

## Review

# The executioners sing a new song: killer caspases activate microglia

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Activation of microglia and inflammation-mediated neurotoxicity are suggested to have key roles in the pathogenesis of several neurodegenerative disorders. We recently published an article in *Nature* revealing an unexpected role for executioner caspases in the microglia activation process. We showed that caspases 8 and 3/7, commonly known to have executioner roles for apoptosis, can promote microglia activation in the absence of death. We found these caspases to be activated in microglia of PD and AD subjects. Inhibition of this signaling pathway hindered microglia activation and importantly reduced neurotoxicity in cell and animal models of disease. Here we review evidence suggesting that microglia can have a key role in the pathology of neurodegenerative disorders. We discuss possible underlying mechanisms regulating their activation and neurotoxic effect. We focus on the provocative hypothesis that caspase inhibition can be neuroprotective by targeting the microglia rather than the neurons themselves.

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### Microglia, the Guardians of the Brain

The brain is partially isolated from the bloodstream by the blood–brain barrier, which prevents infections from affecting several vital brain functions. For this reason, brain and spinal cord were considered ‘immune-privileged’ organs in that they are separated from the rest of the body.

Several different types of neuroglia participate in multiple CNS activities, from structural and metabolic functions to information processing. They also seem to be involved in the progression of neurodegenerative diseases. Neuroglial cells are divided into different subtypes, which include astrocytes, oligodendrocytes, NG2 glial cells and microglia.

Microglia are CNS macrophages first described by Rio-Hortega, a disciple of Ramón y Cajal, in 1919.<sup>1</sup> Microglia populate the mammalian CNS in early embryonic development. By adulthood, microglia are found in all regions of the brain and spinal cord, and comprise 10–15% of all CNS cells. Microglia are the immune cells of the CNS – the guardians of

the brain – constantly scavenging for damaged neurons, plaques and infectious agents by using their multiple surface receptors. In addition to being sensitive to changes in their environment, each microglial cell also regularly physically surveys its domain. While moving through its precinct, if the microglial cell finds any invading viruses, bacteria, damaged cells, apoptotic cells, neural tangles, DNA fragments or plaques it will undergo activation and phagocytize the material or cell. Thereby microglial cells also act as ‘housekeepers’ that clean up the brain. Thus, microglial cells fulfill an astonishing variety of tasks within the CNS (Box 1, FACTS).

Originally, the non-activated microglia were designated as ‘resting’, but nowadays the term ‘surveying’ is preferred, reflecting the more dynamic nature of these cells. When microglia are ‘activated’ they take on an amoeboid shape and increase their gene expression, leading to the production of numerous potentially neurotoxic mediators. During recent years, researchers have tended to differentiate between

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**Abbreviations:** A $\beta$ ,  $\beta$ -amyloid; AD, Alzheimer’s disease; AIM2, absent in melanoma-2; ALS, amyotrophic lateral sclerosis; AMPA receptor,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; AP-1, activator protein-1; APP, amyloid- $\beta$  precursor protein; CoREST, REST co-repressor; COX, cyclooxygenase; DA, dopaminergic; DAMP, danger-associated molecular pattern; fmk, fluoromethyl ketone; GSK3 $\beta$ , glycogen synthase kinase-3 $\beta$ ; HD, Huntington’s disease; HSP, heat-shock proteins; IL, interleukin; IL-1R, IL-1 receptor; IFN- $\gamma$ , interferon- $\gamma$ ; IFNGR, IFN- $\gamma$  receptor; IKK $\epsilon$ , I $\kappa$ B kinase- $\epsilon$ ; iNOS, inducible nitric oxide synthase; IRAK, interleukin receptor-associated kinase; IRF, interferon-regulatory factor; Jak, Janus kinase; LPS, lipopolysaccharide; LTA, lipoteichoic acid; LRR, leucine-rich repeat; MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MS, multiple sclerosis; MyD88, myeloid differentiation factor-88; NEMO, NF- $\kappa$ B essential modulator; NF- $\kappa$ B, nuclear factor  $\kappa$ -light-chain enhancer of activated B cells; NLRP, NLR family pyrin domain containing; NO, nitric oxide; O $_2^-$ , superoxide anion; Nurr-1, nuclear receptor related-1 protein; PAMP, pathogen-associated molecular pattern; PD, Parkinson’s disease; PKC $\delta$ , protein kinase-C $\delta$ ; Q-VD-OPh, quinoly-valyl-O-methylaspartyl-[-2,6-difluorophenoxy]-methylketone; RAGEs, receptors like advanced glycation end products; ROS, reactive oxygen species; SN, substantia nigra; Socs, suppressors of cytokine signaling proteins; STAT, signal transducer and activator of transcription; TANK, TRAF family member-associated NFKB activator; TBK1, TANK-binding kinase-1; TIR, Toll-interleukin-1 receptor; TLR, Toll-like receptor; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; TRAF6, TNF receptor-associated factor-6; TIRAP, TIR domain-containing adaptor protein; TRAM, TRIF-related adapter molecule; TRIF, TIR-domain-containing, adapter-inducing interferon- $\beta$ ; Tyk2, tyrosine kinase-2

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**Box 1****FACTS:**

- Under physiological conditions, microglia cells serve as surveyors of the integrity and function of the brain. However, their function changes under pathological conditions, during which microglia can have either a supportive or a detrimental role for neuronal survival.
- Upon activation, microglia cells release proinflammatory factors (that is, cytokines), reactive oxygen/nitrogen species and proteases that eventually provoke neuronal demise and death.
- Several signaling pathways lead to microglial activation. Depending on the nature of the proinflammatory stimulus they can include TLRs, MyD88, JAK/STAT and NF- $\kappa$ B.
- Recent studies indicate that killer caspases can take on non-apoptotic roles. For example, caspases 8 and 3/7, commonly known as executioners of apoptosis, can promote microglia activation in the absence of cell death.
- Caspases 8 and 3/7 are activated in microglia in the substantia nigra in Parkinson's disease subjects and the frontal cortex of Alzheimer's disease subjects.
- Caspase inhibitors exert neuroprotective effects in several animal models of neurodegenerative disorders, which are characterized by reactive microgliosis.

**Table 1** Innate and adaptive immune responses in chronic neurodegenerative diseases

	Innate immune response	Adaptive immune response	References
Parkinson's disease	<ul style="list-style-type: none"> <li>● Microgliosis</li> <li>● Activation of cleaved caspase-8 and caspase-3 in reactive microglia</li> <li>● Release of matrix metalloproteinase-3, <math>\alpha</math>-synuclein and neuromelanin</li> <li>● Increased proinflammatory cytokines and ROS-generating enzymes: TNF-<math>\alpha</math>, IL-1<math>\beta</math>, IL-2, 4, 6, iNOS, COX-2, NADPH oxidase</li> <li>● Upregulation of CD14, TLR2, TLR5</li> </ul>	<ul style="list-style-type: none"> <li>● Cytotoxic T-lymphocytes in the parkinsonian brain</li> <li>● Suspected FasL-dependent mechanism to trigger cell death of dopaminergic neurons in the substantia nigra</li> </ul>	4,5,8,10,13,14, 18,21,103–105
Alzheimer's disease	<ul style="list-style-type: none"> <li>● Microgliosis</li> <li>● Activation of cleaved caspase-8 and caspase-3 in reactive microglia</li> <li>● Release of proinflammatory toxic A<math>\beta</math> peptides</li> <li>● Increased proinflammatory cytokines and ROS-generating enzymes: IL-1<math>\beta</math>, IL-6, iNOS, IL-18, prostaglandins, COX-2, TNF-<math>\alpha</math></li> <li>● Increased complement components, chemokines and TGF-<math>\beta</math></li> <li>● Upregulation of CD14, TLR2 and TLR4 microglial receptors</li> </ul>	<ul style="list-style-type: none"> <li>● Uncertain</li> </ul>	8,13–18,104, 106–108
Amyotrophic lateral sclerosis	<ul style="list-style-type: none"> <li>● Microgliosis</li> <li>● Increased proinflammatory cytokines IL-6, IL-1<math>\beta</math> and TNF-<math>\alpha</math> elevated in the cerebrospinal fluid and/or spinal cord</li> <li>● Upregulation of COX-2 in spinal cord</li> </ul>	<ul style="list-style-type: none"> <li>● T-lymphocytes at sites of lesion</li> </ul>	109–112
Multiple sclerosis	<ul style="list-style-type: none"> <li>● Microgliosis</li> <li>● Presence of macrophages digesting myelin at the lesion edge</li> <li>● Immunoglobulin and complement deposits found in 50% of cases</li> </ul>	<ul style="list-style-type: none"> <li>● Lymphocytes and activated myeloid cells at sites of lesion</li> </ul>	113–115

Abbreviations: A $\beta$ ,  $\beta$ -amyloid; COX, cyclooxygenase; IL, interleukin; iNOS, inducible nitric oxide synthase; ROS, radical oxygen species; TGF, transforming growth factor; TLR, toll-like receptor; TNF, tumor necrosis factor.

acute inflammation and chronic inflammation, and their effects on neurons. It is widely accepted that sustained inflammation is deleterious to neurons, a typical feature of chronic neurodegenerative diseases including Parkinson's disease (PD) and Alzheimer's disease (AD).

### Microglia Turn Bad: Role for Microglia Activation in Neurodegenerative Diseases

Activated microglia have key roles in neuroinflammation, and depending on the nature of the initial stimulus their actions may be either beneficial or detrimental to neuronal function. In

chronic neurodegenerative diseases, microglia 'turn bad' and remain activated for an extended period during which the production of mediators is sustained longer than usual. This increase in mediators contributes to neuronal death. Activated microglia have been increasingly linked to various neurodegenerative diseases (Table 1). We recently described their involvement in the two most common forms of neurodegenerative disease, PD and AD.

**Parkinson's disease.** Classical reports state that PD is characterized by a clinical syndrome of hypokinesia, rigidity

and tremor. Most probably the motor dysfunction can be largely attributed to the progressive degeneration of dopaminergic (DA) neurons in the substantia nigra (SN).<sup>2</sup> Relatively rare familial forms of PD are associated with mutations in genes encoding several proteins, including  $\alpha$ -synuclein, parkin and ubiquitin C-terminal hydrolase-L1.<sup>3</sup> However, the etiology of idiopathic PD, which accounts for more than 90% of PD, is still not understood.<sup>2</sup> The first indications for a role of inflammation in the pathogenesis of PD came from studies demonstrating the presence of cytotoxic T-lymphocytes and reactive microglia in the parkinsonian brain.<sup>4,5</sup> Studies on brains from humans<sup>4</sup> who had self-administered 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and monkeys<sup>6</sup> receiving the same toxin, revealed a striking microglial activation along with significant accumulation of extraneuronal neuromelanin, which can activate microglia. These findings established that an acute neurotoxic insult to the nigrostriatal system can result in long-term inflammatory response involving a reactive microgliosis and possibly leading to a self-perpetuating process of neurodegeneration.<sup>4</sup> Several lines of evidence suggest that reactive microgliosis is deleterious to the DA neurons in the SN. Thus, a single intra-nigral injection of lipopolysaccharide (LPS), the main component of the cell walls of Gram-negative bacteria and a potent inducer of inflammation, induces an acute inflammatory response with a strong macrophage/microglial reaction. This leads to the progressive death of DA neurons in the SN.<sup>7</sup> Matrix metalloproteinase-3 (MMP3),  $\alpha$ -synuclein and neuromelanin are released by damaged DA neurons, leading to the production of reactive oxygen species (ROS) by over-activated microglia.<sup>8</sup> Mice lacking the protein parkin, which is encoded by a gene associated with autosomal recessive young onset PD, show more severe nigral cell loss following systemic LPS treatment than controls, thus providing a link between a certain inherited form of PD with neuroinflammation.<sup>9</sup> Regardless of the nature of the proinflammatory stimulus (proinflammatory toxin, neuronal death, extracellular neuromelanin or  $\alpha$ -synuclein), the microglial response to neuron damage can lead to further loss of neurons over time. The presence of T-lymphocytes in the midbrain of PD patients supports a role of peripheral inflammation in the etiopathology of PD.<sup>5</sup> Other studies have demonstrated that (i) T-lymphocytes mediate nigral DA neurotoxicity through an FasL-dependent mechanism<sup>10</sup> and (ii) peripheral inflammation induced by a single systemic LPS injection triggers persistent microglia activation in different brain areas, including the SN, and that DA neurons progressively die later.<sup>11</sup>

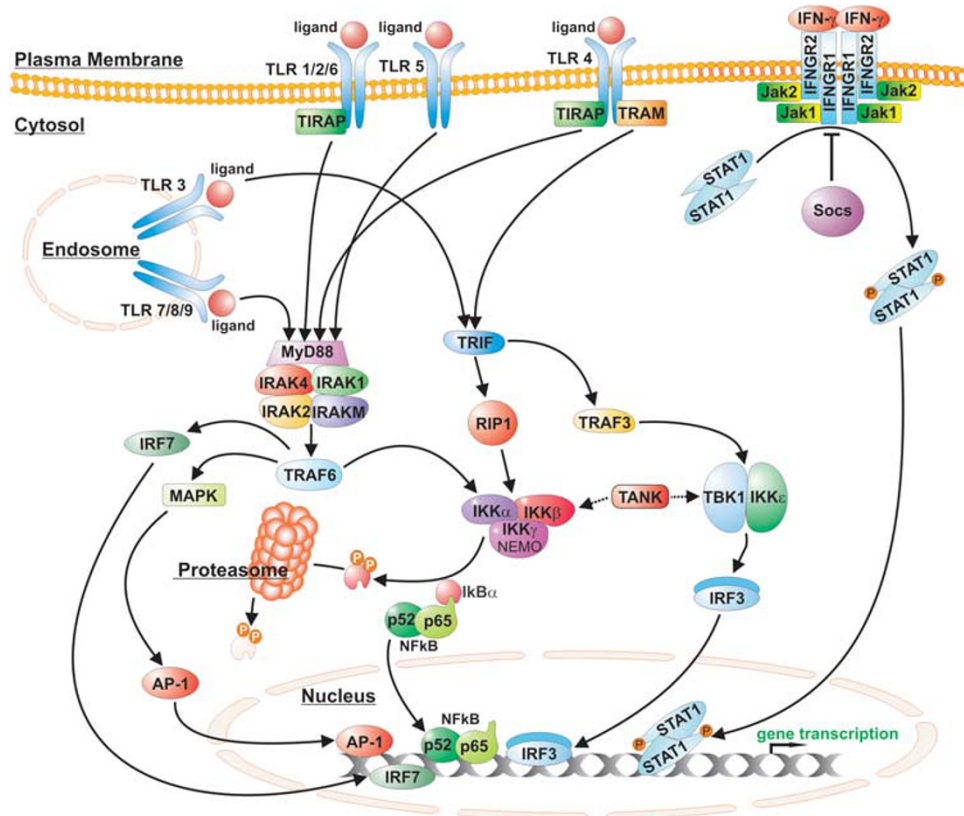
**Alzheimer's disease.** The primary clinical presentation of AD is progressive cognitive decline, with memory loss being an early sign.<sup>12</sup> The AD brain shows prominent atrophic changes in the hippocampal, frontal, parietal and temporal areas as a consequence of extensive synaptic degeneration and neuronal death.<sup>12</sup> Furthermore, the AD brain is characterized by extracellular accumulation of  $\beta$ -amyloid ( $A\beta$ ) aggregates (amyloid plaques) and the appearance of intracellular aggregates of tau, which are called neurofibrillary tangles.<sup>12</sup> Altered cholinergic and glutamatergic neurotransmission, apoptosis, oxidative stress, disrupted calcium homeostasis and neuroinflammation have all been implicated in AD pathogenesis.<sup>12</sup> We will highlight findings of neuroinflammation. In the AD brain, microglia, astrocytes and neurons release various neuroinflammatory mediators, including complement activators and inhibitors, chemokines, cytokines and ROS.<sup>13-15</sup> Historically, inflammation was thought to be secondary to degeneration. However, compelling evidence suggests that inflammatory mediators might have a significant role in the pathogenesis of the disease. For example,  $A\beta$  metabolism and over-activation of microglia are thought to interact and trigger a vicious cycle leading to neuronal death. Thus, toxic  $A\beta$  peptides are generated by the sequential action of two proteases denoted as  $\beta$ -secretase and  $\gamma$ -secretase, which cleave the amyloid precursor protein.  $A\beta$  is toxic to neurons, which in turn activates microglia when they degenerate.<sup>15</sup> The activated microglia, in turn, are deleterious to neurons.<sup>8</sup> In addition, toxic  $A\beta$  peptides show proinflammatory properties.<sup>16</sup> The most prominent activation of microglia in AD brain takes place around  $A\beta$  deposits.<sup>17</sup> The stimulated microglia release a wide variety of proinflammatory mediators, including cytokines, complement factors, free radicals, nitric oxide (NO), chemokines (interleukin-18 (IL-18)) and prostaglandins, all of which potentially contribute to further neuronal dysfunction and death.<sup>13,18</sup> Activated microglia might also stimulate astrocytes that enhance the inflammatory response. These cellular interactions fuel a self-propelling cycle that might drive progressive neurodegeneration in AD.<sup>15</sup>

**Health cost of neurodegenerative disorders associated with a microglia activation component.** Neurodegenerative disorders have a tragic impact for those afflicted and their families. In addition, their socioeconomic impact is enormous, when both direct and indirect costs are considered (Table 2). For both AD and PD the number of affected people is predicted to increase dramatically because of the increasing average age of the population. In 2010, 35.6 million people in the world had dementia (around 60% of

**Table 2** Health cost of some neurodegenerative disorders

	Year/area	Health costs	References
Dementia (60% related to Alzheimer's disease)	● 2010/worldwide	● 604 billion US \$	Annual World Alzheimer Report <sup>116</sup>
Multiple sclerosis	● 2005/Europe	● 12.5 billion €	
Amyotrophic lateral sclerosis	● 2010/Germany	● 36 380 €/patient	
Parkinson's disease	● 2007/USA	● 10.8 billion US \$	

Direct medical costs as well as the indirect costs (i.e. lost working days for the patients and caregivers) are depicted.



**Figure 1** Overview of the TLR-dependent and JAK/STAT-dependent signaling pathways in microglia. TLRs 1, 2, 5, 6, 7, 8 and 9 trigger the classical myeloid differentiation primary response gene (88) (MyD88)-dependent signaling pathway. TLR3 triggers an alternative MyD88-independent, TRIF-domain-containing, adaptor-inducing IFN- $\beta$  (TRIF)-dependent pathway through the TRIF. TLR4 triggers both the MyD88-dependent pathway through TIR domain-containing adaptor protein (TIRAP)–MyD88 interaction and the MyD88-independent pathway through TRIF-related adapter molecule (TRAM)–TRIF interaction. The MyD88-dependent pathway results in the activation of NF- $\kappa$ B, mitogen-activated protein kinase (MAPK) or IRF7 downstream signaling pathways (the latest upon *TLR9* activation) through the IL receptor-associated kinase (IRAK) complex (which includes four subunits: two kinases, IRAK-1 and 4, and two non-catalytic units, IRAK-2 and M) and the TNF receptor-associated factor-6 (TRAF6). The MyD88-independent pathway results in the activation of IRF3 through the use of two kinases (I $\kappa$ B kinase- $\epsilon$  (IKK $\epsilon$ ) and TANK-binding kinase-1 (TBK1)). The TRAF family member-associated NF- $\kappa$ B activator (TANK) interacts with NF- $\kappa$ B essential modulator (NEMO), TBK1 and IKK $\beta$ , and may therefore bridge the MyD88-dependent and MyD88-independent pathways. The ligation of the IFN- $\gamma$  receptor (IFNGR) with IFN- $\gamma$  leads to the activation of Jak1 and Jak2. STAT1 is phosphorylated by Jak kinases and translocates to the nucleus. Socs counteract STAT1 activation

which will be due to AD). It is estimated that this number will increase to 65.7 million by 2030 and 115.4 million by 2050.

### Microglia Plead Guilty to Neuron Death: Triggers of Microglia-Mediated Neurotoxicity

Microglia are guilty of causing neuron death. The underlying molecular mechanisms of neurotoxicity are clearly multiple (Figure 1). In the present section we review each one of them briefly.

**Proinflammatory cytokines.** Immunomodulators referred to as cytokines are particularly important in the genesis of inflammation. Proinflammatory, immunomodulatory and anti-inflammatory cytokines include IL-1 $\beta$ , tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6, IL-8, IL-12, IL-15 and IL-10.<sup>19</sup> The main proinflammatory cytokines are IL-1 $\beta$  and TNF- $\alpha$ . Infusions of LPS into the CNS activate Toll-like receptors (TLRs) on glial cells and induce the activation of microglia.<sup>20</sup> Through this process, LPS stimulates the expression of multiple cytokines, including IL-1, IL-6 and IL-12; cyclooxygenase-2 (COX-2)

and TNF- $\alpha$ .<sup>21</sup> Release of these cytokines creates an environment, which promotes neuronal cell death.<sup>7</sup> Cytokines including IL-1 $\beta$ , interferon- $\gamma$  (IFN- $\gamma$ ) and TNF- $\alpha$  are elevated in postmortem PD brains.<sup>22</sup> A recent meta-analysis study reported significantly higher concentrations of the proinflammatory cytokines IL-6, TNF- $\alpha$ , IL-1 $\beta$ , IL-12 and IL-18 in the peripheral blood of AD subjects as compared with controls.<sup>23</sup> Therefore, these molecules might be actively involved in disease progression in both PD and AD.

**Oxidative stress.** Oxidative stress generated by reactive microglia is supposed to be the most critical factor in inducing the death of neuronal populations.<sup>13</sup> It is highly relevant for the pathogenesis of PD as nigral dopamine neurons are highly vulnerable to oxidative stress.<sup>2</sup> Activated microglia upregulate different enzymes involved in the inflammatory processes mediated by oxidative stress, including inducible NO synthase (iNOS), NADPH oxidase, COX-2 and myeloperoxidase.<sup>8</sup> Direct evidence supporting a neurotoxic role of microglial NADPH activity and NOS-derived NO is supported by studies using intra-parenchymal LPS



injections. Thus, NADPH-deficient mice<sup>24</sup> or pharmacological inhibition of NOS confers protection in the LPS model.<sup>25</sup>

**Proteases.** MMPs are primarily produced and secreted by inflammatory cells. In particular MMP9 is known to significantly digest the basement membrane and negatively impact on barrier integrity.<sup>26</sup> MMP9 exerts also direct neurotoxicity.<sup>26</sup> MMPs are thought to have deleterious roles in different brain diseases, including stroke, multiple sclerosis (MS), infection, AD and PD.<sup>26</sup>

### Contract the Killers: Possible Underlying Mechanisms for Microglia Activation

Microglia are activated in response to neuronal damage, protein aggregate formation and several environmental stimuli/toxins.

**Neuronal damage.** Microglial activation in response to neurodegeneration or neuron injury was initially perceived as a transient event.<sup>27</sup> However, increasing evidences indicate that microglia can become chronically activated in response to dying/damaged neurons, causing a self-propelling cycle of neuron death, which is a proposed mechanism of chronic neuronal loss across diverse neurodegenerative diseases.<sup>6,13</sup> Thus, damaged DA neurons release several factors that seem to activate microglia, such as MMP3,  $\alpha$ -synuclein, neuromelanin and  $\mu$ -calpain.<sup>8</sup>

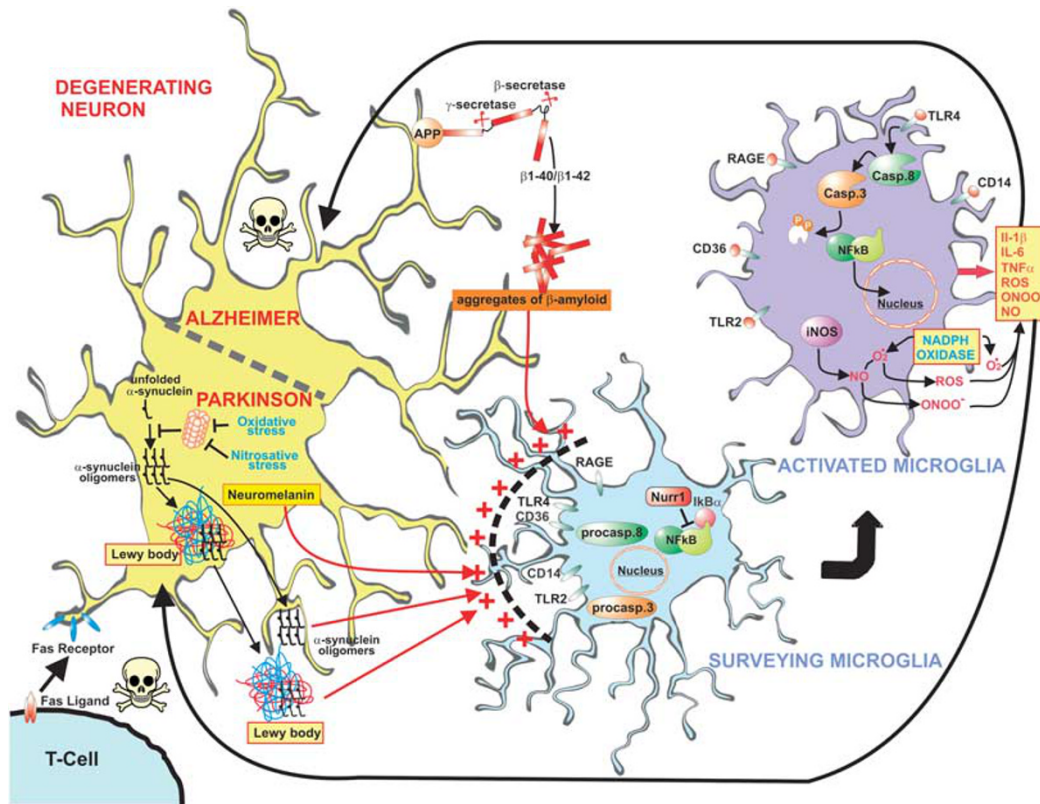
**Protein aggregates.** Protein aggregates have been found to induce neuroinflammation in AD and PD. In AD, *in vitro* experimental evidence indicates that protofibrils and oligomers of A $\beta$ 1–40 and A $\beta$ 1–42 activate microglia by binding to different receptors such as advanced glycation end products (RAGEs),<sup>28</sup> CD14, TLR2 and TLR4,<sup>29</sup> and through heterodimer formation of TLR4 and TLR6, a process dependent on the binding of A $\beta$  to the CD36 scavenger receptor.<sup>30</sup> Further evidence demonstrating a link between A $\beta$  and neuroinflammation comes from studies performed in transgenic animals showing that cerebral amyloid deposition is increased under inflammatory conditions.<sup>15</sup> As mentioned above,  $\alpha$ -synuclein appears to have a pivotal role in the pathogenesis of PD. For example,  $\alpha$ -synuclein is the major protein component of Lewy bodies.<sup>31</sup> Early observations showing that  $\alpha$ -synuclein in the SN are often surrounded by activated microglia suggested that the aggregates activated microglia.<sup>5</sup> In a transgenic mouse model expressing mutant human  $\alpha$ -synuclein, proinflammatory molecules are increased and microglia are activated before SN neurons begin to die.<sup>32</sup> Interestingly neuroinflammation and protein aggregation may be part of the same self-perpetuating vicious cycle, because intra-nigral injections of LPS induce the formation of intra-neuronal  $\alpha$ -synuclein aggregates in mice overexpressing  $\alpha$ -synuclein.<sup>33</sup> Aggregated  $\alpha$ -synuclein activated microglia, leading to enhanced DA neurotoxicity. Microglial phagocytosis of  $\alpha$ -synuclein and activation of NADPH oxidase appeared to be pivotal to the process.

**Environmental factors.** Different environmental factors, including toxins, and pollutants, may influence inflammatory responses that contribute to the etiology of neurodegenerative diseases. Several lines of evidence indicate that environment is a source for compounds that are both directly toxic to neurons and deleterious through direct stimulation of microglia (reviewed by Glass *et al.*<sup>14</sup>).

**Other factors.** Traumatic brain injury activates both microglia and astrocytes, and could potentially induce self-sustaining inflammatory responses in the brain.<sup>34</sup> Accumulating evidence implicates traumatic brain injury as a possible factor predisposing to AD.<sup>34</sup> Activation of microglia cells upon systemic infection might also be involved in the early stages of AD pathogenesis.<sup>35</sup> Recently, a strong correlation between type-2 diabetes and AD was identified, with hyper-insulinemia increasing the risk of AD.<sup>14</sup> Different mechanisms may explain this correlation. Type-2 diabetes is mostly associated with obesity, which courses with a low-grade but chronic form of inflammation in adipose tissue, liver and other organs. Recent evidence suggests that systemic inflammation contributes to the exacerbation of acute symptoms of chronic neurodegenerative diseases, including AD and PD, and may accelerate disease progression,<sup>35</sup> a plausible rationale to explain the correlation between type-2 diabetes and AD.

Once triggered by the factors described above, several intracellular signaling pathways contribute to microglial activation and can be said to sign the 'contract' for the killers (Figure 2).

**Pattern-recognition receptors.** The first paper that implicated TLRs in host immunity was by Lemaitre *et al.* in 1996. They observed that Toll-deficient *Drosophila* are vulnerable to certain fungal infections.<sup>36</sup> TLRs are a family of pattern-recognition receptors in the innate immune system. They are transmembrane proteins, each with an ectodomain with leucine-rich repeats (LRRs) and a cytoplasmic Toll–IL-1 receptor (TIR) motif homologous to that of IL-1 receptor (IL-1R).<sup>37</sup> There are 10 functional TLRs (from TLR1 to TLR10) identified in humans and 11 (from TLR1 to TLR7, TLR9 and from TLR11 to TLR13) in mice.<sup>37</sup> Microglia express TLRs 1–9.<sup>38</sup> TLRs can bind to highly conserved structural motifs, so called pathogen-associated molecular patterns (PAMPs),<sup>39</sup> as well as to several host-derived ligands, for instance, heat-shock proteins (HSP), mRNA, high-mobility group box-1 protein, surfactant proteins A and D, hyaluronan and fibrinogen).<sup>39</sup> All these host-derived ligands can be released during tissue damage in the CNS and they are collectively named danger-associated molecular patterns or DAMPS. Thereby tissue damage due to CNS disease can boost the inflammatory response by activating TLRs, and consequently might further promote cell death. Upon ligand binding, TLRs dimerize and undergo conformational changes that induce a complex cascade of intracellular signaling events, ultimately resulting in the activation of the transcription factors NF- $\kappa$ B (nuclear factor  $\kappa$ -light-chain enhancer of activated B cells), activator protein-1 (AP-1), and IFN-regulatory factor-3 (IRF3) and



**Figure 2** Mechanisms of cell death in AD and PD involving inflammation. A degenerating neuron shows key neuropathological features of PD (left side of the neuron) and AD (right side of the neuron). Unfolded  $\alpha$ -synuclein and proteasomal dysfunction induce the formation of oligomers of  $\alpha$ -synuclein, the main component of Lewy bodies, which are the most distinctive histopathological feature of PD. In AD, sequential action of  $\beta$ -secretase and  $\gamma$ -secretase gives rise to the formation of toxic  $A\beta$  (mainly  $A\beta_{1-40}$  and  $A\beta_{1-42}$ ) from the amyloid- $\beta$  precursor protein (APP). These peptides are proinflammatory and their extraneuronal accumulation form amyloid plaques, a typical histopathological feature of AD. The presence of oxidative stress in PD and other factors in AD commit neurons to die. Degenerating DA neurons in PD release different proinflammatory factors, including  $\alpha$ -synuclein and neuromelanin. All these factors are recognized by different pattern-recognition receptors, including TLRs 2 and 4, CD14, CD36 and RAGE. Binding to these receptors induces the activation of transcription factors such as NF- $\kappa$ B and AP-1 (not shown) leading to microglia activation. Nurr-1 is a repressor of NF- $\kappa$ B, whereas active caspase-3 is an activator. Activation of microglia release different proinflammatory cytokines and activate ROS-producing enzymes such as iNOS, NADPH oxidase and myeloperoxidase (not shown). NADPH oxidase is a major source of extracellular ROS in response to diverse stimuli. It is a membrane-bound enzyme that catalyzes the production of superoxide anion ( $O_2^-$ ) from oxygen and it is strongly induced in response to different proinflammatory stimuli.  $O_2^-$  easily reacts with NO (mainly derived for upregulation of iNOS by reactive microglia) to produce peroxynitrite, the most reactive free radical, thus inducing nitrosative stress. Peroxynitrite has the potential to both initiate and sustain an autotoxic loop considered as a neuronal damaging mechanism in neurodegenerative diseases. Over-activation of microglia is deleterious to neurons, thus enhancing neuronal cell death, which in turn release more proinflammatory factors thus establishing a self-perpetuating process of neuroinflammation and neurodegeneration

IRF7. These factors, in turn, regulate the expression of a wide array of genes involved in inflammatory responses.

**MyD88-dependent and -independent pathways.** Activation of most TLRs mostly results in the recruitment of the adaptor protein myeloid differentiation factor-88 (MyD88) (see Figure 2 for further details), finally leading to the nuclear translocation of NF- $\kappa$ B.<sup>39</sup> In addition, there is an MyD88-independent pathway.<sup>39</sup>

**The Jak-STAT signaling pathway.** Type-I and II cytokine receptors are a conserved family of  $\sim 40$  members that includes the receptors for ILs, IFNs and hormones.<sup>40</sup> These receptors are associated with a cytoplasmic kinase. These cytoplasmic kinases comprise the four members of the Janus kinase (Jak) family: Jak1, Jak2 and tyrosine kinase-2 (Tyk2) transduce signaling from several receptors, whereas Jak3 does so only for one receptor, the common  $\gamma$ -chain, or  $\gamma_c$ .

Upon cytokine binding, the receptor-associated Jaks are activated and in turn phosphorylate the tyrosine residues in the receptor cytoplasmic domain. This event provides a docking site for proteins with Src homology-2 domains, one important class of which is the signal transducer and activator of transcription (Stat) family of transcription factors. With seven members in all (Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b and Stat6), these DNA-binding proteins provide a rapid membrane-to-nucleus pathway for regulation of gene expression.<sup>41</sup> After cytokine stimulation, a family of cytokine-induced inhibitors termed suppressors of cytokine signaling (Socs proteins) is rapidly induced. The predominant function of the Socs proteins is to block the generation of the Stat signal from a cytokine receptor.<sup>42</sup> In canonical IFN- $\gamma$ -Jak-STAT1 signaling (reviewed by Stark<sup>43</sup>) the binding of IFN- $\gamma$  to its receptor leads to the activation of receptor-associated Jak1 and Jak2, and the phosphorylation of a receptor tyrosine residue working as a docking site for

STAT1. STAT1 is then activated by phosphorylation and translocates to the nucleus where it stimulates transcription of STAT1 target genes.

Another mechanism for IFN- $\gamma$  to activate macrophages is by enhancing macrophage responsiveness to other inflammatory stimuli, such as TLR ligands and TNF. This mechanism is known by the name 'priming'. By this 'priming' of IFN- $\gamma$ , the TLR-dependant expression of several proinflammatory cytokines and chemokines is greatly augmented.<sup>44</sup> IFN- $\gamma$  suppresses IL-10 production by increasing the activity of glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ), a serine/threonine kinase that inhibits the function of AP-1 and CREB, two transcription factors critical for IL-10 expression.

**NF- $\kappa$ B: Stress sensor/inflammatory trigger.** The NF- $\kappa$ B signaling pathway has a major role in the development, maintenance and progression of most chronic diseases. NF- $\kappa$ B controls the expression of genes involved in immune inflammatory responses, acute-phase inflammatory responses, oxidative stress responses, cell adhesion, differentiation and apoptosis.<sup>45</sup> Ranjan Sen and David Baltimore first identified NF- $\kappa$ B in 1986. They found that it was bound to an enhancer element of the immunoglobulin  $\kappa$ -light chain gene in the nucleus of B cells.<sup>46</sup> It is conserved during evolution and is ubiquitous in nature, that is, present in all cell type. It belongs to the family of Rel proteins that includes c-Rel, RelA (p65), RelB, NF- $\kappa$ B1 and NF- $\kappa$ B2, all of which can form hetero- or homodimers.<sup>46</sup> NF- $\kappa$ B proteins are present in the cytoplasm in an inactive form together with inhibitory I $\kappa$ B (I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$  and I $\kappa$ B $\epsilon$ ).<sup>46</sup> NF- $\kappa$ B signaling is divided (depending on which proteins are involved) in the canonical (classical) pathway initiated by NF- $\kappa$ B1 and a non-canonical (alternative) pathway initiated by NF- $\kappa$ B2.

Whereas the classical pathway depends on the IKK complex consisting of IKK $\alpha$ , IKK $\beta$ , IKK $\gamma$  and the inhibitory subunit I $\kappa$ Bs, the alternative pathway depends on IKK $\alpha$  homodimers and the NF- $\kappa$ B-inducing kinase (NIK).<sup>47</sup> Once in the nucleus, activated NF- $\kappa$ B undergoes a series of post-translational modifications, including phosphorylation, acetylation and methylation. These modifications regulate both the strength and the duration of NF- $\kappa$ B activity.<sup>48</sup> Activated NF- $\kappa$ B binds to specific DNA sequences in target genes, which are designated as  $\kappa$ B elements, and regulates the transcription of over 500 genes involved, in, for example, immunoregulation.

### Astrocytes as Mediators of Inflammation

Astrocytes, the most abundant cells in the brain, besides having functions in metabolic support, blood-brain barrier integrity, K<sup>+</sup> buffering and regulation of synaptic levels of glutamate, have the ability to work as immunocompetent cells in the CNS.<sup>49</sup> Thus, astrocytes express major histocompatibility complex-II (MHC-II) and present antigen to T-cell lines.<sup>50</sup> In addition, astrocytes express different pattern-recognition receptors, including TLRs, and produce a wide array of chemokines and cytokines that act as immune mediators.<sup>50</sup> More important, selective inactivation of NF- $\kappa$ B in astrocytes reduced inflammation and cell death in different animal models of neurodegeneration, including spinal cord injury,<sup>51</sup> and in an animal model of MS.<sup>52</sup> Keeping this view, it

has been reported that  $\alpha$ -synuclein proteins, whose accumulation is a typical hallmark of PD, released from neuronal cells are readily endocytosed by astrocytes and trigger immune responses.<sup>53</sup> When the internalized  $\alpha$ -synuclein accumulates in astrocytes, the cells produce glial inclusions and inflammatory responses.<sup>53</sup> In AD, astroglial activation is primarily triggered by amyloid deposits in the extracellular space and AGE-modified proteins thus inducing the production of a variety of proinflammatory factors to be released from astrocytes.<sup>49</sup> Several cytokines, including IL-1 $\beta$  and IL-6, have been implicated in the induction and modulation of reactive astrogliosis and pathological inflammatory responses,<sup>50</sup> a demonstration that a crosstalk between microglia and astrocytes activation seems to contribute to the inflammatory responses in the brain. Gage *et al.*<sup>54</sup> have demonstrated in primary cell cultures that LPS-induced expression of factors such as IL-1 $\beta$  and TNF- $\alpha$  by microglia results in a paracrine activation of astrocytes, with the subsequent production of toxic mediators by astrocytes, including NO and ROS. These factors are suggested to act additively or synergistically with neurotoxic factors produced by microglia. The detailed molecular mechanisms involved in this crosstalk are emerging and some of the first important signaling pathways (e.g. nuclear receptor related-1 protein-1 (Nurr1)/REST co-repressor (CoREST<sup>54</sup>)) were described recently. Consequently, under pathological conditions, such as those seen in chronic inflammation and neurodegeneration, it has been proposed that astroglia switch from metabolic support cells to immunological cells capable of inducing inflammation through the production of a variety of proinflammatory factors.<sup>49</sup>

### Caspases, the Usual Suspects: Emerging Non-Apoptotic Role for Killer Caspases

#### Proinflammatory caspase-1 and the inflammasomes.

Caspases, a family of cysteine proteases, were discovered almost two decades ago. In 1992, two laboratories simultaneously identified IL-1 $\beta$ -converting enzyme (ICE), which is involved in the generation of active IL-1 $\beta$ . It was later referred to as caspase-1 being the first mammalian caspase characterized.<sup>55,56</sup> Caspase-1 together with caspase-11 and caspase-12 in mouse, and caspase-4 and caspase-5 in human, comprises the proinflammatory caspases.<sup>57</sup> Caspase-1 catalytic activity is tightly regulated by signal-dependent auto-activation within multi-protein complexes called 'inflammasomes' that mediate caspase-1 autocatalytic activation and the subsequent cleavage of the inactive precursors of IL-1 $\beta$  and IL-18 into bioactive cytokines.<sup>58</sup> For a review of the different inflammasomes, which are, NLR family pyrin domain containing-1 (NLRP1), NLRP3, IPAF and AIM2 (absent in melanoma-2) inflammasomes, see reference Schroder and Tschopp.<sup>58</sup> Importantly, strong associations between dysregulated inflammasome activity, by extension caspase-1 activity, and human inflammatory diseases highlight the importance of this signaling pathway in tailoring the innate immune response. Indeed, the significance of the inflammasome for the initiation of the inflammatory response during systemic

diseases has already been shown, and members of the inflammasome complex were found to be induced in acute brain injury. However, the specific pathophysiological role of the inflammasome in neurodegenerative disorders still remains to be clarified (for a review see reference Trendelenburg<sup>59</sup>). Interestingly, recently it was found that A $\beta$  fibrils can activate NALP3 inflammasomes through the lysosomal damage in mouse microglia<sup>60</sup> and proposed to contribute to AD pathology.<sup>61</sup>

**Apoptotic caspases executioners of cell death.** To date, the mammalian genome encodes 14 caspases, seven of which function in programmed cell death/apoptosis and will henceforth be termed apoptotic caspases, or simply killer caspases. During apoptosis, activated killer caspases cleave selected target proteins to execute cell death.<sup>62</sup> The apoptotic caspases comprise two distinct classes: The upstream initiator caspases, which include caspases 2, 8, 9 and 10, and the downstream effector caspases, which include caspases-3, 6 and 7. Effector caspases cleave a wide range of distinct proteins substrates in different cellular compartments and are responsible for many of the changes typical of apoptosis. They are expressed as zymogens, and are activated when cleaved by the initiator caspases. Initiators caspases are also expressed as zymogens but are activated by recruitment into large multi-protein complexes. Well-defined apoptotic caspase activating complexes include the apoptosome (activating caspase-9), the piddosome (activating caspase-2) and the death-inducing signaling complex (DISC, activating caspases 8 and 10).<sup>63</sup> Two major caspase-dependent pathways of apoptosis signaling have been described, which trigger cell death either by activation of death receptors at the cell surface (i.e. extrinsic pathway) or through the disruption of the outer mitochondrial membrane barrier function, with the simultaneous release of proapoptotic molecules from the mitochondria into the cytosol (i.e., intrinsic pathway).<sup>64</sup>

**Non-apoptotic roles for killer caspases.** Even if killer caspases can be seen as 'the usual suspect' in the death of cells, the opinion that the so-called 'apoptotic caspases'

are more than just killers is supported by several recent studies (Table 3). Miossec *et al.*,<sup>65</sup> as early as 1997, reported that T-lymphocytes upon stimulation acquire caspase-3 activation without evidence of ongoing apoptosis. Shortly thereafter, inhibition of caspase activation was reported to prevent T-cell proliferation.<sup>66</sup> Finally, *in vivo* observations corroborated these initial findings by convincingly proving evidence that the initiator caspase-8 is indispensable for T-cell activation and proliferation.<sup>67</sup> Admittedly, anucleate cell types, namely keratinocytes,<sup>68</sup> lens fiber cells<sup>69</sup> and erythroid cells,<sup>70</sup> are known to require effector caspases such as caspase-3 for the removal of their nucleus, as part of their terminal differentiation. Likewise, nucleate cells, including skeletal muscle cells,<sup>71</sup> osteoblasts,<sup>72</sup> B cells<sup>73</sup> and myelomonocytic cells,<sup>74</sup> are known to employ the effector caspase 3 or 6 for their differentiation. Furthermore, initiator caspases such as caspase-8 and caspase-9 are not always involved in cell death signaling. For example, activation of the apical caspase-9, during myoblastic differentiation, in turn leads to caspase-3 activation, which contributes to myotube formation.<sup>75</sup> Substantial evidence has been built up regarding the non-apoptotic functions of caspase-8, involving embryonic development;<sup>74</sup> NF- $\kappa$ B activation;<sup>76</sup> cell migration;<sup>77</sup> proliferation of T cells, B cells, natural killer cells<sup>76</sup> and hematopoietic progenitor cells;<sup>78</sup> and myeloid or lymphoid differentiation patterns.<sup>74</sup>

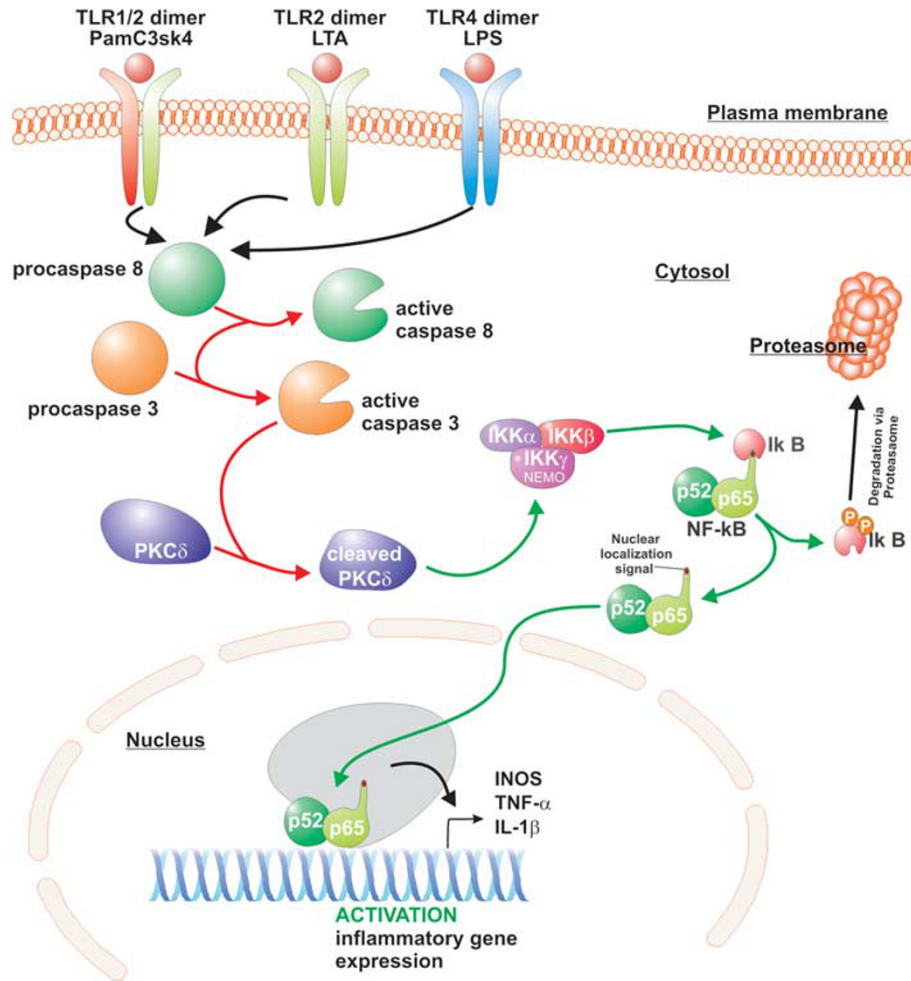
In the brain, which is the focus of this review, activation of killer caspases can occur in various cell types as part of multiple non-apoptotic, essential cell functions. Active caspase-3 has been found in the non-apoptotic, proliferating and differentiating neuronal cells of the forebrain ventricular zone and the external granular layer of the developing cerebellar cortex.<sup>79</sup> In neurons, caspases are present in the dendrites, axons and pre- and postsynaptic terminals, and there is evidence that caspases can be activated in dendrites, synaptosomes and growth cones, without inducing cell death. Several studies have implicated apoptotic caspases in the regulation of synaptic plasticity. Long-term synaptic depression and AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) receptor internalization in mouse hippocampal neurons requires caspase-9 and caspase-3/7

**Table 3** Non-apoptotic roles of apoptotic caspases

Caspases	Non-apoptotic function	References
Caspase-3	<ul style="list-style-type: none"> <li>● Upon stimulation, T-lymphocytes activate caspase-3 without evidence of apoptosis</li> <li>● Removal of nucleus in keratinocytes, lens fiber cells and erythroid cells</li> <li>● Differentiation of skeletal muscle cells, osteoblasts, B cells, myelomonocytic cells and Bergmann glia cells</li> <li>● Long-term synaptic depression and AMPA receptor internalization in hippocampal neuron</li> <li>● Cytoskeletal remodeling associated with astrogliosis</li> </ul>	65 68–70 71–75,82 80,81 83
Caspase-8	<ul style="list-style-type: none"> <li>● Embryonic development</li> <li>● T-cell activation</li> <li>● Proliferation of T cells, B cells, natural killer and hemopoietic progenitor cells</li> <li>● NF-<math>\kappa</math>B activation</li> <li>● Cell migration</li> <li>● Differentiation of myeloid and lymphoid cells</li> <li>● Microglia activation</li> </ul>	74 67,120 67,76,78,120 76 77 74 21
Caspase-9	<ul style="list-style-type: none"> <li>● Differentiation of skeletal muscle cells</li> <li>● Long-term synaptic depression and AMPA receptor internalization in hippocampal neuron</li> </ul>	75 80

Abbreviations: AMPA receptor,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; NF- $\kappa$ B, nuclear factor  $\kappa$ -light-chain enhancer of activated B cells.





**Figure 3** Apoptotic caspases control microglia activation. Activation of TLRs with lipoteichoic acid (LTA, TLR2 agonist), PamC3sk4 (synthetic lipopeptide TLR1/2 agonist) or LPS (TLR4 agonist) leads to the orderly activation of caspase-8 and caspase-3. Caspase-3 activates the NF- $\kappa$ B pathways through processing and activation of PKC $\delta$ . Nuclear accumulation of NF- $\kappa$ B leads to the transcriptional activation of inflammatory gene expression

activity.<sup>80</sup> Caspase-3 cleaves and activates calcineurin, which in turn triggers the dephosphorylation and removal of the GluR1 subunit of the AMPA-type receptor from postsynaptic sites. This caspase-3-dependent mechanism is suggested to promote early synaptic dysfunction in a mouse model of AD at the onset of memory decline.<sup>81</sup> Activation of caspase-3 in the postnatal cerebellum has also been linked to Bergmann glia differentiation.<sup>82</sup> In astroglia, activation of this enzyme, in response to excitotoxic brain damage, is suggested to have role in the cytoskeletal remodeling associated with astrogliosis.<sup>83</sup>

### License to Kill: Caspases Control Microglia Activation

We recently described an unexpected novel function for caspases in the control of microglia activation and thereby neurotoxicity<sup>21</sup> (Figure 3). We showed that orderly activation of caspase-8 and caspase-3 regulates microglia activation through a protein kinase-C $\delta$  (PKC $\delta$ )-dependent pathway. Activation of microglia and inflammation-mediated neurotoxicity are believed to have important roles in the pathogenesis of

several neurodegenerative disorders. In our study, we found that stimulation of microglia with various inflammogens activates caspase-8 and caspase-3/7 in absence of cell death *in vitro* and *in vivo*. Knockdown or chemical inhibition of these caspases hindered microglia activation and importantly reduced neurotoxicity. We also observed that these caspases are activated in microglia in the SN in PD subjects and in the frontal cortex of AD subjects. This novel view of the molecular mechanisms underlying the activation of microglia opens up avenues for new pharmacological interventions aimed at mitigating brain inflammation and subsequent cell death. Such novel strategies might lead to new treatments for neurological disorders where neuroinflammation has a role, such as AD, PD, amyotrophic lateral sclerosis (ALS) and MS.

### Under Arrest: Microglial Caspases are Potential Targets for Future Neuroprotective Strategies

Neuronal cell death is a major feature of many chronic neurodegenerative diseases, such as AD, PD, ALS and Huntington's disease (HD), as well as acute damage following

**Box 2****OPEN QUESTIONS:**

- It is unclear whether inhibition of caspase activation specifically in microglia contributes to the neuroprotective effects of caspase inhibitors. Could caspase inhibition be neuroprotective by targeting the microglia rather than the neurons themselves?
- It is also unclear whether the newly described caspase-dependent signaling pathway controlling microglia activation could also contribute to the beneficial effects of activated microglia.
- Could the selective inhibition of certain caspases in active microglia be a possible therapy to decrease the demise under several neuroinflammatory conditions such as Parkinson's and Alzheimer's diseases?

stroke, spinal cord injury and brain trauma. While effective neuroprotective therapies are still lacking, the most common therapeutic strategies for those brain disorders aim at rescuing the neurons. As caspase activation is considered a feature of apoptosis, synthetic caspase inhibitors have been developed both as research tools, and with the hope that they may eventually be used to prevent cell death in the clinic. Caspase inhibitors have been reported to successfully exert neuroprotective effects in a number of animal models for neurodegenerative disorders, including AD,<sup>84</sup> HD,<sup>85</sup> PD,<sup>86</sup> ALS,<sup>87</sup> as well as acute neurologic diseases, including ischemia, stroke or traumatic injury.<sup>88,89</sup> However, one should also keep in mind these neurodegenerative diseases are also characterized by reactive microgliosis,<sup>8,90</sup> and that our recent study now links apoptotic caspases to the control of microglia activation.<sup>21</sup> Presently, it is unclear whether inhibition of caspase activation specifically in microglia contributes to the neuroprotective effects of caspase inhibitors (Box 2, OPEN QUESTIONS). Therapies that will effectively target microglia activation and thereby their neurotoxicity could significantly improve the therapeutic outcome of these brain diseases. In conclusion, we propose that interfering with caspases that contribute to the activation of microglial can reduce neuronal cells loss.

**Current caspase inhibitors, opportunities and limitations.** The next question is which caspase inhibitors are available and might be suitable to target and control microglia activation? We recently reported that intra-nigral administration of fluoromethyl ketone (fmk)-derivative tetrapeptide caspase inhibitors – DEVD-fmk targeting caspase-3/7 or IETD-fmk targeting caspase-8 – hindered *in vivo* microglia activation.<sup>21</sup> The required administration mode for these caspase inhibitors certainly limits their use in a clinical setting. Furthermore, fmk metabolizes in the liver to form fluoroacetate, an extremely toxic poison.<sup>91</sup>

Quinoly-valyl-O-methylaspartyl-[-2,6-difluorophenoxy]-methylketone (Q-VD-OPh) is a newer, third-generation broad-spectrum caspase inhibitor that has potential as a therapeutic compound.<sup>92</sup> Most importantly, Q-VD-OPh crosses the blood–brain barrier and appears to be far superior to Z-VAD in many respects, including greater potency, selectivity, stability and cell permeability.<sup>92</sup> After acute treatment of mice with Q-VD-OPh, all organs were normal suggesting a lack of toxicity.<sup>92</sup> Evidence for neuroprotection included studies demonstrating efficacy of Q-VD-OPh in animal models of

AD, PD, HD, spinal cord injury and stroke.<sup>84,88,93,94</sup> It remains to be established whether Q-VD-OPh affects microglia activation in those disease models and contributes to the neuroprotective effect in that manner.

Finally, minocycline is particularly interesting in the context of this review. It is a second-generation tetracycline that effectively crosses the blood–brain barrier and shows remarkably broad neuroprotective properties. Indeed, neuroprotection by minocycline has been observed in various animal models of PD, HD, ALS, MS, cerebral ischemia, spinal cord injury and traumatic brain injury.<sup>85,95,96</sup> The precise mechanism of minocycline's neuroprotective effects remains unclear. It is known, however, that minocycline is a potent inhibitor of caspase-related apoptotic pathways.<sup>95</sup> Minocycline has been shown to block the activation and proliferation of microglia in an animal model of global brain ischemia, PD and AD.<sup>90,95</sup> Minocycline not only prevents the activation of microglia, but also the upregulation of caspase-1 and the formation of mature IL-1 $\beta$ ; the activation of NADPH-oxidase and iNOS; and the release of NO metabolites, all of which are key microglial-derived cytotoxic mediators.<sup>90</sup> Thus, minocycline is a potent inhibitor of both caspase activation and microglial activation. However, the link between both awaits further studies. Minocycline shows high bioavailability after oral administration, crosses the blood–brain barrier and has a proven track record for safety in a number of clinical trials involving humans. It is being evaluated in clinical trials in patients with HD, PD, MS and ALS, and for spinal cord injury. A phase-III trial involving minocycline for treatment of ALS was completed recently. Unfortunately, the results of the trial were negative as the patients on minocycline declined more quickly than those on placebo.<sup>97</sup> These results are disappointing and certainly will probably have negative implications for potential future clinical trials with minocycline.<sup>98</sup> In addition, a phase-II trial for PD indicated a decreased tolerability of minocycline in the treatment group.<sup>99</sup> At last, the results of a futility phase-II study on HD with minocycline indicated that further trials with minocycline in HD are not warranted.<sup>100</sup>

**Perspectives**

We have summarized the key aspects of neuroinflammation and its obvious involvement in the etiopathogenesis of different neurodegenerative diseases. The discovery that apoptotic/killer caspases regulate microglia activation and neurotoxicity will open up avenues for new therapeutic strategies, aimed at mitigating neuroinflammation in neurodegeneration. Brain inflammation is typically divided into three phases (acute, chronic and resolution), in which the microglia are largely in three different morphologies (surveying, activated and amoeboid/phagocytic). Considering the presumed dual role of microglia (both beneficial and detrimental), it will be necessary to fully characterize first the role of apoptotic caspases in each activation phase of microglia. Different animal models of neurodegeneration (acute versus chronic inflammation) should help to shed further light on this issue.

We speculate that inhibition of apoptotic/killer caspases might be a viable approach to therapeutic intervention. As mentioned earlier, however, an increasing number of studies

demonstrate that these proteases have important roles in a multitude of physiological conditions. Thus, inhibiting caspases might conceivably inhibit functions that are essential for normal neuronal activity and thereby would preclude the use of such an approach. The disappointing results of minocycline in clinical trials of different neurodegenerative diseases might be taken to suggest that it is important to develop inhibitors, which are selective for microglial caspases. Thus a major challenge will be to develop tools allowing the inhibition of specific caspases in selective cell types. Different strategies might be useful for this purpose: (i) A first approach may be suppression of caspase by targeted delivery of small interfering RNA specifically to macrophage/microglial cells,<sup>101</sup> and (ii) the development of specific non-toxic nanocarriers able to cross the blood–brain barrier and to safely release caspase inhibitors in those areas showing high microgliosis.<sup>102</sup> These are great technical challenges, but the new insights into the non-lethal roles of caspases in microglia certainly support that it could be worthwhile and indicate that the idea of inhibiting caspases as a therapy for neurodegenerative diseases is anything but dead.

### Conflict of Interest

The authors declare no conflict of interest.

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