

Microglia-mediated neurotoxicity: uncovering the molecular mechanisms

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Abstract | Mounting evidence indicates that microglial activation contributes to neuronal damage in neurodegenerative diseases. Recent studies show that in response to certain environmental toxins and endogenous proteins, microglia can enter an overactivated state and release reactive oxygen species (ROS) that cause neurotoxicity. Pattern recognition receptors expressed on the microglial surface seem to be one of the primary, common pathways by which diverse toxin signals are transduced into ROS production. Overactivated microglia can be detected using imaging techniques and therefore this knowledge offers an opportunity not only for early diagnosis but, importantly, for the development of targeted anti-inflammatory therapies that might slow or halt the progression of neurodegenerative disease.

Pattern recognition receptors

(PRRs). Receptors that bind to molecular patterns found in pathogens. Examples include the mannose receptor, which binds to terminally mannose and polymannosylated compounds, and Toll-like receptors, which are activated by various microbial products such as bacterial lipopolysaccharides, hypomethylated DNA, flagellin and double-stranded RNA.

For many years, neurodegenerative diseases such as **Alzheimer's disease** and **Parkinson's disease** have been a major focus of neuroscience research, with much effort being devoted to understanding the cellular changes that underlie their pathology. Microglia, the resident innate immune cells in the brain, have been implicated as active contributors to neuron damage in neurodegenerative diseases, in which the overactivation and dysregulation of microglia might result in disastrous and progressive neurotoxic consequences. Although these concepts have been widely reviewed in recent years^{1–3}, the characteristics defining deleterious microglial activation and the mechanisms by which neurotoxic microglial activation is initiated remain poorly understood. In the current review, we therefore focus on recent reports indicating that pattern recognition receptors (PRRs) are tools used by microglia to identify neurotoxic stimuli and that stimulation of NADPH oxidase activity is the predominant mechanism through which microglia produce neurotoxic reactive oxygen species (ROS). We further explain how the identification of these crucial participants in microglia-mediated neuronal injury could provide the insight necessary for the development of novel markers that specifically define deleterious microglial activation. Furthermore, these mechanisms might be ideal prospects for targeted anti-inflammatory therapy capable of slowing and perhaps preventing neurodegenerative diseases.

Microglia: friend and foe

Microglia are derived from myeloid cells in the periphery and comprise approximately 12% of cells in the brain⁴. Microglia density varies by brain region in the

adult human (0.5–16.6%)⁵ and in adult mice — they predominate in the grey matter, with the highest concentrations being found in the hippocampus, olfactory telencephalon, basal ganglia and substantia nigra⁶. In the mature brain, microglia typically exist in a resting state characterized by ramified morphology, and monitor the brain environment^{7,8}. In response to certain cues such as brain injury or immunological stimuli, however, microglia are readily activated^{7,9}. Activated microglia undergo a dramatic transformation from their resting ramified state into an amoeboid morphology and present an upregulated catalogue of surface molecules^{10,11}, such as CD14, major histocompatibility complex (MHC) molecules, chemokine receptors and several other markers¹². In their activated state, they can serve diverse beneficial functions essential to neuron survival^{13,14}, which include cellular maintenance (for example, clearing toxic cellular debris^{13,15,16}) and innate immunity^{17,18}. Activated microglia are involved in regulating brain development by enforcing the programmed elimination of neural cells^{19,20}, and seem to enhance neuronal survival through the release of trophic and anti-inflammatory factors^{21–23}. In addition, in the mature brain, microglia facilitate repair through the guided migration of stem cells to the site of inflammation and injury²⁴, and might be involved in neurogenesis^{25–28}.

Under other circumstances, however, microglia become overactivated and can induce significant and highly detrimental neurotoxic effects by the excess production of a large array of cytotoxic factors such as superoxide²⁹, nitric oxide (NO)^{30,31} and tumour necrosis factor- α (TNF α)^{32,33}. The stimuli that cause microglial overactivation and dysregulation can be diverse, ranging

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from environmental toxins, such as the pesticide rotenone, to neuronal death or damage. In neurodegenerative disease, activated microglia have been shown to be present in large numbers, a condition termed *microgliosis*, strongly implicating these cells in disease pathology.

Currently, the conditions defining whether microglial activation is detrimental or beneficial to neuronal survival are poorly understood. However, it is becoming more widely accepted that although microglial activation is necessary and crucial for host defence and neuron survival, the overactivation of microglia results in deleterious and neurotoxic consequences²⁶. It is because of this that understanding the causes and defining the characteristics of deleterious microglial activation in neurodegenerative disease has become a recent focus of research.

Microglia and neurodegenerative diseases

Inflammation is an underlying component of a diverse range of neurodegenerative diseases and their associated neuropathology, and increasing evidence suggests that microglia are a key causative factor in this process. In the following sections, evidence for a role of overactivated microglia in certain neurodegenerative diseases will be discussed.

Alzheimer's disease. Alzheimer's disease is the leading cause of dementia in the elderly and one of the first neurodegenerative diseases to be associated with the toxic effects of microglial activation^{34–36}. Neural damage begins in the temporal and parietal lobes of the cerebral cortex and progresses over time to the hippocampus and the amygdala³⁷. Alzheimer's disease results in the progressive impairment of memory and cognitive decline, and microglial activation increases throughout disease progression³⁸. Amyloid- β (A β) protein is implicated in the pathology, both through direct toxicity to neurons^{34,39} and by potentiating neuronal damage by microglial activation^{40,41}. In fact, microglial overactivation is an early pathogenic event that precedes neuropil destruction in patients with Alzheimer's disease⁴²; even before the development of symptoms, activated microglia cluster at sites of aggregated A β and penetrate the neuritic plaques^{35,43}. A β is pro-inflammatory and activates microglia to release neurotoxic factors such as NO⁴⁴, TNF α ⁴⁵ and superoxide⁴¹. So, A β both recruits and activates microglia^{46,47}, indicating a crucial role for microglia in Alzheimer's disease progression⁴⁸.

Parkinson's disease. Parkinson's disease affects approximately 1% of the population at the age of 55 and increases in prevalence to roughly 5% by the age of 85. It is characterized by the loss of the nigrostriatal ascending dopaminergic (DA) pathway, which results in symptoms of motor dysfunction such as rigidity tremor, slowness of motion, difficulty to initiate movements and loss of balance. Numerous activated microglia are present in the vicinity of degenerating neurons in the substantia nigra of patients with Parkinson's disease and of those with other Parkinsonian syndromes^{49–51}. Microglial activation in this disease is not limited to the substantia nigra, but is

also found in the putamen, hippocampus, transentorhinal cortex, cingulate cortex and temporal cortex⁵¹. The selective loss of DA neurons in the substantia nigra might be due to DA neuron glutathione deficiency⁵² (resulting in a reduced antioxidant capacity), high content of DA (a redox active molecule) in neurons in the substantia nigra⁵³, elevated iron concentrations⁵⁴ (redox active elements) and increased numbers of microglia in the substantia nigra^{6,55} compared with other regions. So, DA neurons in the substantia nigra might be particularly vulnerable to inflammatory insult owing to their precarious redox equilibrium and colocalization with a large population of microglia.

Several *in vitro* studies reveal that damaged DA neurons release several factors that seem to activate microglia and are implicated in neuronal degeneration in Parkinson's disease. Matrix metalloproteinase 3, (MMP3), α -synuclein and neuromelanin are released by damaged DA neurons and induce ROS production by overactivated microglia, in addition to other mechanisms. α -Synuclein, a component of Lewy bodies typically found in Parkinson's disease, is able to damage DA neurons at low concentrations in the presence of microglia⁵⁶. MMP3, a proteinase that degrades extracellular matrix, is released on DA neuron damage, activates microglia and induces DA neuron death⁵⁷. In neuron–glia cultures treated with MPP⁺ (1-methyl-4-phenylpyridinium ion), the active metabolite of the selective DA neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), there is an increase of MMP3, microglial activation and DA neurodegeneration. *Mmp3*-knockout mice have reduced DA neuron loss and attenuated microglial activation in response to MPTP⁵⁸.

Neuromelanin is a complex molecule made of melanin, peptides and lipid components that is released in Parkinson's disease by dying DA neurons to activate microglia⁵⁹. Neuromelanin is insoluble and so remains for an extended time in the extracellular space, is loaded with toxins able to activate microglia, and is localized at high concentrations in the human substantia nigra (2–4 mg g⁻¹ tissue)⁵⁹. Human neuromelanin added to neuron–glia cultures is phagocytosed and degraded by microglia with the release of inflammatory factors and ROS, which lead to neuronal death⁶⁰ (L.Z., unpublished observations). So, neuromelanin seems to be a potential candidate for the establishment of the perpetuating cycle of reactive microgliosis in Parkinson's disease.

HIV dementia. Approximately 60% of individuals infected with the human immunodeficiency virus (HIV) have neurological impairment, and post-mortem analysis reveals neuropathology in 90% of autopsied cases⁶¹. HIV-associated dementia (HAD) is a complication marked by cognitive, behavioural and motor dysfunction that develops during the later stages of AIDS. The neuropathological hallmarks of HAD are marked neuronal loss, reactive astrogliosis, activated microglia, multinucleated giant cells and leukocyte infiltration⁶². Microglia are essential to the progression of the dementia, as HIV enters the brain in infected monocytes and is stored in microglia^{63,64}. These cells serve as a lifelong latent

Microgliosis

The generalized microglial response to tissue damage that can be either beneficial or detrimental. The negative and progressive response is also referred to as reactive microgliosis.

MPTP

(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). A contamination product from incorrect synthesis of the abused opiate drug, 1-methyl-4-phenyl-4-propionoxypiperidine. In the brain, MPTP is converted to its active metabolite MPP⁺ (1-methyl-4-phenylpyridinium ion), which is selectively toxic to dopaminergic neurons and results in rapid development of Parkinson's disease symptoms in humans and animals.

reservoir for HIV replication⁶⁵. They are activated in the early stages of the simian immunodeficiency virus (SIV) model of HIV infection, and as the disease progresses this activation increases in intensity⁶⁶. Microglial HIV infection and viral replication results in an increased release of neurotoxic pro-inflammatory cytokines⁶⁷, and the SIV model of HIV infection shows enhanced microglial activation⁶⁸. Microglia are activated in HIV by interaction with viral proteins⁶⁹ such as transactivator of transcription (Tat)⁷⁰ and gp120 (REFS 71, 72), HIV infection and soluble factors released from infected cells⁷³, strongly implicating microglial overactivation in the progression of this disease.

Other neurological disorders. Microglia have been linked to pathology and disease progression in several other neurodegenerative disorders, including prion diseases^{74,75}, **multiple sclerosis**^{76,77}, **amyotrophic lateral sclerosis**^{78,79}, **Huntington's disease**^{80,81} and **Pick's disease**^{82,83}. Although not a neurodegenerative disease *per se*, progressive neuronal damage after stroke and reperfusion is also closely linked to microglial activation^{84,85}.

In Alzheimer's disease and other neurodegenerative disorders, microglia can become overactivated and release ROS, NO and cytokines^{41,44,45,86,87}, which might cause vascular damage in addition to neurodegeneration. In a study of 617 cases of Parkinson's disease, the total frequency of cerebrovascular lesions was higher than that of controls⁸⁸. Damage to the microvasculature in Alzheimer's disease and Parkinson's disease includes fragmentation, atrophy, tortuous structure, basement membrane thickening, vacuolization and pericytic degeneration⁸⁹. In summary, overactivated microglia have a role in neuronal loss and vascular damage in diverse neuropathological conditions.

Aging. Many neurodegenerative diseases increase in prevalence with age. Normal aging in the human brain is accompanied by increasing numbers of activated, interleukin 1 β (IL-1 β)-expressing microglia⁹⁰ and enlarged phagocytic microglial subtypes⁹¹. Analysis of rat cortical sections using electron microscopy at 3, 12, 24 and 27 months (which is equivalent to old age in humans) reveals a progressive increase in microglial activation with age⁹², and the spinal cord seems to be similarly affected⁹³. Microglia from aged rat brains show increases in the microglial protein markers OX42 and OX6 (REF. 94). Additionally, greater DA neuron loss and microglial activation in the substantia nigra in response to MPTP⁹⁵ is seen in aged mice. In fact, several reports propose that the age-associated transformation of microglia is a reflection of a chronic, lifelong accumulation of minor insults leading to increased risk of neurodegenerative disease, such as Alzheimer's disease^{91,96}. So, although the precise mechanisms of action are unclear, aging seems to influence microglial activation, which might contribute to an enhanced inflammatory response in the brain.

Microglia, inflammation and neurotoxicity. As discussed above, microglia-mediated neurotoxicity tends to be progressive^{97–99}, which could contribute to the

progressive nature of several neurodegenerative diseases. This has been most effectively demonstrated in models using lipopolysaccharide (LPS), the polysaccharide component of the cell walls of gram-negative bacteria. Although LPS models cannot not precisely mimic the conditions under which microglia are activated in neurodegenerative disease, these studies demonstrate that LPS is neurotoxic only in the presence of microglia, indicating that microglia can initiate neuronal damage^{100,101}. For example, LPS is reported to induce microglial activation *in vivo* and *in vitro* and cause the progressive and cumulative loss of DA neurons over time^{100,102,103}. Furthermore, embryonic exposure to LPS has an impact on microglial activation and neuron survival into adulthood^{103,104}. Interestingly, once overactivated, microglia can remain in this state, as evidenced by the chronic neuroinflammation that continues years after brief MPTP exposure in humans⁵⁰ and primates⁹⁸.

Some neurotoxins, such as MPTP, require the presence of neurons to induce microglial activation, and effect their damage through direct action on neurons and indirectly by overactivating microglia^{98,99,105}. For example, although MPP⁺ cannot directly activate microglia, the addition of microglia to neuronal cultures enhances MPP⁺-induced DA toxicity⁹⁹. Furthermore, in several animal studies, MPTP toxicity is significantly reduced in mutant mice deficient in pro-inflammatory factors such as superoxide^{105,106}, myeloperoxidase¹⁰⁷, prostaglandins^{108–111}, NO¹¹² and TNF α ^{113–115}. These findings suggest that microglia, when overactivated, serve to enhance and amplify neuronal damage induced by pathological stimuli and toxins, and it seems that this, in turn, induces more widespread damage to neighbouring neurons (reactive microgliosis)^{116,117}. Taken together, these studies indicate that microglia can become overactivated by two types of signal: the direct stimulation of microglia by environmental toxins or endogenous proteins, and neuronal damage and consequent reactive microgliosis (FIG. 1).

However, microglial activation also enhances ongoing neurodegeneration. For example, LPS synergistically enhances both the microglial activation and the neurotoxic effects of rotenone¹¹⁸ and MPTP¹¹⁹, indicating that overactive microglia can amplify neuroinflammation and associated neurotoxicity. Recent reports also indicate that systemic and local inflammation induced using LPS in adult mice exacerbates the neuronal damage associated with additional LPS administration¹²⁰. In addition, LPS exposure during critical periods of microglial development *in utero* also results in an enhanced and progressive response to the administration of LPS in adult mice¹⁰³. So, microglial overactivation initiated by early immunological insult or direct injury to neurons might be propagated and potentially amplified throughout the course of neurodegenerative disease, driving the continuous and cumulative loss of neurons over time.

Fortunately, endogenous protective regulatory signals in the brain have been identified that inhibit microglial overactivation, such as neuropeptides^{121–123}, cannabinoids^{124,125}, anti-inflammatory cytokines (that is, IL-10 and transforming growth factor- β (TGF β))^{126,127},

Lipopolysaccharide

(LPS). An endotoxin that is a complex macromolecule containing a polysaccharide covalently linked to a unique lipid structure, termed lipid A. All gram-negative bacteria synthesize LPS, which is a main constituent of their outer cell membrane.

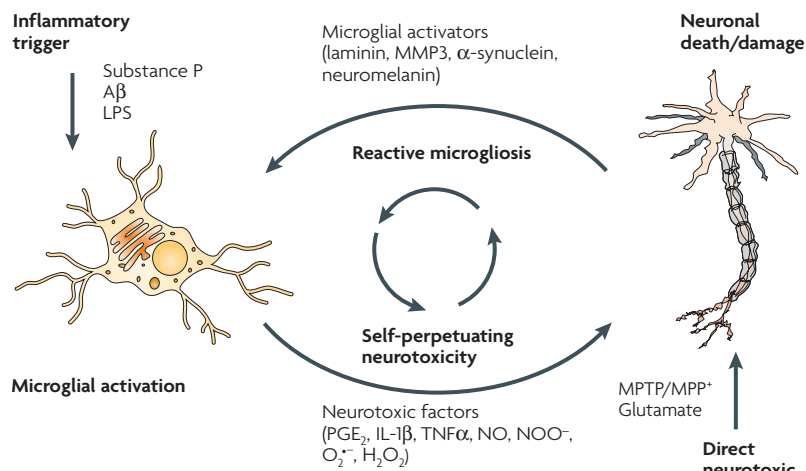


Figure 1 | Reactive microgliosis drives progressive neurotoxicity. Microglia can become overactivated and cause neurotoxicity through two mechanisms. First, microglia can initiate neuron damage by recognizing pro-inflammatory stimuli, such as lipopolysaccharide (LPS), becoming activated and producing neurotoxic pro-inflammatory factors. Second, microglia can become overactivated in response to neuronal damage (reactive microgliosis), which is then toxic to neighbouring neurons, resulting in a perpetuating cycle of neuron death. Reactive microgliosis could be an underlying mechanism of progressive neuron damage across numerous neurodegenerative diseases, regardless of the instigating stimuli. Aβ, amyloid-β; H₂O₂, hydrogen peroxide; IL-1β, interleukin 1β; LPS, lipopolysaccharide; MMP3, matrix metalloproteinase 3; MPP⁺, 1-methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO, nitric oxide; NOO⁻, peroxynitrite; O₂^{•-}, superoxide; PGE₂, prostaglandin E₂; TNFα, tumour necrosis factor-α.

oestrogen¹²⁸, glucocorticoids^{129–131} and even microglial apoptosis^{132,133}. However, it has been proposed that when the ability to activate these protective mechanisms fails, or when they are overwhelmed by an excessive inflammatory response, microglia initiate neuronal death and drive the progressive nature of neurodegenerative disease^{26,121}. A full understanding of the mechanisms underlying microglial dysregulation and overactivation is of pressing interest because of the valuable insight it will provide into the aetiology, pathogenesis and treatment of neurodegenerative diseases.

So, microglial activation is present in diverse neurodegenerative diseases and is closely associated with pathology. Previously, the microglial response to neuronal damage was believed to be passive. However, recent reports indicate that microglial activation is capable of both initiating additional neuronal loss and amplifying ongoing neuronal damage, indicating that microglia might be crucial to the aetiology and the progressive nature of neurodegenerative diseases. Current work is therefore beginning to focus on the stimuli necessary to initiate deleterious microglial function, where studies have revealed several triggers of inflammation-mediated neurodegeneration are present in the environment.

Microglia: linking the environment and disease

Environmental factors are strongly implicated in the aetiology of neurodegenerative diseases, and several lines of evidence indicate that the environment is a source of

stimuli of microglial activation. In particular, Parkinson's disease has one of the strongest known associations between a neurodegenerative disease and environmental toxins. In Parkinson's disease, the cumulative influence of environmental insults on microglial activation has been reported and it has been suggested that multiple environmental exposures could be necessary for development of the disease (the 'multiple hit' hypothesis)¹³⁴. Among the environmental toxins, infectious agents^{135,136}, pesticides^{137,138}, MPTP¹³⁹, proteasome inhibitors^{140,141} and heavy metals^{142–144} have been implicated in its development and progression.

Interestingly, the environment is implicated as a source for compounds that are both directly toxic to neurons and deleterious through direct stimulation of microglia. In fact, multiple environmental toxins such as LPS^{100,104,145}, paraquat¹⁴⁶, rotenone^{147,148}, manganese ethylene-bisdithiocarbamate¹⁴⁹ and diesel exhaust particles (DEP)¹⁵⁰ can affect neuron survival by activating microglia (TABLE 1).

Particulate matter is the ubiquitous particle component of air pollution that has been receiving increasing attention due to its association with numerous inflammation-related conditions and diseases¹⁵¹. The smaller, ultra-fine (< 0.1µm) components of particulate matter can exit the lung and be systemically distributed to multiple organs such as the liver, kidney, spleen, heart and brain¹⁵². In addition to its association with asthma, particulate matter is reported to cause systemic inflammation and is associated with arteriosclerosis¹⁵³. Recently, ambient particulate matter has been linked to ischaemic stroke in humans¹⁵⁴. Ambient particulate matter has been associated with brain inflammation in mice¹⁵⁵, dogs^{156,157} and humans¹⁵⁸. *In vitro*, DEP results in DA neurotoxicity through microglial activation¹⁵⁰. So, increasing evidence indicates that air pollution, such as particulate matter, and several environmental toxins might activate microglia which then have a role in the development of neurodegenerative disease.

Together, these recent reports indicate that the environment is a source of several factors that can activate microglia, leading to either neuronal loss or enhanced vulnerability to further damage. This offers a valuable insight into the potential aetiology of several neurodegenerative diseases. At this time, it is poorly understood why microglia respond to environmental (and endogenous) stimuli in a neurotoxic fashion. However, the answers might reside in the study of the receptors that microglia use to interpret the brain environment and the consequences of their activation.

Microglial PRRs and neurotoxicity

Microglia are 'professional' phagocytes, and have evolved to express multiple, diverse membrane receptors that identify an even wider array of molecular determinants. PRRs are generally constitutively expressed to identify and bind pathogen-associated molecular patterns (PAMPs), and are therefore crucial to the innate immune response¹⁵⁹. Recently, PRRs have been linked to numerous diseases of chronic inflammation such as Crohn's disease, arthrosclerosis, diabetes and cancer¹⁶⁰. PAMPs

Table 1 | **Toxins that cause neurotoxicity through microglia-derived ROS**

Microglia activator (toxin)	NADPH oxidase induced ROS	Pro-inflammatory cytokine release	References
Rotenone	Yes	No	147
Paraquat	Yes	No	146,228
Substance P	Yes	IL-6	121,229
Lipopolysaccharide	Yes	Yes	100,203,210,230
Neuromelanin	Yes	Yes	60
α -Synuclein	Yes	No	56,231
Diesel exhaust particles	Yes	No	150
Gangliosides	Yes	Yes	208
Thrombin	Yes	Yes/No	232–234
Amyloid- β	Yes	Yes	16,41,195
Matrix metalloproteinase 3	Yes	Yes	57,58

ROS, reactive oxygen species

are highly conserved throughout evolution because the sequences are critical to the microorganism's survival¹⁵⁹. However, in the case of microglia-mediated neurotoxicity, receptors responsible for host defence and phagocytosis often mediate neuronal damage in the absence of a microbial pathogen, suggesting that non-pathogenic stimuli are misinterpreted with dire neurotoxic consequences (FIG. 2).

Toll-like receptors. Toll-like receptors (TLRs) recognize PAMPs, initiate innate immune responses on interaction with infectious agents, and are one of the most studied PRR families. In microglia, the expression of TLRs is regulated throughout development and in response to pathogens¹⁶¹. So far, there are twelve members of the TLR family identified in mammals that recognize PAMPs from bacteria, fungi, parasites, viruses and the host itself¹⁵⁹. Microglia are reported to express TLRs 1–9 (REFS 17,162), and the TLR family has been linked to microglial activation and neurotoxicity.

TLR4 — in conjunction with CD14 — is traditionally accepted as the primary LPS receptor¹⁶³, and is reported to be a crucial contributor to the microglial response to LPS^{164–166}, mediating LPS-induced neurodegeneration both *in vitro* and *in vivo*. Additionally, TLR4 is upregulated upon brain inflammation¹⁶⁷ and is reported to mediate LPS-induced neurotoxicity and oligodendrocyte damage¹⁶⁶. In addition, TLR4 is implicated in brain inflammation and microglial activation in response to endotoxemia¹⁶⁵. Independent of LPS, TLR4 also contributes to the initiation of CNS neuroimmune activation and pain neuropathy after transection of the L5 spinal nerve¹⁶⁸. So, in addition to bacterial recognition, microglial TLR4 might recognize additional ligands yet to be identified that contribute to microglial activation after neuronal damage. Interestingly, gangliosides — sialic acid-containing glycosphingolipids found in neuronal cell membranes — are also reported to activate microglia through TLR4 (REF. 169), indicating that neuronal

membrane components are capable of activating a pathway responsible for bacterial pathogen defence. However, in addition to destructive consequences, microglial TLR4 activation has been linked to beneficial repair processes, such as improved remyelination and cerebral tissue protection in the presence of agents with strong cytolytic properties¹⁷⁰.

Other members of the TLR family have been less researched in microglia. However, TLR2 is reported to be important for the microglial response to viruses, such as the herpes simplex virus¹⁷¹. Additionally, the extracellular matrix component peptidoglycan can activate microglia through TLR2 to upregulate chemotactic proteins and increase A β uptake¹⁷². Activation of TLR2, TLR4 and TLR9 induces microglial production of NO through multiple ligands¹⁷³. Furthermore, microglia become activated after recognizing double-stranded RNA through TLR3, indicating the importance of this receptor for the microglial response to viral insult¹⁷⁴. TLR9 recognizes single-stranded unmethylated CpG-DNA (bacterial DNA), which stimulates an increase in the production of microglial NO and TNF α ^{162,175}. So, multiple TLRs are an essential component of the microglial innate immune response and induce the production of neurotoxic factors from microglia, providing a mechanism by which some TLRs might contribute to the microglial response to neuronal damage.

Scavenger receptors. Scavenger receptors are a diverse group of PRRs that are defined by their ability to recognize modified lipoproteins and various polyanionic ligands. They have been categorized into eight classes (A–H) that are expressed on diverse cell types, particularly phagocytes¹⁷⁶. A number of these classes have been shown to affect general microglial function, are linked to neurotoxicity and are often upregulated during neurodegenerative diseases¹⁷⁷. However, not all scavenger receptor subtypes have been identified in microglia, nor have their functions been confirmed.

Scavenger receptor class A1 (SR-A1), SR-B1 and CD36 are cell surface proteins that are differentially regulated in microglia through development and in response to disease. They fulfil multiple functions: recognition and endocytosis of native and pathologically modified substances, initiation of intracellular signalling and defence against bacterial pathogens¹⁷⁷. Microglial expression of SR-A1 (REF. 178) is upregulated in the brains of patients with Alzheimer's disease, and microglia also upregulate scavenger receptors upon injury and in response to cytokines¹⁷⁹. The activation of scavenger receptors can result in ligand internalization and/or production of extracellular superoxide by microglia¹⁷⁷. For example, the class B scavenger receptor CD36 mediates free radical production and tissue injury in cerebral ischaemia¹⁸⁰. Both SR-A¹⁸¹ and SR-B1 (REF. 182) mediate adhesion and endocytosis of fibrillar A β by microglia. Interestingly, SR-A, SR-B1 and CD36 (REF. 183) participate in the microglial production of ROS in response to A β fibrils. Macrophage receptor with collagenous domain (**MARCO**) is an inducible member of the class A scavenger receptor family that is unique to cells of

Endotoxemia

A condition in which endotoxin (a toxin component of the cell wall of gram-negative bacteria that is only released on destruction of the bacterial cell) accesses the blood stream to induce systemic inflammation.

monocytic lineage. MARCO has also been implicated in the adhesion of microglia to Aβ¹⁸⁴, and in the mediation of cytoskeleton rearrangements in microglia¹⁸⁵.

Advanced glycation endproducts (AGEs) are ligands recognized by some PRRs and comprise a common and diverse group of chemically modified proteins that form when sugars non-enzymatically derivitize proteins (glycosylation). Although several scavenger receptors are known to recognize AGEs, the most well-known receptor for AGEs (RAGE) is not a scavenger receptor but another PRR that belongs to an immunoglobulin superfamily¹⁷⁶. Interestingly, RAGE-expressing microglia are upregulated in Alzheimer's disease, and microglial

RAGE is reported to mediate the pro-inflammatory effects of Aβ¹⁸⁶⁻¹⁸⁸. So, scavenger receptors and RAGE might be responsible for both the internalization of noxious toxins in microglia and generation of the pro-inflammatory response, where that response probably depends on the ligand in question and the identity of other PRRs involved.

MAC1 receptor. Integrin CD11b/CD18 (macrophage antigen complex 1, **MAC1**; also known as complement receptor 3, CR3) functions as both an adhesion molecule and a PRR that recognizes several diverse ligands, including the iCR3b fragment of complement^{189,190}. MAC1 is essential for the phagocytosis of multiple compounds and mediates the activation of phagocytes in response to a diverse set of stimuli^{190,191}. The MAC1 receptor is located on microglia and its expression is elevated in the post-mortem brains of patients with Alzheimer's disease¹⁹², suggesting a role for this receptor in neurodegeneration. In addition, MAC1 is associated with activation of the respiratory burst in neutrophils and macrophages^{191,193}. In fact, recent reports indicate that MAC1 might be a key receptor for toxins that activate microglia to produce extracellular superoxide and result in neurotoxicity (J-S.H., unpublished observations; L.Z., unpublished observations). Given that MAC1 is upregulated in neurodegenerative disease, these findings indicate that this molecule could be a key mechanism of microglial-derived oxidative stress during inflammation-mediated neurodegeneration.

Receptor complexes. There is increasing evidence to indicate that neurotoxins might interact with multiple PRRs. For example, microglia recognize fibrillized Aβ through a cell surface receptor complex (CD36, α6β1 integrin and CD47), and antagonists specific for each receptor will block fibrillized Aβ-stimulated phagocytosis¹⁹⁴. This same ensemble of receptors is also required for Aβ-induced production of superoxide from microglia. Specifically, a scavenger receptor antagonist, antibodies specific for CD36 and antibodies against β1 integrin also inhibit the Aβ-stimulated generation of ROS¹⁹⁵. Furthermore, MAC1, SR-A1/2 and Fcγ are known to mediate the phagocytosis of degenerated myelin in macrophages and microglia. However, MAC1 and SR-A1/2, but not Fcγ, mediate phagocytosis after axonal injury¹⁹⁶. Currently, there is little information on the combined receptor mechanisms responsible for the recognition of several diverse neurotoxic/pro-inflammatory ligands, the cellular signalling, the production of extracellular superoxide and their impact on neurotoxicity.

Although it is clear that the microglial response to pathogens and direct signals of innate immunity can differ from the microglial response to neuronal damage in neurodegenerative disease, the study of how microglia respond to PAMPs has been crucial for understanding deleterious microglial activation. Not only have several PRRs been associated with microglial activation in neurodegenerative diseases and neuron damage, but in many cases the ligand responsible is

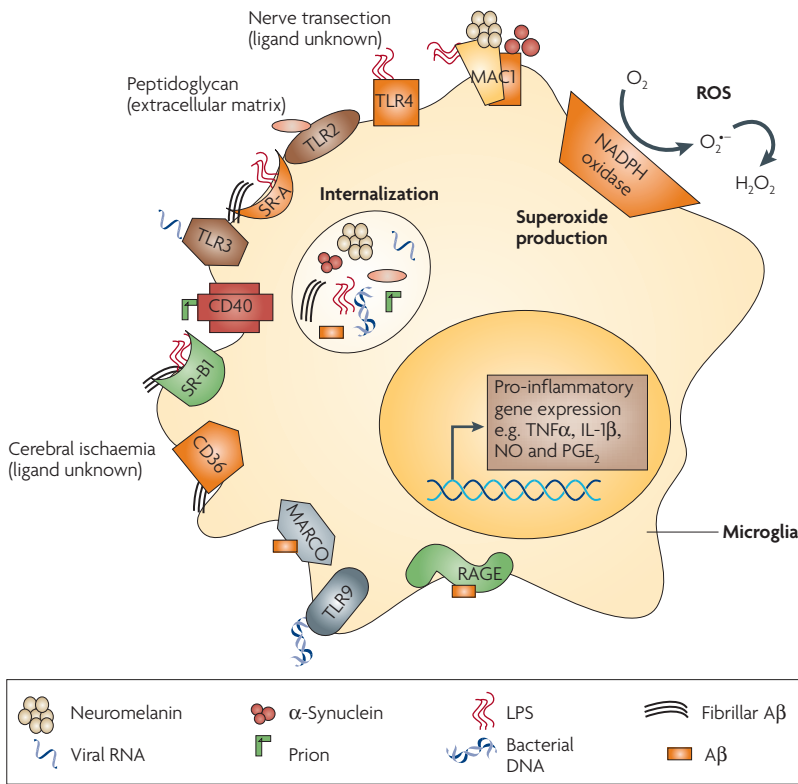


Figure 2 | Microglial PRRs identify neurotoxic and pro-inflammatory ligands.

Microglia actively monitor the brain environment with pattern recognition receptors (PRRs). PRRs are responsible for several phagocyte functions such as the identification of pathogens, the production of extracellular superoxide, the release of pro-inflammatory compounds and the removal and destruction of toxic stimuli through internalization and phagocytosis. Microglia-mediated neurotoxicity occurs through PRRs when pathogen-associated molecular patterns (PAMPs) trigger an excessive immune response, or when stimuli (environmental toxins, endogenous proteins and neuron damage) are misinterpreted as pathogens, where NADPH oxidase is activated and pro-inflammatory cytokines might be produced. The list of pro-inflammatory and neurotoxic ligands that activate microglia through these receptors is extensive and current research is focused on identifying which receptors are responsible for the deleterious microglial response (overactivation and inflammation) versus beneficial maintenance functions (internalization). Given that a single ligand is often recognized by multiple PRRs, the cumulative effect of various receptor combinations might define how microglia respond to neurotoxins and whether the activation is deleterious or beneficial. Aβ, amyloid-β; H₂O₂, hydrogen peroxide; IL-1β, interleukin 1β; LPS, lipopolysaccharide; MAC1, macrophage antigen complex; MARCO, macrophage receptor with collagenous domain (scavenger receptor); NO, nitric oxide; O₂^{•-}, superoxide; PGE₂, prostaglandin E₂; RAGE, receptor for advanced glycation endproducts; ROS, reactive oxygen species; SR-A, scavenger receptor class A; TLR2, Toll-like receptor 2; TNFα, Tumour necrosis factor-α.

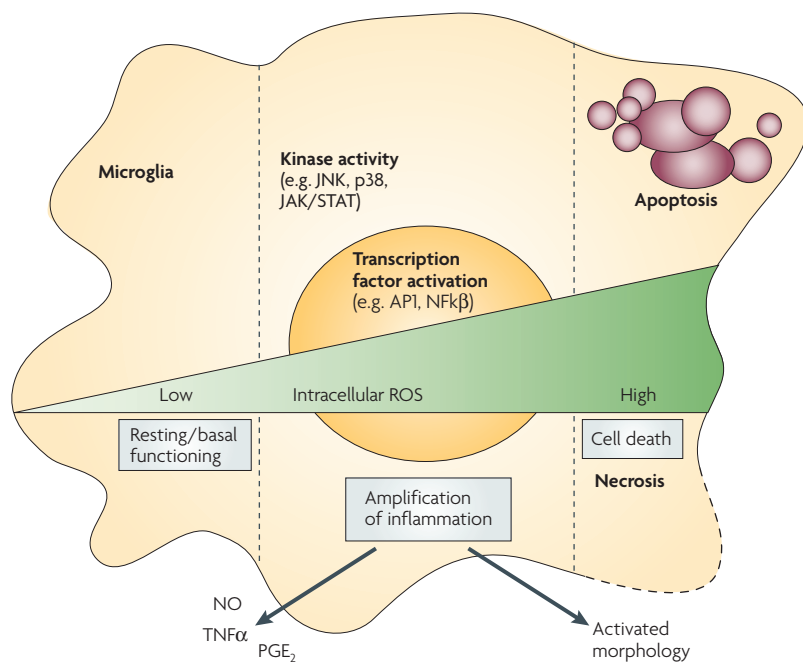


Figure 3 | Intracellular ROS regulate microglial activation. Classic microglial activation results in the production of both extracellular and intracellular reactive oxygen species (ROS). Intracellular ROS are crucial for both microglial pro-inflammatory function and microglial survival. Intracellular ROS act as second messengers capable of modifying gene expression through effects on kinase cascades and transcription factor activation. Cells function normally with a basal level of intracellular ROS that is managed by the cell's antioxidant mechanisms. Upon increasing levels of intracellular ROS and depletion of the cell's antioxidative defence in response to an immunological stimulus, ROS act as second messengers to amplify the pro-inflammatory function of microglia. However, higher levels of intracellular ROS might result in microglial death (predominantly by apoptosis), similar to what occurs in phagocytes in the periphery. The dysregulation of intracellular ROS in microglia to levels that amplify pro-inflammatory gene expression might contribute to overactivation and neurotoxic consequences, and represents an ideal therapeutic target. Interestingly, it is hypothesized that the same protective and inhibitory mechanisms that are in place to prevent the microglial ROS increase that results in neurotoxic microglial activity might also prevent the microglia from progressing into apoptosis and resolution of inflammation once deleterious levels have been achieved, thereby contributing to the progressive nature of microglia-mediated neurotoxicity. JAK, Janus kinase; JNK, c-Jun amino (N)-terminal kinase; NO, nitric oxide; PGE₂, prostaglandin E₂; STAT, signal transducer and activation of transcription; TNF α , tumour necrosis factor- α .

clearly not a pathogen and is unknown. These studies indicate that neurodegenerative diseases might be the result of several misinterpreted patterns (environmental toxins, endogenous disease proteins and in response to neuronal death) that are mistaken for pathogens. Importantly, by identifying PRR mechanisms that cause microglia-mediated neurotoxicity, we could begin to understand the pathways responsible for how microglia exert neurotoxicity.

Neurotoxic microglia: prevalent features

The production of extracellular superoxide is a common consequence of ligand recognition by PRRs and oxidative stress is a prevalent feature of numerous neurodegenerative diseases¹¹⁶, but the source of ROS remains a matter of debate. Microglia are a robust source of free radicals which can induce neuronal damage, and NADPH

oxidase is implicated as both the primary source of microglial-derived extracellular ROS and a mechanism of pro-inflammatory signalling in microglia¹⁹⁷.

NADPH oxidase and superoxide. NADPH oxidase is a membrane-bound enzyme that catalyses the production of superoxide from oxygen. Although dormant in resting phagocytes, NADPH oxidase is activated by various stimuli, including bacterial PAMPs, inflammatory peptides¹⁹⁷ and multiple neurotoxins¹¹⁶. Several PRRs activate NADPH oxidase¹⁹⁸, but the MAC1 receptor might be crucial for microglial NADPH oxidase activation in response to neurotoxic stimuli (L.Z., unpublished data; J-S.H., unpublished observations). NADPH oxidase is composed of several subunits, including a flavocytochrome known as cytochrome b558 (gp91)¹⁹⁷, which is the membrane bound catalytic subunit of the complex. In resting cells, the cytosolic subunits (p47, p67, p40 and Rac2) are distributed between the cytosol and the membranes of intracellular vesicles and organelles¹⁹⁷. When microglia are activated, the cytosolic subunits translocate to membranes, where they bind to the membrane-associated subunits (p22 and gp91) and assemble the active oxidase to produce extracellular superoxide.

NADPH oxidase is associated with neurodegenerative disorders and neuronal damage; it is activated in the brains of patients with Alzheimer's disease¹⁹⁹ and the catalytic subunit (gp91) is upregulated in Parkinson's disease¹⁰⁵. Additionally, neural damage in response to cerebral vascular dysfunction is mediated by NADPH oxidase. Ischaemic stroke is reduced in mice lacking functional NADPH oxidase²⁰⁰, and there is a clear role of NADPH oxidase in intracerebral haemorrhage²⁰¹. Recently, it was reported that overactivated microglia injure oligodendrocytes through NADPH oxidase²⁰². Furthermore, the crucial role of NADPH oxidase in mediating inflammation-related neurotoxicity has been demonstrated in the following toxins: LPS²⁰³, rotenone²⁰⁴, DEP¹⁵⁰, paraquat¹⁴⁶, MPTP^{105,205}, A β ⁴¹, substance P¹²¹, thrombin²⁰⁶ and α -synuclein⁵⁶. Additionally, cortical neurotoxicity from amyloid precursor protein expression is mediated through NADPH oxidase²⁰⁷.

In addition to the production of extracellular ROS, NADPH oxidase is also thought to be a crucial component of microglial signalling. NADPH oxidase-generated superoxide and intracellular ROS are common signalling mechanisms of phagocytes. For example, gangliosides activate microglia through protein kinase C and NADPH oxidase²⁰⁸. Additionally, changes in the morphology²⁰³ and proliferation of microglia are regulated by hydrogen peroxide from NADPH oxidase²⁰⁹. Finally, NADPH oxidase regulates the expression of several pro-inflammatory functions of microglia^{203,210}. In general, the higher the intracellular ROS, the more amplified the inflammatory response^{203,210}, until either apoptosis^{211,212} or necrosis²¹³ occurs (FIG. 3). In some cases, extremely high concentrations of ROS are reported to inhibit pro-inflammatory signalling²¹⁴, and at these higher concentrations ROS will induce cumulative effects harmful for cell survival, such as lipid peroxidation and oxidative modification of proteins²¹⁵.

By altering concentrations of intracellular ROS, NADPH oxidase is also reported to prime the microglial response to additional stimuli. For example, the HIV-1 negative factor (Nef) protein primes the microglial NADPH oxidase response to additional toxins²¹⁶. Neuronal death¹¹⁹ and toxins such as rotenone¹¹⁸ are also shown to prime microglia, and result in synergistic microglial activation and associated neurotoxicity on additional insult with LPS. Therefore, NADPH oxidase and intracellular ROS are crucial to the regulation of microglial activation.

Together, NADPH oxidase and microglia-derived ROS could be essential contributors to the oxidative stress and inflammation associated with neurodegenerative diseases. Because of the essential role of ROS in the mechanisms by which microglia cause neuronal damage, NADPH oxidase could be an ideal therapeutic target for the stages of neurodegenerative disease in which microglia are actively contributing to neuronal loss.

Implications for diagnosis and therapy

In vivo imaging of microglial activation. *In vivo* imaging has made feasible the prospect of monitoring cellular events associated with neurodegenerative disease in a non-invasive manner²¹⁷. Positron emission tomography (PET) imaging reveals microglial activation in patients with neurodegenerative diseases and in animal models by using a specific radioligand of the peripheral benzodiazepine receptor (¹¹C]-PK11195), which is upregulated in activated microglia. Disease specific localization of microglial activation can therefore be detected with PET imaging. For example, in patients with Alzheimer's disease, an increase in microglial activation in entorhinal, temporoparietal and cingulate cortices was found

with PET imaging⁴² (FIG. 4). In patients with Parkinson's disease, microglial activation imaged by PET was increased in the pons, basal ganglia and cortical regions (frontal and temporal)²¹⁸. In early stage Parkinson's disease, PET showed an increase of microglial activation, where the level of activation was inversely correlated with density of DA terminals (measured with a dopamine transport marker) and was positively correlated with motor impairment²¹⁹. In progressive supranuclear palsy and multiple system atrophy (both forms of Parkinsonism), augmented microglial activation was detected by PET in the areas of cortex and basal ganglia where neuropathological changes occur^{218,220}. In a rat model of Parkinson's disease induced by striatal injection of 6-hydroxydopamine, PET showed an increase of microglial activation both in the striatum and substantia nigra²²¹. At 4 weeks post injection, this activation was confirmed by post-mortem immunohistochemistry. The ligand used in the above studies (PK11195) is a marker for the transition of microglia from a resting to an activated state. Importantly, PET imaging of microglial activation in patients has the potential to measure the stage of disease progression. For example, PET imaging shows a correlation between microglial activation and disease severity in patients with Huntington's disease²²². However, much work is needed to identify additional markers to specifically detect the microglial conversion into the deleterious phenotype, as this will greatly enhance the utility of *in vivo* imaging for early detection of inflammation-mediated neurodegenerative diseases.

Microglia and treatment of neurodegenerative disease.

Recent evidence demonstrating the beneficial and neuroprotective profile of microglia indicates that whereas acute microglial overactivation is deleterious, microglia might also be involved in maintenance, repair and possibly protection. Therefore, the ideal therapeutic approach would involve early attenuation of the microglial response to levels that are no longer deleterious, rather than the elimination of the microglial response altogether (FIG. 5). As discussed here, several lines of evidence show that microglial function is regulated by NADPH oxidase and the production of intracellular ROS. Additionally, the production of neurotoxic extracellular ROS is also shown to occur primarily through NADPH oxidase, for multiple, distinct stimuli¹¹⁶, making this enzyme complex an ideal therapeutic target. Recently, several peptides, antibiotics and small molecules have been identified that inhibit NADPH oxidase and are neuroprotective^{123,206,223}. Combined with early diagnosis through PET-based microglial imaging, this approach might provide hope for attenuation of the progression of neurodegenerative disease.

Whereas animal studies show the neuroprotective effects of anti-inflammatory therapy, human studies investigating the neuroprotective effects of anti-inflammatory drugs have shown mixed results. For example, clinical trials treating patients with Alzheimer's disease with non-steroidal anti-inflammatory drugs (NSAIDs) have largely failed²²⁴. However, the use of NSAIDs is associated with a decreased risk of Parkinson's disease^{225,226}, indicating that NSAIDs might be neuroprotective in neurodegenerative

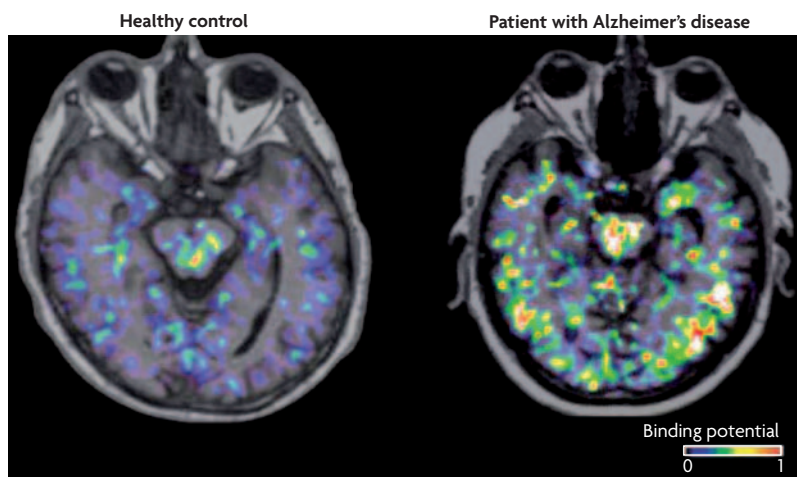


Figure 4 | PET imaging of microglia in neurodegeneration. Positron emission tomography (PET) images depicting microglial activation in the brains of a healthy control (74 years old) and a patient with Alzheimer's disease (65 years old, disease duration of 5 years). The uptake of the [¹¹C]-PK11195 ligand to the peripheral benzodiazepine receptor, which is overexpressed in activated microglia, is shown according to colour scale. In the cortex of the healthy individual there is no significant binding, although low level binding is present in the pons and midbrain. Widespread binding is evident in the cortex of the patient with Alzheimer's disease, with the highest values bilaterally in the temporal lobe. Image courtesy of D. J. Brooks, Medical Research Council Clinical Sciences Centre, London, UK.

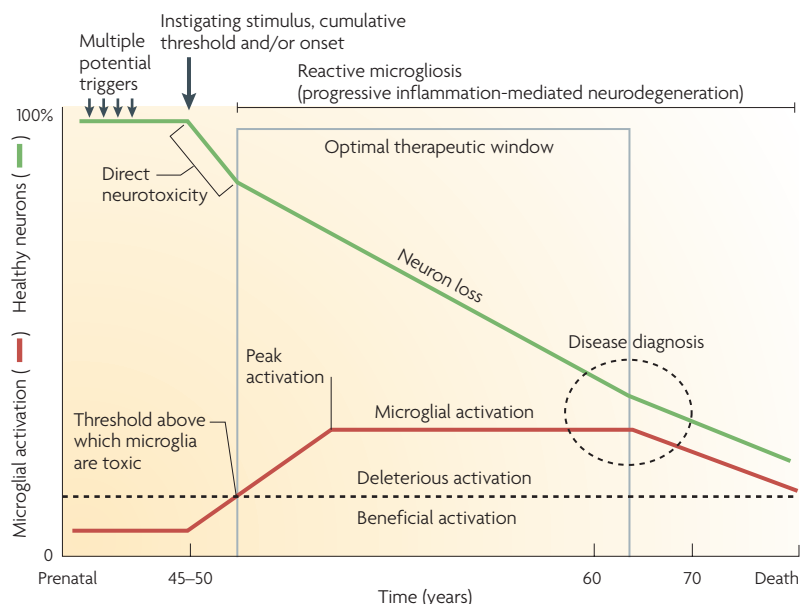


Figure 5 | Microglial activation, neuronal death and the therapeutic relevance. Although the initiating stimuli and specific rate of neuron loss can be individually unique for each patient and neurodegenerative disease, here we depict the generalized hypothesis of the relationship between microglial activation, neuron damage and disease diagnosis. Neuronal damage due to an unknown instigating stimulus can result in microglial activation that will be driven by the further loss of neurons throughout the disease. Microglial activation can pass the threshold of beneficial function and become deleterious, fuelling further neuronal loss (reactive microgliosis) and resulting in a perpetuating cycle of neurotoxicity and a progressive neurodegenerative disease. Currently, disease diagnosis and commencement of therapy occurs outside the optimal therapeutic window, long after extensive damage has occurred. The ideal therapeutic regimen would involve prevention: early detection of microglial activation through *in vivo* imaging and anti-inflammatory therapy to reduce microglial activation to non-deleterious levels holds the promise of slowing and perhaps halting disease progression before extensive irreversible damage and clinical symptoms occur. The advance of technology and the enhancement of *in vivo* imaging techniques offers the hope of testing this proposed relationship of neuronal loss and microglial activation across the human disease course and the opportunity to initiate and monitor therapy during an optimal therapeutic window. Notably, whereas the optimal therapeutic window coincides or precedes the bulk of neuronal damage, which typically occurs before diagnosis for most neurodegenerative diseases, anti-inflammatory therapy might still attenuate the disease progression after diagnosis.

disease. Therefore, the investigation of NSAIDs in human trials and disease incidence emphasizes the importance of pairing anti-inflammatory treatment with the optimal therapeutic window (FIG. 5), during the time when microglia are actively contributing to neuronal loss. The optimal therapeutic window might vary, depending on the disease in question and individual patient differences. Once again, *in vivo* microglial imaging could allow the assessment of disease progression and the state of

microglial activation to assist with this process. Furthermore, NSAIDs, such as cyclooxygenase 2 (COX2) inhibitors, target only a single pro-inflammatory factor (prostaglandin E₂, PGE₂) among a large number of pro-inflammatory factors released from overactivated microglia, which could explain why these anti-inflammatory agents are less efficacious. Recent research indicates that inhibiting NADPH oxidase activation might be a more efficacious approach because this enzyme complex is implicated as the predominant mechanism by which microglia damage neurons, and inhibiting it would simultaneously downregulate multiple pro-inflammatory factors, including PGE₂ (REF. 227). As we understand more about how microglia are toxic, it might be possible to develop targeted anti-inflammatory therapy to be administered during the optimal therapeutic window, offering the hope of slowing or halting the progression of neurodegenerative disease.

Conclusions and future directions

In summary, inflammation-mediated neurotoxicity in neurodegenerative disease can occur as a consequence of microglial dysregulation and overactivation. Microglia monitor the brain environment by interpreting and processing stimuli (environmental toxins, endogenous proteins or reactive microgliosis) through PRRs. Several of these factors might be correctly recognized by microglia as pathogenic. However, misinterpretation of innocuous stimuli through PRRs could be a predominant mechanism through which microglia become overactivated and uncontrolled, and therefore able to exert neurotoxic effects. Although different combinations of receptors might be involved in the recognition of toxic and pro-inflammatory stimuli, there is a common deleterious downstream pathway, involving oxidative stress that both induces neuronal death and amplifies ongoing microglial activation to drive perpetuating neurotoxicity. Given the progressive and cumulative contribution of microglial activation throughout the course of neurodegenerative diseases, imaging microglia might be useful for the early identification of neurodegenerative disease. Monitoring of microglial activation throughout the disease would give an indication of when to begin anti-inflammatory treatment capable of altering disease progression, and provide feedback that allows an individually tailored therapeutic regimen based on response to therapy. Future research will need to focus on detailing the mechanisms responsible for microglial overactivation in an effort to identify more specific markers for *in vivo* imaging and provide the basis for the development of compounds that have greater therapeutic efficacy.

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Competing interests statement

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