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Microglia and inflammation-mediated neurodegeneration: Multiple triggers with a common mechanism

Michelle L. Block *, Jau-Shyong Hong

Neuropharmacology Section, MD F1-01, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709, USA

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Abstract

Inflammation, a common denominator among the diverse list of neurodegenerative diseases, has recently been implicated as a critical mechanism responsible for the progressive nature of neurodegeneration. Microglia are the resident innate immune cells in the central nervous system and produce a barrage of factors (IL-1, TNFα, NO, PGE_2, superoxide) that are toxic to neurons. Evidence supports that the unregulated activation of microglia in response to environmental toxins, endogenous proteins, and neuronal death results in the production of toxic factors that propagate neuronal injury. In the following review, we discuss the common thread of microglial activation across numerous neurodegenerative diseases, define current perceptions of how microglia are damaging neurons, and explain how the microglial response to neuronal damage results in a self-propelling cycle of neuron death.

Contents

Abbreviations: DEP, diesel exhaust particles; PM, particulate matter; PD, Parkinson's disease; AD, Alzheimer's disease; ROS, reactive oxygen species; NO, nitric oxide; TNFα, tumor necrosis factor-alpha; TH, tyrosine hydroxylase; Aβ, beta-amyloid; LPS, lipopolysacharide; HIV, human immunodeficiency virus; MS, multiple sclerosis; AIDS, acquired immune deficiency syndrome; HAD, HIV associated dementia; PHOX; phagocytic oxidase; PGE_2, prostaglandin E2; IR, immuno-reactive; SN, substantia nigra; VTA, ventral tegmental area; FTD, frontotemporal dementias; 6-OHDA, 6-hydroxydopamine; DA, dopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP+, 1-methyl-4-phenylpyridinium ion; NSAID, non-steroidal anti-inflammatory drug; CNS, central nervous system; IR, immunoreactive; NADPH, nicotinamide adenine dinucleotide phosphate; MMP-3, matrix metalloproteinase-3; ECM, extracellular matrix; NFκB, nuclear factor-κB; iNOS, inducible nitric oxide synthase; IL-1β, interleukin-1 beta

* Corresponding author. Tel.: +1 919 541 5169; fax: +1 919 541 0841.
E-mail address: block@niehs.nih.gov (M.L. Block).

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1. Introduction

Inflammation occurs in multiple neurodegenerative diseases, where each disease has unique pathology and symptoms. There is an extensive list of specific triggers of neuronal damage, where each environmental toxin or genetic mutation is specific for a selected disease. However, the gradual accumulation of neuronal death and the increase in disease severity across time is a unifying theme across the diverse classifications of neurodegenerative disease. Previously, inflammation was viewed as only a passive response to neuronal damage. However, increasing reports demonstrate that inflammation is capable of actively causing neuronal death and damage, which then fuels a self-propelling cycle of neuronal death. Thus, while the triggers of various neurodegenerative diseases are diverse, inflammation may be a basic mechanism driving the progressive nature of multiple neurodegenerative diseases. Several cell types have been listed as contributors to inflammation-mediated neurodegeneration, but microglia are implicated as critical components of the immunological insult to neurons. In the following review, we discuss the role of microglia in neuronal death and describe the evidence implicating microglia as a critical mechanism driving the self-propelling nature of neurodegenerative disease.

2. Glial cells are inflammatory mediators of neurodegenerative disease

Early reports described the brain as an immune privileged organ, due to its compartmentalization and separation from the peripheral blood system, as provided by the blood–brain-barrier. However, most neurodegenerative diseases are characterized by both local inflammation from resident cell types in the brain and by the infiltration of leucocytes from the periphery (Kurkowska-Jastrzebska et al., 1999; McGeer et al., 1989). While infiltrating peripheral immune cells can be significantly toxic to neurons (Freude et al., 2002; Wu and Proia, 2004), leukocyte infiltration is not always associated with neurotoxicity (Boztug et al., 2002; Trifilo and Lane, 2003), indicating a critical role for the local glial cells (astroglia and microglia) in the inflammatory response associated with neurodegeneration.

2.1. Astroglia

In the normal brain, astroglia play essential roles in providing glia-neuron contact, maintaining ionic homeostasis, buffering excess neurotransmitters, secreting neurotrophic factors, and serving as a critical component of the blood–brain barrier (Aloisi, 1999; Hansson and Ronnback, 1995; Vernadakis, 1988). Although the pro-inflammatory function of astroglia is not as prominent as that of microglia (Barde, 1989; Lindsay, 1994; Streit et al., 1999), astroglia become activated in response to immunologic challenges or brain injuries (Aloisi, 1999; Tacconi, 1998). Astroglia also produce a host of trophic factors (Friedman et al., 1990; Lindsay, 1994), which are crucial for the survival of neurons. However, activated astroglia become hypertrophic, exhibit increased production of glial fibrillary acidic protein, and form glial scars, which hinder axonal regeneration. While there is a clear relationship between astroglia and microglia in both resting and activated conditions (Kahn MA et al., 1995; Rezaie et al., 2002), efforts to understand the detailed mechanisms of this complex association are ongoing.

2.2. Microglia

Microglia were originally described by del Rio-Hortega (1932) as a unique cell type differing in morphology from other glia and neurons, comprising approximately 12% of the brain. While the precise origin of microglia in the brain is

a source of debate, it is generally accepted that microglia are derived from myeloid origin (del Rio-Hortega, 1932) and are responsible for the innate immune response in the brain. The majority of microglial function goes unnoticed, as they perform general maintenance and clean cellular debris (Beyer et al., 2000). Additionally, microglia have active roles in late embryonic brain development and early postnatal brain maturation, where microglia enforce the programmed elimination of neural cells (Barron, 1995; Milligan et al., 1991). In mature brains, resting microglia exhibit a characteristic ramified morphology and are responsible for immune surveillance. Microglia become readily activated in response to brain injuries or to immunological stimuli (Kreutzberg, 1996; Liu and Hong, 2003; Streit et al., 1988, 1999) and undergo dramatic morphologic alterations upon activation, changing from resting ramified microglia into activated amoeboid microglia (Kreutzberg, 1996). Further, surface molecules, such as complement receptors and major histocompatibility complex molecules, are also upregulated when microglia are activated (Graeber et al., 1988; Oehmichen and Gencic, 1975). In addition, activated microglia are capable of releasing a variety of soluble factors, which are pro-inflammatory in nature and potentially cytotoxic (Table 1). The various mechanisms through which microglia are activated and the identity of the toxic factors released by microglia will be discussed in detail.

Ongoing controversy exists regarding whether microglia are neuroprotective or neurotoxic when activated. In addition to producing cytotoxic factors such as superoxide (Colton and Gilbert, 1987), nitric oxide (Liu et al., 2002; Moss and Bates, 2001), and tumor necrosis factor alpha (TNFα) (Lee et al., 1993; Sawada et al., 1989) in response to immunological stimuli, microglia are also reported to increase neuronal survival through the release of trophic and anti-inflammatory factors (Liao et al., 2004; Morgan et al., 2004; Polazzi et al., 2001) (Table 1). Thus, rather than classify microglia as exclusively beneficial or inherently deleterious, it is likely that microglia can serve both functions, depending on several factors ranging from the progression of the disease state to the type of stimulus. While not all microglial activation results in neuron death, the unregulated response or over-activation of microglia can

Table 1
Microglia-derived factors that influence neuronal survival

Abbrevation	Full length name	Function/effect	Reference
IL-1α/β	Interleukin-1α/β	Pro-inflammatory	Liu et al. (2005a)
IL-6	Interleukin-6	Pro-inflammatory	Laurenzi et al. (2001)
IL-18	Interleukin-18	Pro-inflammatory	Suk et al. (2001)
IP-10	Gamma interferon inducible protein 1	Pro-inflammatory	Kremlev et al. (2004)
TNFα	Tumor necrosis factor alpha	Pro-inflammatory	Si et al. (2004)
IL-16	Interleukin-16	T cell chemotaxis	Zhao et al. (2004)
MCP-1	Monocyte chemoattractant protein 1	Chemotaxis	Nagai et al. (2001)
IL-8	Interleukin-8	Chemokine	Nagai et al. (2001)
MDC	Macrophage-derived chemokine/CCL22	Chemotaxis	Columba-Cabezas et al. (2002)
MIP-1 α	Macrophage inflammatory protein 1 α	Chemotaxis	Nagai et al. (2001)
MIP-1 β	Macrophage inflammatory protein 1 β	Chemotaxis	Nagai et al. (2001)
MIP-2	Macrophage inflammatory protein 2	Chemotaxis	Nagai et al. (2001)
MIP-3 β	Macrophage inflammatory protein 3 β	Chemotaxis	Nagai et al. (2001)
β-Chemokine	Beta chemokine	Chemotaxis	Si et al. (2002)
Gro-α	Growth regulated oncogene	Chemotaxis	Popivanova et al. (2003)
IL-3	Interleukin-3	Proliferation	Laurenzi et al., 2001)
IL-15	Interleukin-15	T-cell regulation/proliferation	Lee et al. (1996)
M-CSF	Macrophage colony stimulating factor	Proliferation	Takeuchi et al. (2001)
IL-2	Interleukin-2	Growth factor/proliferation	Kowalski et al. (2004)
IL-12	Interleukin-12	Proliferation/T-cell differentiation	Nagai et al. (2001)
PGE_2	Prostaglandin E2	Pro-inflammatory, proliferation	Rasley et al. (2004)
TGF β	Transforming growth factor beta	Anti-inflammatory	Hurley et al. (1999)
IL-13	Interleukin-13	Immunosuppressive	Shin et al. (2004)
IL-10	Interleukin-10	Immunosuppressive	Seo et al. (2004)
NGF	Nerve growth factor	Neurotrophic	Elkabes et al. (1996)
BDNF	Brain-derived neurotrophic factor	Neurotrophic	Elkabes et al. (1996)
NT-3	Neurotrophin-3	Neurotrophic	Elkabes et al. (1996)
NT-4	Neurotrophin-4	Neurotrophic	Elkabes et al. (1996)
NO	Nitric oxide	Neurotoxic	Chao et al. (1992)
$O_2^{\bullet -}$	Superoxide	Neurotoxic	Colton and Gilbert (1987)
H_2O_2	Hydrogen peroxide	Neurotoxic	Twig et al. (2001)
OH^-	Hydroxyl radical	Neurotoxic	Chang et al. (2000b)
NOO^-	Peroxynitrite	Neurotoxic	Possel et al. (2002)

Microglia influence the survival of neurons by releasing factors that modulate the functions of surrounding immune cells (chemokines, immunogens, and pro-inflammatory factors), are toxic to neurons (ROS and pro-inflammatory factors), and that are beneficial to neurons (neurotrophic factors). Here we list several factors that microglia are reported to release, listed by their abbreviation, full length name and categorized by function.

have disastrous neurotoxic consequences. Here we summarize the evidence detailing the role of microglial activation in several neurodegenerative diseases.

3. The role of microglia in neurodegenerative disease

3.1. Alzheimer's disease

Alzheimer's disease (AD) is the leading cause of dementia, where neural damage begins in the temporal and parietal lobes of the cerebral cortex and progresses with time to the hippocampus and the amygdala (Braak and Braak, 1994). The result is the loss of language skills, followed by memory decline, and finally delusion in the latter stages. Pathological diagnosis of AD requires identification of insoluble extracellular plaques containing β-amyloid (Aβ) and intraneuronal neurofibrilary tangles in the cortical region of the brain. Early work demonstrated that 100 amino acids of the carboxy terminal of the Aβ peptide can be directly toxic to neurons in vitro and initiated the hypothesis that Aβ was a driving force behind the neurodegeneration of AD (Yankner, 1989; Yankner et al., 1990). Furthermore, pioneering work by McGeer et al. (1987) proposed an additional explanation for neuronal death in AD when they identified the increased presence of activated microglia (those staining more intensively for HLD-DR, a marker for monocytes) around the Aβ containing plaques in postmortem AD tissue, when compared to similar tissue from control brains without neurodegenerative pathology. While the microglial reaction to Aβ was initially perceived as a passive response to neuronal death, ongoing research has shown that the microglial response to Aβ (Combs et al., 2000; Qin et al., 2002) and the senile plaques (Van Everbroeck et al., 2004; Veerhuis et al., 1999) can be detrimental to neuronal survival.

AD was one of the first neurodegenerative diseases associated with neurotoxic microglial activation. As a consequence, the traditionally perceived passive role of microglia as bystander maintenance cells has been questioned and microglia are now accepted as active mediators of neurodegeneration. Aβ will both recruit and activate microglia (Davis et al., 1992; Meda et al., 1995; Sasaki et al., 1997), where Aβ has been reported to result in the release of neurotoxic factors from microglia, such as NO (Ii et al., 1996), TNFα (Dheen et al., 2004) and superoxide (Qin et al., 2002). In fact, use of synthetic Aβ has determined that the amino acids 10–16 are critical for microglial activation (Giulian et al., 1996). Several receptors have been implicated as necessary for the interaction of microglia and Aβ, such as the CD14 receptor (Bate et al., 2004) and the β1-integrin receptor (Koenigsknecht and Landreth, 2004). In fact, Koenigsknecht and Landreth (2004) suggest that microglia interact with Aβ through a cell surface receptor complex consisting of B-class scavenger receptor CD36, α6-β1 integrin, and CD47 (integrin-associated protein). Interestingly, Koenigsknecht and Landreth (2004) also report that this receptor complex is responsible for the internalization of Aβ through a non-traditional pathway. Additionally, the receptor complex reported to be responsible for the internalization of Aβ (CD36, α6-β1 integrin, and CD47) has also been identified as critical for Aβ-induced superoixde production in microglia (Bamberger et al., 2003). While many studies indicate that the phagocytosis of Aβ is neuroprotective (Das et al., 2003), as microglia may take up and degrade Aβ (Paresce et al., 1997), it has been recently suggested that the process of phagocytosis can be associated with neurotoxic consequences (Block et al., 2004; Zhang et al., 2005). This mechanism of phagocytic microglial activation is generalizable to a diverse set of microglia stimuli and is discussed in detail later.

Human clinical trials using Aβ peptide immunization demonstrate the delicate balance between the helpful aspects of microglial activation and potential deleterious consequences of unregulated inflammatory responses (Dodel et al., 2003; Schenk, 2002). The human clinical trials began as a result of earlier promising animal studies demonstrating that immunization with the Aβ peptide in mice over expressing Aβ resulted in protection against AD-like pathology (Schenk et al., 1999), where the mechanism of protection was believed to be through the clearance of Aβ by microglia. However, while Aβ plaques were cleared in human immunized patients, phase IIa clinical trials were halted when 18 of 298 patients immunized with the Aβ peptide demonstrated meningoencephalitis symptoms (Senior, 2002). It has recently been shown that immunization of C57 mice with the Aβ peptide will result in an animal model of autoimmune encephalitis, where these immunized animals display features similar to those reported in the human clinical trials (Furlan et al., 2003). While the cell type responsible for the harmful inflammatory response as a result of Aβ immunization remains a debate (T cells or microglia), these studies support that deleterious potential of microglial activation in the process of Aβ clearance warrants caution and deserves further inquiry.

There has been some success with clinical studies investigating the effects of anti-inflammatory therapy against cognitive decline in AD patients (McGeer and McGeer, 1996; Perry et al., 2003), but the promise of anti-inflammatory treatment for AD is more evident in experimental studies. For example, inhibition of glial activation in animal AD models attenuates neurotoxicity. Murine models employing intraventricular infusion of human Aβ1–42 peptide replicate many of the hallmarks of AD pathology (neuro-inflammation, neuronal and synaptic degeneration, and amyloid deposition). Administration of aminopyridazines is shown to both attenuate glial inflammation and result in reduction of neuronal neurotoxicity in the murine intraventricular human Aβ1–42 infusion model (Craft et al., 2004). This supports the concept that therapeutic inhibition of Aβ-induced inflammation could be neuroprotective. In a separate study, non-steroidal

anti-inflammatory drug (NSAID) treatment in mice overexpressing Aβ was able to lower Aβ deposition, inhibit microglial activation, and provide neuroprotection (Yan et al., 2003), also indicating that inflammation induced by Aβ contributes to neurotoxicity. In vitro studies also support that Aβ is pro-inflammatory, where inhibition of synthetic Aβ-induced microglial activation with dextromethorphan (Liu et al., 2003) results in a reduction of Aβ-induced neurotoxicity. Together, these studies indicate that inflammation and microglia are critical for the ongoing process of neurodegeneration in AD.

3.2. HIV-associated dementia

Dementia associated with human immunodeficiency virus (HIV) infection is a debilitating condition of cognitive, behavioral, and motor dysfunction seen in the later stages of the acquired immunodeficiency syndrome (AIDS). The hallmarks of HIV-associated dementia (HAD) is neuronal loss, reactive astrogliosis, activated microglia, multinucleated giant cells, and leukocyte infiltration (Budka, 1991). Microglia are essential to the progression of the dementia, as HIV will enter the brain on infected circulating monocytes and is stored in microglia (Jordan et al., 1991; Kure et al., 1990; Michaels et al., 1988; Ryzhova et al., 2002). The microglia then serve as a reservoir for viral replication, resulting in an increase in expression of pro-inflammatory factors (Cosenza et al., 2002) and progression of the disease. While the hallmark symptomology of HIV is immunodeficiency, ironically, the later stage of HAD is known to be an inflammation-mediated neurodegenerative complication of HIV infection. Activated microglia are found in the early stages of HIV infection (Chakrabarti et al., 1991), which then increases in intensity with the progression of the disease. The result of microglial HIV infection and viral replication is an increased release of neurotoxic pro-inflammatory cytokines and enhanced microglial activation (Sopper et al., 1996). Microglia are activated by interaction with viral proteins, such as Tat (D'Aversa et al., 2004) and gp120 (Garden et al., 2004; Kong et al., 1996), the HIV infection itself (Sopper et al., 1996), and soluble factors released from infected cells (Lipton and Gendelman, 1995). While the majority of the literature investigating HIV-induced neurotoxicity focuses on the direct neuronal toxicity that occurs and the inflammatory consequence of infiltrating macrophages, it is becoming increasingly evident that microglia play a substantial role in neurodegeneration associated with HAD.

3.3. Multiple sclerosis

Multiple sclerosis (MS) is the most prevalent inflammation-mediated demyelinating disease. MS is typically characterized as a disease of the young adult, where approximately 10% of those diagnosed will display symptoms that worsen over time, despite periods of remission. Initial clinical symptoms begin with vertigo, fatigue, optic neuritis, and weakness in the limb extremities. The disease progresses to further debilitating symptoms including ataxia, paraparesis, limb spasms, and cognitive impairment. The neurodegeneration associated with MS occurs as lesions in the white matter of the CNS. Specifically, the myelin sheath is damaged in MS in what is believed to be an autoimmune response. While the cause of MS is currently unknown, there is a clear inflammatory component. For example, lymphocytes and activated myeloid cells are localized in the area of demyelization (Hill et al., 2004; Rose et al., 2004; Schonrock et al., 1998). Using nuclear magnetic resonance imaging, positron emission tomography, and [^{11}C](R)-PK11195 (a microglia marker) Banati et al. (2000) analyzed the brain of MS patients and showed increased microglia activity around the site of the MS lesion (Banati et al., 2000). The role of microglia in MS is also supported in the MS animal model of experimental autoimmune encephalomyelitis, where microglia are shown to proliferate and increase lysosome activity around active sites of demyelization (Matsumoto et al., 1992). Once at the site of lesion in MS, microglia increase cyclooxygenase 2 (Rose et al., 2004) and inducible nitric oxide synthase (iNOS) (Hill et al., 2004) expression, which is required for the production of neurotoxic PGE_2 and NO, respectively. In addition to being a source of neurotoxic factors upon activation, microglia have been implicated in the initiation and progression of MS as one of the antigen presenting cells that sparks the autoimmune response targeting myelin (Mack et al., 2003). Thus, while infiltrating T-cells and macrophages have a clear role in MS associated demyelination, lesions, and neuronal damage, microglia are also critically involved in this process.

3.4. Frontotemporal lobe dementia

Frontotemporal dementias (FTD) are considered to be a classification of five unique neuropathologies that are categorized by: (1) the presence or absence of Pick's bodies; (2) neuronal tau-positive inclusions; (3) ubiquitin-positive neuronal inclusions; (4) the number of tau repeats in a neuronal inclusion. However, despite variable pathology, FTDs express a similar clinical symptomology and phenotype. In general, FTDs are dementia syndromes that result in both progressive changes in behavior and language dysfunction typically associated with frontal and/or anterior temporal atrophy and loss of neurons (Munoz et al., 2003). Earlier work identified that microglia were indeed activated in frontotemporal dementias (Cooper et al., 1996; Mann, 1998), but not all pathologies were considered. Recent work by Schofield et al. (2003) characterized the inflammatory profiles of these five pathologies to discern a common thread of similarity uniting them. While different protein deposition and degrees of astrogliosis were present in the postmortem patient brain, activated microglia were elevated and present in the white matter of the frontal lobe to similar

degrees across all classifications of neurodegenerative disease (Schofield et al., 2003). Of further interest, at earlier stages of the disease, the microglia activation was also present, providing rare evidence of an early and perhaps initiating role of microglial inflammation in frontotemporal dementias (Schofield et al., 2003). Currently, the specific mechanisms through which microglia are activated in FTDs are unknown.

3.5. Parkinson's disease

Parkinson's disease (PD) is characterized by progressive degeneration of the nigro-striatal dopaminergic (DA) pathway, which regulates body movement (Olanow and Tatton, 1999). Degeneration of the neuronal cell bodies of DA neurons in the substantia nigra (SN) and the nerve terminals in the striatum results in resting tremor, rigidity, bradykinesia, and gait disturbance in the PD patient (Jellinger, 2001). Postmortem analysis of brains from PD patients frequently shows cytoplasmic inclusions (Lewy bodies) in the DA neurons localized in the SN (Holdorff, 2002; Schiller, 2000), which is the pathological hallmark of PD (Takahashi and Wakabayashi, 2001).

Epidemiological studies, pathological analysis, and biochemical characterization indicate that approximately 95% of PD cases are sporadic with late onset (Tanner, 2003) and only 5% of PD cases occur in familial clusters with early onset (Mizuno et al., 2001). Familial PD is associated with mutations in several genes, including parkin, ubiquitin C-terminal hydrolase L1, and α-synuclein (Gwinn-Hardy, 2002). However, idiopathic PD may represent a long and cumulative process, where the final outcome is the result of a complex set of interactions between genetic predisposition, the innate vulnerabilities of the nigro-striatal DA system, and exposure to environmental toxins. Among the environmental toxins, infectious agents (Duvoisin et al., 1963, 1972; Pradhan et al., 1999), pesticides (Barbeau et al., 1985; Elbaz et al., 2004), and heavy metals (Hudnell, 1999; Iregren, 1999; Sadek et al., 2003) have been implicated in the development and progression of PD. Similar to the case with AD, the role of inflammation in the pathogenesis of PD was not extensively questioned until the work of McGeer et al. (1988) where increased HLD-DR staining in the SN of PD patients suggested that microglia may play a role in yet another neurodegenerative disease.

Microglia and inflammation-mediated neurodegeneration have been implicated in numerous other diseases, such as hypoxia (Olson and McKeon, 2004), stroke (Morioka et al., 1993), amyotrophic lateral sclerosis (Banati et al., 1995; Hall et al., 1998), and neuropathic pain (Tsuda et al., 2004). However, the five diverse examples of neurodegenerative diseases presented here detail how the similar feature of microglial activation can result in diverse localization, pathology, and clinical symptoms for each unique disease. To begin to elucidate the common mechanisms of microglia-mediated neurotoxicity, PD will be used as a representative neurodegenerative disease as we address the progressive nature of microglia-mediated neurotoxicity and the common characteristics/mechanism of microglial activation in response to several diverse toxins.

4. Microglia-mediated dopaminergic neurotoxicity

Dopaminergic neurons are inherently susceptible to the deleterious effects of microglial activation. While the detailed mechanism remains debated, one hypothesis is that the selective mechanism of microglia-mediated dopaminergic neurotoxicity is due to the generation of oxidative insult from microglia. In particular, DA neurons possess reduced antioxidant capacity, as evidenced by low intracellular glutathione, which renders DA neurons more vulnerable to oxidative stress and microglial activation relative to other cell types (Loeffler et al., 1994). While oxidative stress is clearly toxic to multiple cell types, according to this hypothesis, DA neurons will succumb first at lower levels of oxidative stress, followed by other neuronal and cell populations. Additionally, the SN contains 4.5 times as many microglia when compared to the cortex and other regions of the brain (Kim et al., 2000), suggesting that the localization of microglia in the SN predisposes them to vulnerability to immunological insult.

Interestingly, there are multiple factors/toxins that are shown to selectively damage DA neurons through microglial activation such as rotenone (Gao et al., 2003a), diesel exhaust particles (DEP) (Block et al., 2004), paraquat (PQ) (Wu et al., 2005), and Aβ (Qin et al., 2002). However, the majority of these toxins are dual mode toxins in that they damage DA neurons primarily through microglia activation at lower concentrations, but directly kill DA neurons at higher concentrations (Fig. 1). The identification of multiple dual mode DA toxins is critical to the understanding the mechanisms of both selective DA neurotoxicity and the general pathways through which microglia become activated. The initial work investigating the role of microglia in selective DA neurodegeneration began with testing the immunogen lipopolysacharide (LPS), where LPS was shown to be toxic to DA neurons only in the presence of microglia (Gao et al., 2002b) and was one of the first microglia-mediated selective DA toxins identified.

4.1. Chronic LPS infusion produces selective and progressive DA toxicity

In an effort to determine whether microglia activation plays an active role in DA neurotoxicity, Gao et al. (2002a,b) infused LPS, a commonly used immunogen derived from the bacterial wall of gram-negative bacteria (for 2 weeks at 5 ng/h) into an area directly above the SN pars compacta of the rat brain. Infusion of LPS induced a delayed, progressive, and selective loss of nigral DA neurons (Gao et al., 2002b). Neuronal loss was absent early in the first days

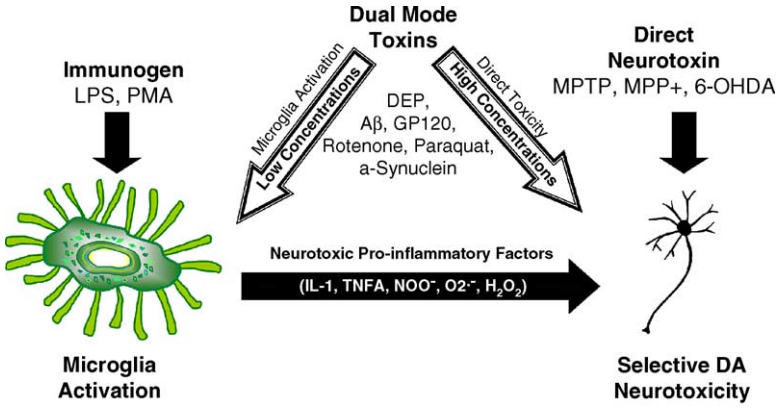

Fig. 1. Mechanisms of selective dopaminergic neurotoxicity. There are multiple pathways through which a toxin can damage DA neurons. Microglial activation by immunogens, such as LPS and PMA, results in selective dopaminergic neurotoxicity through the production of neurotoxic factors from microglia. Alternatively, a toxin may selectively damage the DA neuron directly, such as the case with MPTP and 6-OHDA. However, there is an increasing list of selective DA neurotoxins that work through both microglial activation and direct toxicity, called dual mode toxins. Interestingly, at concentrations when these dual mode toxins are no longer directly toxic to DA neurons, they will still continue to selectively damage and kill DA neurons through their effects on microglial activation.

of the experiment. However, significant DA neuron loss began between 4 and 6 weeks after the start of LPS infusion (40% loss of TH neurons at 6 weeks). The LPS-induced loss of nigral tyrosine hydroxylase immuno-reactive (TH-IR, marker for DA neurons) neurons further progressed over time, where 60% and 70% losses were observed at the 8- and 10-week time point. Interestingly, the LPS-induced neurodegeneration was specific for DA neurons; the loss of Neu-N immuno-reactive (IR) neurons (an index for total number of neurons) in the SN was limited to 20% at 10-week time point, where this decrease was attributed to the loss of DA neurons (Gao et al., 2002b). Furthermore, the LPS-induced DA neuron death was region-specific, as infusion of LPS into an area at the junction between SN and ventral tegmental area (VTA) caused prominent DA neuron loss only in the SN, but not in the VTA. While traditional PD models employing high doses of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) resulted in acute, fast, and selective death of DA neurons, the in vivo LPS model was one of the first to demonstrate this delayed and progressive DA neuron loss, mimicking the disease progression of PD. Of further interest, PD pathology typically spares the VTA, which was also shown in the LPS in vivo model, strongly suggesting that inflammation could play an active role in the selective death of DA neurons in PD.

The Gao et al. (2002a,b) study also showed that in vivo LPS infusion activated microglia and that this activation occurred before the death of DA neurons. In addition to staining for the presence of DA neurons, sections were also immuno-stained with the OX-42 antibody, an antibody against the rat CR3 receptor. At the early time point of 3 days post-LPS infusion, microglia in the SN ipsi-lateral to LPS-infusion exhibited activated morphology. At 1–2 weeks post-LPS infusion, nearly all OX-42-IR microglia were converted to the activated amoeboid form, indicative of a maximal degree of activation. At 4 and 8 weeks after LPS infusion, microglia appeared to remain at the fully activated stage (Gao et al., 2002b). This work defined the temporal relationship of microglial inflammatory insult and neuronal death, as microglial activation preceded DA neuronal death and persisted throughout the experiment, suggesting an active role of microglial activation in inflammation-related DA neurodegeneration.

4.2. Microglia-mediated mechanism of LPS-induced DA toxicity

The cell types and mechanisms responsible for the LPS-induced degeneration of nigral DA neurons was further studied in mixed neuron-glia cultures taken from the ventral midbrain (which encompasses the SN region) (Gao et al., 2002b). Upon stimulation with low concentrations of LPS (0.1–10 ng/ml), this "long-term" (up to 12 days of treatment) in vitro culture system allowed for the study of progressive DA neurodegeneration. Time course study revealed that the lower the concentration of LPS, the longer it took to induce a significant reduction in DA uptake. In vitro comparison of neuron-glia cultures for DA, GABA, or 5-HT neurotoxicity following treatment with LPS showed greater selectivity for DA neurotoxicity, which is consistent with the in vivo findings (Gao et al., 2002b). Immunocytochemical analysis demonstrated that LPS-induced degeneration of DA neurons involved a significant loss of TH-IR perikarya and destruction of TH-IR dendrites, while no significant damage was observed for the other types of neurons (Gao et al., 2002a). Even the surviving TH-IR neurons in the LPS-treated cultures exhibited shrunken cell bodies, rough perimeters, and very short dendrites, in sharp contrast to the appearance of healthy TH-IR neurons in the vehicle-treated cultures.

In addition to reproducing the selective and progressive loss of DA neurons in response to LPS seen in vivo, the in vitro neuron-glia model allowed the ability to individually

determine the cell types responsible for the DA neurotoxicity using ''reconstituted'' cell cultures. In the work by Gao et al. (2002a,b), low concentrations of LPS (1–10 ng/ml) produced damage to DA neurons in the neuron-glia cultures, which are comprised of astroglia, microglia, and neurons. However, across several studies the same concentrations of LPS failed to produce any toxicity in neuron-enriched cultures, which are depleted of microglia and astroglia, indicating that the presence of glia is essential for LPS-induced neurotoxicity (Block et al., 2004; Gao et al., 2002b; Qin et al., 2004). Further, experiments depleting only the microglia indicated that LPS is not toxic in these culture systems (Qin et al., 2004). However, there have been several experiments indicating that by adding enriched microglia to neuron-enriched cultures, or microglia-depleted cultures, the LPS-induced neurotoxicity is reinstated (Gao et al., 2002b; Qin et al., 2004). Together, these results emphasize that microglia, but not astroglia, are necessary for LPS-induced neurotoxicity, which is a critical concept in defining the relative contribution of glial cells to inflammation-mediated neuron death.

Thus, the salient features of these LPS in vivo and in vitro models are: (a) prominent inflammation preceding neuronal death; (b) a delayed and progressive nature of DA neuronal death; (c) a critical role for microglia in neurotoxicity. These models were some of the first to mimic the delayed and progressive nature of the disease symptoms in PD patients and support that microglia activation can actively contribute to neuronal injury and degeneration.

4.3. Early developmental exposure to LPS: critical period of microglia development

Recently, there have been several indications that early life exposure to LPS can activate microglia to cause the loss of dopaminergic neurons (Gayle et al., 2002; Ling et al., 2002, 2004b), where these changes persist from the neonate through to adulthood (Carvey et al., 2003). Ling et al. (2002) defined the critical period of maximal DA neuron cell loss in response to LPS as E10.5, the time period during embryonic development when DA neurons are being born. Additionally, in a separate study, Ling et al. (2004a,b) demonstrated that prenatal LPS can work in concert with other toxins to amplify neurotoxicity, where prenatal (E10.5) LPS exposure produced both a long-lasting DA cell loss and perpetual inflammation, which results in synergistic DA neurotoxicity following subsequent rotenone exposure (Ling et al., 2004a). Interestingly, in another study, prenatal LPS exposure combined with postnatal 6-hydroxydopamine exposure failed to show synergy, suggesting that the mechanisms through which prenatal LPS induces susceptibility to further environmental insult are toxin specific (Ling et al., 2004b). However, neonatal microglial activation has also been linked with the amplification of neurotoxicity, where systemic neonatal exposure to LPS has been shown to significantly amplify neuronal death associated with ischemic insult (Lehnardt et al., 2003). Together, this work suggests that while a critical period of microglial activation exists for maximal impact on DA neuron survival, microglia can respond to LPS throughout development to harm DA neurons and act synergistically with other neurotoxic stimuli, depending on the toxin involved.

5. Triggers of microglia activation and neurodegeneration

It has become increasingly evident that there are diverse triggers through which microglia are activated to exert their neurotoxicity. Interestingly, while these diverse toxins elucidate several mechanisms of microglial activation, NADPH oxidase activation is also a common pathway through which microglia exert neurotoxicity that is shared across these toxins. These diverse triggers of microglial activation include immunological insult, such as LPS; environmental toxins; endogenous disease proteins; neuronal injury.

5.1. Environmental toxins

5.1.1. Rotenone

Rotenone, a common pesticide, is implicated as an environmental risk factor for the development of PD. Betarbet et al. (2000) and Greenamyre et al. (1999) reported that chronic administration of rotenone resulted in a selective destruction of the nigro-striatal DA system, formation of cytoplasmic inclusions in nigral neurons, and induction of hypokinesia and rigidity in rats, reproducing the key features of human PD (Betarbet et al., 2000; Greenamyre et al., 1999). Rotenone's selective DA neuron toxicity has been attributed to the unique vulnerability of DA neurons to oxidative damage, as rotenone is reported to inhibit the activity of complex I of the mitochondrial respiratory chain (Greenamyre et al., 1999; Jenner, 2001). It is generally believed that rotenone directly impacts the neurons to induce toxicity. However, recent work from our laboratory and others has indicated that rotenone can also activate microglia (Gao et al., 2002a; Sherer et al., 2003), which is deleterious to neurons.

Recent work by Gao et al. (2002a,b) indicates that while higher concentrations of rotenone results in direct neurotoxicity, treatment of neuron-enriched cultures (with no microglia present) with up to 20 nM rotenone for 8 days results in little direct DA toxicity. In contrast, neuron-glia cultures (containing both neurons and glia) treated with concentrations of rotenone as low as 1 nM showed selective DA neurotoxicity (Gao et al., 2002a). The enhanced neurodegenerative capacity of rotenone was attributed to the presence of microglia, as the addition of microglia to neuron-enriched cultures markedly increased rotenone-induced DA neurotoxicity. Rotenone was also shown to stimulate superoxide release from microglia. Additionally,

NADPH oxidase inhibition significantly reduced rotenone-induced neurotoxicity (Gao et al., 2002a). Thus, rotenone was shown to exert neurotoxicity by two mechanisms: first, in high concentrations (greater than 25 nM) rotenone will directly damage neurons; second, in much lower concentrations (less than 10 nM), this pesticide will enhance toxicity by activating microglia. At this time, the detailed mechanism through which rotenone activates microglia is unclear.

5.1.2. Paraquat

The herbicide paraquat (PQ, 1,1′-dimethyl-4,4′-bypyridinium) has been implicated as a risk factor for PD, and while there is controversy in the literature as to whether PQ is selectively toxic in vivo, there are increasing reports defining PQ as a trigger for DA neuron cell death. For example, exposure to PQ in early development has been shown to induce long lasting DA neurodegeneration persisting into the adult animal's life (Thiruchelvam et al., 2003). In a separate study, IC injections of PQ directly to the striatum resulted in a dose dependent decrease in DA neurons 2 weeks after the treatment (Liou et al., 1996), where the DA loss was both long lasting and irreversible. Further, Liou et al. (1996) reported glial activation and changes in motor behavior in response to IC injected PQ, as evidenced by rotational behavior. In human cases of fatal PQ poisoning, postmortem analysis revealed microglia and astrocyte activation (Grant et al., 1980). Initially, the herbicide paraquat was assumed to be toxic to DA neurons because of its structural similarity to the selective and direct neurotoxin, 1-methyl-4-phenylpyridinium (MPP+). Thus, while there is evidence of microglial activation in PQ-associated neurotoxicity, the mechanism of neuronal death was initially believed to be through direct interaction with the neuron.

Recent work from our laboratory revealed that PQ (0.5–1 μM) is selectively toxic to DA neurons through the activation of microglial nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and the generation of superoxide (Wu et al., 2005). Microglia-depleted cultures exposed to 1 μM PQ failed to demonstrate a reduction in DA uptake, indicating that microglia are the critical cell type mediating PQ neurotoxicity. Further, neuron-glia cultures treated with PQ failed to generate TNFα and NO. However, microglia-enriched cultures exposed to PQ produced extracellular superoxide, supporting that microglia are an essential source of PQ-derived oxidative stress. Finally, Wu et al. (2005) showed that low concentrations of PQ failed to show toxicity in NADPH oxidase deficient ($PHOX^{-/-}$, phagocytic oxidase, another name for NADPH oxidase) mice, indicating the critical role of NADPH oxidase in PQ neurotoxicity at lower concentrations. NADPH oxidase an enzymatic complex responsible for the production of extracellular superoxide in phagocytes (Babior, 2000). Thus, while higher concentrations of PQ were directly toxic to DA neurons, at lower doses, the indirect superoxide insult generated from microglial NADPH oxidase is the essential factor mediating PQ-induced DA neurotoxicity. However, at this time, how rotenone and PQ activate microglia remains unknown.

5.1.3. Particulate matter and the phagocytic activation of microglia

Air pollution is epidemiologically associated with increased morbidity and mortality in respiratory and cardiovascular disease (Ma and Ma, 2002). Particulate matter (PM) is a ubiquitous particle component of urban air pollution responsible for the deleterious respiratory and cardiovascular effects of air pollution. Diesel exhaust particles (DEP) are a category of PM derived from diesel fossil fuels and combustible engines (Ma and Ma, 2002). DEP is a complex toxin consisting of a carbon core with over 300 potential adsorbed compounds, including polyaromatic hydrocarbons, quinones, and transition metals (Ma and Ma, 2002). However, there are reports that many of the biological effects of PM relate to the physiochemical features of the particles, such as surface charge (Veronesi et al., 2002, 2003). There have been increasing reports that PM can enter the brain and that PM may be associated with neurodegenerative pathology in vivo (Calderon-Garciduenas et al., 2002, 2003; Finch et al., 2002; Jensen et al., 1989). In particular, PM administration has been associated with selective DA neuron loss in the SN in $APOE^{-/-}$ mice (Veronesi et al., 2005). In humans, exposure to high amounts of air pollution in Mexico City is associated with increased markers of brain inflammation (Calderon-Garciduenas et al., 2004). Additionally, mice exposed to concentrated particulate matter showed an increase in TNFα, interleukin-1 beta (IL-1β), and NFκB expression (Campbell et al., 2005). However, until recently, the mechanisms of how particulate matter induces the pro-inflammatory response in the brain and the cell types responsible for the neurotoxicity were unclear.

Recent work from our laboratory reported that mesencephalic neuron-glia cultures treated with diesel exhaust particles (DEP) (<0.22 μM) (5–50 μg/ml) resulted in a selective dose dependent decrease in DA neurons (Block et al., 2004). Microglia were also shown to be a critical component of the neurotoxicity, as was demonstrated by the failure of neuron-enriched cultures (containing only neurons) to exhibit DEP-induced DA neurotoxicity at lower concentrations, where DEP-induced DA neuron death was reinstated with the addition of microglia to neuron-enriched cultures (Block et al., 2004). Further, DEP treatment resulted in activated microglia morphology and the production of intracellular reactive oxygen species and superoxide. Additionally, similar to previously reported toxins, neuron-glia cultures from NADPH oxidase deficient ($PHOX^{-/-}$) mice were insensitive to DEP neurotoxicity when compared to control mice ($PHOX^{+/+}$) (Block et al., 2004). However, unlike other environmental toxins, cytochalasin D inhibited DEP-induced superoxide production in enriched-microglia cultures, implying that DEP induces microglia to produce superoxide through the process of phagocytosis (Block et al.,

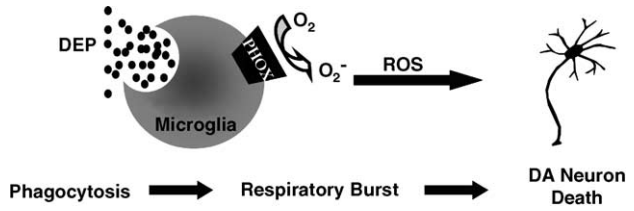

Fig. 2. Phagocytosis-mediated DA neurotoxicity. DEP are phagocytized by microglia, which results in activation of NADPH oxidase (PHOX) and the neurotoxic respiratory burst. DA neurons are particularly vulnerable to oxidative damage and may have an increased sensitivity to ongoing phagocytosis from neighboring microglia compared with other neuronal cell types. Reproduced from (Block et al., 2004).

2004). Together, these in vitro data indicate that DEP selectively damages DA neurons through the phagocytic activation of microglial NADPH oxidase and consequent oxidative insult.

Fig. 2 depicts how DEP initiates the phagocytic activation of microglia. DEP are phagocytized by microglia, which results in the activation of NADPH oxidase and the neurotoxic production of extracellular superoxide. Recently, we have identified a subset of toxins that are selectively toxic to DA neurons through the phagocytic activation of microglia: DEP (Block et al., 2004), LPS (Pei et al., unpublished results), α-synuclein (Zhang et al., 2005), and gp91 (Block et al., unpublished results). These toxins activate microglia through the process of phagocytosis and share the following common features: (1) they tend to exist in aggregates; (2) the neurotoxicity is dependent upon the presence of microglia; (3) they are toxic through the production of superoxide from microglial NADPH oxidase; (4) microglia phagocytize the toxin and it can be found inside the microglia. However, it is clear that not all phagocytosis results in the respiratory burst, as the phagocytosis of apoptotic cells is typically thought to occur without the production of extracellular superoxide (Savill et al., 2003). Whether phagocytosis activates NADPH oxidase is dependent upon the receptors identifying the toxin/target being internalized (Caron and Hall, 1998). The group of toxins that activate microglia through phagocytosis are likely identified by pattern recognition receptors critical to host defense in innate immunity. Currently, the microglial receptors identifying these toxins, such as particulate matter, and the mechanisms through which these receptors activate microglial NADPH oxidase are unknown.

Thus, while phagocytosis is a common and necessary element to maintain homeostasis and remove cellular debris, the deleterious, oxidative collateral-damage of phagocytosis may be another characteristic of the over-activated microglia in the neurodegenerative disease state. This finding has broad reaching implications, as several pathological hallmark proteins associated with neurodegenerative disease, such as beta-amyloid (Mitrasinovic and Murphy, 2003; Mitrasinovic et al., 2003), melanin (von Baumgarten et al., 1980), prions (Jeffrey et al., 1994), and myelin (Rotshenker, 2003) are reported to be phagocytized by microglia.

5.2. Endogenous disease proteins

5.2.1. β-Amyloid

Earlier, Aβ was discussed in detail, as it is a critical mechanism of microglial activation in AD. Interestingly, while Aβ is toxic to mixed cortical cultures and neuronal cell types typically damaged in AD, Aβ is also selectively toxic to DA neurons. In fact, Aβ is another example of a dual mode toxin, where high concentrations are directly toxic to both cortical neurons and DA neurons. Qin et al. (2002) showed that incubation of cortical or mesencephalic neuron-enriched and mixed neuron-glia cultures with high concentrations of Aβ (6.0 μM for cortex and 1.5–2.0 6.0 μM for mesencephalon) directly injured neurons in neuron-enriched cultures. In contrast, lower concentrations of Aβ (1.0–3.0 μM for cortex and 0.25–1.0 μM for mesencephalon) caused significant neurotoxicity in mixed neuron-glia cultures, but not in neuron-enriched cultures (Qin et al., 2002). While low concentrations of Aβ induced activated microglial morphology and superoxide production, the secretion of TNFα, interleukin-1β, and nitric oxide did not occur (Qin et al., 2002). Finally, NADPH oxidase-deficient mutant mice were less sensitive to Aβ DA neurotoxicity when compared to wild-type controls, indicating a critical role of extracellular superoxide in Aβ DA neurotoxicity (Qin et al., 2002). Thus, while Aβ is the hallmark protein associated with AD, it is also a dual mode toxin selective for DA neurons, supporting the assertion that similar mechanisms of microglial activation are responsible for neurotoxicity of multiple cell types.

5.2.2. α-Synuclein

α-Synuclein is a component of lewy bodies, the morphological hallmark of PD (Takahashi and Wakabayashi, 2001). However, the pathophysiological role of this protein in the DA degeneration is not clear. Traditionally, α-synuclein was thought to directly exert damage to DA neurons. However, work from our laboratory has demonstrated that microglia, but not astroglia, enhance α-synuclein-induced DA toxicity (Zhang et al., 2005). Additionally, α-synuclein fails to show DA neurotoxicity in microglia-depleted cultures at low concentrations (Zhang et al., 2005), indicating that α-synuclein is also a dual mode toxin. Further, α-synuclein activates microglia to produce extra-cellular superoxide, increases microglial intracellular ROS concentrations (iROS), and induces morphological changes in microglia (Zhang et al., 2005). Similar to DEP, α-synuclein was shown to be phagocytized by microglia and the production of microglial ROS in response to α-synuclein was inhibited by cytochalsin D, implying that phagocytosis is a critical component of the mechanism of α-synuclein-induced microglial activation (Zhang et al., 2005). This work provides an intriguing hypothesis for the propagation of neuronal death in PD, where damaged neurons releasing α-synuclein could further potentiate neuronal death through microgliosis due to the phagocytosis of aggregated α-synuclein.

5.3. Reactive microgliosis

Microglial activation after CNS injury or in response to neurodegeneration was initially perceived as a transient and self-limited event (Streit et al., 1999). However, it has become increasingly evident that the microglial response to neuronal damage is both long-lived and self propelling (Gao et al., 2003b; Huh et al., 2003; McGeer et al., 2003). The neurotoxic response of microglia to central nervous system (CNS) injury is a critical component of microglia-mediated neurotoxicity across multiple diseases (Eikelenboom et al., 2002; Sanchez-Moreno et al., 2004; Wenk, 2003). In general, dying or damaged neurons have the potential to activate microglia, regardless of how the neurons were damaged (environmental toxin, endogenous disease protein, or reactive microgliosis) or the neurodegenerative disease in question. Fig. 3 depicts the relationship between neuronal damage and microglial activation and characterizes how damaged neurons will activate microglia to initiate a self-propelling cycle of neuron-death. This repeating cycle of the neurotoxic activation of microglia in response to neuron injury is commonly referred to as reactive microgliosis.

In the case of PD, early work by McGeer et al. (1988) first documented the microglial response to the selective loss of DA neurons. However, the persistent activation of microglia in response to DA neuron injury and the active neurotoxic consequences of this microglial activation were only accepted recently, due to further investigation using the selective DA neurotoxin, MPTP. The immediate direct and selective DA neurotoxicity of MPTP was first discovered by Langston et al. (1983), where after the MPTP-model was established as the gold standard animal model of PD. In contrast to LPS, MPTP directly damages DA neurons when it is taken up through the DA transporter, resulting in oxidative mitochondrial damage, which leads to neuronal death. MPTP (or MPP+, the active metabolite of MPTP) does not directly activate microglia (Gao et al., 2003b). However, chronic neuroinflammation has been reported to continue years after MPTP exposure in humans (Langston et al., 1999) and primates (McGeer et al., 2003), despite the fact that the exposure to MPTP was brief. As detailed earlier, most neurodegenerative diseases are delayed and progressive in nature. For example, the time period between the exposure to environmental neurotoxins and the manifestation of the PD symptoms is assumed to be approximately 8–10 years. However, in absence of repeated and continual exposure, it is unlikely that these toxins remain present in the brain at the time of PD diagnosis years later, suggesting that the microglial activation initiated by early toxic insult is propagated and potentially amplified throughout the disease.

Work from our laboratory and others' has documented that microglia also play an active role in the process of neuronal death, as MPTP-induced neurotoxicity is clearly linked with microglial activation (Gao et al., 2003b; McGeer et al., 2003; Wu et al., 2003). For example, the addition of microglia to enriched neuron cultures greatly enhances MPTP-induced DA toxicity (Gao et al., 2003b), demonstrating that the presence of microglia can amplify neuronal damage. In several animal studies, MPTP toxicity is significantly reduced in mutant mice with deficient production of pro-inflammatory factors, such as superoxide (Wu et al., 2003; Zhang et al., 2004), prostaglandins (Feng et al., 2002; Teismann et al., 2003), and TNFα (Sriram et al., 2002). Again, these reports indicate that microglia-mediated neurotoxicity is a component of the toxin-induced cell damage resulting from exposure to direct neurotoxins, such as MPTP. Specifically, the attenuated MPTP neurotoxicity shown in mice deficient in pro-inflammatory function demonstrates that microglial-derived pro-inflammatory factors play a role in overall neurotoxicity.

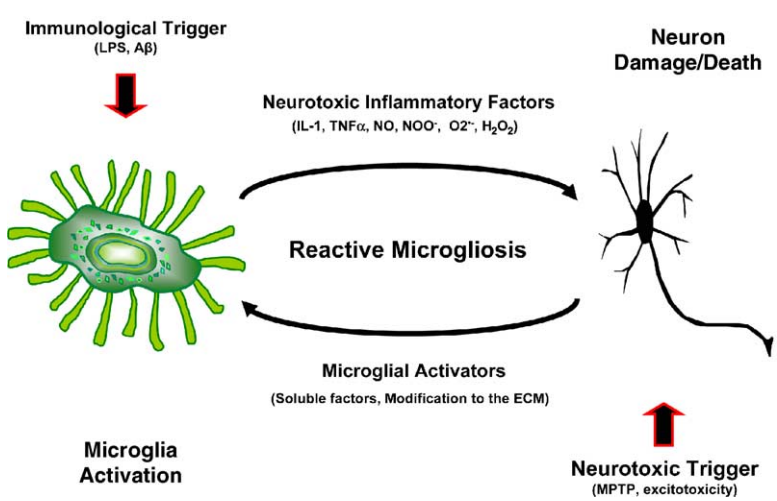

Fig. 3. Reactive microgliosis is a self-propelling cycle of neuronal damage. Regardless of the initial toxic insult (immunological insult from microglia or direct neuronal toxicity), dying or damaged neurons activate microglia to produce neurotoxic factors, which are toxic to surrounding neurons, resulting in perpetuating toxicity. As neuronal death is a common denominator across multiple neurodegenerative diseases, microgliosis may be the common thread responsible for ongoing the microglial activation and the progressive nature of many neurodegenerative diseases.

While the microglia pro-inflammatory response likely involves multiple toxic factors, work from our laboratory and others has identified that microglia-derived ROS are a prominent component of reactive microgliosis (Gao et al., 2003b; Wu et al., 2003). For example, while MPP+ and MPTP do not directly affect microglial activation, Gao et al. (2003b) demonstrated that the addition of both MPP+ and MPTP to neuron-glia cultures induced the production of superoxide at 4 days posttreatment. Gao et al. (2003b) propose that this time delay in superoxide production in response to MPP+ and MPTP occurred due to significant accumulation of neuronal damage at 4 days posttreatment. Further, the extracellular superoxide in response to MPTP was produced only in animals with functioning NADPH oxidase and was attenuated by NADPH oxidase inhibitors (Gao et al., 2003b). However, no detectable amounts of TNFα, NO, or PGE_2 were produced in neuron-glia cultures exposed to MPP+ or MPTP at any time point measured, indicating the essential role of superoxide in microgliosis (Gao et al., 2003b). Gao et al. (2003a,b,c) also showed that in neuron-glia cultures from mice lacking functional NADPH oxidase, MPTP and MPP+ both showed reduced DA toxicity (Gao et al., 2003b), confirming that the production of extracellular superoxide contributes to MPP+ and MPTP-induced neurotoxicity. Further work by Gao et al. (2003c) reports the amplifying nature of microgliosis, where LPS and MPTP administered simultaneously or in tandem resulted in synergystic neurotoxicity. Interestingly, the synergyistic neurotoxicity of LPS and MPTP was also demonstrated to be mediated through NADPH oxidase, again emphasizing the critical role of this enzyme in the microglia activation and DA neurotoxicity associated with reactive microgliosis (Gao et al., 2003c). While there is strong support that microglia become activated by neuronal death to produce neurotoxic superoxide (Gao et al., 2003b), the mechanisms through which neuronal damage induce microglial activation are not completely understood.

5.3.1. Matrix metalloproteinase-3

While it is clear that microglia become activated upon neuronal damage, there is a dearth of information on the neuronal injury signals responsible for the chronic microglial inflammatory response. Recent work suggests that proteases known to modify the extracellular matrix (ECM) may be a critical mechanism through which damaged neurons activate microglia to produce extracellular superoxide. Previous work from our laboratory has emphasized the critical role of ECM proteins in the interactions between microglia and neurons (Chang et al., 2000a). In current work by Kim et al. (2005b) matrix metalloproteinase-3 (MMP-3), a proteinase known to degrade ECM components, was shown to be released upon DA cell damage with MPP+ and to be toxic to DA neurons. Mesencephalic neuron/glia cultures treated with MPP+ resulted in a dose dependent increase in the MMP-3 protein both in cell lysates and in conditioned media, implying that DA neuron death upregulates the expression of MMP-3. Mesencephalic neuron-glia cultures treated with catalytically active MMP-3 showed DA neurotoxicity and activated microglia morphology that preceded neuron death (Kim et al., in review). Moreover, enriched microglia produced extracellular superoxide in response to MMP-3. This finding is critical to understanding the mechanism of microgliosis, as previous studies from our laboratory have identified that the activation of PHOX is a mandatory component of the microglia contribution to MPP+ and MPTP induced death. Further supporting this premise, midbrain neuron-glia cultures from $PHOX^{-/-}$ mice, lacking the catalytic subunit of PHOX and unable to produce the phagocytic respiratory burst, were protected from MMP-3-induced DA neurotoxicity in vitro when compared to control (Kim et al., in review). In vivo experiments showed that MMP-3 deficient mice were less susceptible to SN DA neuronal degeneration and showed a less pronounced microglial response induced in vivo by MPTP. Together, these data suggest that MMP3 is released upon DA neuron damage and activates microglia to further propagate neuronal death.

5.3.2. Neuromelanin

Neuromelanin is also reported to be released by damaged or dying DA neurons to activate microglia (Zecca et al., 2003). In the normal, healthy human SN, neuromelanin is located within dopaminergic neurons, accumulates in the SN with age, and is responsible for the pigmented color of the SN. Functionally, it has been suggested that neuromelanin plays a protective role intracellularly, where neuromelanin will bind toxins (D'Amato et al., 1986; Lindquist et al., 1988; Zecca et al., 1994) and serves as an antioxidant (Fornstedt et al., 1989; Wilczok et al., 1999). However, it has also been suggested that neuromelanin has the potential to be toxic, as excess neruomelanin inhibits the function of the DA neuron proteasome (Shamoto-Nagai et al., 2004). Analysis of the postmortem PD patient SN indicates that neuromelanin levels are significantly reduced, which is consistent with the loss of DA neurons (Hirsch et al., 1988; Zecca et al., 2002). Zecca et al. propose that neuromelanin is released by damaged or dying DA neurons to initiate microglial activation and that neuromelanin may be one of the factors released by DA neurons responsible for the self-propelling cycle of microgliosis (Zecca et al., 2003). Indeed, neuromelanin is insoluble, is localized in high concentrations in the SN (Lindquist, 1972), and it has been found in the extracellular spaces in the SN of PD patients (Calabrese and Hadfield, 1991), presenting an ideal opportunity for neuromelanin to interact with microglia. Using exogenous neuromelanin purified from the human brain, Wilms et al. (2003) have shown that neuromelanin is chemotactic for microglia. Further, Wilms et al. (2003) also showed that neuromelanin added to rat enriched microglia cultures activates microglial NFκB and induces the production of toxic factors, such as TNFα, IL-6, and NO (Wilms et al.,

2003). However, whether neuromelamin induces superoxide production in microglia is unknown.

Currently, MMP3, α-synuclein, and neuromelanin have all been implicated as factors released by damaged DA, resulting in a self-propelling cycle of microglial activation and neuronal death. It seems likely that the identity of the factors released from damaged neurons to signal microglial activation is dependent upon the type of cell damaged and the nature of the cell damage (necrosis versus apoptosis). For example, α-synuclein and neuromelainin are highly expressed by DA neurons in the SN, suggesting that these factors are more likely to be released by injured DA neurons. However, microglial activation is constant across numerous neurodegenerative disorders, where several neuronal cell types die as a result of a diverse set of triggers. It seems likely that there are multiple common neuronal injury signals released from varied neuronal cell types in response to CNS injury, such as the pro-inflammatory characteristics of necrosis. Alternatively, specific toxins may cause the release of unique soluble factors from damaged neurons that serve as propagating pro-inflammatory cues. It is of significant interest for ongoing research to identify soluble factors released from multiple classifications of damaged neurons in response to various toxins, so that we can begin to elucidate how neuronal damage can signal inflammation and propagate further cell death.

6. Common characteristics of microglial activation.

6.1. Temporal relationship of the microglial release of neurotoxic factors

The identification of several potential triggers of microglia activation has allowed a generalizable classification of how microglial respond to stimuli. Firstly, based on in vitro culture data, there is a clear temporal relationship between the released microglial neurotoxic factors. Across several toxins, it is apparent that first event is the production of reactive oxygen species (ROS), which includes the extracellular superoxide anion ($O_2^{\bullet-}$) and an increase in iROS (Gao et al., 2002b; Qin et al., 2002; Zhang et al., 2005). The increase in the production of ROS is rapid, usually occurring within minutes, and is typically measured in microglia 10–30 min after LPS addition. The microglial ROS response is followed by the release of cytokines (such as TNFα and IL-1β), nitric oxide (NO), and prostaglandin E_2 (PGE_2) that peaks around 6–12 h (Gao et al., 2002b; Liu et al., 2003). Experiments using different mutant mice deficient in NADPH oxidase, iNOS, COX-2, or TNFα receptors all resulted in reduced neurotoxicity (Teismann and Ferger, 2001; Wang et al., 2004b), indicating that the individual pro-inflammatory factors released are sufficient, but not mandatory, for neurotoxicity. In fact, individual pro-inflammatory factors, such as IL-1, TNFα, and interferon-γ, work in concert to synergistically induce neuronal damage (Jeohn et al., 1998). Additionally, microglial NADPH oxidase activation ($O_2^{\bullet-}$ production) is shown to enhance the release of other pro-inflammatory factors from microglia (Qin et al., 2004), identifying this enzyme as a key regulating factor of microglia-mediated neurotoxicity.

6.2. NADPH oxidase is the key enzyme for producing ROS in the activation of microglia

Interestingly, microglia consistently generate ROS when activated by multiple pro-inflammatory triggers, such as environmental factors (LPS, DEP, rotenone, paraquat), endogenous protein toxins (β-amyloid peptide, α-synuclein), and neuronal injury (Fig. 4). On the other hand, the

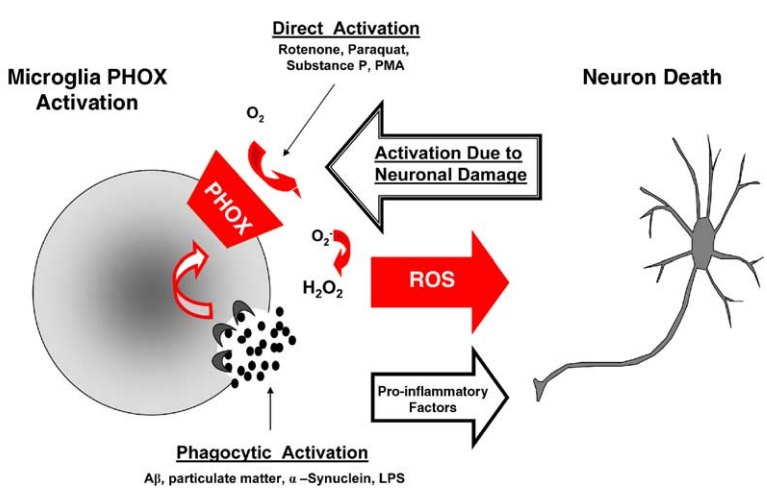

Fig. 4. NADPH oxidase is the common mechanism through which microglia are toxic to neurons. As the resident brain phagocytes, microglia can produce multiple pro-inflammatory factors that are harmful to neurons. Across several toxins studied, the initiating steps of microglial activation are vastly different and often unique combinations of pro-inflammatory factors are released, which are dependent upon the stimulus. However, the mechanism through which these diverse toxins (DEP, rotenone, LPS, PQ, reactive microgliosis, Aβ, and α-synuclein) exert microglia-mediated neurotoxicity has consistently been shown to involve PHOX. At this time, it remains unclear whether pro-inflammatory microglia activation can occur without the consequent activation of PHOX.

production of other factors such as NO, TNFα, or PGE_2 from microglia is much less consistent across a diverse list of toxins (Block et al., 2004; Gao et al., 2002a; Qin et al., 2002), implicating that microglial-derived ROS may be an essential and common factor of microglial activation. In fact, it is unclear whether the microglial pro-inflammatory response can occur without the generation of extracellular or intracellular ROS.

ROS production in phagocytes can originate from several sources, such as PHOX on the surface membrane, peroxidases inside the cell, or oxidative processes of mitochondria. However, NADPH oxidase is the predominant source of microglial extracellular ROS production in response to multiple and diverse stimuli (Gao et al., 2003a; Min et al., 2004; Qin et al., 2004; Wu et al., 2005). NADPH oxidase is a membrane-bound enzyme that catalyzes the production of superoxide ($O_2^{\bullet -}$) from oxygen. This enzyme is dormant in resting phagocytes but is activated when the cell is activated by any of a variety of stimuli, including bacteria and certain inflammatory peptides (Babior, 2000). The enzyme is composed of a number of subunits, including a flavocytochrome known as cytochrome b558 (Babior, 2000). In resting cells, these subunits are distributed between the cytosol and the membranes of intracellular vesicles and organelles (Babior, 2000). When the cell is activated, the cytosolic subunits (p47, p67, p40, and Rac2) migrate to the membranes, where they bind to the membrane-associated subunits (p22 and gp91) to assemble the active oxidase (Babior, 2000). The critical role of PHOX and its ROS products in mediating DA neurodegeneration is discussed below.

6.2.1. Extracellular superoxide is the key factor mediating inflammation-related neurotoxicity

Pro-inflammatory factors released during microglial activation can function synergystically to produce inflammation-related neuronal damage (Jeohn et al., 1998). Among the array of factors released, superoxide is essential for both the amplification and induction of neurotoxicity. This is based on the observation that a host of toxins, such as DEP (Block et al., 2004) rotenone (Gao et al., 2002a), paraquat (Wu et al., 2005), and β-amyloid peptide (Qin et al., 2002), are capable of either producing or enhancing neurotoxicity by generating superoxide, while other factors, such as NO and TNFα, are largely unaffected. While the precise species of ROS responsible for neurotoxicity is unknown, superoxide dismutase/catalase mimetics, which remove superoxide and H_2O_2 respectively, reduce LPS-induced DA toxicity (Wang et al., 2004a) indicating the critical importance of H_2O_2 and superoxide in microglia-mediated neurotoxicity. This is further supported by work by Pawate et al. (2004), where catalase was shown to inhibit the production of pro-inflammatory factors (IL-1, IL-6, iNOS and TNFα) from microglia activated with LPS.

The critical role of PHOX in mediating inflammation-related neurotoxicity was illustrated in our recent paper (Qin et al., 2004), where the LPS-induced loss of nigral DA neurons in vivo and in vitro was significantly less pronounced in PHOX-deficient ($PHOX^{-/-}$) mice, when compared to control ($PHOX^{+/+}$) mice. Reconstituted cell culture experiments confirmed that microglia were the source of ROS production, as both $PHOX^{+/+}$ and $PHOX^{-/-}$ neuron-glia cultures chemically depleted of microglia via leucine-methyl ester failed to show DA neurotoxicity with the addition of LPS (Qin et al., 2004). Second, neuron-enriched cultures (containing no microglia or astroglia) from both $PHOX^{+/+}$ mice and $PHOX^{-/-}$ mice also failed to show any direct LPS-induced DA neurotoxicity. However, the addition of $PHOX^{+/+}$ microglia back to neuron-enriched cultures from either strain resulted in reinstatement of LPS-induced dopaminergic neurotoxicity, supporting the role of microglia as the primary source of PHOX that generates oxidative insult and neurotoxicity (Qin et al., 2004).

Oxidative stress is a common characteristic shared across numerous neurodegenerative diseases (Bahat-Stroomza et al., 2005; Basso et al., 2004; El Kossi and Zakhary, 2000; Liu et al., 2005b; Perluigi et al., 2005), suggesting a basic and similar mechanism underlying diverse neurodegenerative pathology. Interestingly, NADPH oxidase has been linked to microglia-derived oxidative stress from a variety of neurotoxic insults, such as rotenone (Gao et al., 2003a), DEP (Block et al., 2004), α-synuclein (Zhang et al., 2005), Aβ (Qin et al., 2002), PQ (Wu et al., 2005), DA neuronal injury (Gao et al., 2003b; Wu et al., 2003), and cerebral ischemia-reperfusion injury (Green et al., 2001), indicating that microglial NADPH oxidase activation may also be a common denominator of microglial activation associated with neurotoxicity. Further, NADPH oxidase is upregulated in neurodegenerative diseases such as PD (Wu et al., 2003), emphasizing the importance of microglial NADPH oxidase activation for the survival of neurons.

6.2.2. Intracellular ROS regulate the expression of pro-inflammatory factors

Intracellular ROS (iROS) are critical for the activation of microglia and the enhancement of the production of pro-inflammatory factors. The increase in iROS in phagocytes, such as microglia, includes a number of oxygen species, such as superoxide anion, hydroxyl radical, lipid hydroperoxides and their by-products (e.g., H_2O_2) (Li and Trush, 1998). The production of iROS results from the process of normal cellular function and metabolism and may originate from multiple cellular sources, such as xanthine oxidase, mitochondrial electron transport, PHOX, peroxisomes, and endoplasmic reticulum (Li and Trush, 1998). We have previously shown that PHOX activation contributes to at least 50% of the LPS-induced increase in iROS (Qin et al., 2004). There is increasing support that iROS can also function as second messengers to regulate several downstream signaling molecules, including protein kinase C, mitogen activated protein kinase (MAPK) and nuclear factor-κB (NFκB) (Guyton et al., 1996; Konishi et al., 1997;

Schreck et al., 1991). Using PHOX$^{-/-}$ neuron-glia cultures, we have shown that activation of the PHOX initiates an intracellular ROS signaling pathway (Forman and Torres, 2002) that can activate microglia and amplify the production of pro-inflammatory cytokines, such as TNFα (Qin et al., 2004) or PGE_2 (Wang et al., 2004b). Additionally, Min et al. (2004) demonstrated that ganglioside induces the activation of microglia, where the production of IL-1β, TNFα, and iNOS are attenuated by the addition of the NADPH oxidase inhibitor, diphenyleneiodonium. Furthermore, NADPH oxidase inhibitors and catalase are shown to suppress LPS-induced expression of cytokines (IL-1, IL-6, and TNFα), iNOS expression, MAP kinases, and NFκB phosphorylation (Pawate et al., 2004). NADPH oxidase has also been implicated as critical for the morphological changes associated with the early phase of microglial activation, where immuno-staining for F4/80 (marker for mouse microglia) in midbrain neuron-glia cultures treated with LPS revealed that PHOX$^{-/-}$ microglia show much less activated morphology in response to LPS treatment than the PHOX$^{+/+}$ (Qin et al., 2004). While the majority of research has been completed in models of LPS-induced microglial activation, NADPH oxidase is essential across diverse triggers of microglial activation (environmental toxins, endogenous disease proteins, and neuronal injury) indicating that ROS are likely an essential signaling mechanism regulating general microglial activation.

Accumulating evidence supports that NADPH oxidase contributes to microglia-mediated neurotoxicity through two mechanisms (Fig. 5). First, activation of NADPH oxidase results in the production of extracellular ROS that is toxic to neurons. Second, activation of NADPH oxidase causes an increase in the microglial intracellular ROS, which enhances the production of pro-inflammatory factors that are toxic to neurons. This intracellular ROS increase is a critical component of pro-inflammatory signaling in phagocytes (kupher cells, monocytes, microglia, macrophages) and has been called the PHOX-ROS pathway. The dual impact of NADPH oxidase activation on neurotoxicity and the prevalence of NADPH oxidase activation upon microglial activation, regardless of the stimulus, suggest that microglial NADPH oxidase is a critical mechanism of neuronal death across multiple neurodegenerative diseases.

7. Microglial activation as a common mechanism in diverse neuropathology

While we have highlighted the common mechanisms of how microglia respond to various toxins and signals to result in selective DA neuron toxicity, we have also presented evidence indicating that microglial activation contributes to unique pathology associated with multiple neurodegenerative diseases. The intriguing question remains as to how the generalized phenomena of microglial activation can result in diverse and localized neurodegenerative pathology. While this is a complex issue that is likely specific to each neurodegenerative disease, there are several hypotheses as to why microglial activation would be localized in unique brain regions that vary both across and within neurodegenerative disease types.

It is important to note that microglial activation can be localized, where activation of microglia in one brain region does not instigate activation across the entire brain. A classic example is the case of LPS injection into the nigral area, where microglial activation and consequent neuronal damage are limited to this region (Gao et al., 2002b). Thus, the localization of the initiating stimulus (neuronal death, an environmental factor, or an endogenous disease protein) will be a critical factor in determining where microglia will become activated to propagate neuronal toxicity and likely contributes to the regional compartmentalization of many

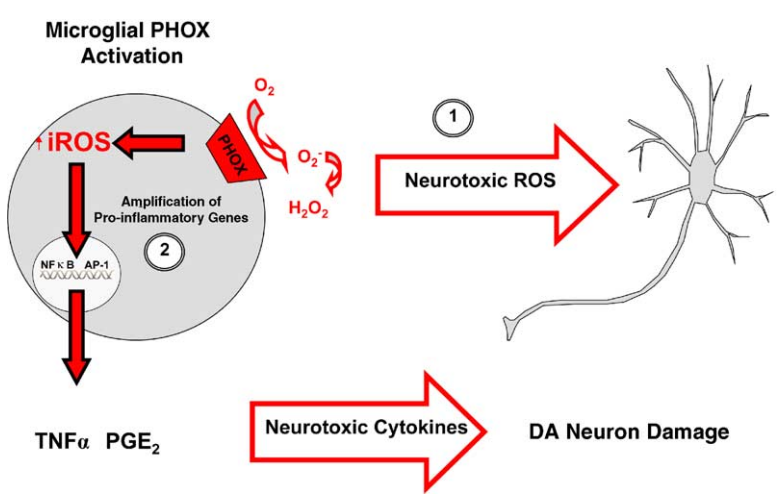

Fig. 5. Dual mechanisms of NADPH oxidase-mediated neurotoxicity. Here we depict the critical mechanisms through which microglial NADPH oxidase (PHOX) mediates neurotoxicity. Extracellular ROS produced as a consequence of PHOX activation is toxic to neurons. Additionally, the increase in intracellular ROS that occurs in microglia as a response to PHOX activation enhances the production of neurotoxic pro-inflammatory factors from microglia.

neurodegenerative disorders. In addition to the location of the original neurotoxic event/trigger, the unique characteristics of the neurotoxic triggers associated with neurodegenerative diseases may determine which cell types will be damaged. For example, while a lower grade inflammatory response selectively kills DA neurons, higher doses of the same toxin (such as LPS) begins to kill multiple cell types (Gao et al., 2002b). Thus, the degree of the microglial inflammatory response may also determine which cell types are damaged, as certain cell types localized in select brain regions may be preferentially vulnerable to microglia-derived insult. Again, while this is particularly evident in the case of DA neurons and PD, the hierarchy of susceptible cell types in the localized region of other neurodegenerative diseases may also play a role in the specificity of disease phenotype.

Finally, regional differences in microglial populations and their pro-inflammatory responses also offer further insight into the potential mechanisms mediating the compartmentalization of both neurodegenerative pathology and microglial activation seen across the numerous neurodegenerative diseases. Again, DA neurons localized in the SN are the extreme example, as there are 4.5 times as many more localized in this region (Kim et al., 2000), making cells there more susceptible to inflammatory insult. In the case of other neurodegenerative disorders, differences of regional microglia density could also explain localized neurotoxicity. In addition, regional differences in subclasses of microglia (Ladeby et al., 2005; Mittelbronn et al., 2001) have been reported, where microglial population differences in reactivity could also offer insight into neurodegenerative disease localization. Furthermore, brain region differences in microglial regulatory factors, such as astrocyte populations, could also explain how the generalized phenomenon of microglial activation could be localized by brain region.

It is not surprising that multiple interacting factors will likely determine the regional activation of microglia and the specific pathology of neurodegenerative diseases. Unfortunately, the identity of the initial neurotoxic triggers remains unknown for most neurodegenerative diseases. As we begin to learn more about the initiating factors for neurodegenerative diseases and further characterize both preserved and affected brain areas, future research will have the ability to address the potential mechanistic role of microglia in specific neurodegenerative pathology in more detail.

8. Conclusions

Microglia are the critical actors of self-propelling mechanisms of neurotoxicity (reactive microgliosis), which is an underlying contributing mechanism to multiple neurodegenerative disorders. Microglia can be activated by two mechanisms: (1) direct stimulation of microglia from environmental or endogenous toxins; (2) activation through a reactive microgliosis process. Oxidative stress is predominant in neurodegenerative disease and microglia are critical sources of oxidative insult. In particular, the activation of microglial NADPH oxidase is a common source of microglial-derived oxidative stress, regardless of the stimulus activating the microglia. Additionally, ROS generated from NADPH oxidase are an essential pathway regulating the microglial expression of neurotoxic pro-inflammatory factors. In summary, while the diversity of neurodegenerative disorders can be explained by unique localized triggers of neuronal death, the concentration of microglia in the brain area of neurodegeneration, the inherent vulnerability of the degenerating neuronal cell type, the severity of the toxic insult, and many other characteristics that will localize the self-propelling neuronal death, it remains constant that once a neuron dies, this death can activate microglia. Thus, multiple triggers of microglial activation may share a common mechanism of microglial-derived oxidative stress fueling the progressive nature of several independent neurodegenerative diseases.

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