# Microglia-mediated neurotoxicity: uncovering the molecular mechanisms

*Michelle L. Block*, Luigi Zecca‡ and Jau-Shyong Hong**

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 mannosylated 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.

**Neuropharmacology Section, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA. ‡Institute of Biomedical Technologies, Italian National Research Council, 20090 Segrate (Milano), Italy. Correspondence to M.L.B. e-mail: Block@niehs.nih.gov*
doi:10.1038/nrn2038

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 brain[4]. 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 nigra[6]. 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 markers[12]. 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 injury[24], 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 superoxide[29], nitric oxide (NO)[30,31] and tumour necrosis factor-α (TNFα)[32,33]. The stimuli that cause microglial overactivation and dysregulation can be diverse, ranging

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 consequences[26]. 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 amygdala[37]. Alzheimer's disease results in the progressive impairment of memory and cognitive decline, and microglial activation increases throughout disease progression[38]. Amyloid-β (Aβ) 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 disease[42]; even before the development of symptoms, activated microglia cluster at sites of aggregated Aβ and penetrate the neuritic plaques[35,43]. Aβ is pro-inflammatory and activates microglia to release neurotoxic factors such as NO[44], TNFα[45] and superoxide[41]. So, Aβ both recruits and activates microglia[46,47], indicating a crucial role for microglia in Alzheimer's disease progression[48].

***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 cortex[51]. The selective loss of DA neurons in the substantia nigra might be due to DA neuron glutathione deficiency[52] (resulting in a reduced antioxidant capacity), high content of DA (a redox active molecule) in neurons in the substantia nigra[53], elevated iron concentrations[54] (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 microglia[56]. MMP3, a proteinase that degrades extracellular matrix, is released on DA neuron damage, activates microglia and induces DA neuron death[57]. 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 MPTP[58].

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 microglia[59]. 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 death[60] (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 cases[61]. 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 infiltration[62]. 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 replication[65]. 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 intensity[66]. Microglial HIV infection and viral replication results in an increased release of neurotoxic pro-inflammatory cytokines[67], and the SIV model of HIV infection shows enhanced microglial activation[68]. Microglia are activated in HIV by interaction with viral proteins[69] such as transactivator of transcription (Tat)[70] and gp120 (REFS 71,72), HIV infection and soluble factors released from infected cells[73], 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 controls[88]. Damage to the microvasculature in Alzheimer's disease and Parkinson's disease includes fragmentation, atrophy, tortuous structure, basement membrane thickening, vacuolization and pericytic degeneration[89]. 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 1β (IL-1β)-expressing microglia[90] and enlarged phagocytic microglial subtypes[91]. 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 age[92], and the spinal cord seems to be similarly affected[93]. 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 MPTP[95] 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 humans[50] and primates[98].

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 toxicity[99]. Furthermore, in several animal studies, MPTP toxicity is significantly reduced in mutant mice deficient in pro-inflammatory factors such as superoxide[105,106], myeloperoxidase[107], prostaglandins[108–111], NO[112] and TNFα[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 rotenone[118] and MPTP[119], 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 administration[120]. 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 mice[103]. 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 (TGFβ))[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.

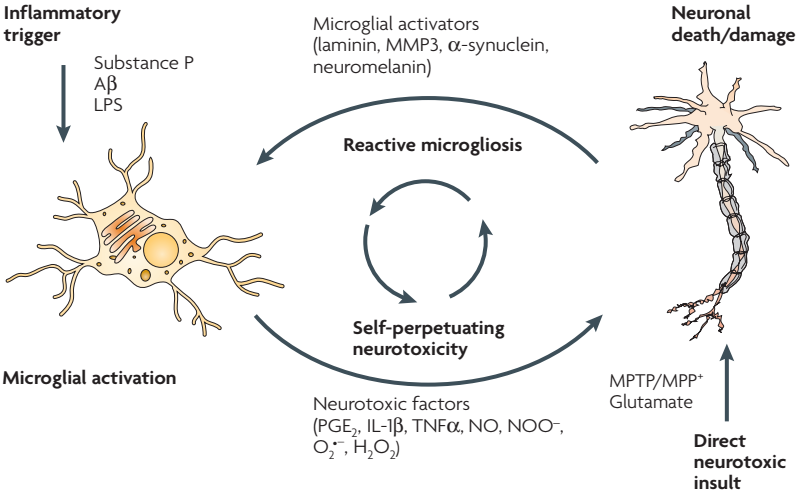


Figure 1 | **Reactive microgliosis drives progressive neurotoxicity.** Microglia can become overactivated and cause neurotoxicity through two mechanisms. First, microglia can initiate neuron damage by recognizing pro-inflammatory stimuli, such as lipopolysaccharide (LPS), becoming activated and producing 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 ; $H_2O_2$, 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; $O_2^{\bullet -}$, superoxide; $PGE_2$, prostaglandin E2; TNFα, tumour necrosis factor-α.

oestrogen[128], 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], MPTP[139], 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], paraquat[146], rotenone[147,148], manganese ethylene-bisdithiocarbamate[149] 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 diseases[151]. 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 brain[152]. In addition to its association with asthma, particulate matter is reported to cause systemic inflammation and is associated with arteriosclerosis[153]. Recently, ambient particulate matter has been linked to ischaemic stroke in humans[154]. Ambient particulate matter has been associated with brain inflammation in mice[155], dogs[156,157] and humans[158]. *In vitro*, DEP results in DA neurotoxicity through microglial activation[150]. 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 response[159]. Recently, PRRs have been linked to numerous diseases of chronic inflammation such as Crohn's disease, arthrosclerosis, diabetes and cancer[160]. 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 survival[159]. 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 pathogens[161]. So far, there are twelve members of the TLR family identified in mammals that recognize PAMPs from bacteria, fungi, parasites, viruses and the host itself[159]. 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 receptor[163], 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 inflammation[167] and is reported to mediate LPS-induced neurotoxicity and oligodendrocyte damage[166]. In addition, TLR4 is implicated in brain inflammation and microglial activation in response to endotoxemia[165]. Independent of LPS, TLR4 also contributes to the initiation of CNS neuroimmune activation and pain neuropathy after transection of the L5 spinal nerve[168]. 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 properties[170].

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 virus[171]. Additionally, the extracellular matrix component peptidoglycan can activate microglia through TLR2 to upregulate chemotactic proteins and increase Aβ uptake[172]. Activation of TLR2, TLR4 and TLR9 induces microglial production of NO through multiple ligands[173]. Furthermore, microglia become activated after recognizing double-stranded RNA through TLR3, indicating the importance of this receptor for the microglial response to viral insult[174]. TLR9 recognizes single-stranded unmethylated CpG-DNA (bacterial DNA), which stimulates an increase in the production of microglial NO and TNFα[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 phagocytes[176]. A number of these classes have been shown to affect general microglial function, are linked to neurotoxicity and are often upregulated during neurodegenerative diseases[177]. 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 pathogens[177]. 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 cytokines[179]. The activation of scavenger receptors can result in ligand internalization and/or production of extracellular superoxide by microglia[177]. For example, the class B scavenger receptor CD36 mediates free radical production and tissue injury in cerebral ischaemia[180]. Both SR-A[181] and SR-B1 (REF. 182) mediate adhesion and endocytosis of fibrillar Aβ by microglia. Interestingly, SR-A, SR-B1 and CD36 (REF. 183) participate in the microglial production of ROS in response to Aβ 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 Aβ[184], and in the mediation of cytoskeleton rearrangements in microglia[185].

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 superfamily[176]. Interestingly, RAGE-expressing microglia are upregulated in Alzheimer's disease, and microglial RAGE is reported to mediate the pro-inflammatory effects of Aβ[186–188]. 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.

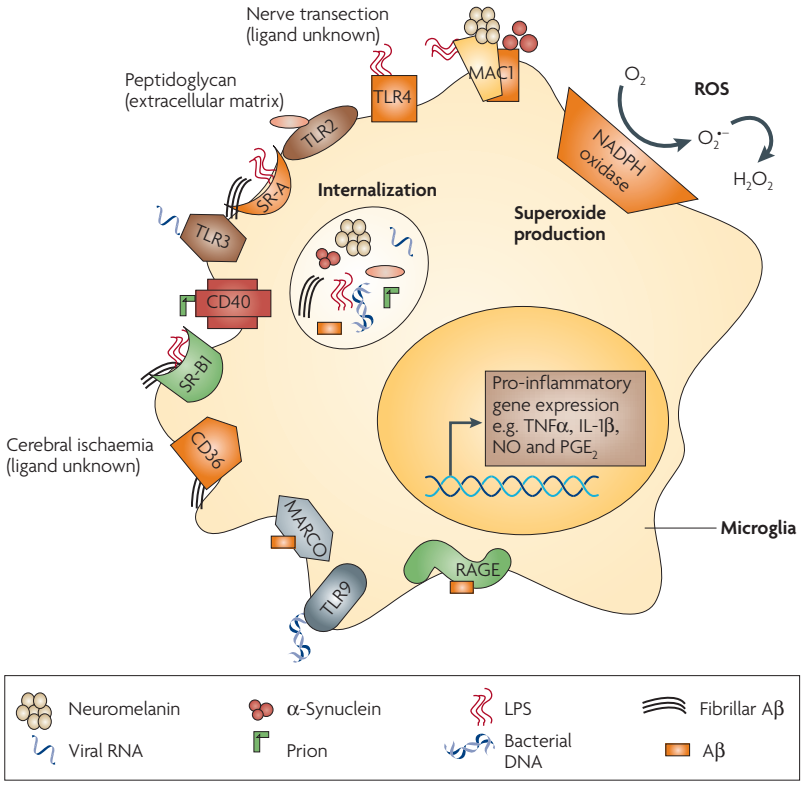


Figure 2 | **Microglial PRRs identify neurotoxic and pro-inflammatory ligands.** Microglia actively monitor the brain environment with pattern recognition receptors (PRRs). PRRs are responsible for several phagocyte functions such as the identification of pathogens, the production of extracellular superoxide, 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; $H_2O_2$, hydrogen peroxide; IL-1β, interleukin 1β; LPS, lipopolysaccharide; MAC1, macrophage antigen complex; MARCO, macrophage receptor with collagenous domain (scavenger receptor); NO, nitric oxide; $O_2^{\bullet -}$, superoxide; $PGE_2$, 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.

***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 disease[192], 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 fibrilized Aβ through a cell surface receptor complex (CD36, α6β1 integrin and CD47), and antagonists specific for each receptor will block fibrilized Aβ-stimulated phagocytosis[194]. 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 ROS[195]. 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 injury[196]. 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

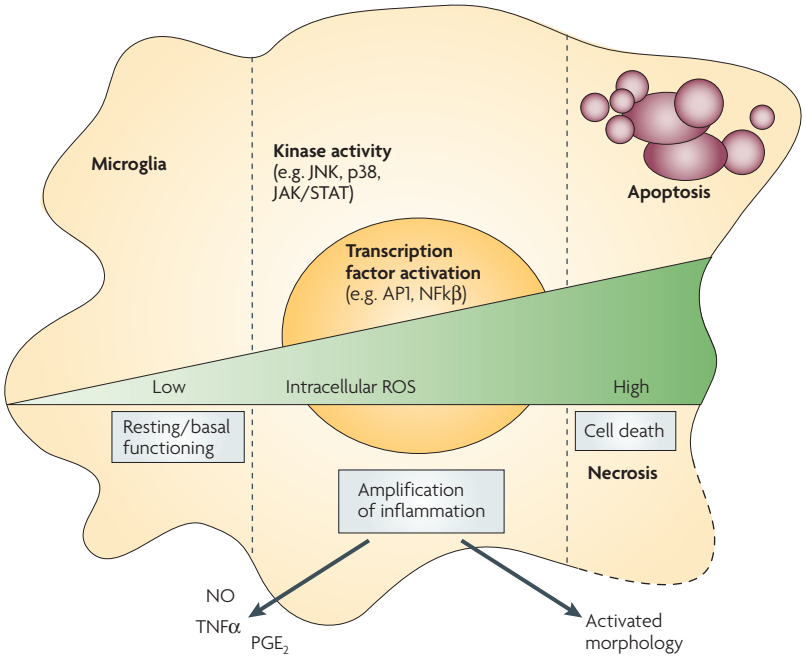


Figure 3 | **Intracellular ROS regulate microglial activation.** Classic microglial activation results in the production of both extracellular and intracellular reactive oxygen species (ROS). Intracellular ROS are crucial for both microglial pro-inflammatory function and microglial survival. Intracellular 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; $PGE_2$, prostaglandin E2; STAT, signal transducer and activation of transcription; TNFα, 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 diseases[116], 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 microglia[197].

***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 peptides[197] and multiple neurotoxins[116]. Several PRRs activate NADPH oxidase[198], 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 organelles[197]. 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 disease[199] and the catalytic subunit (gp91) is upregulated in Parkinson's disease[105]. Additionally, neural damage in response to cerebral vascular dysfunction is mediated by NADPH oxidase. Ischaemic stroke is reduced in mice lacking functional NADPH oxidase[200], and there is a clear role of NADPH oxidase in intracerebral haemorrhage[201]. Recently, it was reported that overactivated microglia injure oligodendrocytes through NADPH oxidase[202]. Furthermore, the crucial role of NADPH oxidase in mediating inflammation-related neurotoxicity has been demonstrated in the following toxins: LPS[203], rotenone[204], DEP[150], paraquat[146], MPTP[105,205], Aβ[41], substance P[121], thrombin[206] and α-synuclein[56]. Additionally, cortical neurotoxicity from amyloid precursor protein expression is mediated through NADPH oxidase[207].

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 oxidase[208]. Additionally, changes in the morphology[203] and proliferation of microglia are regulated by hydrogen peroxide from NADPH oxidase[209]. 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 necrosis[213] occurs (FIG. 3). In some cases, extremely high concentrations of ROS are reported to inhibit pro-inflammatory signalling[214], and at these higher concentrations ROS will induce cumulative effects harmful for cell survival, such as lipid peroxidation and oxidative modification of proteins[215].

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 toxins[216]. Neuronal death[119] and toxins such as rotenone[118] 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 manner[217]. 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 ([$^{11}$C]-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 imaging[42] (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 impairment[219]. 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 nigra[221]. 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 disease[222]. 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.

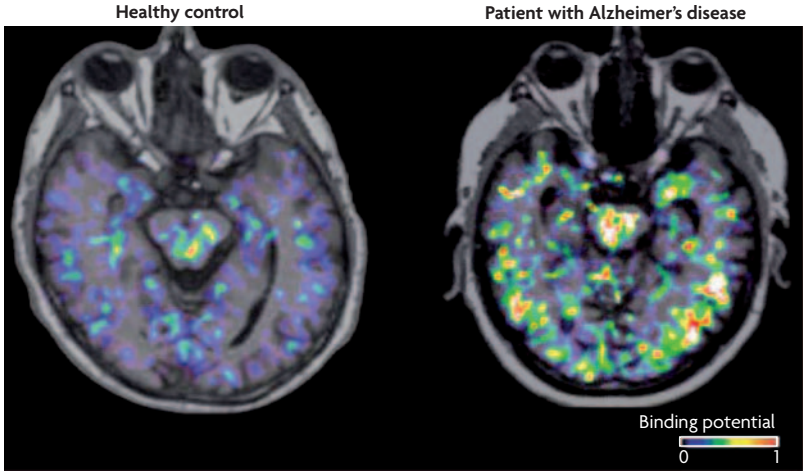


Figure 4 | **PET imaging of microglia in neurodegeneration.** Positron emission tomography (PET) images depicting microglial activation in the brains of a healthy control (74 years old) and a patient with Alzheimer's disease (65 years old, disease duration of 5 years). The uptake of the [$^{11}$C]-PK11195 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.

***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 stimuli[116], 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 failed[224]. 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

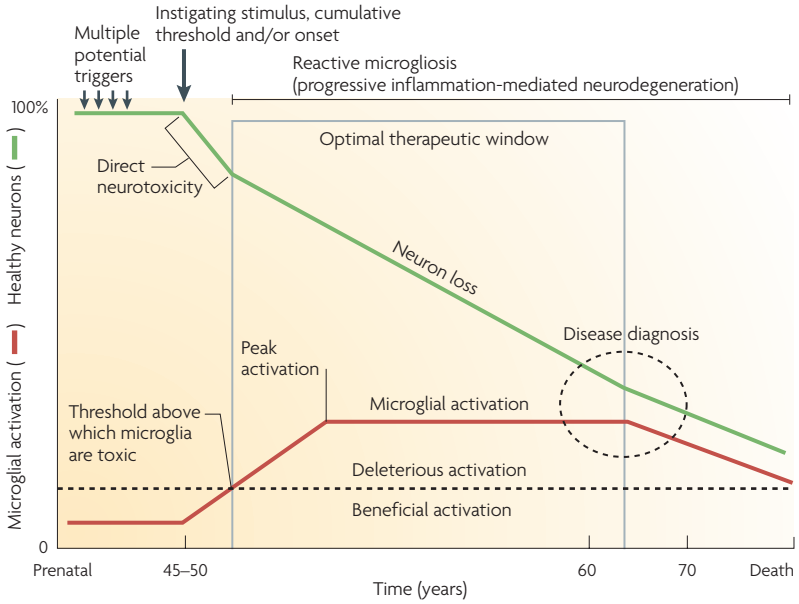


Figure 5 | **Microglial activation, neuronal death and the therapeutic relevance.** Although the initiating stimuli and specific rate of neuron loss can be individually unique for each patient and neurodegenerative disease, here we depict the generalized hypothesis of the relationship between microglial 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 $E_2$, $PGE_2$) 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 $PGE_2$ (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.


1. Zecca, L., Zucca, F. A., Albertini, A., Rizzio, E. & Fariello, R. G. A proposed dual role of neuromelanin in the pathogenesis of Parkinson's disease. *Neurology* **67**, S8–S11 (2006).
2. McGeer, P. L., Rogers, J. & McGeer, E. G. Inflammation, anti-inflammatory agents and Alzheimer disease: the last 12 years. *J. Alzheimers Dis.* **9**, 271–276 (2006).
3. Kim, Y. S. & Joh, T. H. Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. *Exp. Mol. Med.* **38**, 333–347 (2006).
4. del Rio-Hortega, P. *Cytology and Cellular Pathology of the Nervous System* (ed. Hocker, P. P.) (Penfeild Wed, New York, 1932).
5. Mittelbronn, M., Dietz, K., Schluesener, H. J. & Meyermann, R. Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. *Acta Neuropathol. (Berl)* **101**, 249–255 (2001).
6. Lawson, L. J., Perry, V. H., Dri, P. & Gordon, S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* **39**, 151–170 (1990).
7. Nimmerjahn, A., Kirchhoff, F. & Helmchen, F. Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* **308**, 1314–1318 (2005). **A crucial paper that uses novel *in vivo* technology to visualize the extensive vigilance of microglia, as they survey the brain environment.**

8. Davalos, D. *et al.* ATP mediates rapid microglial response to local brain injury *in vivo*. *Nature Neurosci.* **8**, 752–758 (2005).
**An essential paper that documents the ability of microglia to rapidly detect and respond to injury.**
9. Fetler, L. & Amigorena, S. Neuroscience. Brain under surveillance: the microglia patrol. *Science* **309**, 392–393 (2005).
10. Oehmichen, W. & Gencic, M. Experimental studies on kinetics and functions of monuclear phagozytes of the central nervous system. *Acta Neuropathol. Suppl. (Berl) Suppl.* **6**, 285–290 (1975).
11. Cho, B. P. *et al.* Pathological dynamics of activated microglia following medial forebrain bundle transection. *Glia* **53**, 92–102 (2006).
12. Rock, R. B. *et al.* Role of microglia in central nervous system infections. *Clin. Microbiol. Rev.* **17**, 942–964 (2004).
13. Harry, G. J., McPherson, C. A., Wine, R. N., Atkinson, K. & Lefebvre d'Hellencourt, C. Trimethyltin-induced neurogenesis in the murine hippocampus. *Neurotox. Res.* **5**, 623–627 (2004).
14. Streit, W. J. Microglia as neuroprotective, immunocompetent cells of the CNS. *Glia* **40**, 133–139 (2002).
15. Simard, A. R., Soulet, D., Gowing, G., Julien, J. P. & Rivest, S. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. *Neuron* **49**, 489–502 (2006).
16. Wilkinson, B., Koenigsknecht-Talboo, J., Grommes, C., Lee, C. Y. & Landreth, G. Fibrillar β-amyloid-stimulated intracellular signaling cascades require Vav for induction of respiratory burst and phagocytosis in monocytes and microglia. *J. Biol. Chem.* **281**, 20842–20850 (2006).
17. Jack, C. S. *et al.* TLR signaling tailors innate immune responses in human microglia and astrocytes. *J. Immunol.* **175**, 4320–4330 (2005).
18. Town, T., Nikolic, V. & Tan, J. The microglial 'activation' continuum: from innate to adaptive responses. *J. Neuroinflammation* **2**, 24 (2005).
19. Marin-Teva, J. L. *et al.* Microglia promote the death of developing purkinje cells. *Neuron* **41**, 535–547 (2004).
20. Upender, M. B. & Naegele, J. R. Activation of microglia during developmentally regulated cell death in the cerebral cortex. *Dev. Neurosci.* **21**, 491–505 (1999).
21. Muller, F. J., Snyder, E. Y. & Loring, J. F. Gene therapy: can neural stem cells deliver? *Nature Rev. Neurosci.* **7**, 75–84 (2006).
22. Morgan, S. C., Taylor, D. L. & Pocock, J. M. Microglia release activators of neuronal proliferation mediated by activation of mitogen-activated protein kinase, phosphatidylinositol-3-kinase/Akt and delta-Notch signalling cascades. *J. Neurochem.* **90**, 89–101 (2004).
23. Liao, H., Bu, W. Y., Wang, T. H., Ahmed, S. & Xiao, Z. C. Tenascin-R plays a role in neuroprotection via its distinct domains coordinate to modulate the microglia function. *J. Biol. Chem.* **280**, 8316–8323 (2004).
24. Aarum, J., Sandberg, K., Haeberlein, S. L. & Persson, M. A. Migration and differentiation of neural precursor cells can be directed by microglia. *Proc. Natl Acad. Sci. USA* **100**, 15983–15988 (2003).
25. Walton, N. M. *et al.* Microglia instruct subventricular zone neurogenesis. *Glia* **54**, 815–825 (2006).
26. Polazzi, E. & Contestabile, A. Reciprocal interactions between microglia and neurons: from survival to neuropathology. *Rev. Neurosci.* **13**, 221–242 (2002).
27. Ziv, Y., Avidan, H., Pluchino, S., Martino, G. & Schwartz, M. Synergy between immune cells and adult neural stem/progenitor cells promotes functional recovery from spinal cord injury. *Proc. Natl Acad. Sci. USA* **103**, 13174–13179 (2006).
28. Ziv, Y. *et al.* Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nature Neurosci.* **9**, 268–275 (2006).
29. Colton, C. A. & Gilbert, D. L. Production of superoxide anions by a CNS macrophage, the microglia. *FEBS Lett.* **223**, 284–288 (1987).
30. Moss, D. W. & Bates, T. E. Activation of murine microglial cell lines by lipopolysaccharide and interferon-γ causes NO-mediated decreases in mitochondrial and cellular function. *Eur. J. Neurosci.* **13**, 529–538 (2001).
31. Liu, B. *et al.* Role of nitric oxide in inflammation-mediated neurodegeneration. *Ann. NY Acad. Sci.* **962**, 318–331 (2002).
32. Sawada, M., Kondo, N., Suzumura, A. & Marunouchi, T. Production of tumor necrosis factor-α by microglia and astrocytes in culture. *Brain Res.* **491**, 394–397 (1989).
33. Lee, S. C., Liu, W., Dickson, D. W., Brosnan, C. F. & Berman, J. W. Cytokine production by human fetal microglia and astrocytes. Differential induction by lipopolysaccharide and IL-1 β. *J. Immunol.* **150**, 2659–2667 (1993).
34. Yankner, B. A. Amyloid and Alzheimer's disease--cause or effect? *Neurobiol. Aging* **10**, 470–471; discussion 477–478 (1989).
35. McGeer, P. L., Itagaki, S., Tago, H. & McGeer, E. G. Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. *Neurosci. Lett.* **79**, 195–200 (1987).
36. Rogers, J., Luber-Narod, J., Styren, S. D. & Civin, W. H. Expression of immune system-associated antigens by cells of the human central nervous system: relationship to the pathology of Alzheimer's disease. *Neurobiol. Aging* **9**, 339–349 (1988).
37. Braak, H. & Braak, E. Morphological criteria for the recognition of Alzheimer's disease and the distribution pattern of cortical changes related to this disorder. *Neurobiol. Aging* **15**, 355–356; discussion 379–380 (1994).
38. Xiang, Z., Haroutunian, V., Ho, L., Purohit, D. & Pasinetti, G. M. Microglia activation in the brain as inflammatory biomarker of Alzheimer's disease neuropathology and clinical dementia. *Dis. Markers* **22**, 95–102 (2006).
39. Yankner, B. A., Duffy, L. K. & Kirschner, D. A. Neurotrophic and neurotoxic effects of amyloid β protein: reversal by tachykinin neuropeptides. *Science* **250**, 279–282 (1990).
40. Combs, C. K., Johnson, D. E., Karlo, J. C., Cannady, S. B. & Landreth, G. E. Inflammatory mechanisms in Alzheimer's disease: inhibition of β-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARgamma agonists. *J. Neurosci.* **20**, 558–567 (2000).
41. Qin, L. *et al.* Microglia enhance β-amyloid peptide-induced toxicity in cortical and mesencephalic neurons by producing reactive oxygen species. *J. Neurochem.* **83**, 973–983 (2002).
42. Cagnin, A. *et al. In-vivo* measurement of activated microglia in dementia. *Lancet* **358**, 461–467 (2001).
43. Veerhuis, R. *et al.* Cytokines associated with amyloid plaques in Alzheimer's disease brain stimulate human glial and neuronal cell cultures to secrete early complement proteins, but not C1-inhibitor. *Exp. Neurol.* **160**, 289–299 (1999).
44. Ii, M., Sunamoto, M., Ohnishi, K. & Ichimori, Y. β-amyloid protein-dependent nitric oxide production from microglial cells and neurotoxicity. *Brain Res.* **720**, 93–100 (1996).
45. Dheen, S. T., Jun, Y., Yan, Z., Tay, S. S. & Ang Ling, E. Retinoic acid inhibits expression of TNF-α and iNOS in activated rat microglia. *Glia* **50**, 21–31 (2004).
46. Sasaki, A., Yamaguchi, H., Ogawa, A., Sugihara, S. & Nakazato, Y. Microglial activation in early stages of amyloid β protein deposition. *Acta Neuropathol. (Berl)* **94**, 316–322 (1997).
47. Meda, L. *et al.* Activation of microglial cells by β-amyloid protein and interferon-γ. *Nature* **374**, 647–650 (1995).
48. Griffin, W. S. *et al.* Glial–neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. *Brain Pathol.* **8**, 65–72 (1998).
49. McGeer, P. L., Itagaki, S., Boyes, B. E. & McGeer, E. G. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* **38**, 1285–1291 (1988).
50. Langston, J. W. *et al.* Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine exposure. *Ann. Neurol.* **46**, 598–605 (1999).
**A crucial paper, documenting that neurodegeneration in response to a single toxin exposure in humans continued years after the toxin had been metabolized.**
51. Imamura, K. *et al.* Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathol (Berl)* **106**, 518–526 (2003).
52. Loeffler, D. A., DeMaggio, A. J., Juneau, P. L., Havaich, M. K. & LeWitt, P. A. Effects of enhanced striatal dopamine turnover *in vivo* on glutathione oxidation. *Clin. Neuropharmacol.* **17**, 370–379 (1994).
53. Zigmond, M. J., Hastings, T. G. & Perez, R. G. Increased dopamine turnover after partial loss of dopaminergic neurons: compensation or toxicity? *Parkinsonism Relat. Disord.* **8**, 389–393 (2002).
54. Zecca, L., Youdim, M. B., Riederer, P., Connor, J. R. & Crichton, R. R. Iron, brain ageing and neurodegenerative disorders. *Nature Rev. Neurosci.* **5**, 863–873 (2004).
55. Kim, W. G. *et al.* Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. *J. Neurosci.* **20**, 6309–6316 (2000).
56. Zhang, W. *et al.* Aggregated α-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. *FASEB J.* **19**, 533–542 (2005).
57. Kim, Y. S. *et al.* Matrix metalloproteinase-3: a novel signaling proteinase from apoptotic neuronal cells that activates microglia. *J. Neurosci.* **25**, 3701–3711 (2005).
58. Kim, Y. *et al.* A pivotal role of matrix metalloproteinase-3 activity in dopaminergic neuronal degeneration via microglia activation. *FASEB J.* 20 Nov 2006 (doi:10.1096/fj.06–5865com).
59. Zecca, L., Zucca, F. A., Wilms, H. & Sulzer, D. Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics. *Trends Neurosci.* **26**, 578–580 (2003).
**A comprehensive review that explains the role of neuromelanin in reactive microgliosis.**
60. Wilms, H. *et al.* Activation of microglia by human neuromelanin is NF-κB dependent and involves p38 mitogen-activated protein kinase: implications for Parkinson's disease. *FASEB J.* **17**, 500–502 (2003).
61. Ozdener, H. Molecular mechanisms of HIV-1 associated neurodegeneration. *J. Biosci.* **30**, 391–405 (2005).
62. Budka, H. The definition of HIV-specific neuropathology. *Acta Pathol. Jpn* **41**, 182–191 (1991).
63. Jordan, C. A., Watkins, B. A., Kufta, C. & Dubois-Dalcq, M. Infection of brain microglial cells by human immunodeficiency virus type 1 is CD4 dependent. *J. Virol.* **65**, 736–742 (1991).
64. Ryzhova, E. V. *et al.* Simian immunodeficiency virus encephalitis: analysis of envelope sequences from individual brain multinucleated giant cells and tissue samples. *Virology* **297**, 57–67 (2002).
65. Speth, C., Dierich, M. P. & Sopper, S. HIV-infection of the central nervous system: the tightrope walk of innate immunity. *Mol. Immunol.* **42**, 213–228 (2005).
66. Chakrabarti, L. *et al.* Early viral replication in the brain of SIV-infected rhesus monkeys. *Am. J. Pathol.* **139**, 1273–1280 (1991).
67. Ryan, L. A., Cotter, R. L., Zink, W. E., Gendelman, H. E. & Zheng, J. Macrophages, chemokines and neuronal injury in HIV-1-associated dementia. *Cell. Mol. Biol. (Noisy-le-grand)* **48**, 137–150 (2002).
68. Sopper, S. *et al.* The effect of simian immunodeficiency virus infection *in vitro* and *in vivo* on the cytokine production of isolated microglia and peripheral macrophages from rhesus monkey. *Virology* **220**, 320–329 (1996).
69. Sheng, W. S., Hu, S., Hegg, C. C., Thayer, S. A. & Peterson, P. K. Activation of human microglial cells by HIV-1 gp41 and Tat proteins. *Clin. Immunol.* **96**, 243–251 (2000).
70. D'Aversa, T. G., Yu, K. O. & Berman, J. W. Expression of chemokines by human fetal microglia after treatment with the human immunodeficiency virus type 1 protein Tat. *J. Neurovirol.* **10**, 86–97 (2004).
71. Garden, G. A. *et al.* HIV associated neurodegeneration requires p53 in neurons and microglia. *FASEB J.* **18**, 1141–1143 (2004).
72. Kong, L. Y. *et al.* The effects of the HIV-1 envelope protein gp120 on the production of nitric oxide and proinflammatory cytokines in mixed glial cell cultures. *Cell. Immunol.* **172**, 77–83 (1996).
73. Lipton, S. A. & Gendelman, H. E. Seminars in medicine of the Beth Israel Hospital, Boston. Dementia associated with the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **332**, 934–940 (1995).
74. Perry, V. H., Cunningham, C. & Boche, D. Atypical inflammation in the central nervous system in prion disease. *Curr. Opin. Neurol.* **15**, 349–354 (2002).
75. Combrinck, M. I., Perry, V. H. & Cunningham, C. Peripheral infection evokes exaggerated sickness behaviour in pre-clinical murine prion disease. *Neuroscience* **112**, 7–11 (2002).
76. Takeuchi, H. *et al.* Interferon-γ induces microglial-activation-induced cell death: a hypothetical mechanism of relapse and remission in multiple sclerosis. *Neurobiol. Dis.* **22**, 33–39 (2006).

77. Kutzelnigg, A. *et al.* Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* **128**, 2705–2712 (2005).
78. McGeer, P. L. & McGeer, E. G. Inflammatory processes in amyotrophic lateral sclerosis. *Muscle Nerve* **26**, 459–470 (2002).
79. Solomon, J. N. *et al.* Origin and distribution of bone marrow-derived cells in the central nervous system in a mouse model of amyotrophic lateral sclerosis. *Glia* **53**, 744–753 (2006).
80. Sapp, E. *et al.* Early and progressive accumulation of reactive microglia in the Huntington disease brain. *J. Neuropathol. Exp. Neurol.* **60**, 161–172 (2001).
81. Singhrao, S. K., Neal, J. W., Morgan, B. P. & Gasque, P. Increased complement biosynthesis by microglia and complement activation on neurons in Huntington's disease. *Exp. Neurol.* **159**, 362–376 (1999).
82. Schofield, E., Kersaitis, C., Shepherd, C. E., Kril, J. J. & Halliday, G. M. Severity of gliosis in Pick's disease and frontotemporal lobar degeneration: τ-positive glia differentiate these disorders. *Brain* **126**, 827–840 (2003).
83. Paulus, W., Bancher, C. & Jellinger, K. Microglial reaction in Pick's disease. *Neurosci. Lett.* **161**, 89–92 (1993).
84. Zheng, Z. & Yenari, M. A. Post-ischemic inflammation: molecular mechanisms and therapeutic implications. *Neurol. Res.* **26**, 884–892 (2004).
85. Gerhard, A., Schwarz, J., Myers, R., Wise, R. & Banati, R. B. Evolution of microglial activation in patients after ischemic stroke: a [$^{11}$C](*R*)-PK11195 PET study. *Neuroimage* **24**, 591–595 (2005).
86. Mogi, M. *et al.* Tumor necrosis factor-α (TNF-α) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neurosci. Lett.* **165**, 208–210 (1994).
87. Banati, R. B., Gehrmann, J., Schubert, P. & Kreutzberg, G. W. Cytotoxicity of microglia. *Glia* **7**, 111–118 (1993).
88. Jellinger, K. A. Prevalence of cerebrovascular lesions in Parkinson's disease. A postmortem study. *Acta Neuropathol. (Berl)* **105**, 415–419 (2003).
89. Farkas, E., De Jong, G. I., de Vos, R. A., Jansen Steur, E. N. & Luiten, P. G. Pathological features of cerebral cortical capillaries are doubled in Alzheimer's disease and Parkinson's disease. *Acta Neuropathol (Berl)* **100**, 395–402 (2000).
90. Conde, J. R. & Streit, W. J. Microglia in the aging brain. *J. Neuropathol. Exp. Neurol.* **65**, 199–203 (2006).
91. Sheng, J. G., Mrak, R. E. & Griffin, W. S. Enlarged and phagocytic, but not primed, interleukin-1 α-immunoreactive microglia increase with age in normal human brain. *Acta Neuropathol.* (Berl) **95**, 229–234 (1998).
92. Vaughan, D. W. & Peters, A. Neuroglial cells in the cerebral cortex of rats from young adulthood to old age: an electron microscope study. *J. Neurocytol.* **3**, 405–429 (1974).
93. Stuesse, S. L., Cruce, W. L., Lovell, J. A., McBurney, D. L. & Crisp, T. Microglial proliferation in the spinal cord of aged rats with a sciatic nerve injury. *Neurosci. Lett.* **287**, 121–124 (2000).
94. Rozovsky, I., Finch, C. E. & Morgan, T. E. Age-related activation of microglia and astrocytes: *in vitro* studies show persistent phenotypes of aging, increased proliferation, and resistance to down-regulation. *Neurobiol. Aging* **19**, 97–103 (1998).
95. Sugama, S. *et al.* Age-related microglial activation in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration in C57BL/6 mice. *Brain Res.* **964**, 288–294 (2003).
96. Blasko, I. *et al.* How chronic inflammation can affect the brain and support the development of Alzheimer's disease in old age: the role of microglia and astrocytes. *Aging Cell.* **3**, 169–176 (2004).
97. Huh, Y. *et al.* Microglial activation and tyrosine hydroxylase immunoreactivity in the substantia nigral region following transient focal ischemia in rats. *Neurosci. Lett.* **349**, 63–67 (2003).
98. McGeer, P. L., Schwab, C., Parent, A. & Doudet, D. Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine administration. *Ann. Neurol.* **54**, 599–604 (2003).
99. Gao, H. M., Liu, B., Zhang, W. & Hong, J. S. Critical role of microglial NADPH oxidase-derived free radicals in the *in vitro* MPTP model of Parkinson's disease. *FASEB J.* **17**, 1954–1956 (2003).
100. Gao, H. M. *et al.* Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. *J. Neurochem.* **81**, 1285–1297 (2002).
**A crucial paper documenting that microglial activation is progressive and selective for dopaminergic neurons.**
101. Gibbons, H. M. & Dragunow, M. Microglia induce neural cell death via a proximity-dependent mechanism involving nitric oxide. *Brain Res.* **1084**, 1–15 (2006).
102. Ling, Z. *et al. In utero* bacterial endotoxin exposure causes loss of tyrosine hydroxylase neurons in the postnatal rat midbrain. *Mov. Disord.* **17**, 116–124 (2002).
103. Ling, Z. *et al.* Progressive dopamine neuron loss following supra-nigral lipopolysaccharide (LPS) infusion into rats exposed to LPS prenatally. *Exp. Neurol.* **199**, 499–512 (2006).
104. Carvey, P. M., Chang, Q., Lipton, J. W. & Ling, Z. Prenatal exposure to the bacteriotoxin lipopolysaccharide leads to long-term losses of dopamine neurons in offspring: a potential, new model of Parkinson's disease. *Front. Biosci.* **8**, S826–S837 (2003).
**An essential paper demonstrating that microglia have a critical period *in utero*, where immunological perturbation will result in microglial activation and dopaminergic neuron damage that persists into adulthood.**
105. Wu, D. C. *et al.* NADPH oxidase mediates oxidative stress in the 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine model of Parkinson's disease. *Proc. Natl Acad. Sci. USA* **100**, 6145–6150 (2003).
**A crucial paper illustrating the role of microglial NADPH oxidase in a component of MPTP-induced dopaminergic neurotoxicity.**
106. Zhang, W. *et al.* Neuroprotective effect of dextromethorphan in the MPTP Parkinson's disease model: role of NADPH oxidase. *FASEB J.* **18**, 589–591 (2004).
107. Choi, D. K. *et al.* Ablation of the inflammatory enzyme myeloperoxidase mitigates features of Parkinson's disease in mice. *J. Neurosci.* **25**, 6594–6600 (2005).
108. Feng, Z. H. *et al.* Cyclooxygenase-2-deficient mice are resistant to 1-methyl-4-phenyl1,2,3, 6-tetrahydropyridine-induced damage of dopaminergic neurons in the substantia nigra. *Neurosci. Lett.* **329**, 354–358 (2002).
109. Teismann, P. *et al.* COX-2 and neurodegeneration in Parkinson's disease. *Ann. NY Acad. Sci.* **991**, 272–277 (2003).
110. Vijitruth, R. *et al.* Cyclooxygenase-2 mediates microglial activation and secondary dopaminergic cell death in the mouse MPTP model of Parkinson's disease. *J. Neuroinflammation* **3**, 6 (2006).
111. Wang, T. *et al.* $MPP^+$-induced COX-2 activation and subsequent dopaminergic neurodegeneration. *FASEB J.* **19**, 1134–1136 (2005).
112. Wu, D. C. *et al.* Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J. Neurosci.* **22**, 1763–1771 (2002).
113. Sriram, K. *et al.* Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease. *FASEB J.* **16**, 1474–1476 (2002).
114. Sriram, K. *et al.* Deficiency of TNF receptors suppresses microglial activation and alters the susceptibility of brain regions to MPTP-induced neurotoxicity: role of TNF-α. *FASEB J.* **20**, 670–682 (2006).
115. Sriram, K., Miller, D. B. & O'Callaghan, J. P. Minocycline attenuates microglial activation but fails to mitigate striatal dopaminergic neurotoxicity: role of tumor necrosis factor-α. *J. Neurochem.* **96**, 706–718 (2006).
116. Block, M. L. & Hong, J. S. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Prog. Neurobiol.* **76**, 77–98 (2005).
**A comprehensive review summarizing how microglia become activated and contribute to neurodegenerative disease.**
117. Teismann, P. *et al.* Pathogenic role of glial cells in Parkinson's disease. *Mov. Disord.* **18**, 121–129 (2003).
118. Gao, H. M., Hong, J. S., Zhang, W. & Liu, B. Synergistic dopaminergic neurotoxicity of the pesticide rotenone and inflammogen lipopolysaccharide: relevance to the etiology of Parkinson's disease. *J. Neurosci.* **23**, 1228–1236 (2003).
119. Gao, H. M., Liu, B., Zhang, W. & Hong, J. S. Synergistic dopaminergic neurotoxicity of MPTP and inflammogen lipopolysaccharide: relevance to the etiology of Parkinson's disease. *FASEB J.* **17**, 1957–1959 (2003).
120. Cunningham, C., Wilcockson, D. C., Campion, S., Lunnon, K. & Perry, V. H. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J. Neurosci.* **25**, 9275–9284 (2005).
121. Block, M. L. *et al.* Potent regulation of microglia-derived oxidative stress and dopaminergic neuron survival: substance P vs. dynorphin. *FASEB J.* **20**, 251–258 (2006).
122. Brenneman, D. E. & Gozes, I. A femtomolar-acting neuroprotective peptide. *J. Clin. Invest.* **97**, 2299–2307 (1996).
123. Qin, L. *et al.* Microglial NADPH oxidase is a novel target for femtomolar neuroprotection against oxidative stress. *FASEB J.* **19**, 550–557 (2005).
124. Rivest, S. Cannabinoids in microglia: a new trick for immune surveillance and neuroprotection. *Neuron* **49**, 4–8 (2006).
125. Ramirez, B. G., Blazquez, C., Gomez del Pulgar, T., Guzman, M. & de Ceballos, M. L. Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J. Neurosci.* **25**, 1904–1913 (2005).
126. Boche, D., Cunningham, C., Docagne, F., Scott, H. & Perry, V. H. TGFβ1 regulates the inflammatory response during chronic neurodegeneration. *Neurobiol. Dis.* **22**, 638–650 (2006).
127. Boche, D., Cunningham, C., Gauldie, J. & Perry, V. H. Transforming growth factor-β 1-mediated neuroprotection against excitotoxic injury *in vivo*. *J. Cereb. Blood Flow Metab.* **23**, 1174–1182 (2003).
128. Johnson, A. B., Bake, S., Lewis, D. K. & Sohrabji, F. Temporal expression of IL-1β protein and mRNA in the brain after systemic LPS injection is affected by age and estrogen. *J. Neuroimmunol.* **174**, 82–91 (2006).
129. Glezer, I. & Rivest, S. Glucocorticoids: protectors of the brain during innate immune responses. *Neuroscientist* **10**, 538–552 (2004).
130. Nadeau, S. & Rivest, S. Glucocorticoids play a fundamental role in protecting the brain during innate immune response. *J. Neurosci.* **23**, 5536–5544 (2003).
131. Morale, M. C. *et al.* Glucocorticoid receptor deficiency increases vulnerability of the nigrostriatal dopaminergic system: critical role of glial nitric oxide. *FASEB J.* **18**, 164–166 (2004).
132. Peng, G. S. *et al.* Valproate pretreatment protects dopaminergic neurons from LPS-induced neurotoxicity in rat primary midbrain cultures: role of microglia. *Brain Res. Mol. Brain Res.* **134**, 162–169 (2005).
133. Dragunow, M. *et al.* Valproic acid induces caspase 3-mediated apoptosis in microglial cells. *Neuroscience* **140**, 1149–1156 (2006).
134. Carvey, P. M., Punati, A. & Newman, M. B. Progressive dopamine neuron loss in Parkinson's disease: the multiple hit hypothesis. *Cell Transplant* **15**, 239–250 (2006).
**An excellent review explaining the multiple hit hypothesis and how multiple cumulative environmental exposures are likely to result in neurodegenerative disease.**
135. Duvoisin, R. C., Yahr, M. D., Schweitzer, M. D. & Merritt, H. H. Parkinsonism before and since the Epidemic of Encephalitis Lethargica. *Arch. Neurol.* **30**, 232–236 (1963).
136. Pradhan, S., Pandey, N., Shashank, S., Gupta, R. K. & Mathur, A. Parkinsonism due to predominant involvement of substantia nigra in Japanese encephalitis. *Neurology* **53**, 1781–1786 (1999).
137. Elbaz, A. *et al.* CYP2D6 polymorphism, pesticide exposure, and Parkinson's disease. *Ann. Neurol.* **55**, 430–434 (2004).
138. Sherer, T. B., Betarbet, R. & Greenamyre, J. T. Pesticides and Parkinson's disease. *ScientificWorldJournal* **1**, 207–208 (2001).
139. Langston, J. W., Ballard, P., Tetrud, J. W. & Irwin, I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* **219**, 979–980 (1983).
140. Miwa, H., Kubo, T., Suzuki, A., Nishi, K. & Kondo, T. Retrograde dopaminergic neuron degeneration following intrastriatal proteasome inhibition. *Neurosci. Lett.* **380**, 93–98 (2005).
141. McNaught, K. S., Perl, D. P., Brownell, A. L. & Olanow, C. W. Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. *Ann. Neurol.* **56**, 149–162 (2004).

142. Sadek, A. H., Rauch, R. & Schulz, P. E. Parkinsonism due to manganism in a welder. *Int. J. Toxicol.* **22**, 393–401 (2003).
143. Hudnell, H. K. Effects from environmental Mn exposures: a review of the evidence from non-occupational exposure studies. *Neurotoxicology* **20**, 379–397 (1999).
144. Iregren, A. Manganese neurotoxicity in industrial exposures: proof of effects, critical exposure level, and sensitive tests. *Neurotoxicology* **20**, 315–323 (1999).
145. Arai, H. *et al.* Neurotoxic effects of lipopolysaccharide on nigral dopaminergic neurons are mediated by microglial activation, interleukin-1β, and expression of caspase-11 in mice. *J. Biol. Chem.* **279**, 51647–51653 (2004).
146. Wu, X. F. *et al.* The role of microglia in paraquat-induced dopaminergic neurotoxicity. *Antioxid. Redox Signal.* **7**, 654–661 (2005).
147. Gao, H. M., Hong, J. S., Zhang, W. & Liu, B. Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. *J. Neurosci.* **22**, 782–790 (2002).
148. Ling, Z. *et al.* Rotenone potentiates dopamine neuron loss in animals exposed to lipopolysaccharide prenatally. *Exp. Neurol.* **190**, 373–383 (2004).
149. Zhou, Y., Wang, Y., Kovacs, M., Jin, J. & Zhang, J. Microglial activation induced by neurodegeneration: a proteomic analysis. *Mol. Cell Proteomics* **4**, 1471–1479 (2005).
150. Block, M. L. *et al.* Nanometer size diesel exhaust particles are selectively toxic to dopaminergic neurons: the role of microglia, phagocytosis, and NADPH oxidase. *FASEB J.* **18**, 1618–1620 (2004).
151. Nel, A. Atmosphere. Air pollution-related illness: effects of particles. *Science* **308**, 804–806 (2005).
152. Takenaka, S. *et al.* Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environ Health Perspect.* **109**, 547–551 (2001).
153. Sun, Q. *et al.* Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. *JAMA* **294**, 3003–3010 (2005).
154. Wellenius, G. A., Schwartz, J. & Mittleman, M. A. Air pollution and hospital admissions for ischemic and hemorrhagic stroke among medicare beneficiaries. *Stroke* **36**, 2549–2553 (2005).
155. Campbell, A. *et al.* Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. *Neurotoxicology* **26**, 133–140 (2005).
156. Calderon-Garciduenas, L. *et al.* Air pollution and brain damage. *Toxicol. Pathol.* **30**, 373–389 (2002).
157. Calderon-Garciduenas, L. *et al.* DNA damage in nasal and brain tissues of canines exposed to air pollutants is associated with evidence of chronic brain inflammation and neurodegeneration. *Toxicol. Pathol.* **31**, 524–538 (2003).
158. Calderon-Garciduenas, L. *et al.* Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. *Toxicol. Pathol.* **32**, 650–658 (2004).
159. Akira, S., Uematsu, S. & Takeuchi, O. Pathogen recognition and innate immunity. *Cell* **124**, 783–801 (2006).
160. Karin, M., Lawrence, T. & Nizet, V. Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. *Cell* **124**, 823–835 (2006).
161. McKimmie, C. S. & Fazakerley, J. K. In response to pathogens, glial cells dynamically and differentially regulate Toll-like receptor gene expression. *J. Neuroimmunol.* **169**, 116–125 (2005).
162. Olson, J. K. & Miller, S. D. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. *J. Immunol.* **173**, 3916–3924 (2004).
163. Lien, E. *et al.* Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. *J. Clin. Invest.* **105**, 497–504 (2000).
164. Lehnardt, S. *et al.* Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. *Proc. Natl Acad. Sci. USA* **100**, 8514–8519 (2003).
165. Chakravarty, S. & Herkenham, M. Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. *J. Neurosci.* **25**, 1788–1796 (2005).
166. Lehnardt, S. *et al.* The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. *J. Neurosci.* **22**, 2478–2486 (2002).
167. Bsibsi, M., Ravid, R., Gveric, D. & van Noort, J. M. Broad expression of Toll-like receptors in the human central nervous system. *J. Neuropathol. Exp. Neurol.* **61**, 1013–1021 (2002).
168. Tanga, F. Y., Nutile-McMenemy, N. & DeLeo, J. A. The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. *Proc. Natl Acad. Sci. USA* **102**, 5856–5861 (2005).
169. Jou, I. *et al.* Gangliosides trigger inflammatory responses via TLR4 in brain glia. *Am. J. Pathol.* **168**, 1619–1630 (2006).
170. Glezer, I., Lapointe, A. & Rivest, S. Innate immunity triggers oligodendrocyte progenitor reactivity and confines damages to brain injuries. *FASEB J.* **20**, 750–752 (2006).
171. Aravalli, R. N., Hu, S., Rowen, T. N., Palmquist, J. M. & Lokensgard, J. R. Cutting edge: TLR2-mediated proinflammatory cytokine and chemokine production by microglial cells in response to herpes simplex virus. *J. Immunol.* **175**, 4189–4193 (2005).
172. Chen, K. *et al.* Activation of Toll-like receptor 2 on microglia promotes cell uptake of alzheimer disease-associated amyloid β peptide. *J. Biol. Chem.* **281**, 3651–3659 (2006).
173. Ebert, S. *et al.* Dose-dependent activation of microglial cells by Toll-like receptor agonists alone and in combination. *J. Neuroimmunol.* **159**, 87–96 (2005).
174. Town, T., Jeng, D., Alexopoulou, L., Tan, J. & Flavell, R. A. Microglia recognize double-stranded RNA via TLR3. *J. Immunol.* **176**, 3804–3812 (2006).
175. Dalpke, A. H. *et al.* Immunostimulatory CpG-DNA activates murine microglia. *J. Immunol.* **168**, 4854–4863 (2002).
176. Murphy, J. E., Tedbury, P. R., Homer-Vanniasinkam, S., Walker, J. H. & Ponnambalam, S. Biochemistry and cell biology of mammalian scavenger receptors. *Atherosclerosis* **182**, 1–15 (2005).
177. Husemann, J., Loike, J. D., Anankov, R., Febbraio, M. & Silverstein, S. C. Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. *Glia* **40**, 195–205 (2002).
178. El Khoury, J., Hickman, S. E., Thomas, C. A., Loike, J. D. & Silverstein, S. C. Microglia, scavenger receptors, and the pathogenesis of Alzheimer's disease. *Neurobiol. Aging* **19**, S81–S84 (1998).
179. Grewal, R. P., Yoshida, T., Finch, C. E. & Morgan, T. E. Scavenger receptor mRNAs in rat brain microglia are induced by kainic acid lesioning and by cytokines. *Neuroreport* **8**, 1077–1081 (1997).
180. Cho, S. *et al.* The class B scavenger receptor CD36 mediates free radical production and tissue injury in cerebral ischemia. *J. Neurosci.* **25**, 2504–2512 (2005).
181. El Khoury, J. *et al.* Scavenger receptor-mediated adhesion of microglia to β-amyloid fibrils. *Nature* **382**, 716–719 (1996).
182. Husemann, J., Loike, J. D., Kodama, T. & Silverstein, S. C. Scavenger receptor class B type I (SR-BI) mediates adhesion of neonatal murine microglia to fibrillar β-amyloid. *J. Neuroimmunol.* **114**, 142–150 (2001).
183. Coraci, I. S. *et al.* CD36, a class B scavenger receptor, is expressed on microglia in Alzheimer's disease brains and can mediate production of reactive oxygen species in response to β-amyloid fibrils. *Am. J. Pathol.* **160**, 101–112 (2002).
184. Alarcon, R., Fuenzalida, C., Santibanez, M. & von Bernhardi, R. Expression of scavenger receptors in glial cells. Comparing the adhesion of astrocytes and microglia from neonatal rats to surface-bound β-amyloid. *J. Biol. Chem.* **280**, 30406–30415 (2005).
185. Granucci, F. *et al.* The scavenger receptor MARCO mediates cytoskeleton rearrangements in dendritic cells and microglia. *Blood* **102**, 2940–2947 (2003).
186. Arancio, O. *et al.* RAGE potentiates Aβ-induced perturbation of neuronal function in transgenic mice. *EMBO J.* **23**, 4096–4105 (2004).
187. Lue, L. F. *et al.* Involvement of microglial receptor for advanced glycation endproducts (RAGE) in Alzheimer's disease: identification of a cellular activation mechanism. *Exp. Neurol.* **171**, 29–45 (2001).
188. Yan, S. D. *et al.* RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. *Nature* **382**, 685–691 (1996).
189. Ross, G. D. Regulation of the adhesion versus cytotoxic functions of the Mac-1/CR3/αMβ2-integrin glycoprotein. *Crit. Rev. Immunol.* **20**, 197–222 (2000).
190. Ross, G. D. & Vetvicka, V. CR3 (CD11b, CD18): a phagocyte and NK cell membrane receptor with multiple ligand specificities and functions. *Clin. Exp. Immunol.* **92**, 181–184 (1993).
191. Le Cabec, V., Carreno, S., Moisand, A., Bordier, C. & Maridonneau-Parini, I. Complement receptor 3 (CD11b/CD18) mediates type I and type II phagocytosis during nonopsonic and opsonic phagocytosis, respectively. *J. Immunol.* **169**, 2003–2009 (2002).
192. Akiyama, H. & McGeer, P. L. Brain microglia constitutively express β-2 integrins. *J. Neuroimmunol.* **30**, 81–93 (1990).
193. Coxon, A. *et al.* A novel role for the β 2 integrin CD11b/CD18 in neutrophil apoptosis: a homeostatic mechanism in inflammation. *Immunity* **5**, 653–666 (1996).
194. Koenigsknecht, J. & Landreth, G. Microglial phagocytosis of fibrillar beta-amyloid through a β1 integrin-dependent mechanism. *J. Neurosci.* **24**, 9838–9846 (2004).
195. Bamberger, M. E., Harris, M. E., McDonald, D. R., Husemann, J. & Landreth, G. E. A cell surface receptor complex for fibrillar β-amyloid mediates microglial activation. *J. Neurosci.* **23**, 2665–2674 (2003).
196. Reichert, F. & Rotshenker, S. Complement-receptor-3 and scavenger-receptor-AI/II mediated myelin phagocytosis in microglia and macrophages. *Neurobiol. Dis.* **12**, 65–72 (2003).
197. Babior, B. M. Phagocytes and oxidative stress. *Am. J. Med.* **109**, 33–44 (2000).
198. Suh, C. I. *et al.* The phosphoinositide-binding protein p40phox activates the NADPH oxidase during FcγIIA receptor-induced phagocytosis. *J. Exp. Med.* **203**, 1915–1925 (2006).
199. Shimohama, S. *et al.* Activation of NADPH oxidase in Alzheimer's disease brains. *Biochem. Biophys. Res. Commun.* **273**, 5–9 (2000).
200. Walder, C. E. *et al.* Ischemic stroke injury is reduced in mice lacking a functional NADPH oxidase. *Stroke* **28**, 2252–2258 (1997).
201. Tang, J. *et al.* Role of NADPH oxidase in the brain injury of intracerebral hemorrhage. *J. Neurochem.* **94**, 1342–1350 (2005).
202. Li, J., Baud, O., Vartanian, T., Volpe, J. J. & Rosenberg, P. A. Peroxynitrite generated by inducible nitric oxide synthase and NADPH oxidase mediates microglial toxicity to oligodendrocytes. *Proc. Natl Acad. Sci. USA* **102**, 9936–9941 (2005).
203. Qin, L. *et al.* NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. *J. Biol. Chem.* **279**, 1415–1421 (2004). **An essential paper documenting the role of microglial NADPH oxidase in neurotoxicity and pro-inflammatory gene expression.**
204. Gao, H. M., Liu, B. & Hong, J. S. Critical role for microglial NADPH oxidase in rotenone-induced degeneration of dopaminergic neurons. *J. Neurosci.* **23**, 6181–6187 (2003).
205. Gao, H. M., Liu, B., Zhang, W. & Hong, J. S. Critical role of microglial NADPH oxidase-derived free radicals in the *in vitro* MPTP model of Parkinson's disease. *FASEB J.* **17**, 1954–1956 (2003).
206. Choi, S. H. *et al.* Inhibition of thrombin-induced microglial activation and NADPH oxidase by minocycline protects dopaminergic neurons in the substantia nigra *in vivo*. *J. Neurochem.* **95**, 1755–1765 (2005).
207. Qin, B. *et al.* A key role for the microglial NADPH oxidase in APP-dependent killing of neurons. *Neurobiol. Aging* **27**, 1577–1587 (2005).
208. Min, K. J. *et al.* Gangliosides activate microglia via protein kinase C and NADPH oxidase. *Glia* **48**, 197–206 (2004).
209. Mander, P. K., Jekabsone, A. & Brown, G. C. Microglia proliferation is regulated by hydrogen peroxide from NADPH oxidase. *J. Immunol.* **176**, 1046–1052 (2006).
210. Pawate, S., Shen, Q., Fan, F. & Bhat, N. R. Redox regulation of glial inflammatory response to lipopolysaccharide and interferongamma. *J. Neurosci. Res.* **77**, 540–551 (2004).
211. Mayadas, T. N. & Cullere, X. Neutrophil β2 integrins: moderators of life or death decisions. *Trends Immunol.* **26**, 388–395 (2005).
212. Sim, S. *et al.* NADPH oxidase-derived reactive oxygen species-mediated activation of ERK1/2 is required for apoptosis of human neutrophils induced by *Entamoeba histolytica*. *J. Immunol.* **174**, 4279–4288 (2005).
213. Aronis, A., Madar, Z. & Tirosh, O. Mechanism underlying oxidative stress-mediated lipotoxicity: exposure of J774.2 macrophages to triacylglycerols facilitates mitochondrial reactive oxygen species production and cellular necrosis. *Free Radic. Biol. Med.* **38**, 1221–1230 (2005).

214. Li, Q. & Engelhardt, J. F. Interleukin-1β induction of NFκB is partially regulated by $H_2O_2$-mediated activation of NFκB-inducing kinase. *J. Biol. Chem.* **281**, 1495–1505 (2006).
215. Engelhardt, J. F., Sen, C. K. & Oberley, L. Redox-modulating gene therapies for human diseases. *Antioxid. Redox Signal.* **3**, 341–346 (2001). **An informative review detailing redox signalling and human disease.**
216. Vilhardt, F. *et al.* The HIV-1 Nef protein and phagocyte NADPH oxidase activation. *J. Biol. Chem.* **277**, 42136–42143 (2002).
217. Misgeld, T. & Kerschensteiner, M. *In vivo* imaging of the diseased nervous system. *Nature Rev. Neurosci.* **7**, 449–463 (2006).
218. Gerhard, A. *et al. In vivo* imaging of microglial activation with [$^{11}$C](*R*)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol. Dis.* **21**, 404–412 (2006). **An excellent study illustrating how microglia are non-invasively imaged in patients with Parkinson's disease.**
219. Ouchi, Y. *et al.* Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann. Neurol.* **57**, 168–175 (2005).
220. Gerhard, A. *et al.* [$^{11}$C](*R*)-PK11195 PET imaging of microglial activation in multiple system atrophy. *Neurology* **61**, 686–689 (2003).
221. Cicchetti, F. *et al.* Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. *Eur. J. Neurosci.* **15**, 991–998 (2002).
222. Pavese, N. *et al.* Microglial activation correlates with severity in Huntington disease: a clinical and PET study. *Neurology* **66**, 1638–1643 (2006).
223. Cordle, A. & Landreth, G. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors attenuate β-amyloid-induced microglial inflammatory responses. *J. Neurosci.* **25**, 299–307 (2005).
224. Ho, L., Qin, W., Stetka, B. S. & Pasinetti, G. M. Is there a future for cyclo-oxygenase inhibitors in Alzheimer's disease? *CNS Drugs* **20**, 85–98 (2006).
225. Hernan, M. A., Logroscino, G. & Garcia Rodriguez, L. A. Nonsteroidal anti-inflammatory drugs and the incidence of Parkinson disease. *Neurology* **66**, 1097–1099 (2006).
226. Ton, T. G. *et al.* Nonsteroidal anti-inflammatory drugs and risk of Parkinson's disease. *Mov. Disord.* **21**, 964–969 (2006).
227. Wang, T. *et al.* Role of reactive oxygen species in LPS-induced production of prostaglandin E2 in microglia. *J. Neurochem.* **88**, 939–947 (2004).
228. Bonneh-Barkay, D., Reaney, S. H., Langston, W. J. & Di Monte, D. A. Redox cycling of the herbicide paraquat in microglial cultures. *Brain Res. Mol. Brain Res.* **134**, 52–56 (2005).
229. Luber-Narod, J., Kage, R. & Leeman, S. E. Substance P enhances the secretion of tumor necrosis factor-α from neuroglial cells stimulated with lipopolysaccharide. *J. Immunol.* **152**, 819–824 (1994).
230. Gayle, D. A. *et al.* Lipopolysaccharide (LPS)-induced dopamine cell loss in culture: roles of tumor necrosis factor-α, interleukin-1β, and nitric oxide. *Brain Res. Dev. Brain Res.* **133**, 27–35 (2002).
231. Croisier, E. & Graeber, M. B. Glial degeneration and reactive gliosis in α-synucleinopathies: the emerging concept of primary gliodegeneration. *Acta Neuropathol. (Berl)* **112**, 517–530 (2006). **An excellent review outlining how glial cells contribute to neurodegeneration.**
232. Choi, S. H., Lee, D. Y., Kim, S. U. & Jin, B. K. Thrombin-induced oxidative stress contributes to the death of hippocampal neurons *in vivo*: role of microglial NADPH oxidase. *J. Neurosci.* **25**, 4082–4090 (2005).
233. Choi, S. H., Joe, E. H., Kim, S. U. & Jin, B. K. Thrombin-induced microglial activation produces degeneration of nigral dopaminergic neurons *in vivo*. *J. Neurosci.* **23**, 5877–5886 (2003).
234. Kim, K. Y. *et al.* Thrombin induces IL-10 production in microglia as a negative feedback regulator of TNF-α release. *Neuroreport* **13**, 849–852 (2002).



### Acknowledgements

L.Z. was supported with a grant from the Michael J. Fox Foundation and the MIUR-FIRB project on Protein Folding and Aggregation: Metal and Biomolecules in Protein Conformational Diseases. M.L.B. was supported by the National Institutes of Health (NIH) Pathway to Independence Award. This work was also supported in part by the Intramural Research Program of the National Institute of Environmental Health Sciences, NIH.


### Competing interests statement

The authors declare no competing financial interests.

### DATABASES

**The following terms in this article are linked online to:**
**Entrez Gene:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
α-synuclein | MAC1 | MARCO | MMP3 | TLR4
**OMIM:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Alzheimer's disease | amyotrophic lateral sclerosis | Huntington's disease | multiple sclerosis | Parkinson's disease | Pick's disease
**Access to this links box is available online.**