Purine Signaling and Microglial Wrapping

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Abstract Microglial cells are highly dynamic cells with processes continuously moving to survey the surrounding territory. Microglia possess a broad variety of surface receptors and subtle changes in their microenvironment cause microglial cell processes to extend, retract, and interact with neuronal synaptic contacts. When the nervous system is disturbed, microglia activate, proliferate, and migrate to sites of injury in response to alert signals. Released nucleotides like ATP and UTP are among the wide range of molecules promoting microglial activation and guiding their migration and phagocytic function. The increased concentration of nucleotides in the extracellular space could be involved in the microglial wrapping found around injured neurons in various pathological conditions, especially after peripheral axotomy. Microglial wrappings isolate injured neurons from synaptic inputs and facilitate the molecular dialog between endangered or injured neurons and activated microglia. Astrocytes may also participate in neuronal ensheathment. Degradation of ATP by microglial ecto-nucleotidases and the expression of various purine receptors might be decisive in regulating the function of enwrapping glial cells and in determining the fate of damaged neurons, which may die or may regenerate their axons and survive.

Keywords ATP · Adenosine · CD39 · 'Eat-me' signals · Neuronal degeneration · Nerve injury · Microglial migration · Phagocytosis · Purine receptors · Axotomy

Abbreviations

CNS	Central nervous system
PAMPs	Pathogen associated molecular patterns
DAMPs	Damage associated molecular patterns
TLRs	Toll-like receptors

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SAMPs	Self-associated molecular patterns
TREM2	Triggering Receptor Expressed on Myeloid cells 2
PPT	Perforant path transection
ECM	Extracellular matrix

"Resting" Microglia in the Healthy CNS and Their Interaction with the Microenvironment

The term "quiescent" or "resting" microglia, usually used to designate nonactivated microglia in the normal adult central nervous system (CNS), might lead one to think that these cells are in a dormant state with no apparent movement and function. However, nothing could be further from the truth. The combined use of in vivo time-lapse transcranial two-photon microscopy and transgenic mice with green fluorescent protein in resident CNS microglia has made it possible to see microglia interacting with other cortical elements (Davalos et al. 2005; Nimmerjahn et al. 2005). Microglial cells are the most dynamic cells in the healthy CNS, as their morphological changes far exceed those of both neurons (Holtmaat et al. 2008; Knott and Holtmaat 2008) and astrocytes (Hirrlinger et al. 2004). Thus, in the healthy brain, microglial cells are continuously remodeling their shape by extending and retracting their processes, surveying the local microenvironment to scan the surface of the surrounding cells and the interstitial fluid (Davalos et al. 2005; Nimmerjahn et al. 2005) (see Chapters "Glial cells and Integrity of the Nervous System" and "Microglia Function in the Normal Brain"). Under normal conditions, each microglial cell seems to be responsible for checking its own territory, and its highly dynamic processes do not overlap or enter in the territory of neighboring microglial cells. While the microglial soma and main branches remain stable in the nervous parenchyma, with few signs of movement and without any clear relationship to other cells or blood vessels, its motile processes are continuously making direct contacts with nearby neuronal cell bodies, macroglia, and blood vessels (Nimmerjahn et al. 2005; Wake et al. 2009; Tremblay et al. 2010).

Although it might appear at first glance that motility of microglial processes is random (Nimmerjahn et al. 2005), a wide range of studies indicates that microglial cells express a broad variety of surface receptors that allows them to sense subtle changes in the microenvironment (Kierdorf and Prinz 2013). In particular, in the healthy adult brain, movement of microglial processes seems to be closely related to local concentration of some neurotransmitters, neuropeptides, and neuromodulators (Pocock and Kettenmann 2007). Although not conclusive, the current data suggest that microglial motility is increased by global excitatory neurotransmission and decreased by global inhibitory neurotransmission (Nimmerjahn et al. 2005; Fontainhas et al. 2011; Eyo and Wu 2013).

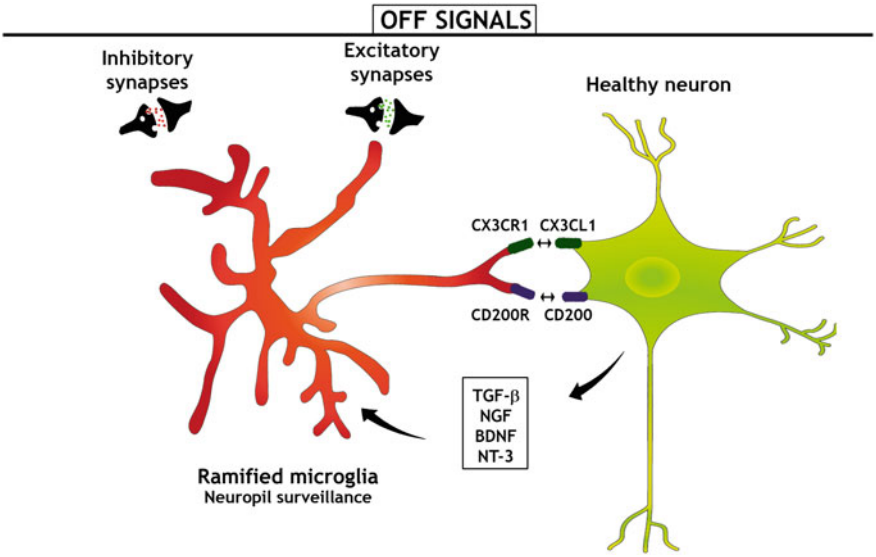

Fig. 1 In the healthy normal brain, ramified microglia is a very dynamic cell and their processes are continuously extending and retracting, monitoring the surface of neurons and having a special predilection for excitatory synapses. Interaction between inhibitory receptors in microglia with both specific ligands in the neuronal surface and neuronal released molecules keeps microglia in a nonactivated state. These signals that are expressed constitutively are know as "Off-signals"

Furthermore, electron microscopic studies demonstrate that, under normal conditions, microglial cell processes directly contact presynaptic and postsynaptic elements and have a special predilection for excitatory synapses (Fig. 1) although the existence of microglial cell interactions with inhibitory synapses under normal physiological conditions remains yet unknown (Perry and O'Connor 2010; Siskova and Tremblay 2013). Microglial cell processes contact synapses about once per hour, remain in a close proximity to presynaptic boutons for 5 min, and then retract (Wake et al. 2009). The interactions between microglia and synapses depend on neuronal activity and, therefore, the frequency of contact declines with decreased synaptic transmission (Wake et al. 2009).

Signaling Mechanisms Involved in Activation of Microglia

As previously discussed in Chapters "Glial cells and Integrity of the Nervous System" and "Microglia Function in the Normal Brain", microglia are activated by various changes in their microenvironment caused by acute insults and chronic disease states (Kettenmann et al. 2011; Chen et al. 2014; Gonzalez et al. 2014).

Transformation of the finely branched resting microglia into enlarged cells with short and stout processes is a hallmark of microglial cell activation (Kettenmann et al. 2011). In addition to morphological changes, microglial activation involves a stereotypical pattern of changes, including proliferation and migration to sites of injury, increased or de novo expression of cytokines and growth factors and, in some circumstances, the full transformation into phagocytes capable of clearing damaged cells and debris (Kettenmann et al. 2011). There is a wide range of molecules promoting microglial activation that can be classified as two main types: PAMPs (Pathogen associated molecular patterns) and DAMPs (damage associated molecular patterns). PAMPs warn of the presence of exogenous material, such as components of bacterial cell walls or repeats of bacterial or viral nucleic acids, whereas DAMPs warn of internal damage to the cells of the own organism and include molecules released by injured cells or modified as a consequence of tissue damage, such as oxidized lipoproteins or fragments of extracellular matrix molecules (Bianchi 2007; Matzinger 2007). Microglial cells possess a wide range of surface molecules, such as toll-like receptors (TLRs) (Lehnardt 2010), scavenger receptors (Husemann et al. 2002) and numerous cytokine and chemokine receptors, whose interaction with DAMPs and PAMPs results in a rapid activation of resting microglia to become motile effector cells (Kierdorf and Prinz 2013).

However, it would be a mistake to think that activation of microglia is a simple event; on the contrary, it is complex and includes still unidentified signaling mechanisms. In the healthy CNS, microglia exhibit a deactivated phenotype due to the interaction of inhibitory receptors ("Off receptors") in their plasma membrane, with the corresponding ligands ("Self-associated molecular patterns" or SAMPs) located on neurons and glial cells that keep microglia in a resting or nonactivated stage (Biber et al. 2007; Eyo and Wu 2013; Kierdorf and Prinz 2013) (Fig. 1). Some of the proposed inhibitory receptors in microglia are CX3CR1 and CD200R, which interact with their respective ligands, CX3CL1 (fractalkine) and CD200 on the surface of healthy neurons (Chertoff et al. 2013; Eyo and Wu 2013). Another proposed microglial inhibitory system is CD45/CD22. Recognition of CD22 on the surface of neurons by CD45 on microglia dampens microglial activation (Mott et al. 2004). Moreover, in addition to displaying membrane bound "Off-signals," neurons also release soluble Off-signals into the extracellular space, such as Transforming growth factor (TGF) β, neurotransmitters and neurotrophins including NGF, BDNF and NT-3 (Biber et al. 2007). If any of these Off-signals are lost, due to changes in the microenvironment, or are downregulated, as may occur in pathological conditions, microglial activation is triggered.

In contrast to Off-signals, which are expressed constitutively in the healthy adult brain, "On-signals" are produced on demand to initiate either a pro- or anti-inflammatory microglial activation program (Kettenmann et al. 2013) (Fig. 2). Some of the On-signals are the so-called "help-me/find-me" molecules (Marin-Teva et al. 2011; Panatier and Robitaille 2012; Xing et al. 2014). When neurons are overactive, impaired or endangered, they release these "alert" signals (Noda et al. 2013) which include nucleotides such as ATP and UTP (Sperlagh and Illes 2007); chemokines such as CCL21 and CXCL10 (Rappert et al. 2004; de Jong et al. 2005);

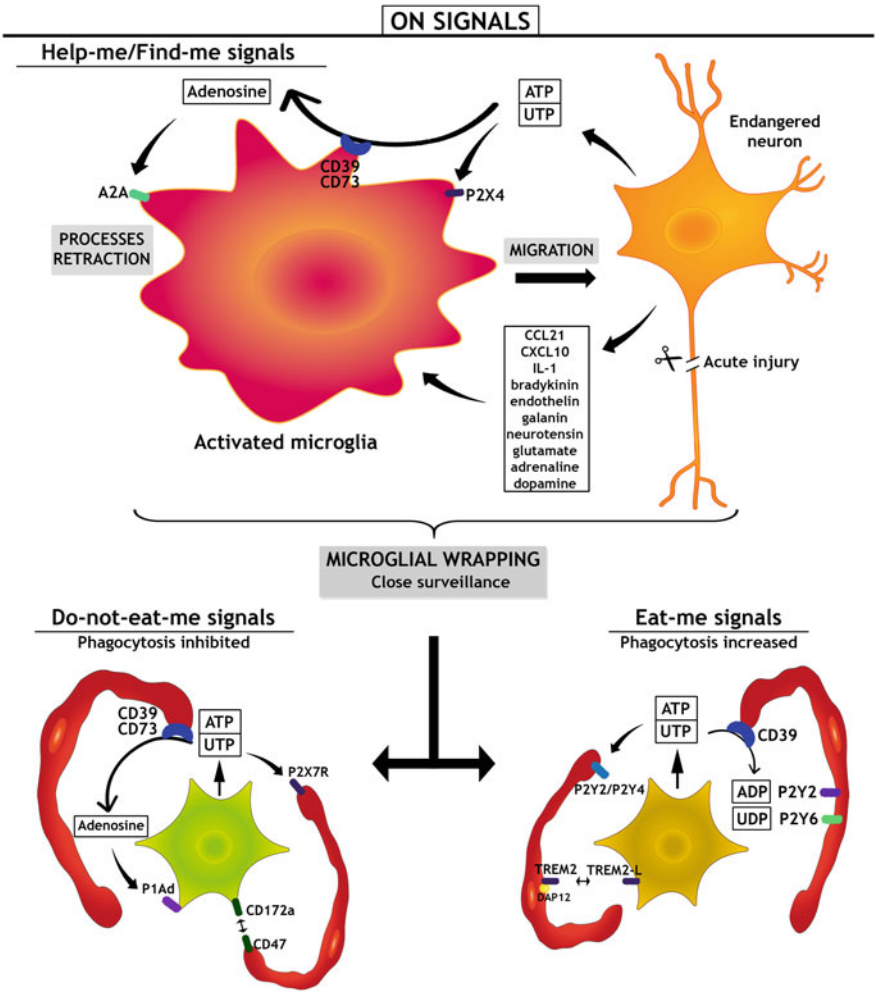

Fig. 2 "On signals" are produced when neurons are damaged and include "Help-me"/"Find-me", "Do-not-eat-me", and "Eat-me" signals. Endangered neurons may release a wide range of alert signals (Help-me/Find-me) including nucleotides that promote microglial activation, process retraction and migration towards neuronal somata. Microglial wrapping in one hand facilitates contact-dependent neuron-microglia interactions but also isolates damaged neurons leaking nucleotides. If Do-not-eat-me signaling predominates, phagocytosis is inhibited and neurons are able to survive. If, on the contrary, Eat-me signaling prevails, an increase in the phagocytic ability of microglia takes place and damaged neurons are removed. Note the importance of the ecto-enzymes CD39 and CD73 regulating the levels of nucleotides and nucleosides in the extracellular space around injured neurons

cytokines like Interleukin (IL) 1 (Cartier et al. 2005); neuropeptides such as bradykinin (Ifuku et al. 2007), endothelin (Fleisher-Berkovich et al. 2010), galanin (Ifuku et al. 2011) and neurotensin (Martin et al. 2005); neurotransmitters such as

glutamate, adrenaline, and dopamine (Farber et al. 2005; Liu et al. 2009); and cannabinoids (Walter et al. 2003) and morphine (Takayama and Ueda 2005). In response to help-me/find-me signals, microglia approach to the source of these molecules and develop either a close surveillance or a phagocytic function, depending on the presence of additional signals in the damaged neuron. If the receptor SIRP-alpha (CD172a) in the membrane of microglia interacts with the ligand CD47 on neurons, a "Do-not-eat-me" signal is presented to microglia (Biber et al. 2007; Ravichandran 2010). However, if the microglial receptor TREM2 (Triggering Receptor Expressed on Myeloid cells 2) recognizes its still-unknown-ligand on the surface of the damaged neuron, this interaction is interpreted as an "Eat-me" signal and therefore the microglial cell is able to initiate an intracellular signaling cascade, through the adaptor protein DAP12, leading to phagocytosis (Linnartz and Neumann 2013). TREM2 expression has been suggested to regulate not only phagocytic but also the migratory capacity of microglia (Melchior et al. 2010).

Migration of Microglia Is Guided by Purinergic Signaling

Release of danger signals that act as chemoattractants at the site of damage, initiates microglial activation and stimulates migration. Time-lapse two-photon imaging demonstrates that, for example, after a small laser ablation in the cerebral cortex, all microglial cells located in the surroundings respond within minutes by enlarging and extending their processes towards the damaged site, converging and forming a spherical shaped containment around it, but without migration of the somata (Davalos et al. 2005; Nimmerjahn et al. 2005). Quick extension of microglial processes to the site of injury without significant displacement of the cell body was previously described using histological sections (Jensen et al. 1994). In this work we showed that, a few hours after a perforant path transection (PPT), microglial cells located in the inner zone of the dentate molecular layer polarize and extend their processes towards and into the denervated PP zone, and it is not until 2–3 days after PPT when microglial cell bodies move to the denervated PP zone, where they accumulate and proliferate (Jensen et al. 1994). Therefore, migration of microglial cells is probably a complex process that involves two stages: a first phase of reconnaissance and damage assessment by microglial cells processes and, if damage persists and is important enough, a second phase where the entire cell body migrates. An intense cross talk, involving the signaling mechanisms referred in the previous section, between extended microglial processes and damaged neurons and glial cells, will determine this microglial cell migration.

Purine nucleotides are among the most potent molecules involved in the migration of microglia. In fact, Davalos et al. (2005) demonstrated that ATP or ADP microinjection in the brain parenchyma was able to mimic the rapid chemotactic response of microglial processes observed following laser ablation. Moreover, lowered ATP extracellular concentration results in reduced microglial

cell process movements, whereas increased ATP gradients stimulate their motility (Haynes et al. 2006).

In the healthy brain, release of ATP to the extracellular space is a common phenomenon, as this nucleotide and its derivatives act both as primary transmitter and as co-transmitter released with other neurotransmitters and peptides in many synapses. The mechanism by which intracellular ATP is released by neurons is a matter of intense debate (Cisneros-Mejorado et al. 2015), because in addition to being released by exocytosis, ATP leakage can also take place through large pores and transporters. Moreover, not only neurons but also glial cells, in particular astrocytes, can release ATP (Butt 2011; Cisneros-Mejorado et al. 2015).

Under pathological conditions when neurons are overexcited, injured or stressed in acute or chronic neurological disorders, a massive release of ATP takes place into the extracellular space (Braun et al. 1998; Melani et al. 2005). As elevated concentrations of extracellular ATP can cause cell death (Matute et al. 2007; Arbeloa et al. 2012), ATP released from endangered or dying cells may aggravate the extent of the ongoing damage. In addition, increased extracellular levels of ATP may over activate the P2X7R in neurons and trigger signaling cascades leading to neurodegeneration (Le Feuvre et al. 2003).

The concentrations of ATP, ADP, AMP and adenosine in the extracellular space are regulated by the activity of ecto-nucleotidases that are located in the plasma membrane of microglia and whose expression is dependent on the development and activation stage of these cells (Dalmau et al. 1998). One of these ecto-nucleotidases is CD39, also called Ecto-nucleoside triphosphate diphosphohydrolase 1 (E-NTPDase1), whose expression in the CNS is restricted to microglial cells and vascular endothelium (Braun et al. 2000). CD39 plays a main role hydrolysing extracellular nucleoside 5′-triphosphates to nucleoside 5′-diphosphates (NTPase enzymatic activity), as well as nucleoside 5′-diphosphates to nucleoside 5′-monophosphates (NDPase enzymatic activity). Nucleoside 5′-monophosphates are further hydrolysed to adenosine by CD73, an ecto-5′-nucleotidase also found, among other cells, in the membrane of microglia (Dalmau et al. 1998; Bulavina et al. 2013). Therefore, microglial cells could be considered as the cells responsible for the regulation of purinergic signaling in the CNS as they can control the rate, extent and timing of nucleotide degradation.

On the other hand, we should consider that microglial cells have several types of purine receptors on their surface (Ohsawa and Kohsaka 2011) whose interactions with changing concentrations of extracellular nucleotides and nucleosides (ATP/adenosine balance) may regulate microglial behavior, including process extension and retraction, microglial migration and even phagocytosis.

Purine receptors are divided into P1 (adenosine receptors) and P2 (ATP receptors). Microglia express the four subtypes of P1 receptors (A1, A3, A2A and A2B) and only some of the different subtypes of P2 receptors cloned, which are divided into ionotropic (seven subtypes: P2X1-7) and metabotropic (eight subtypes: P2Y1, −2, −4, −6, −11, −12, −13, and −14) (Kettenmann et al. 2011). Simultaneous costimulation of P1 and P2 receptors seems to be required for microglial migration (Farber et al. 2008). In particular, microglial process extension is dependent upon

ATP/ADP sensed through microglial P2Y12 receptors (Ohsawa and Kohsaka 2011), which are constitutively expressed on microglia in normal conditions (Sasaki et al. 2003) and upregulated when activated (Tozaki-Saitoh et al. 2008). P2Y12 receptors activate integrin-ß1, which accumulates in the tips of microglial processes, facilitating the adhesion of extended microglial processes with the extracellular matrix (ECM), which is a requisite for subsequent directional microglial migration (Haynes et al. 2006; Kurpius et al. 2007). Further activation of microglia, probably due to continuously elevated levels of ATP and ADP, or both (Kurpius et al. 2007), leads to upregulation of A2A and P2X4 receptors, whereas P2Y12 receptors are downregulated (Haynes et al. 2006; Orr et al. 2009). Signaling through P2X4 receptors enhances migration of microglia. As microglial activation involves increased expression of the ecto-enzymes CD39 and CD73 (causing ATP/ADP degradation), the abnormally increased levels of ATP generated by the pathological situation are gradually reduced, while the adenosine concentration increases and activates A2A receptors. Notably, adenosine causes retraction of microglial processes (Ohsawa and Kohsaka 2011). Therefore, gradually increased levels of adenosine may be the basis of microglial transformation from ramified cells into amoeboid migratory morphologies, usually found in various pathologies.

Microglial Wrapping and Synaptic Stripping

As discussed in Chapters "Glial cells and Integrity of the Nervous System" and "Microglia Function in the Normal Brain", it has been widely reported that activated microglia migrate and accumulate near injured neurons in various pathological conditions. In addition, in certain circumstances, the somata, proximal dendrites and axons of injured neurons become ensheathed by microglia. Microglial wrapping of neuronal cell bodies is one of the most prominent features after peripheral nerve axotomy (Fig. 3). Indeed, the phenomenon of microglial wrapping has been widely described in various CNS areas in several situations involving peripheral nerve axotomy, including the facial nucleus (Moran and Graeber 2004), the hypoglossal nucleus (Sumner and Sutherland 1973; Yamada et al. 2011), the dorsal motor nucleus of the vagus nerve (Masui et al. 2002), and in the spinal cord after sciatic nerve axotomy (Gehrmann et al. 1991). Also, this phenomenon has been reported in experimental models where peripheral nerves are not affected such as hippocampal organotypic cultures after an ischemic insult (Neumann et al. 2006), in the cerebral cortex during either acute focal inflammation (Trapp et al. 2007) or following intraperitoneal LPS injection (Chen et al. 2012), and in the spinal cord after experimental autoimmune encephalomyelitis (EAE) induction (Almolda et al. 2009). Microglial wrapping occurs in parallel with a significant reduction of axosomatic synapses. It was Blinzinger and Kreutzberg (1968) who first described, following facial nerve axotomy, the displacement of presynaptic terminals from the injured motor neuron surface by the interposing of microglial pseudopods and named this phenomenon as "synaptic stripping". Although some

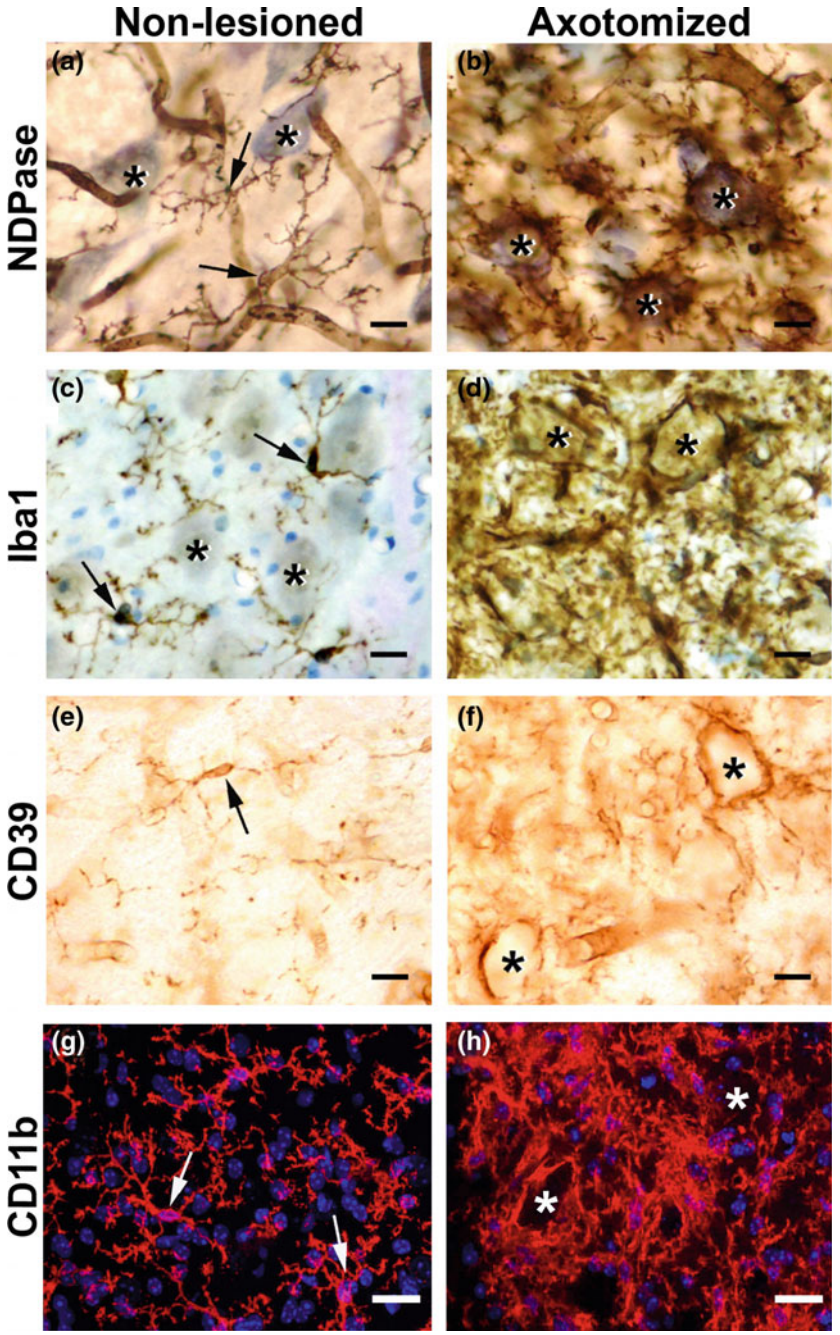

Fig. 3 Microglial wrapping in the facial nucleus of the mouse after facial nerve axotomy. In the normal, non-lesioned facial nucleus, microglia stained with different markers including NDPase histochemistry and immunohistochemistry for Iba1, CD39 and CD11b (**a**, **c**, **e**, **g**), show a ramified morphology (*arrows*) without any particular association with neuronal motor neuron somata (asterisks). After axotomy (**b**, **d**, **f**, **h**), microglia enwrap motor neuron somata (asterisks). In (**a**)–(**d**), sections are counterstained with toluidine blue. In (**g**) and (**h**) nuclei are stained in blue with DAPI. Scale bar = 20 μm

authors claim that reactive microglia spread on the surface of motor neurons to physically disconnect synapses (Moran and Graeber 2004; Yamada et al. 2008), it is still not totally clear whether synaptic stripping is either the cause or the consequence of microglial wrapping. As microglial wrapping and synaptic stripping are associated with motor neuron regeneration, it has usually been considered to be a neuroprotective process (Kreutzberg 1996). As we will discuss below, recent studies support this neuroprotective view (Chen et al. 2014), whereas others suggest that microglial wrapping may reduce neuronal survival (Yamada et al. 2011).

Accumulating evidence indicate that synaptic stripping of either inhibitory or excitatory synapses is beneficial to damaged neurons. Since microglia wrap neuronal cell bodies and the majority of synapses terminating on projection neuron somata in the cerebral cortex are GABAergic inhibitory synapses, it has been proposed that inhibitory axosomatic synapses are preferentially stripped after focal inflammation or peripheral immune challenge (Trapp et al. 2007; Chen et al. 2012). Evidence of microglia-mediated stripping of inhibitory GABAergic presynaptic terminals from cortical neurons in adult mice has been recently confirmed by 3-D electron microscopy (Chen et al. 2014). Reduced axosomatic GABAergic innervation protects neurons against noxious insult (Hardingham et al. 2002) by increasing synchronization of neuronal firing (Woo and Lu 2006), which is critical for synaptic NMDAR-mediated neuronal survival through CREB activation and by increasing neuronal expression of anti-apoptotic and neuroprotective molecules (Hardingham and Bading 2003).

However, it is nowadays clear that microglial wrapping is not always specifically directed to disconnect inhibitory synapses because in other locations, such as the facial nucleus in the rat after nerve axotomy (Raslan et al. 2014) and the spinal cord after either intramedullary axotomy in the cat (Linda et al. 2000) or sciatic nerve transection in the rat (Arbat-Plana et al. 2015), the outcome of microglia-mediated synaptic stripping is the preferential disconnection of excitatory glutamatergic synapses. Removal of the glutamatergic input to the axotomized motor neurons is considered relevant for neuronal survival, as glutamate may exert deleterious excitotoxic effects on nerve cells (Mehta et al. 2013). In support of this possibility, blocking of the NMDA-type glutamate receptor has been reported to increase motor neuron survival after neonatal axotomy in the rat (Mentis et al. 1993). Even assuming that any changes in the synaptic input, either inhibitory or excitatory, to the lesioned neurons may reduce their stress and be beneficial for survival and repair, the question of whether microglia actively participate in this process or if instead nerve terminals simply retract from the surface of neurons remains unsolved (Linda et al. 2000).

It is generally accepted that synaptic stripping does not inevitably mean that the disconnected terminals have to be immediately engulfed by microglia, as they remain in the vicinity of ensheathed neurons and only after axotomized motor neurons regenerate their axons, synapses are restored (Navarro et al. 2007). However, some work indicates that, several weeks after nerve transection, restored synaptic inputs are not normal (Raslan et al. 2014). The usual prevalence of inhibitory over excitatory terminals seems to be shifted for surviving lesioned

motor neurons in various locations (Borke et al. 1995; Linda et al. 2000; Raslan et al. 2014). Although microglia has been suggested to play a main role in regulating these synaptic rearrangements (Raslan et al. 2014), astrocytes might also be involved (Tyzack et al. 2014).

In the healthy brain, neurons, including their synapses, are generally ensheathed by fine processes of astrocytes that participate in the regulation of synapse formation, stability, and elimination. Coverage of synapses by astrocytic processes may change under various physiological conditions (Theodosis et al. 2008; Chung et al. 2013; Perez-Alvarez et al. 2014). Specifically, in the facial nerve of the mouse two weeks after axotomy, thin lamellar astrocyte processes begin to replace microglial wrapping around damaged motor neurons, and by 3 weeks they completely cover the neuron soma (Moran and Graeber 2004). Some authors have suggested that this delayed astrocyte behavior might contribute to synaptic remodeling by engulfing some disconnected presynaptic terminals (Chung et al. 2013) and promoting the rearrangement of synaptic inputs on axotomized motor neurons (Tyzack et al. 2014).

Microglial Wrapping: Detrimental or Beneficial?

Glial wrapping, whether microglial, astroglial or both, may not only cause deafferentation, but might also facilitates contact-dependent neuron–glia interactions that prevent neuron death and promote regeneration. After facial nerve axotomy in the mouse, for example, about 65 % of axotomized neurons regenerate axons and survive, whereas 35 % of neurons degenerate. Research in our laboratory performing facial nerve axotomy on transgenic mice with astrocyte-targeted expression of either IL6 or IL10 in order to investigate how the local expression of those cytokines may affect microglial activation, showed that in addition to changes in the microglial reactivity pattern, there is an altered survival/death ratio of motor neurons (Almolda et al. 2014; Villacampa et al. 2015). Interestingly, higher motor neuron survival in IL10 transgenic mice was not associated with significant changes in microglial wrapping (Villacampa et al. 2015) although increased motor neuronal death in IL6 transgenic mice coincides with reduced microglial wrapping (Almolda et al. 2014). Moreover, ongoing studies performed on IRF8 KO mice indicate that incomplete microglial wrapping of individual axotomized motor neurons correlates with increased motor neuron death (Xie et al. 2014). In agreement with this, some evidence suggests that defects in microglia-neuron attachment after facial nerve axotomy, as occurs in microglial cathepsin deficient mice (Hao et al. 2007) and TGFβ1 deficient animals (Makwana et al. 2007), might lead to more neuron death. These observations support the hypothesis that the intimate association between glial cells and neurons has a neurotrophic rather than neurotoxic function. The close physical proximity of microglia to injured neurons may facilitate the continuous supply of growth factors and other required molecules, thus supporting survival and regeneration (Trapp et al. 2007).

There is however an opposing view holding the possibility that prolonged contact of microglial cells with enwrapped neurons is detrimental (Yamada et al. 2011). Some studies have demonstrated that the survival ratio of injured motor neurons is markedly influenced by the species and the age of animals used (Moran and Graeber 2004; Kiryu-Seo et al. 2005). Interestingly, in this context, facial nerve axotomy in neonatal rats and mice kills damaged motor neurons within a week of lesion. Nevertheless, axotomized motor neurons in adult rats are able to survive, whereas in adult mice there is a slow and progressive motor neuron death after lesion (Kiryu-Seo et al. 2005). Some authors have suggested that these differences among adult rats and mice are due to differences in the ratio of microglial/astroglial wrapping (Yamada et al. 2011). If the astrocytic wrapping predominates, as found in the rat, some protective effects are exerted on axotomized motoneurons, whereas if the wrapping is mainly microglial, as observed in mouse, a slow apoptotic cell death of motor neurons might take place (Yamada et al. 2011).

Microglial wrapping may be the result of a continuous release or leakage of purine nucleotides that act as find-me signals (Fig. 2). Neuron ensheathment by activated microglia expressing ecto-nucleotidases in their plasma membrane effectively isolates damaged neurons leaking purine nucleotides and contributes to their rapid degradation to adenosine around neurons. Increasing concentrations of extracellular adenosine may develop a potentially neuroprotective function on neurons through P1 adenosine receptors (Stone 2002). In addition, adenosine can impair the phagocytic function of peripheral macrophages by binding to the P1 adenosine receptors expressed on their membrane (Hasko et al. 2007). Also, microglial phagocytosis seems to be regulated by purinergic signaling (Bulavina et al. 2013). It has been shown that activation of P1 receptors by a non-hydrolysable analog of adenosine decreases microglial phagocytosis (Bulavina et al. 2013). In the opposite way, activation of P2Y12 receptor by ADP, activation of P2Y6 by UDP and activation of P2Y2/P2Y4 receptors by UTP markedly increase microglial phagocytosis both in vitro and in vivo (Koizumi et al. 2007; Fang et al. 2009). Therefore, the increasing concentration of these nucleotides around injured neurons may be an eat-me signal for wrapping microglia. In agreement with this, CD39-deficient animals presented higher microglial phagocytic activity (Bulavina et al. 2013), suggesting that an increased concentration of extracellular ATP/ADP and UTP/UDP, due to the lack of CD39 enzymatic activity, leads to a chronic stimulation of the microglial phagocytic activity. However, other studies indicate that activation of P2X7 receptors by exposure to ATP induced inhibition of microglial phagocytic activity even if microglia are cotreated with UDP (Fang et al. 2009). Taken together, these observations suggest that a fine control of the levels of nucleosides and nucleotides in the extracellular space around injured neurons together with a fine regulation of purine receptors may be decisive to control phagocytosis and hence in determining the fate of damaged neurons wrapped by microglia.

Concluding Remarks

The meaning of microglial wrapping around injured neurons is not completely understood. Microglial wrapping partially isolates endangered neurons from the adjacent neuropil, leading to an important deafferentation from synaptic inputs. Besides, the wide area of contact between microglia and neuronal surfaces enables an intense exchange of molecular signals between them. Injured neurons circumscribed by microglia may survive or die and their fate will depend on a plethora of signals. In this scenario, nucleosides and their phosphorylated nucleotides may play a key role, as they can be involved in regulation of apoptosis, in the synthesis and release of different trophic factors by astrocytes (Rathbone et al. 1999), in promotion of axonal growth (Heine et al. 2006), and in modulation of microglial phagocytosis (Inoue 2008). Although programmed neuronal cell death can result from axonal injury, cell regeneration and axonal outgrowth programs are also activated (Raivich and Makwana 2007; Kiryu-Seo and Kiyama 2011). The putative involvement of microglia and astroglia in the activation of these regenerative programs are still poorly understood and will be a challenge for researchers in the coming years.

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