Title: Glia-specific autophagy dysfunction in ALS

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Abstract

Neuronal cell death is the main pathological feature of chronic neurodegenerative diseases (NDs) such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). As age is strongly linked to NDs, these diseases are one of the leading medical and societal challenges faced by the rapidly aging western societies. Despite the increasing prevalence, the causes and mechanisms behind most NDs are still vague. A common hallmark of several NDs is the accumulation and aggregation of proteins. Prominent examples are amyloid beta and tau in Alzheimer's disease, α–synuclein in Parkinson's disease and transactive response DNA binding protein 43 kDa (TDP-43) in ALS and FTD. Under physiological conditions, protein quality control systems, namely the ubiquitin proteasome system and the autophagy machinery, eliminate such aberrant protein forms and thereby prevent proteotoxic stress. However, as proteins must unfold to undergo proteasomal degradation, aggregated proteins are poor substrates for the proteasome. Such proteins are thought to be primarily turned over by autophagy. Therefore, autophagy is considered a critical ND-protective pathway, which opens up potential new therapeutic interventions. One drawback is that the majority of research in NDs has been focused on elucidating the underlying pathomechanisms in neurons. However, neurons make up only about half of the brain cells with neuroglia being the other major central nervous system (CNS) cell type. Due to the ubiquitous presence of disease-causing mutations in all cells of the CNS, it is likely that non-neuronal cells contribute to the disease onset and/or progression. While our understanding of the roles of autophagy and its contribution to neurodegeneration in neurons deepened considerably over the last years, still comparatively little is known about the functions and disease contribution of the autophagy machinery in glia cells.

1. Autophagic Pathways

Autophagy, Greek for "self-eating", is a highly conserved recycling process, which is present in all eukaryotes [1]. This term describes the delivery of cytosolic material to the lysosome and its subsequent lysosomal degradation. Three different autophagic pathways (microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy) are distinguished in mammals based on the delivery mode of the cargo to the lysosome. In microautophagy smaller portions of cytoplasm are invaginated into the lysosomal membrane, which then pinch off inwards as vesicles. In contrast to this mainly unselective pathway stands the highly selective CMA. Chaperones recognize specific substrate proteins and shuttle them to the lysosome where they are unfolded and translocated through the membrane. During macroautophagy endogenous or exogenous cytosolic material is engaged by a cup-shaped membrane (coined phagophore) that expands to surround the cargo entirely. Membrane closure results in the formation of autophagosomes, which deliver their content for bulk degradation upon fusion with lysosomes [2-4] (Figure 1). Depending on the cellular condition, macroautophagy can degrade vast portions of the cytosol or exclusively target selective constituents of the cytoplasm. Since macroautophagy (referred to as autophagy hereafter) is by far the most studied autophagic pathway in the context of glia cells, we focus on this process.

1.1. Autophagy Machinery and Regulation

The formation of autophagosomes is tightly controlled by autophagy-related (ATG) proteins. While most cell types exhibit basal levels of autophagy, stress stimuli such as nutrient or growth factor deprivation, hypoxia, DNA damage or protein aggregates can further activate autophagy [5]. For example, amino-acid deprivation leads to an inactivation of the target of rapamycin 1 complex (TORC1) kinase and allows its substrate ATG13 to be rapidly dephosphorylated [6]. This induces the assembly of the unc-51-like kinase 1 (ULK1) and ULK2 complex at the endoplasmic reticulum (ER). In addition to ULK1 and ULK2, this complex consists of ATG13, ATG101 and RB1-inducible coiled-coil protein 1 (RB1CC1) and coordinates the recruitment of other initiation factors including ATG9 vesicles and the autophagy specific class III phosphatidylinositol 3-kinase (PI3K-III) complex [7, 8]. ULK-dependent phosphorylation of the PI3K-III complex, which is composed of the subunits PIK3C3, PIK3R4, BECN1 and ATG14, in turn induces the generation of phosphatidylinositol 3-phosphate (PI3P). As a result, a PI3P-enriched domain of the ER is formed which morphologically resembles the Greek letter omega and has thus been termed omegasome [9]. PI3P-binding proteins including the zinc-finger FYVE domain-containing protein 1

(DFCP1) and WD repeat domain phosphoinositide-interacting protein 2 (WIPI2) are subsequently recruited to these specialized ER domains and direct the conjugation of the ubiquitin (Ub)-like human ATG8 proteins to phosphatidylethanolamine incorporated in the phagophore membrane [10, 11]. Exactly how phagophores emerge from such omegasomes is less well understood. A central question is the origin of the autophagosomal membrane. Recent data suggest that next to the ER, mitochondria, the Golgi apparatus and the plasma membrane contribute lipids to the phagophore [10, 12-14]}. On the molecular level, the ATG8 conjugation machinery controls the phagophore expansion. This machinery comprises the E1 activating enzyme ATG7, the E2 conjugating enzyme ATG3 and the E3 ligase scaffolding ATG12–ATG5-Atg16L1 complex [15, 16]. Human cells contain six ATG8 family members that can be grouped into two subfamilies: i) microtubule-associated proteins 1A/1B light chain 3A (LC3A), LC3B and LC3C and ii) γ-aminobutyric acid receptor-associated protein (GABARAP), GABARAPL1 and GABARAPL2 [17]. While the subfamily of LC3 proteins has been demonstrated to act early in phagophore expansion, GABARAP proteins were implicated later in autophagosome closure and maturation [18]. ATG8-PE conjugates are incorporated into the inner and outer surface of autophagosomes where ATG8 provides a docking site for cargo receptors and regulatory adaptor proteins. While the former facilitate delivery of autophagosomal cargo to lysosomes, the latter contribute to autophagosome assembly and maturation as well as transport and lysosomal fusion. Furthermore, inner-leaflet ATG8 remains a part of the autophagosome through the whole process of its formation and maturation and is finally degraded in the lysosome, whereas ATG8 molecules conjugated to the outer autophagosome membrane are cleaved off by protease ATG4. The scission of the extremities of the growing phagophore results in a completed and closed double-layered autophagosome [19]. Finally, the fusion of autophagosomes with lysosomes results in autolysosomes and the subsequent cargo degradation [20-22].

1.2. Bulk versus Selective Autophagy

Autophagy is an essential process due to divers, indispensable functions. Initially, it was described as a catabolic pathway with the purpose of providing new building blocks through turnover of cellular constituents [23-25]. Cells are in constant need of a pool of metabolites to be able to generate new proteins, lipids and carbohydrates. If this pool cannot be replenished from extracellular sources, cells need to compensate for the lack of these nutrients. Therefore, cells have sensors, which continuously monitor metabolic stress levels to sense for example nutrient and energy deficits. The three central sensing pathways include sirtuin 1 (SIRT1), AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR). SIRT1 and AMPK sense changes in intracellular NAD^+ and AMP levels, respectively [26]. The master regulator mTOR processes nutrients, growth factors, energy and stress inputs, partially by integrating AMPK and SIRT1 signals [27]. As a consequence, these sensors activate autophagy in metabolic stress periods. Since under these conditions it is the highest priority to overcome starvation, any cytoplasmic material is rather non-selectively degraded by so called bulk autophagy.

On the other hand, autophagy also plays a major role in the cellular quantity and quality control by degrading surplus or damaged organelles (e.g. mitophagy), aberrant or aggregated proteins (aggrephagy) as well as cytosol-invading pathogens (xenophagy) [28-30]. These selective forms of autophagy are characterized by the labelling of the cargo with specific “eat-me” signals, which are then recognized by autophagic receptors. The most prominent “eat-me” signal is Ub, which is for example conjugated to proteins within aggregates or outer mitochondrial membranes by different ubiquitination machineries. Additionally, lipid and sugar moieties that accumulate on the organelle surface are also able to function as autophagic degrons [29, 31]. Autophagy receptors recognize these signals with the help of degron-specific domains such as Ub-binding domains (UBD) in the case of Ub and physically link the bound cargo to the nascent autophagosome through interaction with members of the ATG8 family. This latter interaction is mediated by a short linear sequence with the consensus motif $F/W/Y_1$-X_2-X_3-$I/L/V_4$ (where X can be any amino acid) known as LIR (LC3-interaction region) or AIM (ATG8 family-interacting motif) which is common to all autophagy receptors and that non-covalently engages a highly conserved binding site on ATG8 family proteins [32, 33]. Several UBD-containing autophagy receptors exist, namely Sequestosome-1 (SQSTM1, also known as p62), next to BRCA1 gene 1 protein (NBR1), Calcium-binding and coiled-coil domain-containing protein 2 (CALCOCO2, also known as nuclear dot protein 52 (NDP52)), Optineurin (OPTN), Tax1-binding protein 1 (TAX1BP1) and Toll-

interacting protein (TOLLIP). To what extend these receptors are functionally redundant or act synergistically in the distinct Ub-dependent selective autophagy pathways is not fully understood.

1.3. Extended Functions of the Autophagy Machinery: More than Degradation

Besides the quantity and quality control functions of autophagy, autophagy-related processes recently moved into the spotlight. These processes involve parts of the autophagic machinery but either the formation of autophagosome or the lysosomal degradation of cytosolic constituents is absent. So far, it was shown that ATG proteins play inter alia roles in cell survival, apoptosis, signaling, cellular transport and secretion [34]. A relatively well-described autophagy-related pathway known as secretory autophagy mediates the unconventional secretion of leaderless proteins under certain stress conditions. Proteins, which lack a signal for entering the conventional ER-Golgi-membrane pathway are exported outside the cell via translocation across the membrane of an autophagosome-like vesicular intermediate which eventually fuses with the plasma membrane [35-37]. Another example of an autophagy-related process is autophagic cell death. Although autophagy is known to block the induction of apoptosis, it is substantiated that autophagy is also able to activate cell death. Here, the autophagosomal membrane serves as a scaffold for the assembly of the intracellular death-inducing signaling complex mediating for instance the activation of caspase-8 and thereby the initiation of the apoptotic cascade [38, 39].

2. Roles of Autophagy in Different CNS Specific Cell Types

In 1856, R. Virchow described a cell population in the brain, distinct from neurons, which he believed functions to embed the neurons and to hold everything together. Therefore he named them “Neuroglia”, according to the ancient Greek word “glia” for glue. More than 50 years later, Río-Hortega firstly distinguished between the three main glial cell types: microglia, astrocytes and oligodendrocytes. Nowadays, it is known that glia are far more than only bystanders and cement of neurons. Their functions in tissue homeostasis, myelin formation, development regulation and synaptic communication are broadly known. With the appreciation of their fundamental roles in the CNS, it became clear that they are likely important players in disease states as well. Defective autophagy is thought to contribute to neuronal degeneration and to the development of NDs. Therefore, we wanted to review the roles of autophagy in glia cells during neurodegeneration, exemplified for ALS and FTD.

2.1. ALS and FTD

ALS is the most common adult-onset motor neuron disease with degeneration of upper and lower motor neurons. This disease is characterized by rapid progression of muscle weakness, which leads to death within one to five years after diagnosis. Often ALS patients also present cognitive changes ranging from mild abnormalities to severe FTD [40], which comprises a group of disorders caused by a more widespread cortical affection, which results in cognitive and language deficits or changes in personality and behavior [41]. Despite the distinct neurological and psychiatric symptoms, ALS and FTD are tightly linked by shared neuropathological markers (i.e. RNA-binding protein TDP-43-positive inclusions) [41, 42] and mutations in common genes [43, 44]. Approximately 10% of all ALS cases are familial and 90% are sporadic. While the molecular basis of ALS and FTD remains largely unknown, many ALS-causing genes are directly or indirectly connected to autophagy (Figure 2). Some of them function in distinct or even multiple steps of the autophagy pathway whereas for others this is not formally established. Still, a rough classification is possible for several ALS genes. Optineurin (OPTN), ubiquilin-2 (UBQLN2) and SQSTM1 are known autophagy receptors, while the TANK-binding kinase 1 (TBK1), the Rab guanine nucleotide exchange C9ORF72, the Ub E3 ligase substrate adaptor cyclin F (CCNF), the Rho guanine nucleotide exchange factor ALS2 and the vesicle-associated membrane protein-associated protein B/C (VAPB) are associated with autophagy regulation and initiation [45-56]. TAR DNA binding protein (TDP-43) and the multifunctional AAA-type ATPase valosin-containing protein (VCP, alias p97) have functions in autophagosome maturation [57, 58]. Furthermore, charged multivesicular body protein 2b (CHMP2B), ALS2, phosphoinositide phosphatase (FIG4), 1-phosphatidylinositol 3-phosphate 5-kinase (PIKFYVE) and VCP have functions in autophagosome-lysosome fusion and cytoplasmic dynein 1 intermediate chain 1 (DCTN1) in the transport of autophagosomes [59-65]. In addition, the ALS gene spatacsin (SPG11) was observed to play a role in lysosome reformation [66]. Importantly, mutated superoxide

dismutase 1 (SOD1) and RNA-binding protein fused in sarcoma (FUS), which can be found in ALS-associated inclusions, are also known to interfere with several events in autophagy [53, 67, 68]. However, how the aggregates formed in ALS affect the autophagic pathway have been acquired using (motor)neuronal cells and data from glia cells are lacking. ALS mutations are mostly inherited in an autosomal-dominant manner [69] and range from point mutations (in all genes except C9ORF72), frame shifts (e.g. in OPTN) to GGGGCC (G_4C_2) hexanucleotide repeat expansions in a non-coding region (C9ORF72). For most of these genes it remains to be determined if the underlying disease mechanism is due to loss of function, gain of one or more toxic properties or both. Expanded C9ORF72 G4C2 repeats, which are most often found in ALS/FTD patients, are an example for the latter as haploinsufficiency, aberrant RNA interactions and production of toxic translation products (dipeptide repeat proteins; DRPs) and aggregates have been suggested as non-mutually exclusive toxicity mechanisms [70]. Evidently, defective autophagy plays a crucial role in ALS/FTD and inclusion bodies in motor neurons are considered a pathological hallmark of ALS. However, protein aggregates are also present in astrocytes, microglia and oligodendrocytes of ALS patients [71-73].These observations together with several studies showing non-cell autonomous mechanisms in ALS/FTD, indicate the involvement of defective glial autophagy in the disease [74-79].

2.2. Microglia

2.2.1. General Functions of Microglia

Microglia are the immunocompetent cells of the CNS and account to 10-15% of its cells. They originate from a pool of primitive macrophages derived from the embryonic yolk sac, which proliferate in the brain and maintain themselves by local self-renewal. Adult microglia are equally distributed throughout the brain and the spinal cord [80-82]. The main functions of microglia include synaptic pruning during development, regulation of synaptic activity, neurogenesis and immune response [83]. Homeostatic microglia have a ramified appearance with branches and processes with which they constantly screen their environment for example by contacting neurons, astrocytes and blood vessels [84]. Various microglial receptors such as neurotransmitters and immune receptors are essential for this surveillance function [85-88]. Disturbances in tissue homeostasis are registered by these receptors whereupon microglia shift from their homeostatic state to a disease associated state [89]. Concurrently, microglia acquire an amoeboid shape, proliferate and migrate towards the origin of the disturbances. Microglial activation can be accompanied by production of neurotoxic, excitatory and pro-inflammatory signals (i.e. cytokines, chemokines and reactive oxygen species (ROS)), generation of anti-inflammatory cues, activation of adaptive immunity and phagocytosis of apoptotic cells, pathogens, extracellular protein aggregates and other cell debris. Although the classical distinction between a proinflammatory M1 phenotype and an anti-inflammatory M2 phenotype is still common, recent technical advances in single-cell sequencing in fact revealed a variety of microglial gene expression profiles in different physiological or pathological conditions which likely reflects a spectrum of different microglial states [90, 91].

2.2.2. Autophagic Functions in Microglia

Autophagy participates in the regulation and the execution of innate as well as adaptive immune responses. Autophagy can be activated by pattern recognition receptors (PRRs), which recognize pathogen-associated molecule patterns (PAMPs) and damage-associated molecule patterns (DAMPS). Upon detections of pathogens or other exogenous ligands by PAMPs/DAMPs, autophagy is activated followed by the elimination of intracellular pathogens [92-94]. This has been demonstrated for example for toll-like receptor 7 (TLR7) in macrophages [95]. Furthermore, autophagy operates as a topological inverter which ferries cytosolic antigens into the lumen of major histocompatibility complex (MHC)-II compartments for antigen presentation [96, 97]. On the inflammasome, autophagy has a two-sided effect. On the one side, autophagy restricts inflammasome activation. Saitoh *et al.* have shown that deletion of the autophagy protein ATG16L1 results in increased levels of proinflammatory, endotoxin-induced interleukin (IL-1β) in murine fetal liver macrophages. By the removal of damaged mitochondria, autophagy prevents the accumulation of cytosolic mitochondrial DNA and overproduction ROS, thus preventing inflammasome activation [98-101]. Houtman *et al*. observed an increase of IL-1β and IL-18 in neonatal primary microglia with as well genetic as chemical induced defects in autophagy. They show

evidence indicating that Family Pyrin Domain Containing 3 (NLRP3), the PRR of the NLRP3 inflammasome, is degraded by autophagy and thereby provide another mechanism explaining the connection between autophagy and the inflammatory response. On the other side, autophagy controls IL-1β and IL-18 activation, release and signaling by contributing to its biogenesis and unconventional secretion [102, 103] (Figure 3). However, beside autophagy mediated IL-1β secretion, pyroptosis, a form of osmotic cell death, and pore-forming protein Gasdermin D-dependent secretion are in the focus of recent studies [104, 105]. Furthermore, in 2011 it was described by Harris *et al*. that autophagy also negatively regulates IL-1β secretion by degrading pro-IL-1β [106]. More recent data present contradictory results, showing that pro-IL-1β is rather degraded by the proteasome than by autophagy [107, 108]. Eldrige *et al*. determined the ubiquitin E2 enzyme UBE2L3 as a regulator of pro-IL-1β protein levels in human and murine cells. UBE2L3 promotes the ubiquitylation and thereby the proteosomal degradation of pro-IL-1β. UBE2L3 itself is indirectly targeted for degradation by caspase-1, Therefore, an increase of IL-1β after autophagy inhibition is possibly mediated by the reduction of the inflammasome and the subsequent increase of UBE2L3.Based on the various roles of autophagy in microglia, it is expected that mutations in autophagy genes linked to ALS not only affect neuronal health but also impact on the functionality of microglia.

Box: Inflammasome

Inflammasomes are a group of cytosolic multimeric protein complexes which form molecular platforms. A typical inflammasome consists of specific pattern recognition receptors (PRRs), which are connected to inflammatory caspases via adaptor proteins. The PRRs sense pathogenic insults resulting in the cleavage and activation of the inflammatory caspases. These activated caspases in turn induce proteolytic cleavage of pro-inflammatory cytokines into their mature form and their secretion, which induces an inflammatory response [109, 110].

2.2.3. Microglial Autophagy in ALS

Neuroinflammation is known to play an important role in ALS with more than thousand inflammatory genes differentially expressed in ALS patients compared to healthy control individuals [111]. Moreover, it was shown that microglia reactivity precedes symptom onset in SOD1 transgenic mice and that disease progression is accompanied by microgliosis in patient brain sections [112-114]. Accordingly, transplanted wild-type microglia are able to slow down disease progression in ALS transgenic animals [115]. In line with the immunological pathogenesis tumor necrosis factor alpha (TNFα) levels are elevated in ALS patients [116-118]. A recent study by Jin *et al*. reported decreased autophagic flux in microglia upon TNFα exposure. Concomitantly, an elevation of pro-inflammatory markers was observed, suggesting that downregulation of autophagy in murine BV2 microglia cells leads to increased neurotoxicity [119]. This finding was substantiated by the observation that activation of autophagy in BV2 cells led to a reduction of lipopolysaccharides (LPS) or α-synuclein induced pro-inflammation [120]. Recently, Yoon and colleagues demonstrated the importance of autophagy in microglia for synaptic homeostasis and proper synaptic function in mice [121] (Figure 3). Therefore, impaired autophagy in microglia could at least partially contribute to the observed synaptic abnormalities in motor neurons of ALS patients. Furthermore, a C9orf72 loss of function study emphasized the role of autophagy in microglia in ALS. Intriguingly, deletion of C9orf72 in mice did not lead to a motor neuron phenotype but instead gave rise to lysosomal accumulation and altered immune response in microglia [122]. In contrast, loss of motor neurons has been shown in several C9orf72 loss-of function and gain-of function models [49, 51, 71, 123-125]. Interestingly, a number of laboratories independently reported that C9orf72 functions in different steps of the autophagy pathway in conjunction with SMCR8 and WDR41 [49, 50, 126, 127]. However, it is still unclear whether defective autophagy caused for example by C9orf72 haploinsufficiency is a primary cause of ALS or a modulator of the disease phenotype. Overexpression of a mutated form of SOD1 in microglia gave further insight into the role of autophagy in microglia during ALS. In this study, primary rat microglia accumulated SOD1, displayed impaired autophagy, shifted to an activated phenotype and induced neurotoxicity in co-cultures with neurons [78]. Together, there is increasing evidence that defective autophagy in microglia contributes to ALS pathogenesis, most likely through changes in microglial inflammasome activation and protein clearance.

2.3. Astrocytes

2.3.1. General Function of Astrocytes

Astrocytes were first mentioned by Lenhossek in 1891 and include all cells which match the characteristics of star-like cells with numerous fibrils in their cytoplasm. As a result, astrocytes present the most diverse cell population in the CNS. They are divided into two main subtypes: protoplasmic astrocytes, found in the grey matter, and fibrous astrocytes, which are located in the white matter. Interestingly, astrocytes are organized in separate spatial domains, which do not overlap under physiological conditions [128, 129]. Astrocytes are found in close proximity of blood vessels and their processes often envelop synapses [130]. This location allows inference on their function. They cover blood stream regulation, modulation of synaptic function, neurotransmitter recycling as well as provision of trophic, antioxidant and metabolic support to neurons [131-134] (Figure 3). Astrocytes are able to release transmitter precursors and transmitters, purines, growth factors, and energy substrates like lactate. For example, in response to glutamate release by neurons, astrocytes increase their glucose utilization and provide lactate to neurons to fuel their metabolism [135, 136]. Astrocytes are able to enhance their glucose uptake from blood vessels or to break down their glycogen granules and generate lactate [137, 138]. Although astrocytes are non-excitable cells, they express ion channels and neurotransmitter receptors and exhibit a Ca^{2+} based “intrinsic excitability” [139-141]. Due to these properties, they can serve to clear transmitters from the synaptic space in order to prevent their extrasynaptic accumulation and consequential excitotoxicity [142-144]. In a similar manner, astrocytes regulate ion homeostasis in the synaptic space [145]. Furthermore, the detection of synaptic activity induces an elevation of Ca^{2+} in astrocytes, resulting in the release of synaptically active molecules such as glutamate, d-serine and adenosine triphosphate (ATP) from these astrocytes. These so-called gliotransmitters in turn allow feedback regulation of neuronal activity and synaptic strength by acting on neuronal receptors [146, 147]. In addition, astrocytes are active players in neuroinflammation. They have the capability to secret a wide range of pro- and anti-inflammatory chemokines and cytokines, including TNFα, IL-6, IL-10 and CC-chemokine ligand 2 [148-151]. The central role of astrocytes in the regulation of neuroinflammation was further substantiated by *in vivo* data of mice with inhibited NF-kB in astrocytes [152, 153]. In response to any forms of CNS insult, astrocytes change their morphology, function and molecular profile in a highly graduated continuum, referred to as reactive astrogliosis [154-156]. Astrogliosis is defined by upregulation of intermediate filaments, mainly glial fibrillary acidic protein (GFAP), vimentin and nestin, and by cellular hypertrophy [157]. Besides, reactive astrocytes have elevated phagocytic capacity [158-161]. Severe astrogliosis additionally comprises proliferation, leads to disruption of individual spatial domains, and causes inflammation and glial scar formation [162-164]. Taken together, astrocytes are key for CNS homeostasis due to their comprehensive roles. However, in disease states the beneficial functions of astrocytes can revert to detrimental effects.

2.3.2. Autophagic Functions in Astrocytes

In contrast to the recent advances in the understanding of autophagic functions in microglia, still relatively little is known about its role in astrocytes. Based on the widespread functions of astrocytes especially in the uptake and release of molecules as well as in immune reactions, it seems plausible that autophagy likewise exerts crucial functions in these cells. However, support for this notion is only anecdotal. For example, given that astrocytes have the capacity to phagocyte and to secrete inflammatory molecules like IL-1β and IL-18 in a similar manner than microglia, these two cell types might also share the involvement of autophagy in immune-related processes [149, 165]. However, evidence for this hypothesis is lacking. In addition, a function of autophagy in glutamate responses in immortalized rat astrocytes was reported [166]. Glutamate uptake by astrocytes leads to a Ca^{2+} release from lysosomes and to autophagy induction. While Ca^{2+} elevations trigger gliotransmitter release, autophagy induction could provide metabolic support not only for astrocytes but also for the nearby neurons. Furthermore, parts of the autophagy machinery could play a role in the release of molecules from astrocytes as described for the insulin-degrading enzyme in murine primary astrocytes [167, 168]. This notion is supported by the finding that ATP release to the extracellular space involves autophagic

vesicles in HeLa cells, melanoma cells and rat primary astrocytes [169-172] (Figure 3). However, the exact role of autophagy in unconventional secretion in astrocytes remains to be established.

2.3.3. Astrocytic Autophagy in ALS

Although our knowledge about autophagy in astrocytes is very limited, interest in its role in ALS is increasing since the contribution of astrocytes to ALS has been substantiated by several studies. In a mutant SOD1 mouse model it was shown that inclusions appear in astrocytes first and also to a higher extend than in neurons [173]. Remarkably, astrocyte-specific expression of mutant SOD1 did not cause motor neuron degeneration but astrocyte-specific attenuation of mutant SOD1 expression slowed down disease progression in transgenic mice [75, 79]. Moreover, pro-inflammatory conditions as they occur in NDs can induce profound changes in the mitochondrial network of astrocytes. Under these conditions, fragmentation of mitochondria and decreased respiratory capacity was reported. Therefore, functional autophagy is indispensable for astrocytes in an inflammatory environment to maintain the mitochondrial architecture and to prevent an accumulation of ROS [174]. In ALS mice models with mutated FIG4 and VAC14, the majority of p62-containing inclusions were found in astrocytes, highlighting the relevance of autophagic clearance in these cells [61]. Moreover, astrocytes from mice with autophagic-lysosomal dysfunction were shown to contribute directly to neurodegeneration due to an impaired ability to metabolically support neurons as shown for lysosomal storage disorder [175]. Astrocytes seem to assist neurons also in the degradation of their waste. Neurons were reported to extrude protein aggregates and damaged organelles, which were subsequently taken up and degraded by C. elegans astrocytes and astrocytes of the murine optic nerve head[176, 177] (Figure 3). A recent study by Gomez-Arboledas *et al*. further expanded the spectrum of neuronal recycling targets of astrocytes from metabolites (ascorbate) and organelles (mitochondria) to dystrophic neurites, which were shown to be cleared by astrocyte-mediated phagocytosis from injured neurons in murine AD models [158]. The notion that several of the supportive roles of astrocytes seem to involve the autophagy pathway highlights the importance of assessing the impact of ALS-causing mutations in autophagy components on astrocyte function in ALS.

2.4. Oligodendrocytes

2.4.1. General Functions of Oligodendrocytes

Oligodendrocytes are the glial cells of the CNS responsible for axon myelination. Besides electrical insulation of the axon, myelin plays an important function in providing trophic and metabolic supports to axons [178]. Myelinated fibers were already described in 1717 by van Leeuvenhock but at this time the myelin origin was controversial. In the 1920s, Pío del Río-Hortega discovered oligodendrocytes, cells with few processes, and proposed them as myelin producing cells [179]. This theory was not proven until first electron microscopic studies demonstrated a cytoplasmic connection between oligodendrocytes and myelin sheaths [180]. After an initial contact between oligodendrocyte and axon, the oligodendrocyte processes start to extend into myelin sheaths. The leading innermost “tongue” of the myelin sheath wraps around the axon by laying the newly formed myelin layers underneath the existing layer of myelin. Afterwards, all myelin sheaths extend laterally towards the nodes of Ranvier, also known as myelin sheath gaps. After a few wraps of myelin around the axon, compaction starts from the outermost layer extending inwards [181]. Besides securing rapid conduction of action potentials through myelin isolation, oligodendrocytes metabolically support axons. This support is essential, since the compacted myelin limits the metabolic supply from the extracellular space. Within compacted myelin, cytoplasm-rich channels enable the transport of cargo from the oligodendrocyte cell body to the innermost myelin layer facing the axon (adaxonal tongue of myelin) and vice-versa [182]. As a result, oligodendrocytes and myelinated axons are metabolically coupled. Oligodendrocytes are able to import glucose, transform it to lactate or pyruvate and provide it via monocarboxylic acid transporters (MCT1, MCT2) to the axon [183, 184] (Figure 3). This is finely regulated by N-methyl-D-aspartate (NMDA) receptors on oligodendrocytes, which recognize neuronal activity and sequentially mediate the incorporation of additional glucose transporters and thereby increase glucose import [185]. This

metabolic support of axons by oligodendrocytes is reminiscent of the astrocyte-neuron lactate shuttle and essential as depletion of MCT1 in oligodendrocytes led to motor neuron death [184].

2.4.2. Autophagic Roles in Oligodendrocytes

Studies on autophagy in oligodendrocytes are scarce. However, interest in the functional role of autophagy in oligodendrocytes are rising lately. As in most cell types, autophagy can be induced by rapamycin and exerts protein quality control functions in oligodendrocytes. This was shown for example in the rat oligodendroglial cell lines OLN-t40 and OLN-93 [186, 187]. However, under apoptotic conditions rapamycin treatment led to augmented cell death in rat primary oligodendrocytes [188]. In addition, autophagy is involved in myelination, which is one of the main functions of oligodendrocytes. Duncan and colleagues demonstrated that autophagy increases myelination, more specifically myelin thickness and the number of myelinated axons in Long–Evans shaker rats [189]. Furthermore, autophagy was observed to be essential for myelination during development in mice [190]. Clearance of damaged myelin is necessary for maintenance of healthy myelin and normal nerve function. Interestingly, Schwann cells, the counterparts of oligodendrocytes in the peripheral nervous system, are able to degrade myelin after injury by autophagy in a process called myelinophagy. While injury promotes autophagy in Schwann cells in mice, oligodendrocytes appear to be unable to digest myelin, possibly reflecting the poor regenerative capability of the CNS tissue after myelin injury [191]. The conditional deletion of *Atg5* in murine oligodendrocytes by Whittemore and colleagues was instrumental to demonstrate the protective role of autophagy. In this model, the authors observed a reduction of cell viability in response to ER stress [190]. Similarly, in a human oligodendrocyte cell line (MO3.13) with a vanishing white matter disease mutation, autophagy plays a role in ensuring cell survival of oligodendrocytes under ER stress conditions [192]. Given that autophagy is involved in the trophic support of neurons by astrocytes and that oligodendrocytes maintain this function at myelinated axons, it can be assumed that autophagy is also linked to this process in oligodendrocytes. Indeed, Roy *et al.* supported this hypothesis by showing that autophagy promotes the retromer-dependent cell surface trafficking of the nutrient transporters glucose transporter 1 (GLUT1) and MCT1 in mouse embryonic fibroblasts [193] (Figure 3). Therefore, autophagy seems to play an important role in metabolic support of neurons by astrocytes and oligodendrocytes via facilitating increased glucose uptake and increased lactate release.

2.4.3. Oligodendrocytic Autophagy in ALS

While oligodendrocytes and myelin are central to the pathomechanisms of inflammatory diseases such as multiple sclerosis, they are also implicated in the pathology of several NDs. For example, TDP-43- and FUS-positive inclusions were found among other cells in oligodendrocytes in postmortem tissues of ALS patients [194-196]. The occurrence of aggregates in oligodendrocytes in ALS suggests an impairment of autophagy in these cells. Importantly, Rothstein and colleagues observed that MCT1 expression, which is high in oligodendrocytes in otherwise unperturbed conditions, is reduced in in patients with, and mouse models of ALS and correlated with neuronal death in animal and cell culture models [184]. Given that autophagy has been shown to be essential for MCT1 translocation, defective autophagy might at least partially be responsible for the disruption of MCT1 in ALS oligodendrocytes. However, an oligodendrocyte-specific role of autophagy in ALS remains to be formally demonstrated.

Concluding Remarks

Neuroimmune changes and non-cell autonomous toxicity are tightly linked to the pathology of ALS [197]. For example, ALS-related mutant SOD1 expression in microglia and astrocytes was demonstrated to be the decisive source that drives disease progression after onset [115, 198]. Intriguingly, rare mutations in the triggering receptor expressed on myeloid cells 2 (TREM2) gene have recently been linked to increased risk of ALS [199]. Hence, glial modulation emerged as a strategy to slow disease progression in ALS/FTD. Given the crucial role of autophagy and its components in balancing beneficial and detrimental effects of immunity, inflammation and metabolism in other cell types [200], autophagy appears an attractive target for the titration of glial activity to ultimately slow down disease progression. However, despite the progress that has been made in understanding the basic mechanistic principles

underlying autophagy (which has primarily been described in cells culture under extreme conditions of starvation), many unanswered questions remain, in particular with respect to cell type-specific roles of autophagy and autophagy-related pathways as well as with regard to the contribution of defects in these processes to onset and/or progression of NDs. Continuing studies in this area are needed to define and delineate the function of the autophagy machinery in glia cells and its disturbance in ALS/FTD.

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Figure legend

Figure 1. The autophagic pathway. The ULK protein and the PI3K-III lipid kinase complex control the formation of phagophores. Cargo is recruited to these phagophores by ATG8 family proteins and autophagic receptors. Subsequent membrane elongation results in closed, mature autophagosomes. These autophagosomes undergoes several fusion events with late endosomal compartments and lysosomes. In these autolysosomes, cargo is broken down by lysosomal hydrolases.

Figure 2. ALS genes and their possible involvement in the autophagic pathway. A large number of ALS genes (indicated in green) have direct or indirect functions in the autophagy pathway including roles as autophagic receptors (dark green). In addition, some mutated forms of ALS-associated proteins interfere with autophagy (red).

Figure 3. Degradative and non-degradative roles of the autophagic machinery in glial cells. (a) Degradation of endogenous and exogenous toxic proteins and organelles. (b) Unconventional secretion. (c) Regulation of the inflammasome. (d) Trafficking of the nutrient transporters glucose transporter 1 (GLUT1) and monocarboxylic acid transporter 1 (MCT1). (e) Transport of cytosolic antigens into the lumen of major histocompatibility complex (MHC)-II compartment. (f) Regulation of the secretion of soluble factors, which influence synaptic pruning. Not all functions take exclusively place in the indicated cell type.

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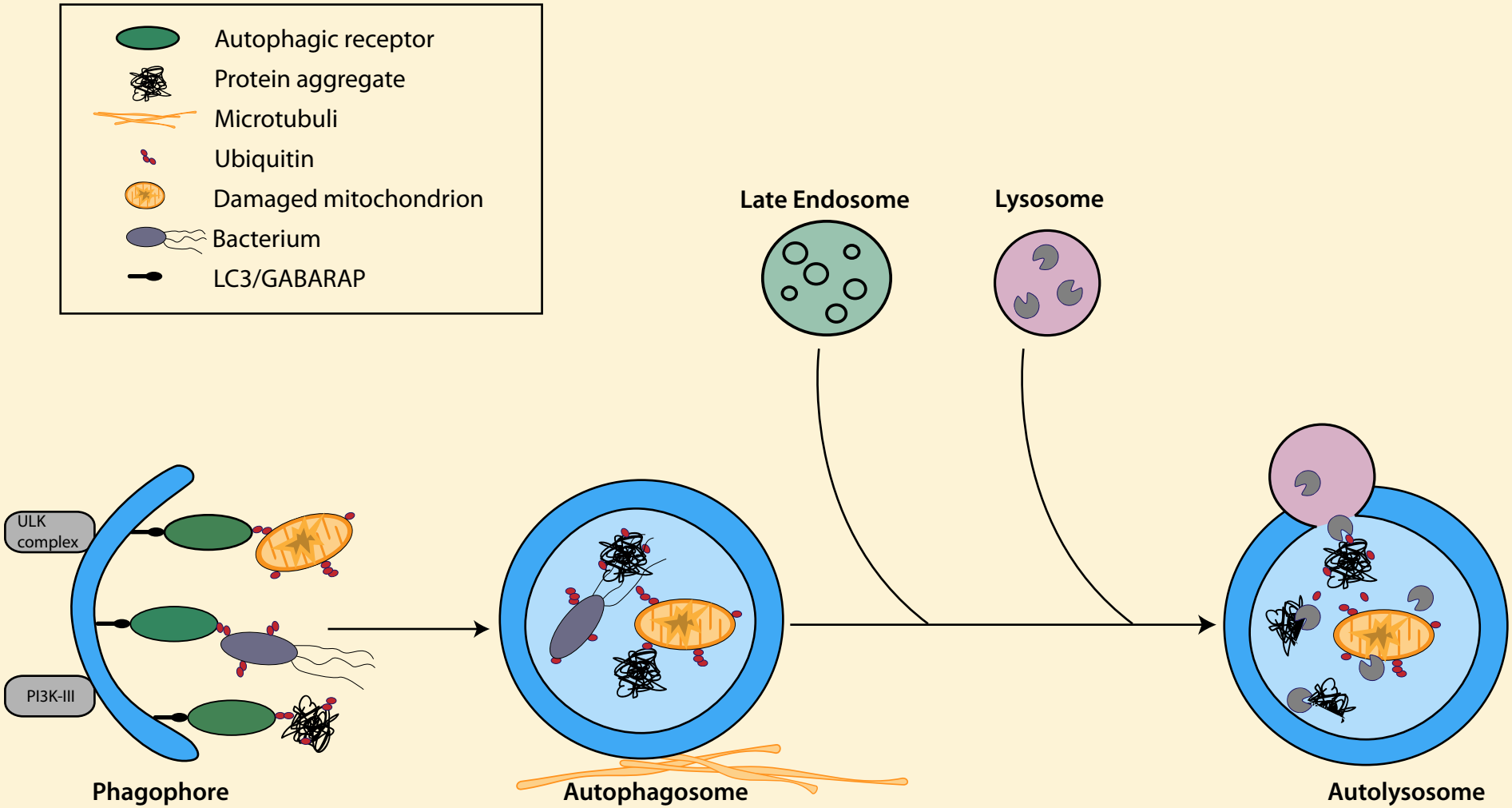
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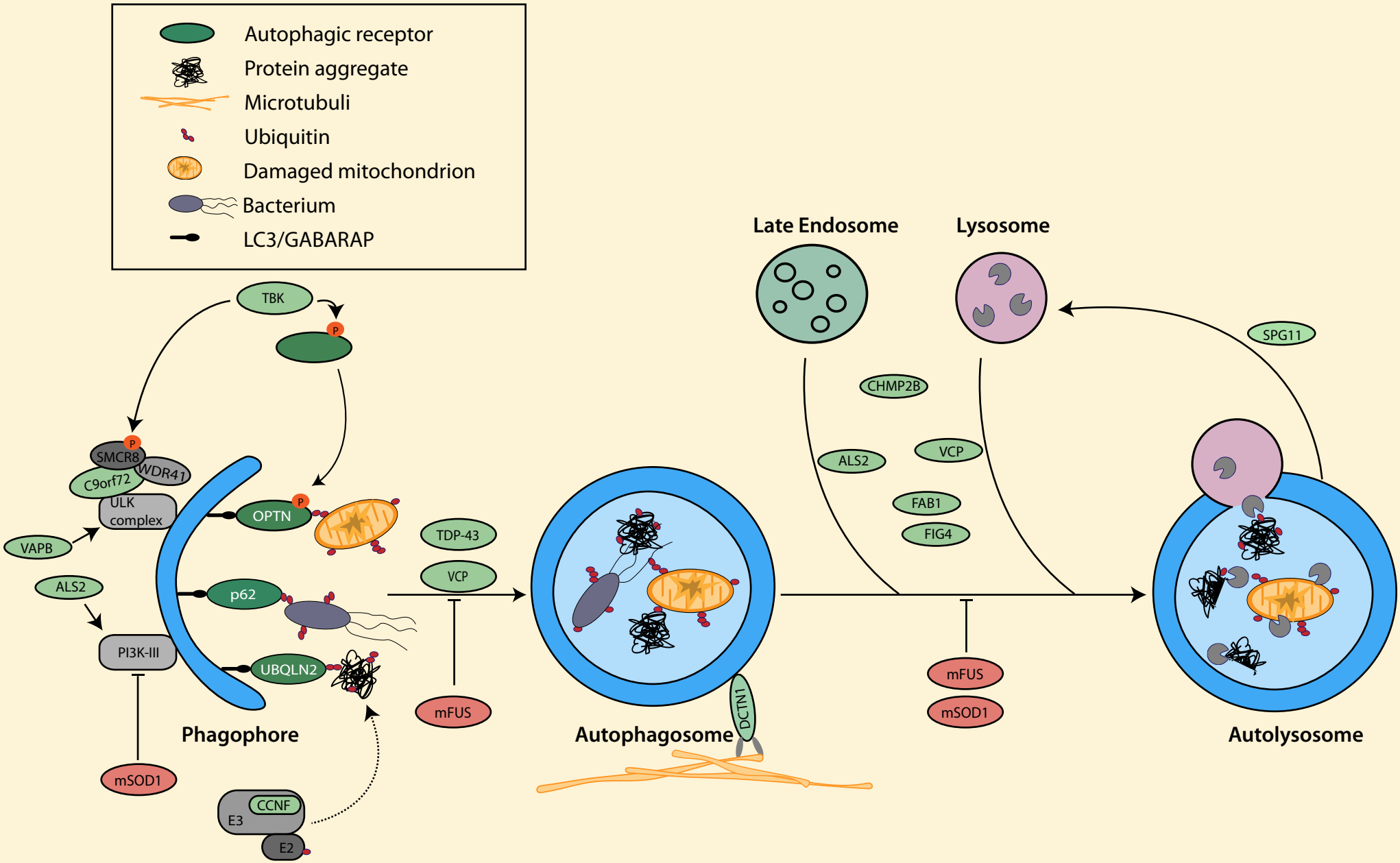
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ULK complex
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ALS2
PI3K-III
mSOD1
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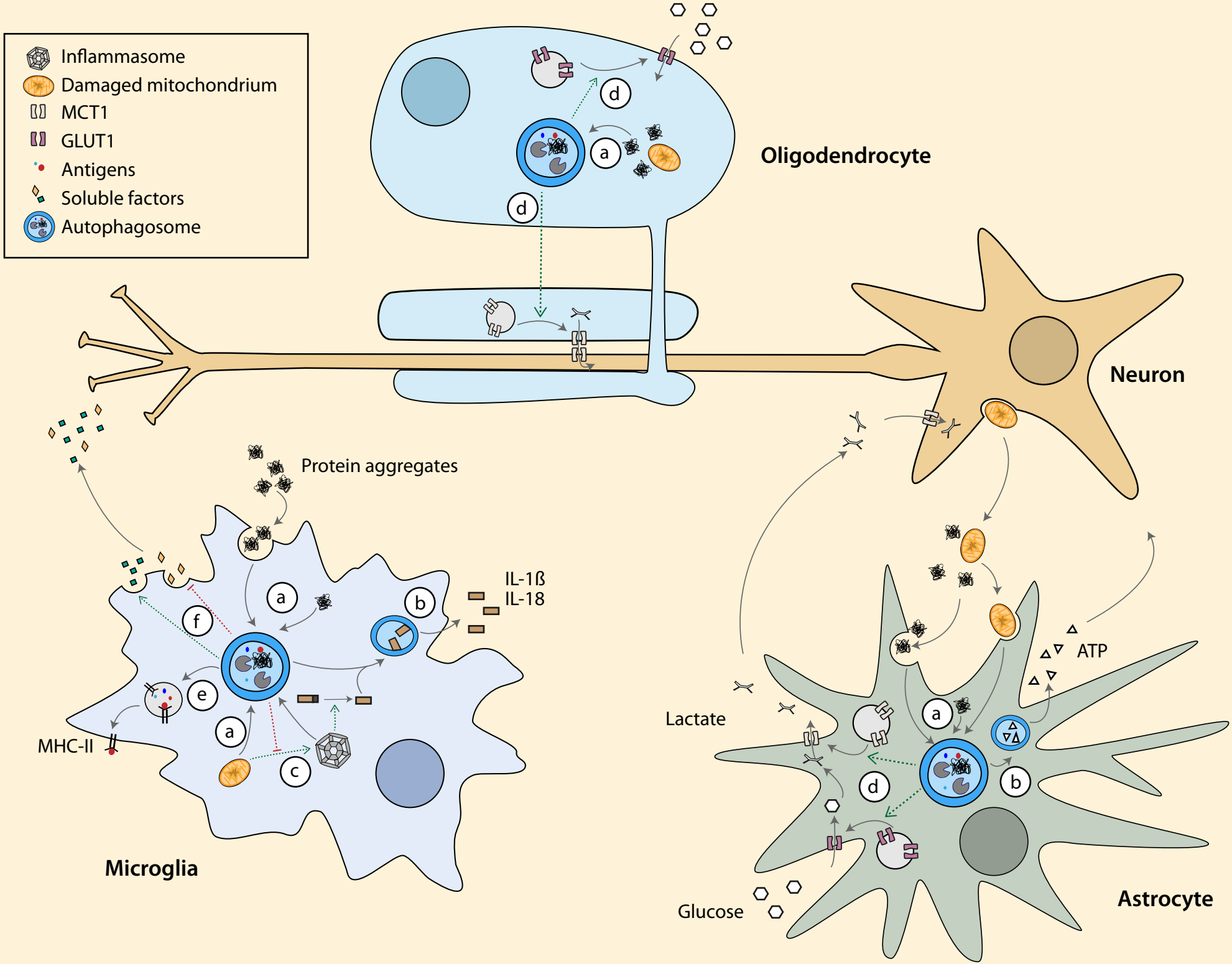
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Oligodendrocyte
Neuron
Protein aggregates
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Microglia
Lactate
ATP
Glucose
Astrocyte
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