



Primary phagocytosis of neurons by inflamed microglia: potential roles in neurodegeneration

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Microglial phagocytosis of dead or dying neurons can be beneficial by preventing the release of damaging and/or pro-inflammatory intracellular components. However, there is now evidence that under certain conditions, such as inflammation, microglia can also phagocytose viable neurons, thus executing their death. Such phagocytic cell death may result from exposure of phosphatidylserine (PS) or other eat-me signals on otherwise viable neurons as a result of physiological activation or sub-toxic insult, and neuronal phagocytosis by activated microglia. In this review, we discuss the mechanisms of phagocytic cell death and its potential roles in Alzheimer's Disease, Parkinson's Disease, and Frontotemporal Dementia.

Keywords: microglia, phagocytosis, inflammation, neurodegeneration, phosphatidylserine, MFG-E8, lactadherin, reactive oxygen and nitrogen species

INTRODUCTION

Phagocytosis of host cells is generally thought to be *secondary* to the target cell dying by some means such as apoptosis (Savill et al., 2002; Ravichandran, 2003). However, phagocytosis can execute cell death of viable cells, and we shall refer to this form of cell death as “*primary* phagocytosis,” with the defining characteristic that inhibition of phagocytosis prevents cell death. Examples of primary phagocytosis outside the brain include macrophage phagocytosis of “aged” erythrocytes (Föller et al., 2008; Lee et al., 2011) and activated neutrophils (Lagasse and Weissman, 1994; Jitkaew et al., 2009; Stowell et al., 2009; Bratton and Henson, 2011). In *C. elegans*, primary phagocytosis has been shown to contribute to programmed cell death of neuronal precursors during development (Hoeppner et al., 2001; Reddien et al., 2001), the elimination of cells subjected to sub-toxic insults (Neukomm et al., 2011) or simply as a result of phosphatidylserine (PS) exposure on the surface of cells (Darland-Ransom et al., 2008). In this review, we will briefly discuss the mechanisms of phagocytosis of cells, current evidence for primary phagocytosis in the central nervous system (CNS) and the resulting implications for neurodegenerative disease.

MECHANISMS OF PHAGOCYTOSIS OF HOST CELLS

The process of phagocytosis is normally initiated by the release of attractive signals from the target cell (referred to as “come-get-me” signals) leading to chemotaxis of a nearby macrophage. Upon reaching the target cell, the macrophage recognizes cell-surface signals on the target cell (“eat-me” signals), which then induce its

uptake. The best characterized “eat-me” signal is the cell-surface exposure of phosphatidylserine (PS; Fadok et al., 1992; Martin et al., 1995), although display of proteins such as calreticulin has also been implicated (Gardai et al., 2005). In healthy cells, PS is found exclusively on the inner leaflet of the plasma membrane, because the aminophospholipid translocase removes PS from the outer leaflet. However, a second enzyme, the phospholipid scramblase, can cause PS exposure by randomizing phospholipid distribution between the inner and outer leaflets. PS exposure may occur as a result of: (i) apoptosis (by unknown mechanisms), (ii) necrosis (due to plasma membrane rupture), (iii) calcium elevation (which stimulates the phospholipid scramblase and inhibits the aminophospholipid translocase), (iv) ATP depletion (which inhibits the aminophospholipid translocase), (v) oxidative stress (which inhibits the aminophospholipid translocase and stimulates the scramblase), and/or (vi) fusion of intracellular vesicles with the plasma membrane (Bratton et al., 1997; Gleiss et al., 2002; Tyurina et al., 2007).

While PS display has generally been regarded as an early sign of apoptotic cell death, it is now clear that PS exposure can be reversible and independent of apoptosis (Dias-Baruffi et al., 2003; Mackenzie et al., 2005; Tyurina et al., 2007; Jitkaew et al., 2009; Neher et al., 2011), and therefore may lead to the phagocytosis of viable host cells in the presence of macrophages. For example, galectin-1 induces PS exposure on the surface of neutrophils, which is fully reversible when galectin-1 is removed and does not lead to cell death. However, when macrophages are present at the time of PS exposure these cells are phagocytosed and thus killed (Dias-Baruffi et al., 2003; Stowell et al., 2009). Whether exposure of PS by itself is sufficient for recognition and removal is not entirely clear: PS exposure may be sufficient for some cells (Fadok et al., 2001), while others may require PS oxidation or other co-stimulatory signals to induce phagocytosis (Borisenko et al., 2003, 2004). Furthermore, some healthy cells actively protect themselves from phagocytic removal by displaying signals on their surface,

Abbreviations: AD, Alzheimer's disease; DAP12, DNAX adaptor protein-12; FTD, Frontotemporal dementia; MerTK, Mer receptor tyrosine kinase; MFG-E8, milk fat globule EGF-like factor 8; MS, multiple sclerosis; PD, Parkinson's disease; PS, phosphatidylserine; Tim4, T-cell immunoglobulin- and mucin-domain-containing molecule-4; TLR, Toll-like receptor; TREM2, triggering receptor expressed by myeloid cells-2; VR, vitronectin receptor.

which inhibit phagocytosis (“don’t-eat-me” signals, such as CD200 or CD47; see below).

A range of receptors on the macrophage can mediate recognition of PS on target cells, implying redundancy on first glance. However, not all receptors are expressed by a macrophage at any one time, but rather are dependent on its activation state, e.g., classically activated/pro-inflammatory vs. alternatively activated/anti-inflammatory macrophages. For example, resting peritoneal mouse macrophages express the PS receptors Tim4 (T-cell immunoglobulin- and mucin-domain-containing molecule-4; Kobayashi et al., 2007; Miyanishi et al., 2007), and alternatively activated human monocyte-derived macrophages express stabilin-1 (Park et al., 2009) and stabilin-2 (Park et al., 2008). In contrast, inflammatory activated (thioglycollate-elicited) peritoneal macrophages upregulate the PS-binding protein MFG-E8 (Milk fat globule EGF-like factor 8, also known as lactadherin, SED1; Hanayama et al., 2002) and the Mer receptor tyrosine kinase (MerTK), which recognizes PS or other eat-me signals through bridging molecules Gas6, protein S, galectin-3, tubby, and Tulp1 (Scott et al., 2001; Seitz et al., 2007; Shao et al., 2009; Caberoy et al., 2011). At the same time, activated (thioglycollate-elicited) macrophages downregulate expression of other phagocytic proteins such as Tim4 (Miyanishi et al., 2007). Interestingly, it therefore appears from the literature that resting macrophages express receptors that bind PS directly (i.e., Tim4, stabilin-1/2), whereas activated/pro-inflammatory macrophages utilize phagocytic pathways that recruit bridging proteins (e.g., MFG-E8, Gas6), which bind both PS on the target cell and a receptor on the macrophage [e.g., vitronectin receptor (VR), MerTK].

Microglia are resident brain macrophages, which continuously and actively survey their microenvironment (Nimmerjahn et al., 2005). Microglia represent a distinct population of tissue macrophages as they arise at least in part from primitive myeloid progenitors during early embryonic stages and are thought to be maintained by self-replication throughout life in the healthy brain (Ginhoux et al., 2010). However, under specific conditions, such as brain injury and inflammation, peripheral monocytes can be recruited to the brain. This occurs for example after stroke, but whether peripheral monocytes modify the pathology of chronic neurodegenerative diseases is disputed. In Alzheimer’s disease (AD) for example, recent evidence indicates that peripheral monocytes do not invade the brain, but contribute to removal of amyloid- β (A β) in the perivascular environment (Mildner et al., 2011). While it is beyond the scope of this review to discuss this issue in detail, it appears that invading peripheral monocytes may mediate effects distinct from resident microglia (Prinz et al., 2011). Nevertheless, microglia share a number of similarities with peripheral macrophages, including their recognition of both exogenous “non-self” ligands (e.g., lipopolysaccharide, LPS) and endogenous “self” ligands (such as A β , HSP60, and HMGB1) through pattern recognition receptors (such as Toll-like receptor-2 and -4; Kawai and Akira, 2010). Recognition of these ligands results in inflammation and associated neurodegeneration due to inflammatory activation of microglia, which also renders the microglia highly phagocytic (Babcock et al., 2006; Boivin et al., 2007; Hanisch and Kettenmann, 2007; Neher et al., 2011; Neniskyte et al., 2011). Other macrophage populations in the brain, with phenotypes distinct

from microglia, include those associated with the perivascular space, the circumventricular organs, the choroid plexus, and the meninges (Ransohoff and Perry, 2009).

Although data on microglia is limited, they appear to express similar phagocytic receptors and bridging proteins as peripheral macrophages. For example, primary microglia in culture express MFG-E8 (Neher et al., 2011) and MerTK (Grommes et al., 2008), possibly consistent with a partially activated microglial phenotype induced by cell-isolation procedures (Streit et al., 1999) as expression levels of these two proteins are low in homogenates of the naïve brain (Binder et al., 2008; Fuller and Van Eldik, 2008). In support of this notion, it has been reported that stimulation of microglia with the TLR4 ligand LPS increases MerTK expression in culture, and MerTK and its ligand Gas6 are also upregulated after a demyelinating insult in the brain (Binder et al., 2008). The expression of PS receptors in resting microglia has not been investigated in detail, but the mRNA levels of Tim4 appear to be low in brain homogenates (Miyanishi et al., 2007; Tanaka et al., 2009). Importantly, in the CNS, surrounding astrocytes may support microglial phagocytic function by producing bridging proteins such as MFG-E8 (Boddaert et al., 2007; Cahoy et al., 2008; Kranich et al., 2010) and protein S (Stitt et al., 1995), thus enabling efficient clearance of PS-exposing neurons.

A different phagocytic receptor, triggering receptor expressed by myeloid cells-2 (TREM2), mediates microglial clearance of debris and apoptotic neurons *in vitro* after activation by TREM2 ligands expressed on neuronal cells. Accordingly, knockdown of TREM2 impairs phagocytic function of microglia and increases the generation of pro-inflammatory cytokines *in vitro* (Takahashi et al., 2005). The function of TREM2 and its signaling partner DNAX adaptor protein-12 (DAP12) are essential for CNS immune homeostasis as loss-of-function mutations cause Nasu–Hakola disease (also known as polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy, PLOSL), which presents with inflammation and neurodegeneration (Neumann and Takahashi, 2007). This supports the idea that microglial phagocytosis of dead and dying cells (rather than viable cells) can be protective and anti-inflammatory. The only identified TREM2 agonist is the endogenous “self” ligand HSP60, which upon binding to TREM2 strongly stimulates microglial phagocytosis (Stefano et al., 2009). Interestingly, HSP60 is also a ligand for TLR4, and TLR4 activation by HSP60 can cause microglial activation and inflammatory neurodegeneration *in vitro* (Lehnardt et al., 2008). Thus, TLR4 activation by HSP60 may contribute to the inflammation and neurodegeneration seen in Nasu–Hakola disease, where the anti-inflammatory signaling via HSP60 and TREM2 would be missing.

Wang and Neumann (2010) identified Siglec-11 as a microglial receptor, which binds polysialylated proteins on the surface of neurons (in particular neuronal cell adhesion molecule, NCAM) resulting in inhibition of inflammation and phagocytosis. Transfection of mouse microglia with human Siglec-11 reduced the spontaneous phagocytosis of neurites and neuronal cell bodies occurring in neuronal–microglial co-cultures, and this was dependent on the presence of polysialylated proteins on the surface of neurons. Thus polysialylation can act as a “don’t-eat-me” signal for neurons *in vitro*.

Several other molecules can downregulate microglial activity. CD200 and CD47 are both expressed on the surface of neurons, and are known to act as “don’t-eat-me-signals.” The CD200 receptor (OX2) is found on microglia (Wright et al., 2000), and CD200 suppresses brain inflammation in experimental autoimmune encephalomyelitis and after facial nerve transection (Hoek et al., 2000). CD47 expression on cells and myelin sheaths inhibits phagocytosis by microglia via a CD47 receptor, SIRP α (Gitik et al., 2011).

Another signaling pathway that has been shown to modulate microglial activity is the CX3CL1 (fractalkine)/CX3CR1 pathway. CX3CL1 is expressed by neurons, while its receptor is predominantly expressed by microglia in the brain (Harrison et al., 1998). Inflammatory microglial activity can be suppressed by the neuronal chemokine CX3CL1 (also known as fractalkine) via the microglial chemokine receptor CX3CR1 as shown in neuron–microglia co-cultures (Zujovic et al., 2000) and in CX3CR1-deficient mice after systemic injection of LPS, in the MPTP model of Parkinson’s Disease (PD), as well as in a transgenic model of amyotrophic lateral sclerosis (Cardona et al., 2006). Furthermore, CX3CR1 knockout restricts natural killer cell recruitment in experimental autoimmune encephalomyelitis thereby worsening disease outcome (Huang et al., 2006). However, in other circumstances fractalkine/fractalkine receptor knockout has been shown to improve neuropathology. For example, CX3CR1 deficiency resulted in improved outcome after spinal cord injury possibly due to enhanced recruitment of bone marrow derived macrophages, which also displayed reduced release of inflammatory mediators (Donnelly et al., 2011). Further, it has been reported that CX3CL1 deficient mice displayed smaller infarcts (about 30%) 24 h after middle cerebral artery occlusion (MCAo; Soriano et al., 2002), although the mechanisms of this effect were not analyzed in this study. However, a different group showed an even more pronounced reduction of infarct size (more than 50%), reduced blood brain barrier damage, leukocyte infiltration, neuronal death, and levels of IL-1 β (Denes et al., 2008) between 1 and 3 days after MCAo. Importantly, a recent study analyzed the influence of the fractalkine/fractalkine receptor pathway after permanent MCAo (Cipriani et al., 2011). Similar to the two studies described above, they found reduced infarct size in both CX3CL1 and CX3CR1 knockout mice 24 h after the insult. Interestingly, *icv* infusion of recombinant CX3CL1 in rats also reduced infarct size and this effect persisted for up to 56 days. When analyzing the *in vitro* responses of wildtype and CX3CL1 knockout microglia to medium from oxygen–glucose deprived neurons, the authors found that microglial phagocytic activity was suppressed only in wildtype, but not in CX3CL1 knockout microglia. In the same experiment, the release of TNF- α was reduced in CX3CL1 knockout but not in wildtype microglia demonstrating a changed microglial response resulting from fractalkine knockout.

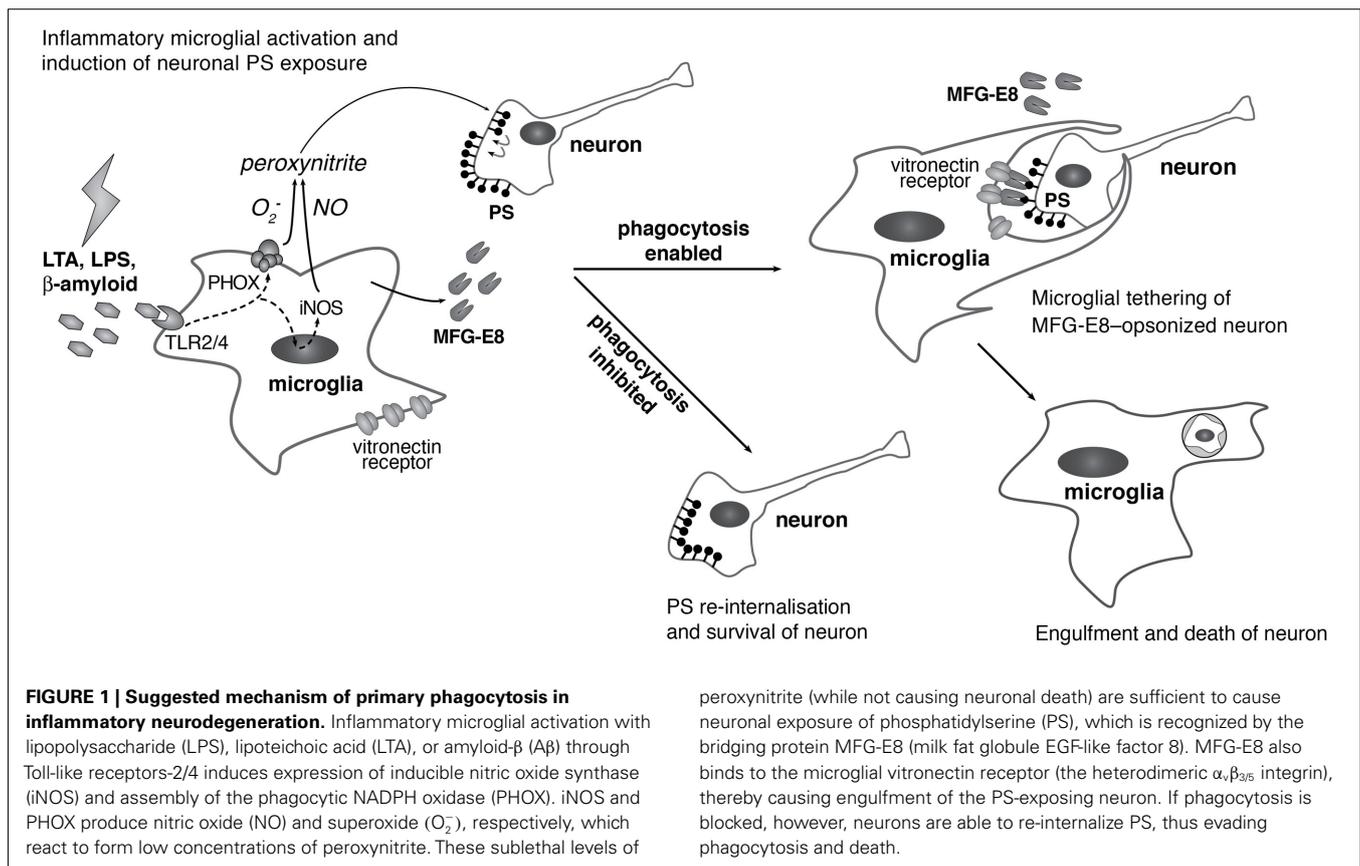
Fractalkine is normally displayed on the cell surface of neurons, but its release is induced by stress such as nerve injury or excitotoxicity, when it may suppress microglial inflammation but can also act as a chemokine for leukocyte infiltration as well as microglial recruitment. Additionally, soluble fractalkine may also promote microglial phagocytosis of neuronal debris by

stimulating microglial production and release of MFG-E8 (Harrison et al., 1998; Cook et al., 2010; Fuhrmann et al., 2010; Noda et al., 2011) and induces upregulation of microglial integrin β_5 expression, which is one of the subunits of the receptor for MFG-E8, the VR (Leonardi-Essmann et al., 2005). Interpretation of experiments in CX3CL1 or CX3CR1 knockout animals are therefore difficult as the outcome may be due to any of the above mechanisms or combinations thereof. However, from the literature described above, it appears that suppression of leukocyte recruitment and microglial inflammation may dominate the outcome.

EVIDENCE FOR PRIMARY PHAGOCYTOSIS IN THE CNS

Activation of microglial phagocytosis is generally considered to be beneficial via removal of pathogens or potentially pro-inflammatory debris and apoptotic cells (Neumann et al., 2009). However, we and others have shown that microglia can also phagocytose viable synapses and neurons. For example, during development microglia may be involved in synaptic pruning, i.e., elimination of synapses, and mice lacking the fractalkine receptor, CX3CR1, show higher densities of spines and functional synapses during early postnatal development, which the authors attributed to temporarily reduced microglial density (Paolicelli et al., 2011). Furthermore, microglia kill developing neurons in cerebellar organotypic slices leading to an increase in the number of fully differentiated Purkinje cell clusters (Marín-Teva et al., 2004). Similarly, two phagocytosis-related proteins, CD11b and DAP12, appear to mediate developmental neuronal death in the hippocampus *in vivo* (Wakselman et al., 2008). In animals with a loss-of-function mutation in DAP12 as well as by inhibition of the complement receptor 3 subunit CD11b, neuronal death was reduced at postnatal day 1 by about 25%. Both in cerebellar slices and in the hippocampus, this effect appeared to be mediated through the release of reactive oxygen species, although it is unclear whether phagocytosis is required for this developmental death. These data are reminiscent of findings in the nematode, where knockdown of phagocytic genes was shown to result in the survival of neuronal precursor cells that would otherwise die during development (Hoepfner et al., 2001; Reddien et al., 2001) indicating a potential role for primary phagocytosis of immature neurons.

Importantly, we and others have found that stressed but viable cells can reversibly expose PS *in vitro* (Tyurina et al., 2007; Jitkaew et al., 2009; Kim et al., 2010; Neher et al., 2011) and therefore macrophages may potentially phagocytose viable cells. In particular, we have shown that microglia activated with LPS (a bacterial TLR4 agonist), lipoteichoic acid (LTA, a bacterial TLR2 agonist), or nanomolar concentrations of A β (an endogenous TLR2/4 agonist) phagocytose viable neurons *in vitro* (Figure 1). We found that microglial activation with these ligands greatly increases their phagocytic activity, and video imaging of the inflamed glial-neuronal cultures showed highly mobile microglia phagocytosing large numbers of neurons appearing morphologically healthy (Neher et al., 2011). Mechanistically, we showed that inflammatory activated microglia release reactive oxygen and nitrogen species, which induce reversible PS exposure on neurons and neuronal phagocytosis by microglia (Neher et al., 2011; Neniskyte et al.,



2011). Specifically, when inflammatory activated microglia were co-cultured with neurons but physically separated by a transwell membrane, neurons showed increased PS exposure but no signs of cell death. When microglia were then allowed to interact with these neurons, the neurons were phagocytosed and thus killed. However, if the activated microglia were removed from the transwell co-culture, the neuronal PS exposure was fully reversed and neurons remained viable for as long as they were cultured. This implied that inflamed microglia release soluble mediators that cause reversible PS exposure on neurons. We identified these soluble mediators to be reactive oxygen or nitrogen species, probably peroxynitrite, based on the findings that the reversible PS exposure and neuronal loss in glial-neuronal cultures is prevented by inhibitors of the NADPH oxidase (PHOX) or nitric oxide synthases (NOS), or addition of superoxide dismutase or a peroxynitrite scavenger. Furthermore, addition of 5–10 μ M peroxynitrite alone caused neuronal loss in the presence of microglia, but reversible PS exposure and no neuronal loss or death in the absence of microglia (Neher et al., 2011).

We further identified a pathway consisting of neuronal PS exposure, recognition of PS by the bridging molecule MFG-E8, and MFG-E8 binding to the VR to be essential for neuronal loss. Again, blocking different components of the PS/MFG-E8/VR pathway (proteins blocking the exposed PS, antibodies to MFG-E8, VR antagonists) rescued neurons, leaving apparently healthy cells behind (Neher et al., 2011; Neniskyte et al., 2011; Fricker et al., 2012). If the neurons had been killed first and then eaten,

then blocking phagocytosis would leave dead neurons. Instead, blocking phagocytosis in LPS or LTA-treated cultures prevented essentially all neuronal loss and death, leaving neurons with intact plasma and mitochondrial membrane potentials, and these neurons remained alive for as long as untreated cultures (Neher et al., 2011).

More recently we have found that LPS-induced neuronal loss is absent in glial-neuronal cultures from *Mfge8* knockout mice, but can be reconstituted by adding purified MFG-E8 to these cultures (Fricker et al., 2012). The absence or presence of MFG-E8 had no apparent effect on microglial inflammation, and this is very strong evidence that the inflammatory neuronal death is mediated by the MFG-E8 pathway of phagocytosis. Further, we have shown that LPS (5 μ g and 1 μ g, respectively) injection into the striatum of rats and mice *in vivo* causes strong microglial inflammation and loss of 20–30% of neurons, which is reduced by approximately 50% through co-injection of a VR inhibitor and in *Mfge8* knockout mice (Fricker et al., 2012).

Similarly, McArthur et al. (2010) recently reported that a microglial cell-line (BV2), when activated with LPS or $A\beta$ phagocytosed viable neuron-like cells (PC12). Although a rescue of neurons by blocking phagocytosis was not shown in this study, these data further support the idea that inflammatory activated microglia may phagocytose stressed but viable neurons, thereby executing neuronal death. Altogether, this suggests the possibility that inflammatory neuronal loss in human pathologies may be prevented by inhibitors of specific phagocytic pathways.

POTENTIAL ROLES OF PRIMARY PHAGOCYTOSIS IN NEURODEGENERATIVE DISEASES

Alzheimer's disease is characterized by extracellular plaques of which the main constituent is A β . These plaques are associated with inflammatory activated microglia, and there is evidence that inflammation may contribute to the disease (Griffin et al., 1998; Combs, 2009). Some studies have indicated a beneficial role of microglia in the disease process, which may be due to phagocytic removal of A β , although it is possible that this effect is mostly mediated by invading (CCR2+) peripheral monocytes rather than resident microglia (Simard et al., 2006; El Khoury et al., 2007). However, it has recently become clear that these data may have been confounded by the fact that many studies investigating monocyte recruitment to the brain used whole-body irradiation, which may prompt invasion of peripheral monocytes. By shielding the brain, a recent study found that peripheral monocytes did not invade the brain and that amyloid clearance was mediated by perivascular myeloid cells (Mildner et al., 2011).

In humans, AD is also characterized by extensive loss of neurons and synapses by means that are not entirely clear. In contrast, in most animal models of AD neuronal loss is limited, restricted to specific brain regions and negatively correlated to plaque load. However, interestingly in these models neuronal loss occurs predominantly in the hippocampus, the area affected most severely in the human condition (Calhoun et al., 1998; Fuhrmann et al., 2010; Rupp et al., 2011).

High concentrations of A β (μ M) can induce direct toxicity to neurons, but low concentrations (nM, which may be more relevant to AD) induce neuronal loss via inflammatory activation of glia *in vitro* (Maezawa et al., 2011). We found that nanomolar concentrations of A β caused microglia to phagocytose viable neurons and synapses in culture, and if we blocked this phagocytosis then all neuronal loss and death was prevented (Neniskyte et al., 2011). This suggests the possibility that microglial phagocytosis of synapses and neurons contributes to AD.

In line with this hypothesis, A β can induce PS exposure on neurons (Mohammad Abdul and Butterfield, 2005), and there appears to be an increase in PS-exposed neurons in AD and mild cognitive impairment, as evidenced by PS-exposing synaptosomes isolated from patients' brains (Bader Lange et al., 2008, 2010). We have found that the PS-binding bridging protein MFG-E8 and its VR mediate inflammatory neuronal loss by primary phagocytosis *in vitro* (Neher et al., 2011; Neniskyte et al., 2011; Fricker et al., 2012). Levels of MFG-E8 are reduced in the brains of AD mice and patients with AD (Boddaert et al., 2007; Fuller and Van Eldik, 2008) possibly due to phagocytosis and degradation, while integrin β_3 , a subunit of the MFG-E8 recognizing VR is strongly upregulated on reactive microglia in AD (Akiyama et al., 1991). However, it has also been reported that MFG-E8 can interact directly with A β (Boddaert et al., 2007), and it therefore remains to be determined whether in AD MFG-E8 mediates phagocytosis of A β or of PS-exposing synapses and/or neurons.

Microglia have been shown to contribute to neuronal loss in an Alzheimer's mouse model (Fuhrmann et al., 2010). Two-photon imaging of neuronal loss in the brain of living triple transgenic APP/PS1/tau AD mice revealed an involvement of microglia in neuron elimination. Microglial number and migration velocity

was increased prior to loss of neurons and knockout of the microglial chemokine receptor CX3CR1, which is critical for microglial chemotaxis, prevented this neuron loss (Fuhrmann et al., 2010). However, testing whether phagocytosis of neurons in AD is primary or secondary to neuronal death by other means *in vivo* is challenging. Primary phagocytosis (unlike apoptosis or necrosis) leaves no cell corpse to diagnose the cause of death. Furthermore, most mouse models expressing mutant APP and presenilins lack significant neuronal loss unless they also include mutant tau, and we currently lack suitable phagocytosis inhibitors that cross the blood–brain barrier. However, in principle, mouse models deficient in phagocytic genes could be used to test for primary phagocytosis.

More support for an involvement of phagocytosis of cells in AD comes from recent genome wide association studies. These analyses revealed that variants in a number of phagocytosis-related genes promote late onset AD, including ApoE, ApoJ (clusterin), ABCA7, and CR1 (Jun et al., 2010; Morgan, 2011). ApoE genotype has the largest effect on AD incidence, and is known to modulate phagocytosis of apoptotic cells *in vitro* and *in vivo* (Grainger et al., 2004). Clusterin expression is upregulated by exposure to phosphatidylserine and it facilitates the uptake of cellular debris through a pathway mediated by the LDL receptor-related protein (LRP), megalin, and other yet undefined endocytic receptors (Bach et al., 2001; Bartl et al., 2001). Optimal ligand-induced signaling through LRP requires the presence of another protein related to increased AD risk, namely ABCA7. After stimulation, ABCA7 is transported to the cell membrane, localizes to the phagocytic cup and enhances the clearance of apoptotic cells *in vitro* and *in vivo* (Jehle et al., 2006). Altogether, accumulating evidence suggests that microglial phagocytosis of neurons may have a role in AD. Since impaired phagocytosis of cells can lead to inflammation, it remains to be determined whether phagocytosis may contribute to AD pathology through primary phagocytosis of viable synapses/neurons or through inflammation resulting from impaired phagocytosis of dying cells.

Parkinson's disease is characterized by motor dysfunction, resulting from progressive loss of neurons, particularly dopaminergic neurons of the pars compacta region of the substantia nigra (SN). The causes of neuronal loss may include: α -synuclein inclusions (which may be directly toxic to neurons), mitochondrial dysfunction, and glial inflammation (Tansey et al., 2007). A role for glial inflammation is suggested by the findings of: (i) inflamed glia and pro-inflammatory cytokines in the SN of patients and animal models, (ii) pro-inflammatory agents, e.g., LPS, causing loss of dopaminergic neurons in culture and *in vivo*, and (iii) anti-inflammatory drugs being protective in patients and animal models (McGeer et al., 1988; Herrera et al., 2005; Block et al., 2007; Tansey et al., 2007). We have found that LPS injection into rodent striatum causes microglial inflammation and neuronal loss reminiscent of the dopaminergic neuronal loss in LPS-induced PD. Using this model, we found that neuronal loss and death is reduced in *Mfge8* knockout mice or by co-injection of phagocytosis inhibitors (Fricker et al., 2012), suggesting that primary phagocytosis may contribute to inflammatory neuronal loss as it may occur in PD.

Substantia nigra neurons are black because they contain neuromelanin. Neuromelanin granules are found within activated microglia of the SN in PD patients, suggesting microglial phagocytosis of neurons (McGeer et al., 1988; Banati et al., 1998), even though there is little evidence of neuronal apoptosis in PD (Banati et al., 1998). Furthermore, addition of human neuromelanin causes microglial activation and dopaminergic neuronal loss in culture and *in vivo*, and this neuronal loss is prevented if a microglial phagocytosis receptor (Mac-1/CR3) is genetically deleted (Zhang et al., 2011b). Thus the neuromelanin-induced neuronal loss could be due to primary phagocytosis, however these results were interpreted in terms of a Mac-1 requirement for phagocytosis of neuromelanin (Zhang et al., 2011b). Interestingly, A β -induced neuronal loss is also dependent on Mac-1 (Zhang et al., 2011a), suggesting either that Mac-1 is an A β receptor or that neuronal loss is occurring by primary phagocytosis.

Importantly, it has been reported that in the 6-hydroxydopamine model of PD microglia become activated before any significant decrease in the number of dopaminergic neurons. Phagocytic microglia are found attached to morphologically intact neurons with normal chromatin distribution, suggesting that microglia may engulf pre-apoptotic neurons precluding the possibility of their recovery (Marinova-Mutafchieva et al., 2009), in accordance with a role for primary phagocytosis in PD.

Frontotemporal lobar degeneration (FTD) has a strong genetic component, and one of the major causes is inactivating mutations in the progranulin gene (Baker et al., 2006; Cruts et al., 2006). Progranulin deficiency leads to exaggerated microglial response after insult as well as to age-dependent accumulation of activated microglia (Ahmed et al., 2007; Yin et al., 2010a,b).

Importantly, progranulin was recently found to inhibit phagocytosis of apoptotic/PS-exposed cells in culture and *in vivo*, and it was suggested that neuronal loss in FTD was due to primary phagocytosis that progranulin normally suppressed (Kao et al., 2011). Strikingly, polymorphisms in the progranulin gene are also associated with AD, PD, and amyotrophic lateral sclerosis (Brouwers et al., 2007, 2008; Sleegers et al., 2008). Therefore it is possible that premature neuronal phagocytosis as observed in the FTD model may also contribute to pathogenesis of other neurodegenerative diseases.

CONCLUSION

We have recently found that inflammatory activated microglia can execute neuronal death through phagocytosis. This primary phagocytosis is a potential mechanism for neuronal loss in neurodegenerative diseases that present with inflammation and microglial activation, and we have reviewed evidence here suggestive of such a mechanism. Primary phagocytosis may have been overlooked as a form of cell death as it is difficult to assess whether a neuron was viable prior to its phagocytosis and because the general assumption has always been that a cell must die before it is phagocytosed. The only way to determine whether phagocytosis mediates cell death is by comparing neuronal survival in the presence and absence of phagocytosis. It will be important to test the contribution of primary phagocytosis to neuronal death using models of neurodegenerative disease in mice that are deficient in phagocytic components.

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