Chapter 7
Developmental Neuronal Elimination

José L. Marín-Teva, Julio Navascués, Ana Sierra, and Michel Mallat

Abstract Microglia, the brain's innate immune cell type, are cells of mesodermal origin that populate the central nervous system (CNS) during early development. Their functions which are best characterized in the developing CNS are related to programmed cell death (PCD), a physiological process that massively affects neural cell lineages and contributes to brain morphogenesis and neuronal network maturation. Although relatively scarce before advanced developmental stages, microglia can remove dead cells in an effective manner due to their migratory and phagocytic behavior. Recent studies indicate that microglia do not only scavenge cell corpses, but also eliminate neural progenitors cells and trigger or induce PCD in different types of developing neurons. Conversely, microglia were also found to promote the neuronal survival by their release of trophic factors. In this chapter we shall discuss the functional involvement of microglia in the loss of neural cells during normal development and review the mechanisms and cell signalling that underlie microglial regulation of PCD and elimination of dead cells.

Keywords Microglia • Neural progenitors • Apoptotic neural cells • Programmed cell death • Survival • Physiology • Development • Phagocytosis • Trophic factors

Bullet Points

- Microglial cells remove apoptotic neural cells by phagocytosis in different central nervous system (CNS) regions from early to advanced development stages.
- The contribution of microglia to the elimination of apoptotic neural cells varies depending on the species, developmental stage, and CNS region.

J.L. Marín-Teva, Ph.D. (✉) • J. Navascués, Ph.D. • A. Sierra
Facultad de Ciencias, Departamento de Biología Celular, Universidad de Granada,
Avda. Fuentenueva s/n, Granada E-18071, Spain
e-mail: jlmarin@ugr.es; navascue@ugr.es; asierra@ugr.es

M. Mallat, M.D. Ph.D.
Centre de Recherche de l'Institut du Cerveau et de la Moelle Epinière,
UPMC/InsermUMR_S1127, CNRS UMR 7225, Hôpital de la Salpêtrière,
47 boulevard de l'Hôpital, Paris 75013, France
e-mail: michel.mallat@upmc.fr

M.-È. Tremblay, A. Sierra (eds.), *Microglia in Health and Disease*,
DOI 10.1007/978-1-4939-1429-6_7

- Secreted “find-me” and exposed “eat-me” signals comprise the central elements of apoptotic cell removal by microglial cells.
- Microglia not only scavenge cell corpses, but also eliminate neural progenitors and trigger or induce programmed cell death in different types of developing neurons.
- Microglia can also promote the survival of some neurons during development through the release of trophic factors.

7.1 Introduction

Microglia are the resident macrophages of the central nervous system (CNS). Since the first studies by del Rio-Hortega, it has generally been accepted that ramified microglia are differentiated cells in the CNS that arise from precursor cells of mesodermal origin with ameboid morphology, which are called ameboid microglia and are present in the developing CNS (Cuadros et al. 1992; del Rio-Hortega 1932; Cuadros and Navascués 1998; Chan et al. 2007; Prinz and Mildner 2011; Prinz et al. 2011). It is now established that most if not all microglia originate from yolk sac-derived primitive myeloid progenitors that seed the CNS during early developmental stages (Alliot et al. 1999; Herbomel et al. 2001; Chan et al. 2007; Ginhoux et al. 2010, 2013; Mizutani et al. 2012; Prinz et al. 2011; Schulz et al. 2012; Kierdorf et al. 2013). During development, microglia infiltrate all CNS regions through extensive cell migration and proliferation. They progressively differentiate into mature ramified cells and become spread throughout the adult CNS. Ramified microglia appear to continuously scan the extracellular environment by extending and retracting their cell processes. Contact inhibition between microglial cells leads to a characteristic spatial arrangement in which microglial cells occupy non-overlapping territories (Davalos et al. 2005; Nimmerjahn et al. 2005). Microglia are highly plastic cells that strongly react to any lesion or pathologies affecting the CNS, and they play key roles in neuroinflammatory reactions (Hanisch and Kettenmann 2007; Saijo and Glass 2011).

Since the discovery of microglial cells, their biology has been widely considered with relation to programmed cell death (PCD) taking place in the CNS during normal development. Histological studies have addressed temporal and spatial relationships between the spread of microglial cells and PCD in the CNS of developing vertebrates (Perry et al. 1985; Ferrer et al. 1990; Ashwell 1990, 1991; Perry and Gordon 1991; Marín-Teva et al. 1999; Peri and Nusslein-Volhard 2008; Calderó et al. 2009; Rigato et al. 2011). Microglial phagocytosis of dying cells has been described in various regions of the developing CNS (Ferrer et al. 1990; Marín-Teva et al. 1999; Dalmau et al. 2003; Peri and Nusslein-Volhard 2008). Besides their debris clearance function, microglial cells actively contribute to cell–cell interactions that trigger or ensure the execution of PCD (Mallat et al. 2005; Bessis et al. 2007; Marín-Teva et al. 2011). Lately, microglial cells were also shown to promote neuronal survival in the developing CNS (Ueno et al. 2013).

In this chapter, we shall review current knowledge on the role of microglial cells in neuronal elimination/survival during normal development.

7.2 Programmed Cell Death of Neurons in the Developing CNS: Where, When, and Which

The large-scale loss of neural cells occurs as a normal and essential stage in CNS maturation during the embryonic and postnatal development of many vertebrate species (Oppenheim 1991; Kuan et al. 2000; Yuan and Yankner 2000; Buss et al. 2006). Cell death affects neuronal and glial cell lineages at different stages of development. The loss of neural cells contributes to the morphological sculpting of the developing CNS and is one of the regressive events involved in the remodelling and functional adaptation of neuronal networks (Oppenheim 1991; Roth and D'Sa 2001). Although a variety of forms of cell death have been reported depending on morphological and biochemical criteria (Edinger and Thompson 2004; Lockshin and Zakeri 2002), this physiological PCD appears to occur by two major pathways: apoptosis, recognized by cell rounding, DNA fragmentation, externalization of phosphatidyl serine (PS), caspase activation, and the absence of inflammatory reaction; and autophagy, characterized by the presence of large vacuoles and the fact that cells can be rescued from death until very late in the process. The boundary between apoptosis and autophagy is not sharply defined, and a complex interplay between the two forms of cell death has been described (reviewed in Booth et al. 2013). However, apoptosis is the dominant type of PCD during normal CNS development (Clarke 1990). The major effect of PCD on projecting neurons during development is well-documented. Thus, during the period in which neuronal connections are established, up to 50 % of numerous types of differentiated neurons undergo cell death (Oppenheim 1991; Raff et al. 1993). This neuronal death is thought to be in part triggered by the shortage of neurotrophic factors (neural growth factor, NGF; brain-derived neurotrophic factor, BDNF; etc.) released by the target cells innervated by these neurons (Barde 1989; Oppenheim 1989; Raff et al. 1993; Snider 1994). Even though less documented, PCD is also observed earlier in development, before synaptogenesis, in populations of undifferentiated cells such as the proliferating neuroepithelial cells and newly postmitotic neuroblasts, where it participates to specific functions (e.g., morphogenic sculpting of the early CNS) and involves various regulatory mechanisms (Kuan et al. 2000; de la Rosa and de Pablo 2000; Yeo and Gautier 2004; Valenciano et al. 2009). It is generally considered that the PCD of neural cells during development requires relatively conserved molecular pathways. These include "proapoptotic" genes of the Bcl-2 family, the apoptosome (cytochrome c, Apaf-1, caspase-9) and downstream caspases (e.g., caspase-3), which lead to the formation of apoptotic bodies that are rapidly phagocytosed and digested by different types of "professional" and "non-professional" phagocytes. Some studies on the in vivo role of caspases in the normal PCD of developing neurons have shown that caspase activation is involved in most cases of neuronal PCD, but is only necessary for the PCD of immature neurons or neuronal precursors at early developmental stages, when neurogenesis is ongoing (Kuan et al. 2000; Oppenheim et al. 2008). Genetic deletion or pharmacological inhibition of caspases was shown to prevent this early type of PCD in the CNS, while being ineffective at

preventing the normal PCD of postmitotic neurons at later developmental stages during the establishment of synaptic connections (Oppenheim et al. 2001, 2008; Yaginuma et al. 2001; Boya et al. 2008). In the absence of caspases, these postmitotic neurons undergo quantitatively normal amounts of PCD by a different caspase-independent pathway that exhibits signs of autophagy (Oppenheim et al. 2008). Recent studies have also reported that the authophagic machinery provides the energy required for proper cell corpse removal and further degradation of apoptotic cells during the neurogenesis period (Mellén et al. 2008; Boya et al. 2008; Aburto et al. 2012).

7.3 Scavenger Role of Microglia in Different CNS Regions from Early to Advanced Development

Cell apoptosis is marked by DNA fragmentation and caspase-triggered cleavage of the cellular proteome (Nicholson 1999), which lead to cell shrinkage but spare the integrity of the plasma membrane up to an advanced stage of the death process. Swift elimination of apoptotic cells by tissue phagocytes is important to prevent secondary cell necrosis involving plasma membrane disruption and leakage of intracellular compounds in the extracellular space, which may be responsible for inflammatory reactions or autoimmune diseases (Nagata et al. 2010). Microglial cells remove apoptotic neural cells by phagocytosis in different CNS regions from early (Sorokin et al. 1992; Cuadros et al. 1991, 1993; Herbomel et al. 1999, 2001; Lichanska and Hume 2000) to more advanced development stages (Hume et al. 1983; Ashwell et al. 1989; Ashwell 1990, 1991; Pearson et al. 1993; Perry et al. 1985; Ferrer et al. 1990; Perry and Gordon 1991; Thanos 1991; Moujahid et al. 1996; Egensperger et al. 1996; Marín-Teva et al. 1999; Upender and Naegele 1999; Rakic and Zecevic 2000; Dalmau et al. 2003; Peri and Nusslein-Volhard 2008; Calderó et al. 2009). In the adult mouse CNS, phagocytic microglia ensure the elimination of dying neuroblasts that derive from neural stem cells in the hippocampal neurogenic niche (Sierra et al. 2010) (see Chap. 10).

The contribution of microglia to the elimination of apoptotic neural cells appears to vary depending on the species, developmental stage, and CNS region studied (Dalmau et al. 2003; Calderó et al. 2009; Sierra et al. 2013). For example, in the cerebral cortex, subcortical white matter, and hippocampus of the in vivo perinatal rat brain, microglial cells engulf virtually all cells undergoing PCD (Dalmau et al. 2003). However, low microglial cell density may be limiting for scavenging activity, especially at the earliest stages of CNS development, when microglial cells remain scarce. During early development of the CNS, in which the phagocytic capacities of microglia appear to be overwhelmed by the number of dying cells, other non-professional phagocytes, such as neighboring neuroepithelial cells, neuroblasts, retinal Müller cells, cerebellar Bergmann glia, and spinal cord astrocytes also contribute to the elimination of apoptotic cells or bodies (O'Connor and Wyttenbach 1974; García-Porrero and Ojeda 1979; Kálmán 1989; Cuadros et al. 1991; Egensperger et al. 1996; Marín-Teva et al. 1999; Parnaik et al. 2000; Mellén et al. 2008).

7.4 How Do Microglia Find and Selectively Engulf Dead Cells in the Developing CNS?

The molecular signalling that triggers microglial phagocytosis during normal development remains a key question to be solved, but this issue clearly benefits from the recent discovery of signals that are generated by apoptotic cells and sensed by phagocytes.

Secreted "find-me," exposed "eat-me," and disabled "don't-eat-me" signals comprise the central elements of apoptotic cell removal by professional phagocytes (Savill and Fadok 2000; Lauber et al. 2004; Ravichandran 2010). Soluble forms of lysophosphatidylcholine (LPC), as well as the chemokine and adhesion molecule CX3CL1 (also known as fractalkine) and the nucleotides ATP and UTP, are known to act as "find-me" signals that are released by apoptotic cells and attract phagocytes (Lauber et al. 2004; Ravichandran 2010). LPC has an attractive chemotactic effect on human monocytic cells and macrophages (Lauber et al. 2003). Moreover, it is released from apoptotic cells through the caspase-3-mediated activation of calcium-independent phospholipase A_2 and is recognized by the phagocyte G-protein-coupled receptor G2A (Lauber et al. 2003; Peter et al. 2008). On the other hand, fractalkine was found to be released before the loss of plasma membrane integrity by apoptotic lymphocytes via a caspase-regulated mechanism and to have an attractive effect on macrophages expressing the fractalkine receptor CX3CR1, as inferred from in vitro and in vivo studies carried out in humans and mice (Truman et al. 2008). Microglial cells are known to be the only CNS cells that express CX3CR1 (Jung et al. 2000). Interestingly, fractalkine released from cultured neurons damaged by glutamatergic excitotoxicity promotes the phagocytosis of cell debris by microglial cells (Noda et al. 2011). However, the actual role of G2A or CX3CR1 in the elimination of neural cell corpses during development has not yet been established. Finally, it was reported that the caspase-dependent release of ATP and UTP during the early stages of apoptosis in thymocytes acts as a "find-me" signal to promote phagocytic clearance by human monocytes (Elliott et al. 2009). Nucleotides could possibly act as "find-me" signals in the normal developing CNS, but this hypothesis needs to be experimentally tested. In particular, extracellular ATP/ADP has chemotactic effects on microglial cells by binding to $P2Y_{12}$ purinergic receptors (Davalos et al. 2005; Nimmerjahn et al. 2005; Haynes et al. 2006; Ohsawa and Kohsaka 2011). Costimulation of microglial P2Y receptors and adenosine (A1 type) receptors appears to be required for microglial chemotaxis towards ATP. This cell response is prevented in purified microglia, obtained by shaking mixed glial cell cultures, which are derived from the brain of newborn mice deficient in the expression of CD39, an ectonucleotidase that degrades nucleotides to nucleosides (Farber et al. 2008).

Once recruited in the vicinity of apoptotic cells, phagocytes can selectively recognize the dead cells as prey to be engulfed due to their expression of "eat-me" signals. In mammals, the best-characterized "eat-me" signal is PS, a cell membrane component that translocates from the inner to outer leaflet of the plasma membrane

during the apoptotic process (Fadok et al. 2000). Externalized PS can stimulate phagocytosis of apoptotic cells through direct binding to phagocyte receptors such as T cell immunoglobulin- and mucin-domain containing molecule 4 (Tim-4) (Miyanishi et al. 2007), brain-specific angiogenesis inhibitor-1 (BAI1) (Park et al. 2007) or stabilin-2 (Park et al. 2008). PS recognition by phagocytes can also involve several "bridging" molecules, binding to both PS and phagocyte receptors, which are released in extracellular fluid (Erwig and Henson 2008). Among these, milk fat globule epidermal growth factor-8 (MFG-E8) and C3bi can bind to PS and are then recognized by integrins expressed by macrophages/microglia such as vitronectin receptor (αvβ3) and complement-receptor-3 (CR3/CD11b), respectively (Hanayama et al. 2002; Mevorach et al. 1998; Savill and Fadok 2000). The bridging molecules and receptors responsible for PS-mediated clearance of dead cells (see Sierra et al. 2013 for a review) are not yet clearly defined in the developing CNS, but the capacity of purified microglial cells to engulf PS-coated cells or particles is well-documented (Witting et al. 2000; Konduru et al. 2009; Neher et al. 2011; Liu et al. 2013).

Another possible "eat-me" signal is the endogenous cellular ligand for the triggering receptor expressed on myeloid cells 2 (TREM2). TREM2 is specifically expressed by microglial cells, as demonstrated by in vivo immunocytochemical studies in different regions of the mouse brain (Hsieh et al. 2009). Its ligand is upregulated on apoptotic neurons, mediates signal transduction by association with DNAX adaptor protein-12 (DAP12) on microglia, and promotes the phagocytosis of dying neurons in cell cultures (Takahashi et al. 2005; Hsieh et al. 2009; Neumann et al. 2009). Finally, another in vivo and in vitro study demonstrated that activation of $P2Y_6$ receptors in microglial cells by UTP/UDP released from damaged neurons triggers the clearance of dying cells by phagocytosis (Koizumi et al. 2007). Hence, in addition to PS, the ligands for TREM2 and UTP/UDP may be considered as putative "eat-me" signals promoting microglial phagocytosis during development.

7.5 PCD and Microglial Cell Distribution in the Developing CNS

Besides its role in the elimination of dead cells, apoptotic cell-to-phagocyte signalling clearly fulfills the expected criteria for a mechanism that underlies microglia distribution in the developing CNS. Consistent with the recent demonstration that dying cells release chemotactic "find-me" signals, the codetection of microglia and dying cells provided evidence that local PCD may account for the entry, local spread, or transient clustering of macrophages/microglia in different CNS regions of embryonic vertebrates, including the retina (Santos et al. 2008), spinal cord (Rigato et al. 2011), choroid plexus (Swinnen et al. 2013), cerebellum, or cerebral cortex (Ashwell 1990, 1991). However, microglia infiltration in cell layers of the developing CNS is not always correlated with the occurrence of PCD (Marín-Teva et al. 1999), and it involves different molecular cues unrelated to the signalling

generated by dying cells. In particular, developing neural cells can produce chemoattractants that target macrophages/microglia, such as chemokines (Ransohoff 2009), macrophage colony-stimulating factor 1 (M-CSF), or vascular endothelial growth factor A (VEGF-A) (Breier et al. 1992; Nandi et al. 2012; Lelli et al. 2013). In zebra fish embryos, M-CSF receptor expressed by mononuclear phagocytes is required for the early infiltration of yolk-sac-derived phagocytes in the CNS (Herbomel et al. 2001), whereas VEGF receptor 1 signalling promotes early postnatal infiltration of microglia in deep layers of the mouse cerebral cortex (Lelli et al. 2013). Furthermore, microglia appear to use radially oriented processes of glial or neural progenitors cells as a substrate for cell migration in the retina (Sánchez-López et al. 2004), spinal cord (Rigato et al. 2011), and cerebral cortex (Swinnen et al. 2013). Strikingly, ameboid microglial cells migrate in embryonic quail retina following well-defined routes that are not altered when they pass close to regions of abundant cell death, and they only engulf apoptotic bodies encountered along these routes (Marín-Teva et al. 1999). This observation suggests that cell guiding cues unrelated to PCD may outcompete "find-me" signals emitted by dead cells. Alternatively, the capacity of microglial cells to sense "find-me" (and/or "eat-me") signals may vary according to their localization in the developing CNS. In connection with this possibility, the heterogeneity of microglial cell phenotypes during normal development is well-documented (Hristova et al. 2010; Verney et al. 2010; Scheffel et al. 2012; Arnoux et al. 2013). Moreover, recent studies have emphasized that the functional implications of microglial recruitment are not limited to the scavenging of dead cells. Microglia fulfill other roles during development, including the remodelling of developing synapses (Hoshiko et al. 2012; Tremblay et al. 2010; Paolicelli et al. 2011; Schafer et al. 2012), homeostatic regulation of neuronal firing (Ji et al. 2013) (Chap. 9), stimulation of angiogenesis (Checchin et al. 2006; Fantin et al. 2010) (Chap. 8), and the induction or prevention of developmental cell death, which is discussed below.

7.6 Switching Programmed Cell Death On and Off in the Developing CNS: A Dual Role for Microglia

Various studies have demonstrated that microglial cells not only have a scavenger role during development, but can also trigger or promote PCD in different types of developing neurons. The mechanisms involved in the induction of PCD by microglial cells are diverse and can be classified into two groups: phagocytosis-unrelated PCD induction; and PCD triggering/execution involving phagocytosis, designated as "engulfment-promoted PCD."

The former group relies in part on the capacity of microglia to produce neuronal growth factors such as neurotrophins (Mallat et al. 1989; Elkabes et al. 1996). Frade and Barde (1998) showed that nerve growth factor (NGF) is produced in vivo during early chick embryo development by primitive macrophages and microglial cells, which are present in the vitreous body and scattered within the

retinal neuroepithelium between embryonic day 3 (E3) and E6. In addition, in vitro experiments in cultured eye cups demonstrated that macrophage/microglial production of NGF triggers PCD in retinal progenitor cells expressing neurotrophin receptor p75 at E3 (Fig. 7.1a). Another type of phagocytosis-independent involvement of macrophages in PCD was observed in the embryonic rat spinal cord, in which motoneurons undergo PCD from E15. Experiments in cultured explants of ventral horns of rat embryo spinal cord showed that embryonic motoneurons acquire the competence to undergo PCD between E12 and E13, 2 days before the onset of cell death (Sedel et al. 2004). This neuronal commitment to a death fate appears to be driven by tumor necrosis factor α (TNF-α) released from primitive macrophages that invade the surrounding somitic mesenchyme at these developmental stages (Fig. 7.1b). Macrophage-derived TNF-α signals through the TNF-α receptor 1 expressed by embryonic motoneurons (Sedel et al. 2004). Subsequent in vivo studies provided evidence that these somitic macrophages eventually infiltrate the spinal cord and therefore contribute to microglial development (Rigato et al. 2011).

Engulfment-promoted PCD was observed in postmitotic neurons in the cerebellum of 3 day-old (P3) mice (Marín-Teva et al. 2004); at this developmental stage, Purkinje cells initiate a death program marked by caspase-3 activation. In vivo observations in the P3 mouse cerebellum and in vitro experimental studies in P3 cerebellar slices showed that the majority of these neurons are engulfed by microglial cells that actively prevent abortion of the death program in the engulfed cells (Marín-Teva et al. 2004). In the developing mouse CNS, microglia express all of the genes encoding multimeric Nox1- and Nox2-dependent NADPH oxidases that catalyze the formation of superoxide ions (Bedard and Krause 2007; Chéret et al. 2008). Activation of microglial NADPH oxidases is detectable at early postnatal stages and is required for microglia-promoted Purkinje cell death (Fig. 7.1c$_1$) (Marín-Teva et al. 2004; Lelli et al. 2013). This mechanism is reminiscent of the innate immune response in which bacteria or fungi ingested by neutrophils or macrophages are neutralized by reactive oxygen species derived from the activation of phagocyte Nox2-dependent NADPH-oxidase (Bedard and Krause 2007; Lam et al. 2010). It is noteworthy that the involvement of engulfing cells in the execution of developmental cell death is not limited to vertebrates (Mallat et al. 2005). It is well documented

Fig. 7.1 (continued) MiCs promote neuron death by the engulfment of differentiated neurons (DNs) undergoing early reversible steps of cell death (**c$_1$**) and healthy neural progenitor cells (**c$_2$**). (**c$_1$**). In the developing cerebellum (Marín-Teva et al. 2004) and hippocampus (Wakselman et al. 2008), DNs in early stages of cell death express activated caspase-3 and show putative "eat-me" signals on their surface. These signals are recognized by the β2-integrin CD11b and the immunoreceptor DAP12 on the MiC surface, thereby triggering neuron engulfment and the progression of late stages of cell death, which are promoted by microglial-derived apoptotic effectors such as superoxide ions ($O_2^{\bullet-}$). (**c$_2$**). MiCs colonize proliferative zones in the developing cerebral cortex and phagocytose postmitotic neural progenitors (NPs) that show no sign of cell death, thereby contributing to regulate the size of the NP pool (Cunningham et al. 2013). (**d**) In the developing cerebral cortex, MiCs recruited in the subcortical white matter limit the extent of PCD in DNs of the cortical layer V through production of insulin-like growth factor 1 (IGF-1) (Ueno et al. 2013)

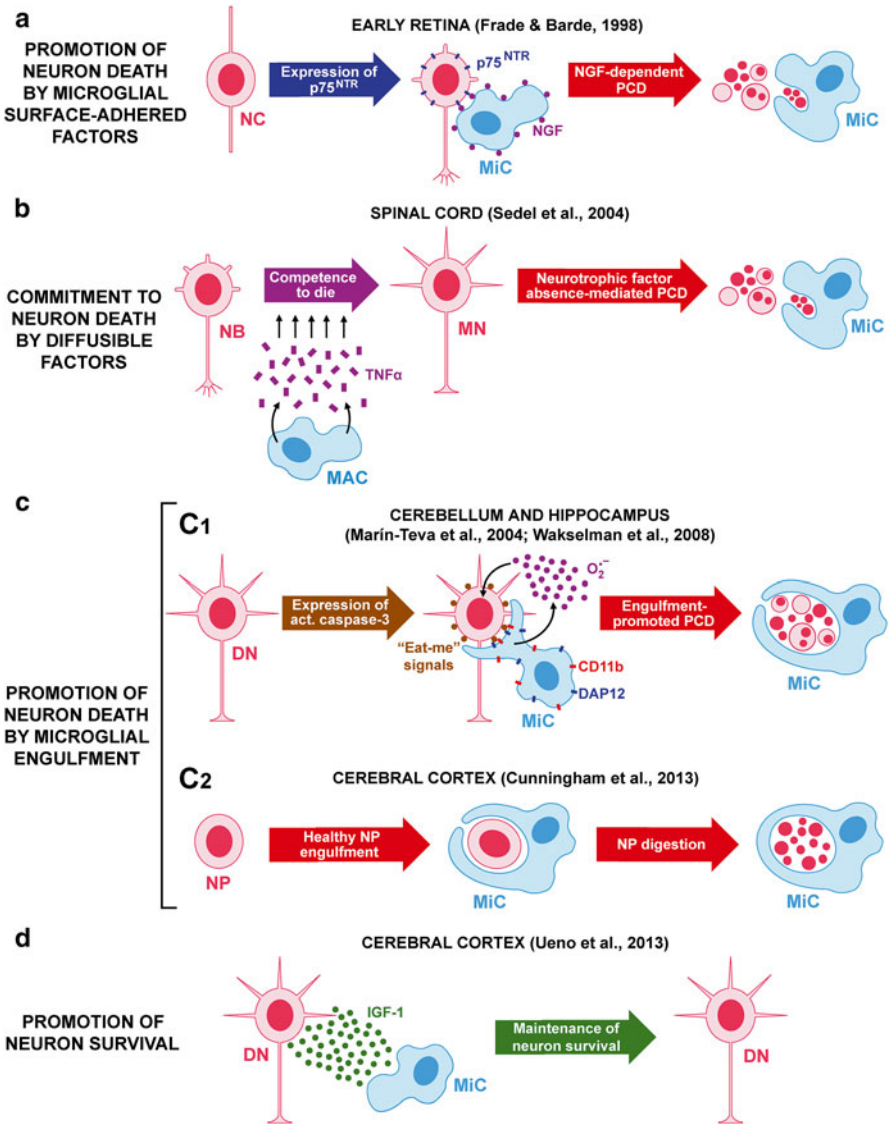

Fig. 7.1 Dual role of microglial cells in promoting either the death or survival of neurons and neural progenitor cells, as demonstrated by studies performed in different parts of the developing central nervous system (CNS). (**a**) In the early retina, ameboid microglial cells (MiCs) are involved in the death of some neuroepithelial cells (NCs) expressing the p75 neurotrophin receptor ($p75^{NTR}$) by producing nerve growth factor (NGF). The NGF secreted by MiCs remains bound to the cell surface and promotes NC death after $p75^{NTR}$ stimulation (Frade and Barde 1998). (**b**) Primitive macrophages (MACs) have a role in committing differentiating motoneurons (MNs) in the developing spinal cord to death (Sedel et al. 2004). These MNs acquire competence to die through signalling via the tumor necrosis factor α (TNF-α) released from MACs in adjacent somites. Two days after TNF-α signalling, committed MNs undergo neurotrophic PCD, and cell debris is phagocytosed by ameboid MiCs invading the spinal cord. (**c**) In other developing CNS regions,

in the nematode *Caenorhabditis elegans*, in which "death-committed" cells expressing activated CED-3 (homologue to vertebrate caspase-3) can be rescued by inactivation of genes that control the engulfment of cell corpses (Hoeppner et al. 2001; Reddien et al. 2001). Therefore, it was suggested that live Purkinje cells committed to a death fate readily express "eat me" signals, which stimulate engulfing behavior and superoxide production in microglia (Marín-Teva et al. 2004).

Further insights into this issue came from an in vivo study in newborn mice, which showed that, similar to developing Purkinje cells, hippocampal neurons in contact with microglia undergo PCD through a mechanism requiring the microglial production of superoxide ions (Wakselman et al. 2008). Both microglial superoxide generation and neuronal death were found to be reduced in newborn mice bearing a mutation preventing expression of the CD11b integrin subunit or activity of the DAP12 signalling protein. CD11b/CD18 (αMβ2) functions as an engulfment receptor of prey tagged with the C3bi complement component (Bohana-Kashtan et al. 2004). The involvement of complement components in developmental neuronal death has not yet been established, whereas a recent investigation shows that C3bi-CD11b signalling contributes to microglia-mediated synapse elimination during normal development (Schafer et al. 2012). DAP12 is a transmembrane-anchored signalling adaptor containing an immunoreceptor tyrosine-based activation motif that transmits signals from CD11b/CD18 and other immunoreceptors (Ivashkiv 2009). Among these, TREM2 and β3 integrins are expressed by microglia and can directly or indirectly bind to "eat-me" signals, including PS. Whether these receptors contribute to the DAP12-dependent loss of hippocampal neurons (Wakselman et al. 2008) has not been addressed. However, recent in vitro studies show that a transient PS externalization can occur in neurons cultured in the presence of microglial cells under proinflammatory conditions. Although these PS-tagged neurons remain viable, they are recognized and engulfed by microglia via mechanisms involving the cross-linking of neuronal PS and microglial αvβ3 integrin (vitronectin receptor) by the MFG-E8 bridging protein, and in fact, neuronal death is executed by microglial phagocytosis (Neher et al. 2011; Fricker et al. 2012).

In the developing CNS, the engulfment-mediated loss of cells is not restricted to postmitotic neurons. Thus, a study carried out in vivo, as well as in in vitro cultured slices, revealed that microglia limit the production of cells in the developing cerebral cortex of macaques and rats by phagocytosing neural progenitor cells, mostly during late stages of cortical neurogenesis (Cunningham et al. 2013). Interestingly, this study found that most neural progenitor cells contacted or engulfed by microglial cells in the cortical proliferative zones show no signs of cell death or apoptosis as defined by the expression of cleaved caspase-3, TUNEL-labelling of fragmented DNA, PS exposure, or nuclear breakdown. The mechanisms by which microglia recognize these healthy progenitor cells as a prey to be engulfed have not been determined. Nevertheless, these findings reveal a new type of engulfment-promoted cell elimination during development, with microglial cells eliminating viable neural progenitor cells through a process unrelated to apoptosis (Fig. 7.1c$_2$).

Besides engulfing behavior and the generation of prodeath signals, microglia can produce various neuronal growth factors that may directly support the survival

of neurons or the growth of their processes. These functional capacities were documented in culture studies more than 20 years ago (Mallat and Chamak 1994), and their relevance to normal brain development was recently demonstrated in an in vivo and in vitro study of the mouse cerebral cortex (Fig. 7.1d) (Ueno et al. 2013). It was observed that, during early postnatal stages (P3–P7), microglial cells recruited in the subcortical white matter limit the extent of PCD in corticospinal and callosal neurons, the cell bodies of which are localized in the cortical cell layer V. This neurotrophic influence is mediated by the microglial production of insulin-like growth factor 1 (IGF-1), as shown in vitro in cocultures of cortical neurons and microglia (Ueno et al. 2013). The apparently contradictory roles of microglia (elimination of viable cells versus trophic effect on neuron survival) revealed by the two studies performed in the rat and mouse cerebral cortex (Cunningham et al. 2013; Ueno et al. 2013, Fig. 7.1c$_2$, d) are in agreement with the current view that microglia have a dual role on cell death depending on the microenvironment and interactions with other cell types (Hanisch and Kettenmann 2007; Mallat and Chamak 1994; Czeh et al. 2011). Microglia analyzed in the above studies act in different microenvironments and at different developmental periods. Thus, the study by Cunningham et al. (2013) reported that microglial phagocytosis of neural precursor cells occurs in the proliferative ventricular zone of E19-P2 rat embryo cerebral cortex, whereas Ueno et al. (2013) observed microglia-promoted survival of differentiated corticospinal neurons, through IGF-1 signaling, in the cortical layer V of the P3–P5 mouse brain. These differences may reflect functional heterogeneity of microglia driven by diverse environmental cues. They may also arise from the selective capacities of target cells to express compounds required for microglial phagocytic or neurotrophic activities, such as “eat-me” signals, the IGF-1 receptor or other signaling components.

7.7 Concluding Remarks

Taken together, current evidence indicates that microglia not only eliminate dead cells, but also play an important role in cell–cell interactions that regulate PCD in the developing CNS. Notably, the signalling by which microglia can promote PCD is diversified. Studies of the developing cerebral cortex have revealed that microglia exert a dual influence on local cell production, promoting the elimination of progenitor cells but preventing the loss of differentiated neurons. It is now clear that the role of microglial phagocytosis extends beyond the elimination of dead cells, as it plays a part in the mechanisms that determine the death fate of neural cells during brain development. The engulfment of apoptotic cells was also shown to impact on the ability of the engulfing cells to produce cytokines or lipid mediators (Elliott and Ravichandran 2010). Therefore, microglial phagocytosis could additionally modulate microglial capacity to influence neural cell survival, growth, or differentiation during normal development. This issue warrants further investigation. The variety of mechanisms by which microglia regulate developmental neural cell death, as

reviewed here, is consistent with the high functional plasticity of microglia, whose behavioral repertoire appears to be location- and time-specific. The physiological effects of microglia on neural cell survival is most likely tuned by the local cell environment or by specific interactions between microglia and target cells, which may change according to the CNS region and developmental time.

Acknowledgments This study was supported by grants from the Spanish Ministry of Science and Innovation (BFU2010-19981) and from Agence Nationale de la Recherche (ANR-06-NEURO-028). The authors thank Richard Davies for improving the English.

Statement of Interest: None.

References

Aburto MR, Sánchez-Calderón H, Hurlé JM, Varela-Nieto I, Magariños M (2012) Early otic development depends on autophagy for apoptotic cell clearance and neural differentiation. Cell Death Dis 3:e394

Alliot F, Godin I, Pessac B (1999) Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. Brain Res Dev Brain Res 117:145–152

Arnoux I, Hoshiko M, Mandavy L, Avignone E, Yamamoto N, Audinat E (2013) Adaptive phenotype of microglial cells during the normal postnatal development of the somatosensory "Barrel" cortex. Glia 61:1582–1594

Ashwell K (1990) Microglia and cell death in the developing mouse cerebellum. Brain Res Dev Brain Res 55:219–230

Ashwell K (1991) The distribution of microglia and cell death in the fetal rat forebrain. Brain Res Dev Brain Res 58:1–12

Ashwell KW, Holländer H, Streit W, Stone J (1989) The appearance and distribution of microglia in the developing retina of the rat. Vis Neurosci 2:437–448

Barde YA (1989) Trophic factors and neuronal survival. Neuron 2:1525–1534

Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87:245–313

Bessis A, Bechade C, Bernard D, Roumier A (2007) Microglial control of neuronal death and synaptic properties. Glia 55:233–238

Bohana-Kashtan O, Ziporen L, Donin N, Kraus S, Fishelson Z (2004) Cell signals transduced by complement. Mol Immunol 41:583–597

Booth LA, Tavallai S, Hamed HA, Cruickshanks N, Dent P (2013) The role of cell signalling in the crosstalk between autophagy and apoptosis. Cell Signal 26:549–555

Boya P, Mellén MA, de la Rosa EJ (2008) How autophagy is related to programmed cell death during the development of the nervous system. Biochem Soc Trans 36:813–817

Breier G, Albrecht U, Sterrer S, Risau W (1992) Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development 114:521–532

Buss RR, Sun W, Oppenheim RW (2006) Adaptive roles of programmed cell death during nervous system development. Annu Rev Neurosci 29:1–35

Calderó J, Brunet N, Ciutat D, Hereu M, Esquerda JE (2009) Development of microglia in the chick embryo spinal cord: implications in the regulation of motoneuronal survival and death. J Neurosci Res 87:2447–2466

Chan WY, Kohsaka S, Rezaie P (2007) The origin and cell lineage of microglia: new concepts. Brain Res Rev 53:344–354

Checchin D, Sennlaub F, Levavasseur E, Leduc M, Chemtob S (2006) Potential role of microglia in retinal blood vessel formation. Invest Ophthalmol Vis Sci 47:3595–3602

Chéret C, Gervais A, Lelli A, Colin C, Amar L, Ravassard P, Mallet J, Cumano A, Krause KH, Mallat M (2008) Neurotoxic activation of microglia is promoted by a Nox1-dependent NADPH oxidase. J Neurosci 28:12039–12051

Clarke PG (1990) Developmental cell death: morphological diversity and multiple mechanisms. Anat Embryol (Berl) 181:195–213

Cuadros MA, Navascués J (1998) The origin and differentiation of microglial cells during development. Prog Neurobiol 56:173–189

Cuadros MA, García-Martín M, Martin C, Ríos A (1991) Haemopoietic phagocytes in the early differentiating avian retina. J Anat 177:145–158

Cuadros MA, Coltey P, Carmen Nieto M, Martin C (1992) Demonstration of a phagocytic cell system belonging to the hemopoietic lineage and originating from the yolk sac in the early avian embryo. Development 115:157–168

Cuadros MA, Martin C, Coltey P, Almendros A, Navascués J (1993) First appearance, distribution, and origin of macrophages in the early development of the avian central nervous system. J Comp Neurol 330:113–129

Cunningham CL, Martínez-Cerdeño V, Noctor SC (2013) Microglia regulate the number of neural precursor cells in the developing cerebral cortex. J Neurosci 33:4216–4233

Czeh M, Gressens P, Kaindl AM (2011) The yin and yang of microglia. Dev Neurosci 33: 199–209

Dalmau I, Vela JM, González B, Finsen B, Castellano B (2003) Dynamics of microglia in the developing rat brain. J Comp Neurol 458:144–157

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8: 752–758

de la Rosa EJ, de Pablo F (2000) Cell death in early neural development: beyond the neurotrophic theory. Trends Neurosci 23:454–458

del Rio-Hortega P (1932) Microglia. In: Penfield W (ed) Cytology and cellular pathology of the nervous system, vol 2. Hafner, New York, pp 483–534

Edinger AL, Thompson CB (2004) Death by design: apoptosis, necrosis and autophagy. Curr Opin Cell Biol 16:663–669

Egensperger R, Maslim J, Bisti S, Holländer H, Stone J (1996) Fate of DNA from retinal cells dying during development: uptake by microglia and macroglia (Müller cells). Brain Rev Dev Brain Res 97:1–8

Elkabes S, DiCicco-Bloom EM, Black IB (1996) Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. J Neurosci 16: 2508–2521

Elliott MR, Ravichandran KS (2010) Clearance of apoptotic cells: implications in health and disease. J Cell Biol 189:1059–1070

Elliott MR, Chekeni FB, Trampont PC, Lazarowski ER, Kadl A, Walk SF, Park D, Woodson RI, Ostankovich M, Sharma P, Lysiak JJ, Harden TK, Leitinger N, Ravichandran KS (2009) Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature 461:282–286

Erwig LP, Henson PM (2008) Clearance of apoptotic cells by phagocytes. Cell Death Differ 15: 243–250

Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM (2000) A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature 405:85–90

Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C (2010) Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood 116:829–840

Farber K, Markworth S, Pannasch U, Nolte C, Prinz V, Kronenberg G, Gertz K, Endres M, Bechmann I, Enjyoji K, Robson SC, Kettenmann H (2008) The ectonucleotidase cd39/ENTPDase1 modulates purinergic-mediated microglial migration. Glia 56:331–341

Ferrer I, Bernet E, Soriano E, del Rio T, Fonseca M (1990) Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. Neuroscience 39: 451–458

Frade JM, Barde YA (1998) Microglia-derived nerve growth factor causes cell death in the developing retina. Neuron 20:35–41

Fricker M, Neher JJ, Zhao JW, Thery C, Tolkovsky AM, Brown GC (2012) MFG-E8 mediates primary phagocytosis of viable neurons during neuroinflammation. J Neurosci 32:2657–2666

García-Porrero JA, Ojeda JL (1979) Cell death and phagocytosis in the neuroepithelium of the developing retina. A TEM and SEM study. Experientia 35:375–376

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841–845

Ginhoux F, Lim S, Hoeffel G, Low D, Huber T (2013) Origin and differentiation of microglia. Front Cell Neurosci 7:45

Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S (2002) Identification of a factor that links apoptotic cells to phagocytes. Nature 417:182–187

Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci 10:1387–1394

Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, Julius D (2006) The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat Neurosci 9: 1512–1519

Herbomel P, Thisse B, Thisse C (1999) Ontogeny and behaviour of early macrophages in the zebrafish embryo. Development 126:3735–3745

Herbomel P, Thisse B, Thisse C (2001) Zebrafish early macrophages colonize cephalic mesenchyme and developing brain, retina, and epidermis through a M-CSF receptor-dependent invasive process. Dev Biol 238:274–288

Hoeppner DJ, Hengartner MO, Schnabel R (2001) Engulfment genes cooperate with ced-3 to promote cell death in Caenorhabditis elegans. Nature 412:202–206

Hoshiko M, Arnoux I, Avignone E, Yamamoto N, Audinat E (2012) Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. J Neurosci 32:15106–15111

Hristova M, Cuthill D, Zbarsky V, Acosta-Saltos A, Wallace A, Blight K, Buckley SM, Peebles D, Heuer H, Waddington SN, Raivich G (2010) Activation and deactivation of periventricular white matter phagocytes during postnatal mouse development. Glia 58:11–28

Hsieh CL, Koike M, Spusta SC, Niemi EC, Yenari M, Nakamura MC, Seaman WE (2009) A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia. J Neurochem 109:1144–1156

Hume DA, Perry VH, Gordon S (1983) Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. J Cell Biol 97:253–257

Ivashkiv LB (2009) Cross-regulation of signaling by ITAM-associated receptors. Nat Immunol 10:340–347

Ji K, Akgul G, Wollmuth LP, Tsirka SE (2013) Microglia actively regulate the number of functional synapses. PLoS One 8:e56293

Jung S, Aliberti J, Graemmel P, Sunshine MJ, Kreutzberg GW, Sher A, Littman DR (2000) Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Mol Cell Biol 20:4106–4114

Kálmán M (1989) Dead cells can be phagocytosed by any neighboring cell in early developing rat brain. Int J Neurosci 46:139–145

Kierdorf K, Erny D, Goldmann T, Sander V, Schulz C, Perdiguero EG, Wieghofer P, Heinrich A, Riemke P, Hölscher C, Müller DN, Luckow B, Brocker T, Debowski K, Fritz G, Opdenakker G, Diefenbach A, Biber K, Heikenwalder M, Geissmann F, Rosenbauer F, Prinz M (2013) Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat Neurosci 16:273–280

Koizumi S, Shigemoto-Mogami Y, Nasu-Tada K, Shinozaki Y, Ohsawa K, Tsuda M, Joshi BV, Jacobson KA, Kohsaka S, Inoue K (2007) UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature 446:1091–1095

Konduru NV, Tyurina YY, Feng W, Basova LV, Belikova NA, Bayir H, Clark K, Rubin M, Stolz D, Vallhov H, Scheynius A, Witasp E, Fadeel B, Kichambare PD, Star A, Kisin ER, Murray AR, Shvedova AA, Kagan VE (2009) Phosphatidylserine targets single-walled carbon nanotubes to professional phagocytes in vitro and in vivo. PLoS One 4:e4398

Kuan CY, Roth KA, Flavell RA, Rakic P (2000) Mechanisms of programmed cell death in the developing brain. Trends Neurosci 23:291–297

Lam GY, Huang J, Brumell JH (2010) The many roles of NOX2 NADPH oxidase-derived ROS in immunity. Semin Immunopathol 32:415–430

Lauber K, Bohn E, Kröber SM, Xiao YJ, Blumenthal SG, Lindemann RK, Marini P, Wiedig C, Zobywalski A, Baksh S, Xu Y, Autenrieth IB, Schulze-Osthoff K, Belka C, Stuhler G, Wesselborg S (2003) Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. Cell 113:717–730

Lauber K, Blumenthal SG, Waibel M, Wesselborg S (2004) Clearance of apoptotic cells: getting rid of the corpses. Mol Cell 14:277–287

Lelli A, Gervais A, Colin C, Chéret C, Ruiz de Almodovar C, Carmeliet P, Krause KH, Boillée S, Mallat M (2013) The NADPH oxidase Nox2 regulates VEGFR1/CSF-1R-mediated microglial chemotaxis and promotes early postnatal infiltration of phagocytes in the subventricular zone of the mouse cerebral cortex. Glia 61:1542–1555

Lichanska AM, Hume DA (2000) Origins and functions of phagocytes in the embryo. Exp Hematol 28:601–611

Liu Y, Yang X, Guo C, Nie P, Ma J (2013) Essential role of MFG-E8 for phagocytic properties of microglial cells. PLoS One 8:e55754

Lockshin RA, Zakeri Z (2002) Caspase-independent cell deaths. Curr Opin Cell Biol 14:727–733

Mallat M, Chamak B (1994) Brain macrophages: neurotoxic or neurotrophic effector cells? J Leukoc Biol 56:416–422

Mallat M, Houlgatte R, Brachet P, Prochiantz A (1989) Lipopolysaccharide-stimulated rat brain macrophages release NGF in vitro. Dev Biol 133:309–311

Mallat M, Marín-Teva JL, Chéret C (2005) Phagocytosis in the developing CNS: more than clearing the corpses. Curr Opin Neurobiol 15:101–107

Marín-Teva JL, Cuadros MA, Calvente R, Almendros A, Navascués J (1999) Naturally occurring cell death and migration of microglial precursors in the quail retina during normal development. J Comp Neurol 412:255–275

Marín-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M (2004) Microglia promote the death of developing Purkinje cells. Neuron 41:535–547

Marín-Teva JL, Cuadros MA, Martín-Oliva D, Navascués J (2011) Microglia and neuronal cell death. Neuron Glia Biol 7:25–40

Mellén MA, de la Rosa EJ, Boya P (2008) The autophagic machinery is necessary for removal of cell corpses from the developing retinal neuroepithelium. Cell Death Differ 15:1279–1290

Mevorach D, Mascarenhas JO, Gershov D, Elkon KB (1998) Complement-dependent clearance of apoptotic cells by human macrophages. J Exp Med 188:2313–2320

Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura T, Nagata S (2007) Identification of Tim4 as a phosphatidylserine receptor. Nature 450:435–439

Mizutani M, Pino PA, Saederup N, Charo IF, Ransohoff RM, Cardona AE (2012) The fractalkine receptor but not CCR2 is present on microglia from embryonic development throughout adulthood. J Immunol 188:29–36

Moujahid A, Navascués J, Marín-Teva JL, Cuadros MA (1996) Macrophages during avian optic nerve development: relationship to cell death and differentiation into microglia. Anat Embryol (Berl) 193:131–144

Nagata S, Hanayama R, Kawane K (2010) Autoimmunity and the clearance of dead cells. Cell 140:619–630

Nandi S, Gokhan S, Dai XM, Wei S, Enikolopov G, Lin H, Mehler MF, Stanley ER (2012) The CSF-1 receptor ligands IL-34 and CSF-1 exhibit distinct developmental brain expression patterns and regulate neural progenitor cell maintenance and maturation. Dev Biol 367:100–113

Neher JJ, Neniskyte U, Zhao JW, Bal-Price A, Tolkovsky AM, Brown GC (2011) Inhibition of microglial phagocytosis is sufficient to prevent inflammatory neuronal death. J Immunol 186:4973–4983

Neumann H, Kotter MR, Franklin RJ (2009) Debris clearance by microglia: an essential link between degeneration and regeneration. Brain 132:288–295

Nicholson DW (1999) Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ 6:1028–1042

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308:1314–1318

Noda M, Doi Y, Liang J, Kawanokuchi J, Sonobe Y, Takeuchi H, Mizuno T, Suzumura A (2011) Fractalkine attenuates excito-neurotoxicity via microglial clearance of damaged neurons and antioxidant enzyme heme oxygenase-1 expression. J Biol Chem 286:2308–2319

O'Connor TM, Wyttenbach CR (1974) Cell death in the embryonic chick spinal cord. J Cell Biol 60:448–459

Ohsawa K, Kohsaka S (2011) Dynamic motility of microglia: purinergic modulation of microglial movement in the normal and pathological brain. Glia 59:1793–1799

Oppenheim RW (1989) The neurotrophic theory and naturally occurring motoneuron death. Trends Neurosci 12:252–255

Oppenheim RW (1991) Cell death during development of the nervous system. Annu Rev Neurosci 14:453–501

Oppenheim RW, Flavell RA, Vinsant S, Prevette D, Kuan CY, Rakic P (2001) Programmed cell death of developing mammalian neurons after genetic deletion of caspases. J Neurosci 21:4752–4760

Oppenheim RW, Blomgren K, Ethell DW, Koike M, Komatsu M, Prevette D, Roth KA, Uchiyama Y, Vinsant S, Zhu C (2008) Developing postmitotic mammalian neurons in vivo lacking Apaf-1 undergo programmed cell death by a caspase-independent, nonapoptotic pathway involving autophagy. J Neurosci 28:1490–1497

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Ferreira TA, Guiducci E, Dumas L, Ragozzino D, Gross CT (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333:1456–1458

Park D, Tosello-Trampont AC, Elliott MR, Lu M, Haney LB, Ma Z, Klibanov AL, Mandell JW, Ravichandran KS (2007) BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. Nature 450:430–434

Park SY, Jung MY, Kim HJ, Lee SJ, Kim SY, Lee BH, Kwon TH, Park RW, Kim IS (2008) Rapid cell corpse clearance by stabilin-2, a membrane phosphatidylserine receptor. Cell Death Differ 15:192–201

Parnaik R, Raff MC, Scholes J (2000) Differences between the clearance of apoptotic cells by professional and non-professional phagocytes. Curr Biol 10:857–860

Pearson HE, Payne BR, Cunningham TJ (1993) Microglial invasion and activation in response to naturally occurring neuronal degeneration in the ganglion cell layer of the postnatal cat retina. Brain Res Dev Brain Res 76:249–255

Peri F, Nusslein-Volhard C (2008) Live imaging of neuronal degradation by microglia reveals a role for v0-ATPase a1 in phagosomal fusion in vivo. Cell 133:916–927

Perry VH, Gordon S (1991) Macrophages and the nervous system. Int Rev Cytol 125:203–244

Perry VH, Hume DA, Gordon S (1985) Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. Neuroscience 15:313–326

Peter C, Waibel M, Radu CG, Yang LV, Witte ON, Schulze-Osthoff K, Wesselborg S, Lauber K (2008) Migration to apoptotic "find-me" signals is mediated via the phagocyte receptor G2A. J Biol Chem 283:5296–5305

Prinz M, Mildner A (2011) Microglia in the CNS: immigrants from another world. Glia 59: 177–187

Prinz M, Priller J, Sisodia SS, Ransohoff RM (2011) Heterogeneity of CNS myeloid cells and their roles in neurodegeneration. Nat Neurosci 14:1227–1235

Raff MC, Barres BA, Burne JF, Coles HS, Ishizaki Y, Jacobson MD (1993) Programmed cell death and the control of cell survival: lessons from the nervous system. Science 262:695–700

Rakic S, Zecevic N (2000) Programmed cell death in the developing human telencephalon. Eur J Neurosci 12:2721–2734

Ransohoff RM (2009) Chemokines and chemokine receptors: standing at the crossroads of immunobiology and neurobiology. Immunity 31:711–721

Ravichandran KS (2010) Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums. J Exp Med 207:1807–1817

Reddien PW, Cameron S, Horvitz HR (2001) Phagocytosis promotes programmed cell death in C. elegans. Nature 412:198–202

Rigato C, Buckinx R, Le-Corronc H, Rigo JM, Legendre P (2011) Pattern of invasion of the embryonic mouse spinal cord by microglial cells at the time of the onset of functional neuronal networks. Glia 59:675–695

Roth KA, D'Sa C (2001) Apoptosis and brain development. Ment Retard Dev Disabil Res Rev 7: 261–266

Saijo K, Glass CK (2011) Microglial cell origin and phenotypes in health and disease. Nat Rev Immunol 11:775–787

Sánchez-López A, Cuadros MA, Calvente R, Tassi M, Marín-Teva JL, Navascués J (2004) Radial migration of developing microglial cells in quail retina: a confocal microscopy study. Glia 46: 261–273

Santos AM, Calvente R, Tassi M, Carrasco MC, Martín-Oliva D, Marín-Teva JL, Navascués J, Cuadros MA (2008) Embryonic and postnatal development of microglial cells in the mouse retina. J Comp Neurol 506:224–239

Savill J, Fadok V (2000) Corpse clearance defines the meaning of cell death. Nature 407: 784–788

Schafer DP, Lehrman EK, Kautzman AG, Koyama R, Mardinly AR, Yamasaki R, Ransohoff RM, Greenberg ME, Barres BA, Stevens B (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron 74:691–705

Scheffel J, Regen T, Van Rossum D, Seifert S, Ribes S, Nau R, Parsa R, Harris RA, Boddeke HW, Chuang HN, Pukrop T, Wessels JT, Jurgens T, Merkler D, Bruck W, Schnaars M, Simons M, Kettenmann H, Hanisch UK (2012) Toll-like receptor activation reveals developmental reorganization and unmasks responder subsets of microglia. Glia 60:1930–1943

Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SE, Pollard JW, Frampton J, Liu KJ, Geissmann F (2012) A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 336:86–90

Sedel F, Béchade C, Vyas S, Triller A (2004) Macrophage-derived tumor necrosis factor alpha, an early developmental signal for motoneuron death. J Neurosci 24:2236–2246

Sierra A, Encinas JM, Deudero JJ, Chancey JH, Enikolopov G, Overstreet-Wadiche LS, Tsirka SE, Maletic-Savatic M (2010) Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. Cell Stem Cell 7:483–495

Sierra A, Abiega O, Shahraz A, Neumann H (2013) Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. Front Cell Neurosci 7:6

Snider WD (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. Cell 77:627–638

Sorokin SP, Hoyt RF Jr, Blunt DG, McNelly NA (1992) Macrophage development: II. Early ontogeny of macrophage populations in brain, liver, and lungs of rat embryos as revealed by a lectin marker. Anat Rec 232:527–550

Swinnen N, Smolders S, Avila A, Notelaers K, Paesen R, Ameloot M, Brône B, Legendre P, Rigo JM (2013) Complex invasion pattern of the cerebral cortex by microglial cells during development of the mouse embryo. Glia 61:150–163

Takahashi K, Rochford CD, Neumann H (2005) Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J Exp Med 201:647–657

Thanos S (1991) The relationship of microglial cells to dying neurons during natural neuronal cell death and axotomy-induced degeneration of the rat retina. Eur J Neurosci 3:1189–1207

Tremblay ME, Lowery RL, Majewska AK (2010) Microglial interactions with synapses are modulated by visual experience. PLoS Biol 8:e1000527

Truman LA, Ford CA, Pasikowska M, Pound JD, Wilkinson SJ, Dumitriu IE, Melville L, Melrose LA, Ogden CA, Nibbs R, Graham G, Combadiere C, Gregory CD (2008) CX3CL1/fractalkine is released from apoptotic lymphocytes to stimulate macrophage chemotaxis. Blood 112: 5026–5036

Ueno M, Fujita Y, Tanaka T, Nakamura Y, Kikuta J, Ishii M, Yamashita T (2013) Layer V cortical neurons require microglial support for survival during postnatal development. Nat Neurosci 16:543–551

Upender MB, Naegele JR (1999) Activation of microglia during developmentally regulated cell death in the cerebral cortex. Dev Neurosci 21:491–505

Valenciano AI, Boya P, de la Rosa EJ (2009) Early neural cell death: numbers and cues from the developing neuroretina. Int J Dev Biol 53:1515–1528

Verney C, Monier A, Fallet-Bianco C, Gressens P (2010) Early microglial colonization of the human forebrain and possible involvement in periventricular white-matter injury of preterm infants. J Anat 217:436–448

Wakselman S, Béchade C, Roumier A, Bernard D, Triller A, Bessis A (2008) Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. J Neurosci 28:8138–8143

Witting A, Muller P, Herrmann A, Kettenmann H, Nolte C (2000) Phagocytic clearance of apoptotic neurons by microglia/brain macrophages in vitro: involvement of lectin-, integrin-, and phosphatidylserine-mediated recognition. J Neurochem 75:1060–1070

Yaginuma H, Shiraiwa N, Shimada T, Nishiyama K, Hong J, Wang S, Momoi T, Uchiyama Y, Oppenheim RW (2001) Caspase activity is involved in, but is dispensable for, early motoneuron death in the chick embryo cervical spinal cord. Mol Cell Neurosci 18:168–182

Yeo W, Gautier J (2004) Early neural cell death: dying to become neurons. Dev Biol 274: 233–244

Yuan J, Yankner BA (2000) Apoptosis in the nervous system. Nature 407:802–809