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MINIREVIEW

Canonical and Noncanonical Autophagy Pathways in Microglia

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ABSTRACT Besides the ubiquitin-proteasome system, autophagy is a major degradation pathway within cells. It delivers invading pathogens, damaged organelles, aggregated proteins, and other macromolecules from the cytosol to the lysosome for bulk degradation. This so-called canonical autophagy activity contributes to the maintenance of organelle, protein, and metabolite homeostasis as well as innate immunity. Over the past years, numerous studies rapidly deepened our knowledge on the autophagy machinery and its regulation, driven by the fact that impairment of autophagy is associated with several human pathologies, including cancer, immune diseases, and neurodegenerative disorders. Unexpectedly, components of the autophagic machinery were also found to participate in various processes that do not involve lysosomal delivery of cytosolic constituents. These functions are defined as noncanonical autophagy. Regarding neurodegenerative diseases, most research was performed in neurons, while for a long time, microglia received considerably less attention. Concomitant with the notion that microglia greatly contribute to brain health, the understanding of the role of autophagy in microglia expanded. To facilitate an overview of the current knowledge, here we present the fundamentals as well as the recent advances of canonical and noncanonical autophagy functions in microglia.

KEYWORDS canonical autophagy, noncanonical autophagy, LC3-associated endocytosis, LC3-associated phagocytosis, microglia, neurodegeneration

AUTOPHAGY PROCESSES

Maintaining cellular homeostasis involves controlled protein, organelle, and metabolite degradation systems, which are conserved among all eukaryotic cells. Autophagy, Greek for "self-eating," is an intracellular recycling process by which cytoplasmic material is targeted for lysosomal degradation (1). Depending on the type of cargo delivery to the lysosome, three different autophagy processes can be classified. (i) Microautophagy describes the internalization of smaller cytosolic portions by inward-budding vesicles from the lysosomal membrane (2), whereas in (ii) chaperone-mediated autophagy, cytosolic proteins are specifically recognized by chaperones and taken up by the lysosome in a transporter-dependent manner (2). Finally, (iii) macroautophagy (here abbreviated autophagy) is characterized by the engulfment of cytosolic constituents by double-membrane structures, termed autophagosomes, which fuse with lysosomes (Fig. 1). Regardless of the delivery route, autophagic cargo is eventually digested by lysosomal enzymes, and the degradation products are released to the cytosol as new building blocks (3).

During autophagy, autophagy-related (ATG) proteins regulate the formation of the autophagosome in a hierarchical manner through different phosphorylating and ubiquitin-like conjugating events (Fig. 1). Initiation of autophagy requires assembly of the unc-51-like kinase 1 (ULK1) complex, including its subunits ULK1, ATG13, ATG101, and RB1-inducible coiled-coil protein 1 (RB1CC1), with dephosphorylation of ATG13 being a main triggering factor (4). Following activation, ULK1 phosphorylates and

Citation Jülg J, Strohm L, Behrends C. 2021. Canonical and noncanonical autophagy pathways in microglia. Mol Cell Biol 41:e00389-20. https://doi.org/10.1128/MCB.00389-20.

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Accepted manuscript posted online 2 November 2020
Published 23 February 2021

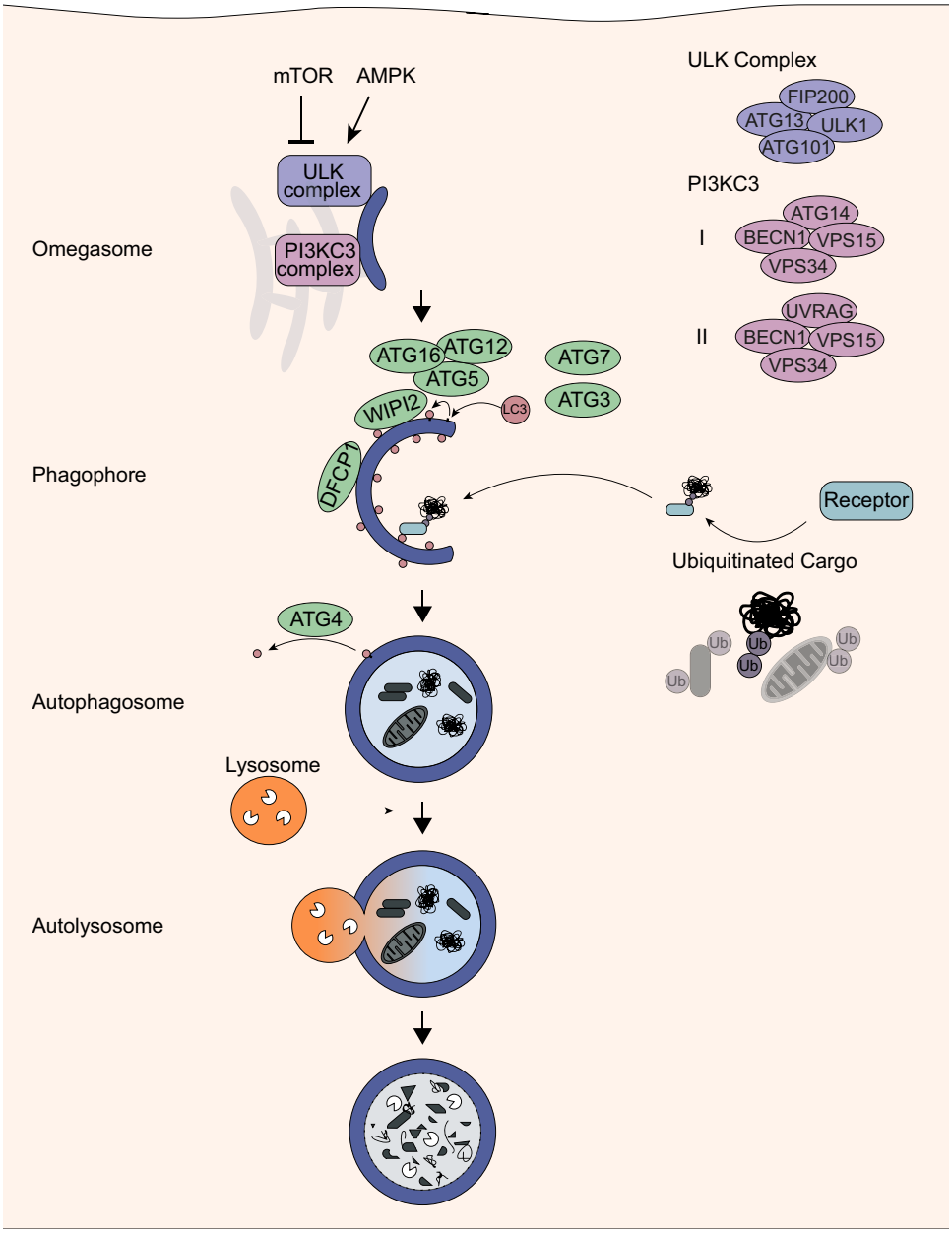

FIG 1 Autophagy pathway. The energy sensors mTORC1 and AMPK control autophagy activation via the ULK1 complex. Following activation, the ULK1 and PI3KC3 complex regulate the formation of the omegasome, and cargo is then recruited by autophagic receptors to the phagophore. Finally, the mature autophagosome fuses with lysosomes to autolysosomes, and the cargo is degraded.

recruits the transmembrane protein ATG9 and class III phosphatidylinositol 3-kinase (PI3KC3) complex I, which in turn promote autophagosome biogenesis (5–7). Mammalian PI3KC3 complex I consists of a core composed of hVps34, hVps15, beclin-1 (BECN1), and ATG14 as well as associated factors such as activating molecule in beclin-1-regulated autophagy (AMBRA1) and nuclear receptor-binding factor 2 (NRBF2) (8, 9). PI3KC3 produces phosphatidylinositol 3-phosphate (PI3P), which then induces the formation of the omegasome, a membrane platform tightly associated with the endoplasmic reticulum (ER) that gives rise to a preautophagosomal structure termed phagophore or isolation membrane (10, 11). Enrichment of PI3P leads to the recruitment of WD repeat domain phosphoinositide-interacting protein 2 (WIPI2) and FYVE domain-

containing protein 1 (DFCP1) to the omegasome (11, 12). Subsequently, WIPI2 orchestrates the conjugation of ubiquitin-like human ATG8 (hATG8) proteins to phosphatidylethanolamine (PE), which anchors them to the nascent phagophore (12). The family of hATG8 proteins comprises seven members which are categorized in two protein subfamilies: (i) the microtubule-associated proteins 1A/1B light chain 3 (LC3A, LC3B, LC3B2, and LC3C) and (ii) the γ-aminobutyric acid receptor-associated proteins (GABARAP, GABARAPL1, and GABARAPL2) (13). This conjugation is exerted by a ubiquitin-like machinery, where ATG7 is equivalent to an E1 activating enzyme, ATG3 to an E2 conjugating enzyme, and the ATG12-ATG5-ATG16 complex to an E3 scaffolding ligase (14). Upon lipidation to the concave phagophore membrane, hATG8 proteins are retained in the autophagosome and degraded along with its engulfed cargo, whereas hATG8-PE conjugates on the outer membrane of autophagosomes are eventually removed by the family of ATG4 hydrolases (15). The dynamics of LC3 and GABARAP conjugation play a central role in autophagosome formation by controlling the tethering and hemifusion of membranes (16, 17). In addition, LC3 was found to participate in transporting autophagosomes to lysosomes by forming a complex with Rab7 and its effector protein FYCO1, which facilitates microtubule transport of vesicles (18).

Importantly, the hierarchical orchestration of ATG proteins is not absolutely mandatory for autophagic degradation of cytosolic content. BECN1-independent autophagy, for example, can be induced, among other conditions, by resveratrol and was shown to be insensitive to the knockdown of PI3KC3 complex components such as BECN1 or hVps34 (19). In the following years, other independent forms were discovered, including the bypassing of ATG3, ATG5, ATG7, or ULK1 and ULK2 (20–22).

Most cell types exert basal levels of autophagy but can upregulate the pathway in response to a variety of stimuli, including nutrient deprivation. Thereby, autophagy provides the cell with metabolic building blocks for the synthesis of proteins, carbohydrates, and lipids. Key energy sensors are the mammalian target of rapamycin complex I (mTORC1) and AMP-activated protein kinase (AMPK), which induce bulk autophagic degradation of cytosolic constituents according to the nutrient status of the cell (23). As a negative autophagy regulator, mTORC1 couples sensing of amino acids, growth factors, and metabolic stress to the phosphorylation-mediated repression of the ULK1 complex at high-nutrient stages (24, 25). Decline of intracellular ATP levels triggers the activation of AMPK, which in turn positively controls ULK1 complex activation by direct phosphorylation of ULK1 and suppression of mTOR activity (26). The urge of the cell to overcome starvation and to promote cell survival requires autophagic activity to be elevated above basal levels and represents a rather nonselective pathway.

In contrast, selective autophagic degradation pathways exert cellular quality and quantity control through a number of soluble and membrane-bound receptors which mediate autophagic engulfment of specific substrates, including protein aggregates, damaged organelles, or pathogens (27–29). The ability of these autophagy receptors to recognize and tether their targets to the forming autophagosome is mediated by distinct functional domains. Here, polyubiquitination of cargo is a major signal for selective autophagy, allowing the binding of cargo by receptors via their ubiquitin-binding domain (UBD) (30, 31). It remains unclear how different polyubiquitin chains confer specificity for different autophagy receptors. Several studies demonstrated lysine-63 ubiquitination to be predominantly associated with autophagic turnover. However, in autophagy-deficient mice, several different types of polyubiquitin chains accumulate, suggesting that substrate oligomerization rather than ubiquitin topology is the driving signal for receptor selectivity (32–34).

Apart from target recognition, autophagy receptors directly interact with members of the hATG8 family, causing the autophagosome to zip around specific cargo (35). This interaction is mediated by the so-called LC3 interaction region (LIR), a characteristic linear motif among autophagy receptors and other hATG8-interacting proteins (36). Several autophagy receptors have been identified to carry both the UBD and LIR,

including sequestosome 1 (SQSTM1; also known as p62), next to BRCA1 gene 1 (NBR1), optineurin (OPTN), calcium-binding and coiled-coil domain-containing protein 2 (CALCOCO2; alias, NDP52), Tax1-binding protein 1 (TAX1BP1), and Toll-interacting protein (TOLLIP) (37). Genetic mutations within some of these autophagy receptor domains are linked to neurodegenerative diseases, thus assigning selective autophagy as a contributor to preserving neuronal homeostasis (38).

Throughout the brain and spinal cord, tissue maintenance and removal of cellular debris is accomplished by parenchymal macrophages, the microglia (39). Given the vital role of microglia in the central nervous system (CNS), this article discusses the latest findings regarding microglial autophagy in context of the inflammatory response and different degradation pathways. Besides the degradation of cytosolic components by canonical autophagy, recently identified noncanonical pathways participate in the clearance of extracellular entities. Here, we focus on the molecular aspects of both autophagy pathway variants in microglia.

MICROGLIA

Microglia are the major resident immune cells of the central nervous system (CNS) that constitute up to 15% of all CNS cells. They arise early in development and originate from a pool of primitive macrophages derived from the embryonic yolk sac. During development as well as during adulthood, they contribute in multiple ways to overall brain function (40). Microglia are capable of orchestrating tissue homeostasis by releasing inflammatory and neurotrophic factors as well as by phagocytosis (41, 42). Phagocytosis is the ingestion and digestion of extracellular particles, for example, bacteria or dead cells. In an intact brain, microglial processes undergo rapid extensions and retractions, thereby scanning their environment for tissue abnormalities (43). They can sense disturbances due to a variety of neurotransmitter and immune receptors as well as ion channels (44–50). As a response, microglia can undergo multiple morphological and functional changes depending on microenvironmental factors and migrate to the site of injury. These transformations include the retraction of processes accompanied by a more amoeboid appearance as well as changes in signaling transduction, receptor expression, and phagocytic capacity. When microglia are faced with potentially harmful entities, they can recognize these through Toll-like receptors (TLRs), triggering receptor expressed on myeloid cells 2 (TREM2) or other receptors such as the mannose receptor (51–53). The various activated receptors trigger different signaling pathways, which initiate reorganization of the actin cytoskeleton and phagosome formation (54, 55). First, the target is engulfed by the plasma membrane, leading to interiorization, also called ingestion (56). Thereby, a membrane vesicle containing the particle, termed phagosome, buds inwards and fuses with lysosomes (Fig. 2A). This phagocytic mechanism is needed for the clearance of all kinds of harmful targets. Furthermore, microglial activation can result in the release of a broad range of pro- and anti-inflammatory factors such as cytokines or chemokines (57, 58). Interestingly, accumulating evidence indicates that autophagy plays a crucial role in the immune functions of microglia.

CANONICAL AUTOPHAGY IN MICROGLIA

Canonical autophagy pathways follow a stepwise variable assembly of the autophagic machinery. While in most cells, there is no strict dependency on distinct components, autophagy induction often includes the association of the ULK1 kinase complex, the recruitment of the PI3KC3 complex, ATG9, PI3P effector proteins, and the conjugation of hATG8s to PE on the expanding phagophore. In this way, constituents from the cytosol are engulfed and delivered to lysosomes. Whether these autophagy components are compulsory for microglial autophagy is unknown. Within the CNS, autophagic activity has been mainly examined in neurons, leaving unanswered questions regarding microglia as the key players in neuronal immune responses in the brain (59).

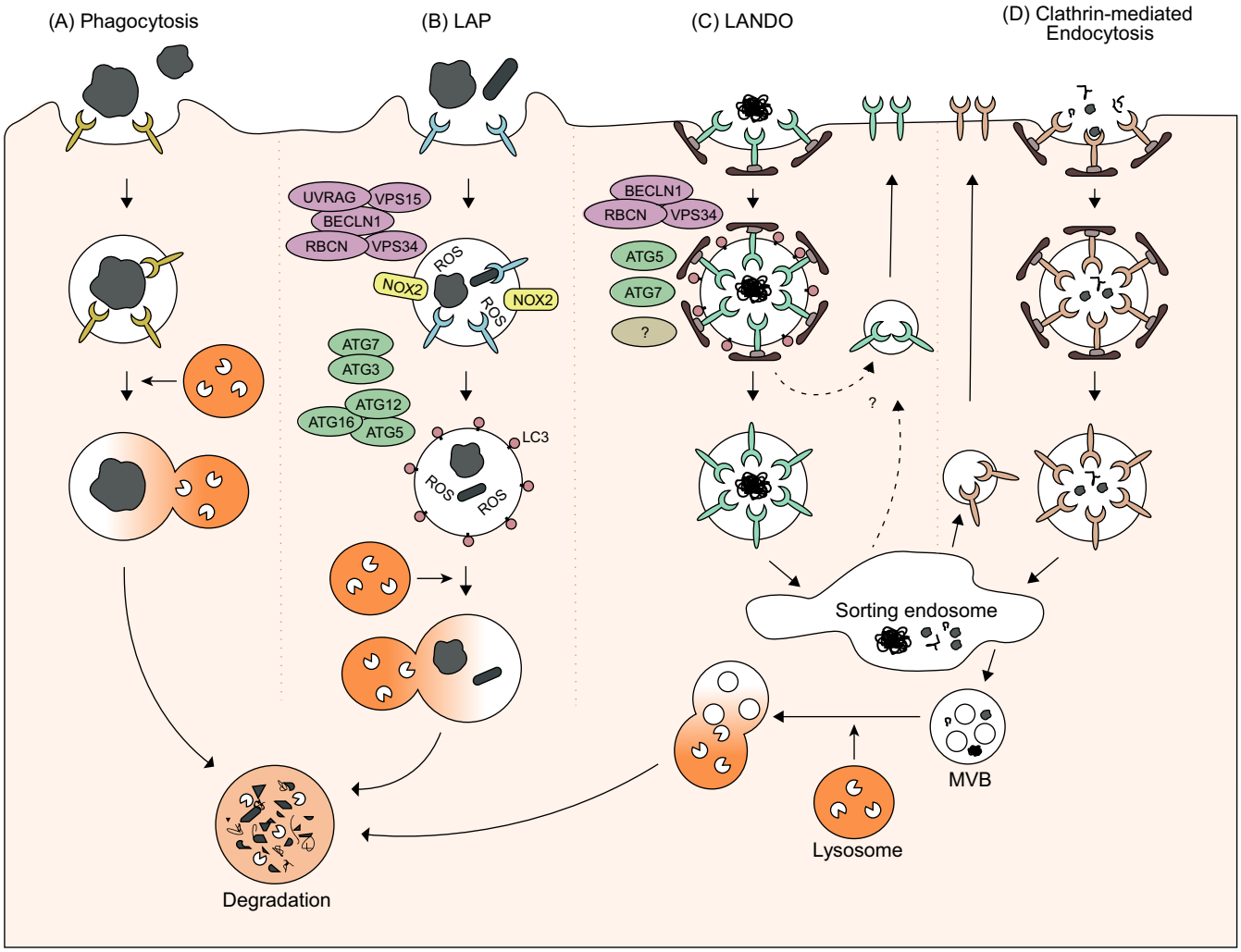

FIG 2 Overview of phagocytosis, LC3-associated phagocytosis (LAP), LC3-associated endocytosis (LANDO), and clathrin-mediated endocytosis. (A) During phagocytosis, large extracellular particles are recognized by specific receptors, engulfed by the plasma membrane, and finally internalized by phagosomes. (B) When LC3 is recruited via the PI3KC3 II complex and other autophagy proteins to the phagosome before its fusion with the lysosome, the pathway is referred to as LAP. (C) When conjugation of LC3 by autophagy proteins to Rab5- and clathrin-positive endosomes is necessary for receptor recycling, the pathway is called LANDO. (D) Clathrin-mediated endocytosis describes the uptake of smaller extracellular cargo into clathrin-coated vesicles. After uncoating, the nascent vesicle is further transported within the cell, for example, to the sorting endosome.

Microglial autophagy and intracellular aggregates. Accumulation of protein aggregates is a pathological hallmark of neurodegenerative diseases (60). Intracellular aggregates are predominantly found in neurons, whereas they are remarkably less abundant in microglia. Interestingly, recent studies showed that extracellular aggregates, which reach the cytosol of microglia, can be cleared by autophagy.

Synucleinphagy, the degradation of neuron-released α-synuclein by selective autophagy in microglia, was discovered by Choi and colleagues studying *in vivo* and *in vitro* models (61). α-Synuclein is thought to function in vesicular trafficking and is predominantly known due to its pathological contribution to Parkinson's disease (PD), where it aggregates in neuronal inclusions called Lewy bodies (62, 63). Following overexpression of human α-synuclein in transgenic mice, microglia are activated and engulf extracellular α-synuclein deposits (61). Binding of α-synuclein to TLR4 on the microglial cell surface is accompanied by a significant increase of p62, whereas levels of other autophagy receptors remain unchanged (61). This process is mediated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which regulates the transcription of p62 (61). Upregulated p62 is thought to associate with internalized α-synuclein to promote its sequestration in autophagosomes (61). This selective bind-

ing is assumed to be mediated by p62's recognition of ubiquitinated α-synuclein (64). The mechanism of how α-synuclein is taken up by microglia and released into the cytosol remains unclear. Different from other TLR4 signaling, binding of α-synuclein does not stimulate TLR4 endocytosis, thus excluding phagocytosis or receptor-mediated endocytosis as an internalization mechanism (61, 65). Alternatively, α-synuclein might permeate the microglial cell membrane in a lipid raft-dependent manner (66). When exposed to neuronal SH-SY5Y cells, biochemically generated α-synuclein fibrils were shown to escape intracellular vesicles by rupturing their membrane after endocytosis (67). Consistent with this study, exogenous fibrillary α-synuclein is able to trigger lysosomal rupture following internalization by microglia-derived BV2 cells and primary microglia (68). In response to this damage, TANK-binding kinase 1 (TBK1), OPTN, and LC3 are recruited to ubiquitin earmarked rupture sites and drive the removal of irreparable lysosomes by autophagy (68).

Moreover, Cho and colleagues described a function of autophagy in the clearance of extracellular β-amyloid (Aβ) fibrils using primary microglia and BV2 cells (69). Here, autophagy initiation is thought to be dependent on AMPK and serine/threonine-protein kinase 11 (STK11) signaling (69, 70). OPTN and LC3B were found to coimmunoprecipitate with Aβ, and deletion of OPTN resulted in higher intracellular Aβ concentrations (69). Accordingly, OPTN was suggested to be an autophagy receptor that recruits the autophagy machinery to cytosolic Aβ via binding to LC3B (69). How Aβ aggregates are selectively targeted by OPTN is not yet understood. However, OPTN was also found to associate with protein aggregates in a ubiquitin-independent manner and to mediate their autophagic clearance (71). While the uptake of extracellular Aβ can be accomplished by receptor-mediated phagocytosis (72, 73), Aβ might leak through the phagosomal membrane, causing its release into the cytosol and thereby allowing the binding of OPTN for autophagic degradation (69).

Understanding the molecular dependencies between internalization of extracellular Aβ and microglial autophagy requires further investigations. For instance, TREM2 is capable of sensing Aβ through lipoproteins and mediates the phagocytic uptake of Aβ in microglia (74). Deletion of TREM2, in turn, has a severe impact on mTOR signaling and causes increased activation of autophagy (75).

Microglial autophagy and pathogen defense. Autophagy is able to modulate the immune response, as it functions as an intracellular defense mechanism by capturing cytosol-invading pathogens and delivering them for lysosomal degradation (76), a selective autophagy pathway termed xenophagy. In macrophages, recognition of pathogen-associated molecular patterns and damage-associated molecular patterns stimulates phagocytosis and subsequent elimination of pathogens by autophagy (77). Comparable to selective degradation of other cytosolic cargo, xenophagy involves the core autophagic machinery, autophagosome formation, and ubiquitination as an "eat-me" signal. Several autophagy receptors (p62, NDP52, and OPTN) can target ubiquitinated pathogens or damaged pathogen-containing phagosomes to the autophagosome via binding to LC3 (29, 78–80). In addition, NDP52 interacts with galectin-8, a cytosolic lectin and danger receptor that recognizes cytosol-accessible β-galactosides on damaged phagosomes (81, 82). This specific interaction represents an alternative way to selectively target invading bacteria for autophagic degradation.

As host cell invasion serves for microbe replication, several bacterial pathogens possess strategies to escape xenophagy. For instance, after infection of epithelial MDCK cells with the Gram-negative bacterium *Shigella flexneri*, binding of the *Shigella* virulence factor VirG to ATG5 triggers autophagy induction (83). By secreting the bacterial effector IscB, *Shigella flexneri* is capable of concealing the presence of its virulence factor VirG (83). IscB binds VirG in a competitive manner, thereby ultimately preventing the autophagic capture of *Shigella flexneri* (83). Disrupting lysosomal degradation by inhibiting components of the xenophagy machinery is another strategy of pathogens to ensure bacterial multiplication and survival. During invasion of HEK 293T cells and primary macrophages, *Legionella pneumophila* utilizes the bacterial effector RavZ, a

cysteine protease, to hydrolyze the linkage of LC3 to PE (84). This irreversible cleavage results in a conjugation-deficient LC3 protein and inhibits autophagy in the host cell (84, 85). Furthermore, the *Salmonella enterica* serovar Typhimurium virulence factors SseF and SseG inhibit autophagy initiation by interfering with Rab1A signaling (86). Rab1A belongs to the family of small GTPases and is involved in translocation of the ULK1 kinase complex to the phagophore (87). In murine macrophage-like cells infected with SseF- or SseG-deficient *Salmonella* variants, bacterial replication was impaired, whereas downregulation of Rab1A restored this effect (86).

To what extent microglia deploy autophagy as a pathogen defense mechanism and in what way bacterial effectors impact microglial autophagy remain largely elusive. Given that several bacterial pathogens are able to cross the blood-brain barrier and infect cells in the CNS, microglial xenophagy could serve as an important neuroprotective mechanism.

Microglial autophagy and proinflammatory response. Microglia-dependent secretion of cytokines is a key event in regulating the stimulation of proinflammatory responses after brain injury. Evidence shows that autophagy proteins modulate microglial inflammation by exhibiting either inductive or suppressive effects (69, 88, 89). In general, proinflammatory signaling is executed by the activation of the inflammasome, a cytoplasmic tripartite protein complex consisting of a sensory component for recognizing ligands, an adaptor component for the binding of caspases, and caspases for proteolytic processing of cytokines (90).

Belonging to the inflammasome sensors, NACHT, LRR, and PVD domain-containing protein 3 (NLRP3) can be activated in the presence of Aβ followed by oligomerization of the adaptor component apoptosis-associated speck-like protein containing a CARD (ASC) and the intrinsic cleavage of caspase-1 (CASP1) (91–93). These molecular events, in turn, promote the secretion of the cytokine interleukin-1β (IL-β) and the proinflammatory response to Aβ plaques (Fig. 3) (91). Studies on the Aβ-induced activation of the NLRP3 inflammasome indicate that dysfunctional autophagy enhances the inflammasomal activation state (69). For example, primary microglia treated with Aβ showed intensified cleavage of CASP1 and increased release of IL-1β when the autophagy proteins LC3B and ATG7 were depleted (69). A constitutive and abnormally high inflammatory response can also lead to neuronal toxicity and cell damage. In this context, elevated autophagic activity points toward a reduction of Aβ-induced inflammation, thereby possibly promoting cell survival (69, 94). Which autophagy proteins directly participate in the regulation of microglial inflammation is poorly understood. Houtman and colleagues described a novel function of microglial autophagy in regulating the NLRP3 inflammasome via BECN1 (88). In comparison to microglia from wild-type mice, the activation of BECN1-deficient microglia resulted in larger amounts of IL-1β secretion and higher NLRP3 protein levels (88). Furthermore, NLRP3 colocalizes with LC3 and NDP52 at autophagosomes (88). It is hypothesized that NDP52 recruits NLRP3 for autophagosomal turnover, since downregulation of NDP52 leads to elevated levels of IL-1β (Fig. 3) (88). In addition, Shi and colleagues showed p62-dependent colocalization of ubiquitinated inflammasomes with autophagosomes in macrophages (95). Hence, microglia presumably modulate the activity of inflammasomes via autophagosomal degradation of NLRP3. Saitoh and colleagues demonstrated ATG16L1-dependent regulation of endotoxin-induced inflammation in macrophages (89). ATG16L1 is essential for the formation and phagophore localization of the ATG12-ATG5-ATG16L1 E3 ligase scaffold and, thus, for the lipidation of LC3 to the autophagosomal membrane (96). Upon endotoxic activation, ATG16L1-depleted macrophages elicit higher expression of IL-1β than wild-type macrophages (89). In what way this observation translates to microglial function needs to be further elucidated. However, clearly, these findings suggest that autophagy has an important role in balancing microglial proinflammatory responses.

Given the morphological changes accompanied by frequent encounters with invading pathogens and toxic aggregates, activation of microglia is in urgent need of high

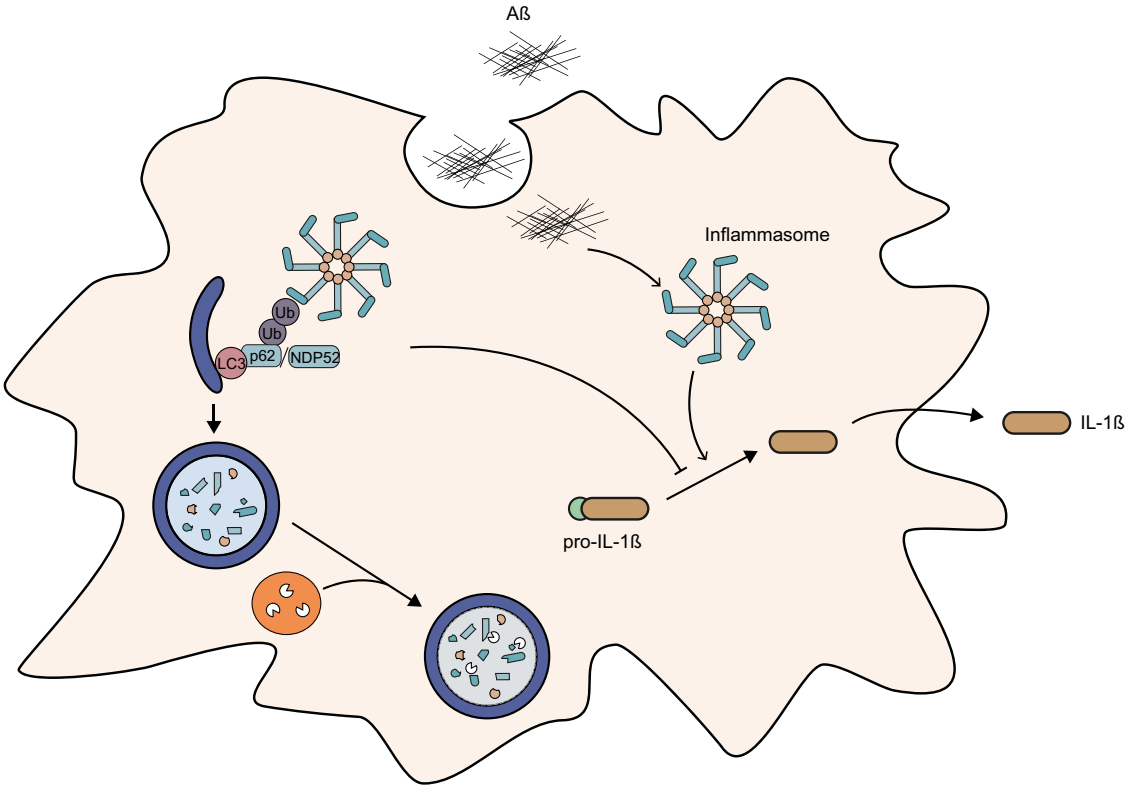

FIG 3 Selective autophagy as a possible regulator of inflammasome activity in microglia. Presence of Aβ causes activation of proinflammatory response and release of the cytokine IL-1β. Autophagy receptors p62 and NDP52 might recognize and target ubiquitinated inflammasomes for lysosomal degradation, thereby controlling Aβ-induced inflammation and survival of the cell.

energy levels in order to maintain microglial function. Therefore, autophagy not only is critical for the regulation of proinflammatory responses and elimination of extracellular aggregates but also needs to be considered a catabolic process for permanently supplying microglia with essential nutrients and controlling intracellular protein and organelle quality.

NONCANONICAL AUTOPHAGY IN MICROGLIA

In noncanonical autophagy processes, components of the autophagy machinery are deployed to fulfill functions which do not involve lysosomal delivery of cytosolic entities. Initial indicators for these pathways in microglia were provided by Lucin and colleagues (97), who were able to show that BECN1 is required for phagocytic uptake and subsequent degradation of Aβ. In *ex vivo* studies, deletion of BECN1 in microglia-derived BV2 cells exposed to APP transgenic mouse brain slices resulted in insufficient phagocytosis of Aβ (97). More importantly, isolated microglia from postmortem human Alzheimer's disease (AD) brains exhibit reduced levels of BECN1, suggesting a link between autophagic regulation and phagocytic uptake of Aβ deposits (97). Recently, two pathways with key roles in myeloid cells deepened the understanding of noncanonical autophagy: LC3-associated phagocytosis (LAP) and LC3-associated endocytosis (LANDO) (98, 99). During LAP and LANDO autophagy, machinery components are used to conjugate LC3 to the membranes of phagosomes and endosomes, respectively. Due to their important function in myeloid cells, these two pathways will be discussed below in more detail.

LC3-associated phagocytosis. The LAP pathway makes use of components of the canonical autophagy machinery to conjugate LC3 to the phagosome (Fig. 2B). Several membrane receptors can initiate this process, which is mainly studied in macrophages. Among others, these receptors include TLRs (1/2, 2/6, and 4) that are able to detect pathogen-associated patterns, immunoglobulin receptors that recognize antigens and

pathogens opsonized with IgG, and T-cell immunoglobulin mucin receptor 4 that binds to phosphatidylserines on the surfaces of dead cells (100–103). Binding to the receptor mediates the phagocytosis of the extracellular target. Upon complete engulfment of the cargo and the sealing of the phagosome, LAP effectors are recruited. However, it remains unclear how exactly this recruitment is triggered. The most upstream autophagy complex present in LAP is the PI3KC3 complex II, which shares the core subunits hVps34 and hVps15 with PI3KC3 complex I, but instead of ATG14, it contains UV radiation resistance-associated gene protein (UVRAG) and Rubicon (RUBCN) (8, 9). While RUBCN is a known negative regulator of PI3KC3 during canonical autophagy (104), it is also compulsory for LAP (99). During LAP, RUBCN is, on one side, essential for PI3KC3-mediated PI3P generation and, on the other side, it recruits and stabilizes the NADPH oxidase 2 (NOX2) complex at the phagosome (99, 105). NOX2 activity itself as well as NOX-induced reactive oxygen species (ROS) production are crucial for the following LC3 lipidation. However, the underlying mechanism remains unknown (99, 101, 106). PI3P generation and NOX2 association to the phagosome result in conjugation of LC3 to PE on the phagosomal membrane. Notably, lipidation of LC3 and GABARAP proteins during LAP differs in two important mechanistic aspects from its use in canonical autophagy. First, WIPI2 is not necessary but probably substituted by another, yet-undiscovered effector protein (107). Second, the WD40 domain of ATG16L1 is indispensable (107, 108). One other main difference between canonical autophagy and LAP is the timing and the location of LC3 lipidation. In canonical autophagy, LC3 lipidation occurs during autophagosome formation on the inner and outer autophagosomal membranes (109). In contrast, LC3 conjugation during LAP occurs after formation of the single-membrane phagosome, leading to LC3 lipidation on the outer leaflet only. The resulting structure is called a LAPosome (103). Consequently, the function of conjugated LC3 does not entirely overlap between canonical autophagy and LAP. However, a potential common role of LC3-PE in both pathways is to facilitate the fusion of the respective transient organelle with lysosomes, leading to the degradation of the engulfed material (99, 110, 111).

Due to the targeting of several pathogens, LAP is critical for fighting bacterial as well as fungal infection (106, 112–114). Martinez et al. highlighted an additional importance of LAP by showing ineffective efferocytosis, the phagocytosis of dead cells, accompanied by an increase in proinflammatory cytokines in LAP-deficient mice (115). Furthermore, LAP plays also a role in major histocompatibility complex (MHC) class II-restricted antigen presentation. MHC class II proteins present antigens from extracellular proteins to $CD4^+$ T cells and are found on a number of professional antigen-presenting cells, including macrophages and microglia. Antigenic peptides derived from partially degraded exogenous particles taken up by phagocytosis or endocytosis are loaded onto MHC class II molecules in late endosomes. Intriguingly, it was shown that recruitment of LC3 to the phagosome facilitates MHC class II presentation of fungal antigens in a murine macrophage cell line (116). This process is conserved in human macrophages, in which compromised fungal antigen presentation was reported when LAP was inhibited (117). Mechanistically, the coupling of LC3 to phagosomes is thought to result in prolonged antigen presentation by MHC class II molecules in human macrophages.

So far, LAP has mainly been studied in macrophages. Although microglia are distinct from other macrophages, they share many features such as the ability to phagocytose and to release cytokines. Therefore, it was generally assumed that LAP occurs in a similar or even the same manner in microglia. This was recently confirmed by two independent studies, which observed a role of LAP in the uptake of zymosan in microglia (98, 118). However, the physiological targets of microglial LAP still remain unknown.

LC3-associated endocytosis. Remarkably, in microglia, another uptake pathway was recently demonstrated to employ autophagy proteins and therefore, in analogy to LAP, termed LANDO (98). Endocytosis is a ubiquitous cellular process that is defined by the active uptake of extracellular materials as well as components of the plasma

membrane into the cytoplasm. It is important for many physiological processes, including nutrient uptake and cell signaling. Generally, endocytic pathways are differentiated as clathrin mediated and independent, with clathrin-independent endocytosis processes classified as macropinocytosis and phagocytosis. In comparison to phagocytosis, clathrin-mediated endocytosis (CME) is common in all eukaryotic cells and describes the uptake of smaller cargo into clathrin-coated vesicles (Fig. 2D) (119, 120). The early endosome matures to a late multivesicular endosome which then finally fuses with lysosomes, resulting in the degradation of the cargo (121). This process is driven by constant fusion and fission with other vesicles. Importantly, a considerable subset of the cargo escapes lysosomal degradation. For example, a large fraction of membrane components, including receptors, is often recycled back to the plasma membrane, while other cargo is targeted to the *trans*-Golgi network (122–124).

LANDO is characterized by LC3 conjugation to clathrin- and Rab5-positive endosomes (Fig. 2C). As in LAP, the conjugation process is dependent on BECN1, Vps34, ATG5, ATG7, and RUBCN. In contrast to LAP, the absence of LC3 conjugation does not result in defects in cargo degradation but in reduced receptor recycling. Therefore, secondary uptake of the cargo is diminished. So far, roles for LANDO have been observed for the recycling of the putative Aβ receptors TREM2, CD36, and TLR4 in microglia (98). It is conceivable that this noncanonical function of autophagic proteins plays a role not only for other receptors but also in other cell types. Notably, previous studies reporting a link between autophagy, retromer trafficking, and receptor recycling are in line with the proposed role of LANDO. The retromer complex sorts endosomal cargo back to the plasma membrane, the *trans*-Golgi network, or other compartments. Popovic et al. reported that induction of autophagy results in dissociation of the retromer-associated Rab GTPase-activating protein TBC1D5 from the retromer complex (125). This partition was observed to be due to an activation of Rab7a and resulted in enhanced retromer receptor recycling (126, 127). Furthermore, Lucin et al. described a dependency of TREM2 as well as CD36 receptor recycling on BECN1 and Vps34 in microglia (97). Taken together, LANDO is a newly discovered noncanonical pathway in microglia involved in receptor recycling. Although, so far, evidence is limited to one report, several previous studies are in accordance with such a pathway. Clearly, there is a need for further investigation to dissect the mechanisms of LANDO, in microglia and other cell types, in detail.

RELEVANCE IN DISEASE

The human brain comprises only 2% of total body weight but consumes 20% of total body oxygen (128). This highlights its relevance for the human body. Among others, it senses and coordinates information and controls body movements and personality as well as thoughts. Accordingly, disruption of brain functions has severe consequences such as impaired mental abilities, memory damage, and loss of muscle coordination and strength or vision and language impairment. Therefore, CNS repair and protection strategies are indispensable. The brain is protected not only by the skull and the blood-brain barrier but also by a multitude of molecular mechanisms. Among others, these include enzymatic antioxidant systems, the ubiquitin-proteasome pathway, neuroinflammation, and autophagy. Due to the role of autophagy in organelle and protein quality control, its impairment results in accumulation of aggregated proteins and damaged organelles, common hallmarks of neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and PD. The essentiality of autophagic processes for the CNS is substantiated by the multiplicity of autophagic genes which are known to be linked to neurological diseases, ranging from those encoding autophagic receptors, regulators, and adaptors (including OPTN, SQSTM1, TBK1, C9orf72, UBQLN2, HTT, and PICALM), which are risk genes for neurodegenerative diseases, to that encoding AMBRA1, which has been linked to autism and schizophrenia (129–139). Furthermore, mutations in the hATG8-binding protein tectonin β-propeller containing protein 2 (TECPR2) are known to result in hereditary spastic paraplegias, while absence of the autophagic protein WD repeat domain phosphoinositide-

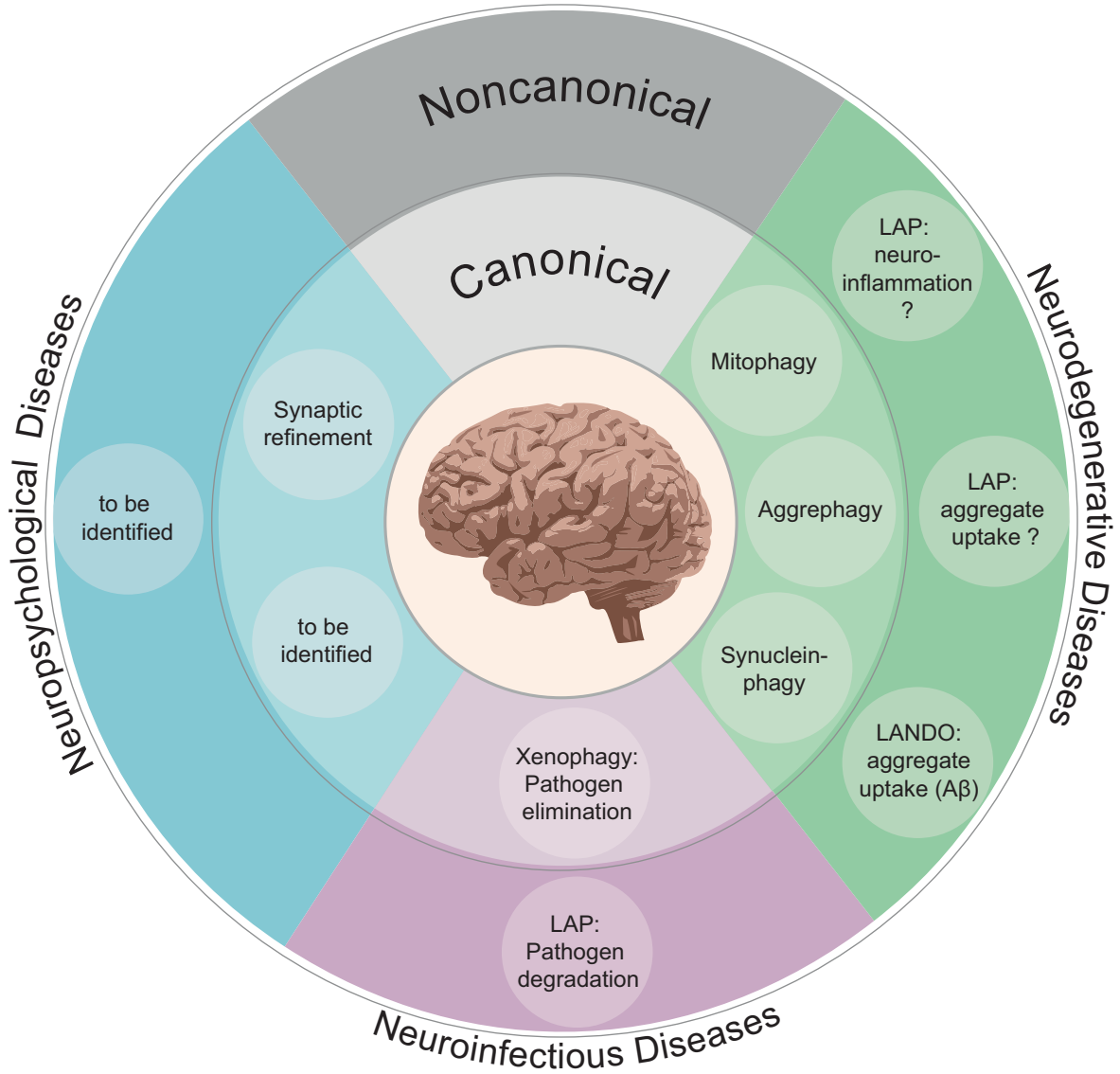

FIG 4 Canonical and noncanonical autophagy functions associated with neurological diseases. The inner and outer circles present canonical and noncanonical autophagy processes, respectively. So far, these pathways were most extensively studied in neurodegenerative diseases. However, only little is known about their functions in neuropsychological diseases. Further aspects remain to be identified. Concerning neuroinfectious diseases, either canonical xenophagy or LAP can eliminate the invading particle, depending on its pathogenic characteristics.

interacting protein 4 (WIPI4) causes beta-propeller protein-associated neurodegeneration (140, 141). Although the value of autophagic processes for brain health is widely appreciated, they were mainly studied in neurons. However, the contribution of canonical as well as noncanonical autophagy functions in microglia increasingly receives more attention in several CNS diseases (Fig. 4).

Relevance of microglial autophagy in diseases. As aging progresses, loss of protein homeostasis is a generic consequence (142). The tight regulation of inflammatory responses and degradation of extracellular aggregates by microglial autophagy supports a link between disease development and impaired autophagic processes at different stages. During AD progression, alterations in AMPK signaling are a central issue (143, 144). As a homeostasis sensor, AMPK activates microglial autophagy in the presence of Aβ plaques, consequently leading to their lysosomal degradation (69). Inactivation of AMPK with compound C decreases Aβ clearance, thereby making AMPK a potential target for AD treatment. In PD, neuron-released α-synuclein and its accu-

mulation in Lewy bodies result in degeneration of the dopaminergic system (145). Intriguingly, impaired microglial autophagy provokes a decrease of dopaminergic neurons when α-synuclein is expressed in mice (61). Hence, removal of extracellular α-synuclein by microglial autophagy is suggested to have a vital role in maintaining neuronal protection and function.

Modulation of proinflammatory responses by autophagy was found to be crucial in Crohn's disease, where excessive production of cytokines results in chronic inflammation, compromising the entire gastrointestinal tract (146, 147). Among patients suffering from Crohn's disease, a genetic missense variant of ATG16L1 was identified (148). This disease-related polymorphism shows an elevated sensitivity to cleavage by caspase-3, which eventually leads to the degradation of ATG16L1 (146, 149). Following ATG16L1 loss of function in macrophages, autophagic activity is reduced and, as a consequence, levels of cytokines increase (146). Therefore, microglial autophagy could own a similar role in controlling proinflammatory responses within the CNS.

Despite neurodegeneration and inflammatory diseases, microglia were shown to have a critical impact on neuropsychological behaviors (150). In autism spectrum disorders, impaired microglial autophagy pathways studied in mice led to defective synaptic pruning, which becomes visible by an abnormal high dendritic spine density (151). Synapsis formation and elimination is fundamental, as they ensure proper brain development (152). Here, autophagy could have a modulatory role in neuronal connectivity by clearing excessive and dysfunctional synapses (151). Uncovering the molecular basis of autophagy pathways in microglia is an essential objective in order to provide a wide-ranging platform for neurotherapeutic approaches.

Relevance of microglial noncanonical autophagy in diseases. LANDO contributes to the clearance of Aβ plaques. Furthermore, an upregulation of proinflammatory cytokines was detected in LANDO-deficient cells, which resulted in microglial hyperactivation in mice harboring several mutations associated with human familial AD. These mice also presented accelerated tau phosphorylation. In this model, the deficiency of microglial LANDO caused neuronal cell death accompanied by behavioral and memory impairments, emphasizing the role of microglia as well as LANDO in AD (98). Although the relevance of microglial LANDO has so far only been reported for AD, it is likely that this process targets other molecules or particles besides Aβ, especially since LANDO supports the recycling of the receptors CD36, TLR4, and TREM2, which can bind a broad range of ligands besides Aβ. Although LANDO is suggested to be a major player in Aβ clearance, it is not known to what extent this process is physiologically relevant. Still, it is tempting to speculate that stimulation of LANDO could be a promising target for therapeutic interventions in neurodegenerative diseases, especially in AD. However, it needs to be clarified to what extend increased LANDO is beneficial for microglia and at what point negative effects arise. In contrast to LANDO, to date, LAP was not investigated in detail in microglia. Interestingly, LAP is firmly entangled with inflammation. Mice, which lack LAP-obligatory proteins, release more proinflammatory cytokines and are defective in the clearance of apoptotic cells (115). Since microglia are responsible for the phagocytosis of apoptotic cells in the CNS and neuroinflammation is a common feature of neurodegenerative disorders, it is plausible that defective LAP could play an important role in the onset or progression of neurodegenerative diseases (153, 154). Another known function of LAP in macrophages is the elimination of pathogens. Several of these LAP-targeted pathogens, including *Streptococcus pneumoniae* and *Listeria monocytogenes*, can penetrate the blood brain barrier to enter the CNS and are common causes for bacterial meningitis (155, 156). While it remains to be firmly established that microglial LAP is part of the defense against these pathogens in the CNS, increasing LAP activity in microglia could represent a potential target for antibacterial therapeutic approaches. However, due to the two-sided role of neuroinflammation in neurodegenerative diseases, beneficial as well as detrimental impacts could result from an overactivation of LAP.

CONCLUSION

As resident immune cells of the brain and spinal cord, microglia accomplish vital functions, including synaptic pruning, neurogenesis, and immune responses. Growing evidence indicates that microglia utilize autophagy to meet CNS homeostasis, thereby strictly controlling proinflammatory responses and removal of protein aggregates as well as damaged organelles from the cytosol. Compared to canonical autophagy, noncanonical pathways allow microglia to target also noncytosolic entities for lysosomal degradation. LAP and LANDO are two noncanonical pathways which were shown to be present in microglia. While it was observed that microglial LANDO is crucial for the uptake of Aβ, the exact role of LAP in microglia remains unknown. Regarding the decline of protein function during aging and various neuronal diseases, these noncanonical features contribute to the maintenance of CNS homeostasis, and their defect can have detrimental consequences. With regard to the energetic aspect, microglia may omit and exploit specific signaling pathways to activate autophagy, thereby recycling nutrients and sustaining energy. Taken together, canonical as well as noncanonical autophagy pathways play key parts in microglia functioning, most likely in complementary ways. Yet, several questions as to the role of LAP in microglia and the detailed interplay between canonical and noncanonical autophagy remain unanswered and should be elucidated by further research.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (DFG; German Research Foundation) within the frameworks of the Munich Cluster for Systems Neurology (EXC 2145 SyNergy identifier [ID] 390857198) and of the Collaborative Research Center 1177 (ID 259130777).

J.J., L.S., and C.B. contributed to the drafting of the article. J.J. and L.S. wrote the manuscript and designed the figures. L.S. realized digital illustrations. All authors reviewed the final manuscript. C.B. acquired funding.

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