by which ROS induce LB formation and their relationship with neurodegeneration represent a current focus of research in this area. Recently, Liu et al. demonstrated that ROS induced by mitochondrial defects in microglia stimulated the formation of LBs and promoted neurodegeneration [12]. In fact, a number of seemingly autonomous observations indicate the close relationship between LBs and neurodegeneration. These observations include the discovery of mitochondrial mutants that are closely associated with neurodegenerative diseases and aging [26] as well as the correlation between LB accumulation and increased ROS, an effect that is ameliorated by a reduction in ROS. Furthermore, elevated ROS levels promote JNK/SREBP activation, which leads to LB accumulation and most importantly, to a reduction in LBs in microglia resulting in delayed neurodegeneration. Based on these observations, it was concluded that ROS and mitochondrial dysfunction stimulates neuronal c-Jun-N-terminal kinase (JNK) and sterol regulatory element binding protein (SREBP) activity, leading to LB accumulation in microglia, and probably resulting in neurodegeneration [12].
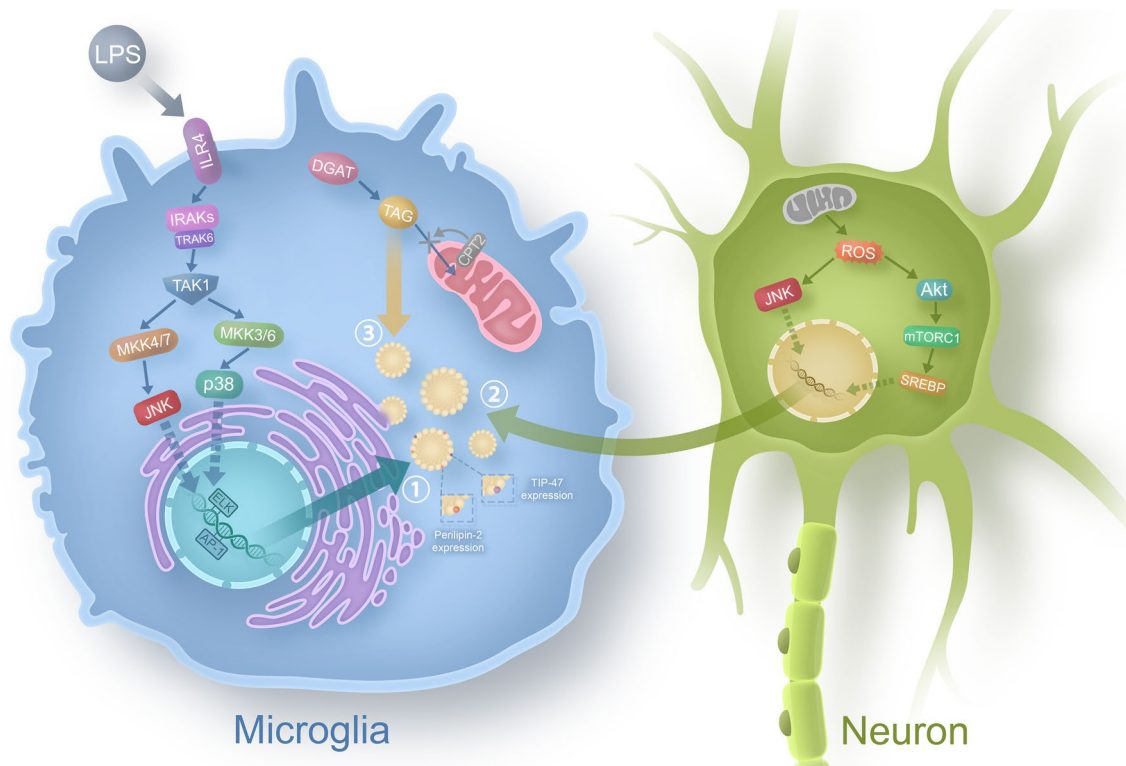
Fig. 1 Three postulated models of lipid body formation cell. LBs consist of a monolayer of amphipathic phospholipids decorated with various proteins (such as perilipin-2 and TIP-47 as shown in above). The figure shows three possible models for LB formation. (*1*) The first model is characterized by LPS-induced perilipin-2 expression. ILR4 phosphorylates TAK1 following LPS stimulation, and induces MKK phosphorylation. Phosphorylated MKKs activate the JNK and p38 pathways, leading to LB formation through expression of per-

ilipin-2 by activating the downstream targets, AP-1 and ELK1. (*2*) In the second model, mitochondrial defects increase ROS production, which further activates the JNK and Akt-mTORC1-SREBP pathways in neurons, leading to accumulation of LBs in the surrounding microglia. (*3*) In the third model, the absence or dysfunction of CPT2 prevents the entry of TAG into the mitochondria for β-oxidation, leading to cytosolic accumulation and LB formation

Thus, we postulate three possible mechanisms for LB formation. The first mechanism proposed for LB formation is based primarily on experiments involving LPS-induced ROS produced in microglia. LPS is an outer cell wall component of Gram-negative bacteria that is widely used to induce ROS generation both in vivo and in vitro [27]. LPS is also often used as a proinflammogen to stimulate the microglial activation that occurs as part of the aging process [28]. Normally, LPS binds to TLR4 to activate downstream signaling of mitogen-activated protein kinase (MAPK), JNK and p38 [29]. MAPK kinases (MKK) phosphorylate p38 and JNK, with resulting activation of the downstream targets activator protein-1 (AP-1) transcription complex (c-Jun/ATF2) and ELK1. This activation of AP-1 induces proinflammatory cytokine expression, which is the key characteristic of microglia in the aging process [30]. Activated AP-1 binds to the Ets/AP-1 binding site, increasing the expression of perilipin-2, a key regulator of LB formation [31]. This increased perilipin-2 expression stimulates LB formation not only in macrophages, but also in

microglia [25, 31]. In addition to LPS, oleic acids or low-density lipoproteins have also been shown to induce perilipin-2 expression [32, 33]. Although the detailed mechanism by which perilipin-2 influences LB formation remains to be fully elucidated, a number of hypotheses have been proposed. One is based on the location of perilipin and other enzymes in the ER, where triglycerides accumulate at privileged sites to form nascent LD [11]. The binding of perilipin-2 to LBs does not require other LD proteins, which suggests that perilipin-2 is probably one of the first proteins to bind to LBs [34]. However, there is no direct evidence that perilipin-2 is located in the ER. Alternatively, binding of perilipin-2 to phospholipids at the surface of the nascent LD occurs via hydrophobic and electrostatic interactions. This binding results in accumulated binding of more lipoproteins and continuously increased size until the LD becomes large enough to bud from the ER, forming a new LB [35, 36]. This hypothesis is supported by several studies showing that perilipin-2 in the cytosol binds to LD in vitro without the assistance of other LD proteins, and

also that perilipin-2 interacts directly with other LD proteins [34, 37–39].

The second proposed mechanism of LB formation involves defects in neuronal mitochondria that lead to elevated levels of ROS, resulting in activation of the JNK signaling pathway and also SREBP. This important regulator of LB biogenesis is a highly conserved, membrane-bound, basic helix-loop-helix leucine zipper transcription factor that is crucial for lipid homeostasis [12, 40]. This activation of JNK and SREBP in neurons induces accumulation of LBs in glial cells, including microglia. However, the specific mechanism of LB formation in response to this stimulation by surrounding neurons remains unknown and it is noteworthy that mitochondrial defects in neurons, but not in glial cells, induce downstream LB accumulation in microglia [12].

In addition to non-cell-autonomous LB accumulation, LBs can also accumulate in microglia in a cell-autonomous manner. Thus, a third explanation for LB accumulation in microglia is raised. Triglycerides (TAGs) accumulate to form abundant LBs in glial cells, including microglia, which lack the mitochondrial enzyme carnitine palmitoyltransferase 2 (CPT2) that is necessary for β-oxidation of long-chain FAs [41, 42]. This accumulation of LBs has also been observed in vitro [43]. CPT2 catalyzes the replacement of carnitine by CoA, which is catabolized by β-oxidation and generates ATP in brain tissues [44, 45]. In the absence of CPT2 in microglia, TAGs do not undergo β-oxidation in mitochondria, resulting in LB accumulation in the microglial cytoplasm and a reduction in the energy supply to the surrounding neurons. This suggests a possible relationship between LB accumulation and neurodegenerative diseases via a mechanism that does not involve SREBP, which is an important molecule in non-cell-autonomous LB accumulation [41].

Lipid Bodies and Neurodegenerative Diseases

LBs are associated with neurodegenerative diseases, although LB accumulation has not been reported in patients with neurodegenerative diseases such as Leigh Syndrome or in corresponding animal models resulting from LBs' transient accumulation and intensive occurrence during the presymptomatic stages [12]. Nevertheless, an association between LB accumulation and neurodegenerative diseases is indicated based primarily on evidence that LBs release proinflammatory molecules. Some studies have shown an increasing number of LBs and alterations in lipid metabolism in autosomal dominant neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD), although the specific mechanisms remain unknown.

Alzheimer's Disease

AD, which is the most common neurodegenerative disease, is closely associated with aging, microglia and the metabolism of lipids, such as cholesterol, resulting in amyloid β accumulation. However, the association of LBs with AD has recently been revealed with the discovery of protein-O-linked N-acetyl-β-D-glucosaminidase (O-GlcNAcase, OGA). Evidence has shown that OGA accumulates on the surface of the nascent LD along with perilipin-2. Furthermore, OGA knockout causes increased levels of perilipin-2 and perilipin-3, suggesting that OGA is a regulator of LD formation. OGA has also been linked with AD via the hexosamine signaling pathway. Therefore, these findings indicate that LB assembly and mobilization are associated with AD [46].

The three isoforms of apolipoprotein E (ApoE) encoded by the ε2, ε3 and ε4 alleles play important roles in lipid metabolism and transport from one tissue or cell to another in the brain. The ε4 allele of the apolipoprotein E gene (ApoE4) is a major risk factor for later onset AD. Studies have revealed that the ApoE4-lipoproteins bind to Aβ with lower affinity than that of the ApoE3-lipoproteins, which may lead to less efficient Aβ clearance by microglial cells [47, 48]. Furthermore, ATP-binding cassette sub-family A member 1 (ABCA1)-deficiency is associated with reduced efflux of cholesterol to exogenous ApoE in the microglia. Expression of both ABCA1 and ApoE is induced following LXR stimulation, which is closely related to the amyloid plaque load [49]. Taken together, these observations indicate the close relationship between lipid metabolism in microglial cells and AD.

Parkinson's Disease

PD is the second most common neurodegenerative disorder associated with microglia and aging. Studies have shown that activated microglia accumulate in damaged areas of the brain in patients with PD [50, 51]. The release of proinflammatory cytokines, such as IL-1β, by activated microglia has been demonstrated in animal models of PD induced by MPTP, 6-OHDA and LPS [52]. LBs play a role in this process as a site of proinflammatory cytokine storage. In addition, LBs interact with α-synuclein, a key player in PD and triglyceride turnover. Normally, α-synuclein exists in the soluble state in the cytosol and adopts an α-helical structure when it interacts with membranes containing acidic phospholipids [53]. The A53T mutant of synuclein, which causes PD [54], is redistributed to the lipid droplet surface, where it forms oligomers. This is an initial step in the formation of α-synuclein patches, which are a characteristic of PD [55].

Hereditary Sensory Neuropathy Type 1(HSN-1)

Hereditary sensory neuropathy type 1(HSN-1), an autosomal dominant neurodegenerative disease, is caused by *SPTLC1 gene missense mutations* [56] which encodes the serine palmitoyltransferase long-chain subunit of serine palmitoyltransferase (SPT). This ubiquitously expressed, and highly regulated ER-bound membrane enzyme maintains sphingolipid concentrations and is involved in lipid metabolism and signaling [57–59]. Studies have shown increased LB accumulation in HSN-1 patient-derived lymphoblasts, which is consistent with earlier reports that HSN-1 is caused by the accumulation of two neurotoxic sphingolipids [57]. Although co-localization with mitochondria was not detected [56], this evidence is controversial because it was based on immunoblotting of markers of these two organelles in fractions rather than observation in unbroken cells in vivo. This method does not exclude the possibility that these interactions are weak and may be broken during the purification of the fractions.

No research into LBs has yet been conducted in HSN-1 patient-derived microglia. However, since microglia and lymphoblasts are both immune cells, we assume that the results obtained by studying lymphoblasts can be applied in microglia. Thus, we propose the hypothesis that mutations in the *SPTLC1* gene result in the production of toxic lipids by the mutated SPT enzyme. These toxic lipids accumulate in LBs, which interact with mitochondria to promote neurodegeneration and induce the death of peripheral neurons to cause HSN-1.

Remodeling of Lipid Bodies in Microglia

So far, we have discussed LB accumulation during the aging process and the potential mechanisms. However, this process involves the active formation of large LBs in preparation for storage of various inflammatory mediators [60]. This remodeling represents the morphological and functional changes in organelles as a result of several types of stimulation, including docosahexaenoic acid (DHA). Furthermore, emerging evidence suggests that the LB remodeling is an early biomarker of neuroinflammation and modulator of neurodegenerative diseases [6]. In this review, we describe the morphological changes and transforming interactions between LBs and other organelles during LB remodeling.

Morphological Changes

LPS is used to stimulate microglia to mimic the state occurring in aging and neuroinflammatory conditions. Using confocal microscopy and transmission electric microscopy (TEM), Tremblay showed that LBs accumulate in various sizes in N9 microglial cells exposed to LPS. Under LPS stimulation, the newly formed LBs were in larger in size than those in the control cells. The electron density was also found to vary, with electron-lucent content observed in LBs in LPS-stimulated microglia. However, following the addition of DHA, LBs reorganized and aggregated, with the formation of medium-sized LBs and electron-dense bodies. This remodeling could be induced by the activation and phosphorylation of p38 [12, 25, 61]. This result was observed in both human and mouse microglia [6].
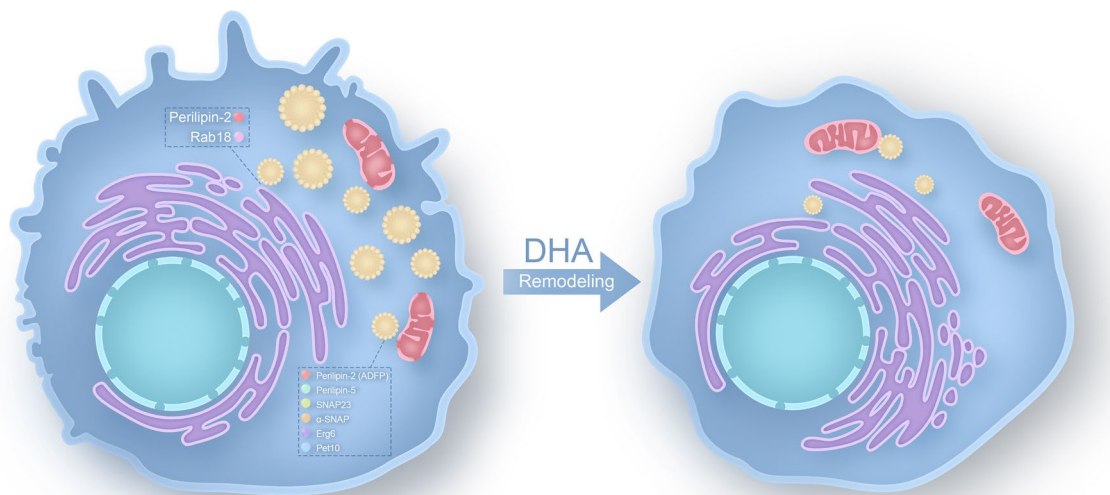
Interactions with Other Organelles

LB remodeling also influences their spatial relationship with other organelles, as shown in Fig. 2.

Mitochondria

In LPS-stimulated microglia, LBs and mitochondria are in close proximity, but with few direct contacts and no fusion. DHA induces numerous direct contacts with mitochondria and LB remodeling. Interestingly, LPS stimulation results in partial or even complete loss of mitochondrial cristae and the double membrane, leading to decreased mitochondrial membrane potential and ROS generation. ROS not only stimulates the formation of LBs and release of TNFα, IL-1β and NO, leading to inflammation and neurodegeneration, but also causes damage to mtDNA, proteins and lipids, and influences phagocytosis. Dysregulated phagocytosis promotes cytokine production, leading to mitochondrial modification and LB remodeling [61–63]. DHA rescues mitochondrial integrity and ameliorates the defects associated with mitochondrial damage, probably through LB remodeling [61].

The molecules involved in the LB-mitochondrial interaction in microglia have not yet been fully clarified, although this interaction has been elucidated in other cells. LBs play an important role in cells under conditions of starvation stress by functioning as storage and transportation compartments for FAs. Furthermore, the LB-mitochondria interaction is beneficial in starved cells, which use LBs as a conduit for β-oxidation of FAs by mitochondria [24]. However, the mechanisms underlying the responses of microglia under nutrient stress conditions and the proteins involved in the fusion of LBs and mitochondria remain to be clarified. Perilipin-2 (ADFP) is found in significant levels in the mitochondrial fraction, suggesting a potential interaction with mitochondria. Synaptosome-associated protein 23 (SNAP23) and the soluble NSF adaptor protein receptor (α-SNAP) are also found in the LB-mitochondria interactional area. Ablation of SNAP23 reduces perilipin-2 levels, indicating a decrease in LB-mitochondria complex

Fig. 2 Remodeling of lipid bodies and interactions with other organelles. The *left-hand* schematic shows LPS-stimulated mimicry of the aging state in microglia, while the *right-hand* schematic shows DHA-induced remodeling of microglia. Striking differences occur: stretches decrease obviously through this remodeling; the number and size of LB reduces significantly; mitochondrial function is greatly rescued with increasing cristae and membrane integrity through remodeling; interaction of LB with mitochondria and ER increases. Possible regulators are shown in the box (perilipin-2 and Rab18; perilipin-2, perilipin-5 and SNAP23, α-SNAP, Erg6 and Pet10)

formation [64]. Perilipin-5, also known as LB-associated protein, was first detected in isolated mitochondria and recruits mitochondria to the LB surface. Moreover, perilipin-5 inhibits LB hydrolysis and prevents excessive fatty acid (FA) oxidation in the mitochondria by storing FAs transiently in the LBs [65]. Erg6 and Pet10 are two proteins located in LBs that have been shown to interact with a series of mitochondrial proteins, including Mcr1, Mdv1, Om14 and Cox7, using bimolecular fluorescence complementation (BiFC) assays [66], but further underling mechanisms still need to be elucidated.

Endoplasmic Reticulum

In contrast to the interaction between LBs and other organelles, the LB–ER interaction has been regarded as the result of budding of LBs from the ER [67–69]. Excess FAs and cholesterol are stored in the ER as neutral lipids, such as triglycerides and cholesterol esters. The terminal enzymes involved in neutral lipid synthesis, including acyl-CoA: cholesterol acyltransferase (ACAT) and acyl-CoA: diacylglycerol acyltransferase (DGAT), are also localized in the ER [70]. Acyl-CoA synthetase 3 (ACSL3) is another enzyme that is redistributed from the ER to LBs and is necessary for the formation of LBs in the early stages [11]. The hydrophobic nature of neutral lipids is also important for the budding of lipid droplets from the ER; this widely accepted hypothesis for LBs formation is reviewed elsewhere [20]. There is, however, evidence also showing that LB–ER interactions are independent of LB formation [71, 72]. There is a lack of direct evidence to support both of the hypotheses that have been proposed previously. Although the detailed molecular mechanism of the LB–ER interaction in microglia remains to be elucidated, molecules involved in this process have been identified in other non-adipocyte cells. Proteomic analyses have revealed a number of Rab proteins in LBs. Among them, Rab18 plays a central role in the LB–ER interaction and Rab18 overexpression significantly reduces perilipin-2 levels. However, in contrast to the effects in the LB–mitochondrial interaction, reduced perilipin-2 induces membrane apposition between LBs and the ER, as well as the ER-derived membrane [73]. Furthermore, regulation of the LB–ER interactions is dependent on the GTP/GDP state of Rab18 [74].

Few focal contacts between LBs and stretches of ER occur in response to LPS stimulation. However, following the addition of DHA, electron microscopy showed stretches of ER running parallel to the LBs and their intermingling over long distances, suggesting the occurrence of specifically orientated functional interactions induced by LB remodeling [6]. Studies have shown that LBs are related to, and probably derive from the ER, which contains enzymes involved in LB formation and accumulation. Budding from the ER occurs once the LBs reach a certain size [18, 75, 76]. Furthermore, this remodeling is critical for neurodegeneration. DHA treatment increases the direct contacts between these two organelles as well as the levels of phosphatidyl serine (PS), which is the most abundant negatively

charged phospholipid in the eukaryotic membrane [77]. PS is critically involved in signal transduction, suggesting that DHA-induced remodeling plays a role in releasing proinflammatory cytokines in the microglia [78].

Lipofuscin Granules

Lipofuscin granules, which are formed in microglia and neurons during the aging process in animals as a result of oxidative stress [79], interfere with several metabolic processes [80] and are implicated in the development of neurodegenerative diseases. After remodeling, LBs are juxtaposed to very large lipofuscin granules, with some regions of fusion [6]. However, the significance of this interaction in neurodegeneration remains to be clarified.

Perspective

In the main, we have discussed LB formation and remodeling in relation to neurodegenerative diseases. Studies have shown that LB accumulation results in the release of proinflammatory molecules and neuroinflammation, which serves as a signaling platform and indicates the potential of LBs as therapeutic targets in neurodegenerative diseases [81, 82]. Two aspects of LB function are implicated as targets for the treatment of aging-related neurodegenerative diseases; LB remodeling and reduction in LB numbers, especially large LBs. As we have discussed earlier in this review, LB remodeling induced by DHA restores normal LB function in aging microglia by increasing contacts with other organelles that are required for normal function of activated microglia [57]. DHA treatment has also been shown to reduce the number of large LBs [6, 83], thus, indicating the marked potential of ω-3 polyunsaturated fatty acids (PUFAs) for the treatment of neurodegenerative diseases.

Even with these exciting advances in our understanding of the role of microglia in aging and neurodegeneration, significant challenges remain. First, relevant animal models of neurodegenerative disease have not yet been established. Moreover, reports of LBs in humans with neurodegenerative diseases are scarce, probably because LB formation and degradation is a highly dynamic process, which is therefore difficult to monitor. However, overcoming the difficulties associated with investigations of neurodegenerative diseases will enable researchers to gain a further understanding of the role of LBs in these diseases.

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