Role of inflammation in neurodegenerative diseases

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Purpose of review

Inflammation is a self-defensive reaction aimed at eliminating or neutralizing injurious stimuli, and restoring tissue integrity. In neurodegenerative diseases inflammation occurs as a local response driven by microglia, in the absence of leucocyte infiltration. Like peripheral inflammation, neuroinflammation may become a harmful process, and it is now widely accepted that it may contribute to the pathogenesis of many central nervous system disorders, including chronic neurodegenerative diseases. This review addresses some of the most recent advances in our understanding of neuroinflammation.

Recent findings

The presence of activated microglia surrounding amyloid plaques and increased levels of complement elements, cytokines, chemokines and free radicals support the existence of a self-propagating toxic cycle and provide a rationale for anti-inflammatory approaches to prevent or delay neurodegeneration. Nonetheless, recent studies have provided evidence that chronic stimulation leads microglia to acquire an anti-inflammatory phenotype, characterized by activated morphology and induction of neuroprotective and immunoregulatory molecules. The causes and consequences of this atypical phenotype have just begun to be unravelled.

Summary

Although significant advances have been made in our knowledge of degenerative diseases, there remains controversy regarding whether neuroinflammation and microglial activation are beneficial or detrimental. Strategies aimed at both preventing and boosting microglial activation are presently under investigation, and these studies might reveal new potentially effective treatments for these neurological disorders.

Keywords

cytokine, microglia, neurodegeneration, neuroinflammation, nonsteroidal anti-inflammatory drug

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Abbreviations

α7 nAChR	α7 nicotinic acetylcholine receptor subunit
AD	Alzheimer's disease
COX	cyclo-oxygenase
IL	interleukin
LPS	lipopolysaccharide
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NO	nitric oxide
NSAID	nonsteroidal anti-inflammatory drug
PD	Parkinson's disease
TNF	tumour necrosis factor

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Introduction

Emerging evidence indicates that inflammation represents a potential pathogenetic factor in many central nervous system diseases, including chronic neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and Creutzfeldt–Jakob disease. In these diseases, inflammation is atypical [1] and occurs in the absence of overt leucocyte infiltration. The major players are the resident cellular elements. Microglia, the macrophages of brain parenchyma, are central to the inflammatory response. In healthy normal brain microglia are present in a down-regulated state as compared with other tissue macrophages, but subtle microenvironmental alterations can induce microglia rapidly to react, change morphology and acquire an array of functions, including phagocytosis and secretion of inflammatory mediators [2••]. In addition to microglia, reactive astrocytes contribute to the process by limiting the area of lesions and releasing local mediators. This localized process, clearly distinct from inflammation of peripheral tissues, is often referred to as 'neuroinflammation'.

Like peripheral inflammation, neuroinflammation is a two-edged sword and it must be tightly regulated because both deficient and excessive responses will result in pathological conditions. It is clear that the non-inflammatory state is the result of a process requiring the positive actions of specific gene products either to suppress or to promote reactions, as necessary for survival of the organism. Regardless of the nature of primary pathogenetic events, inflammation *per se* remains one of the main therapeutic targets and is often the best choice target in the treatment of disease.

Neuroinflammation in the pathogenesis of neurodegenerative diseases

The past decade has witnessed an explosive increase in research into neurodegenerative diseases [3••]. Converging lines of evidence revealed that aggregation of

misfolded proteins, leading to progressive amyloidosis, is a common feature of these diseases. Although the primary causes of protein misfolding are varied and disease specific, they invariably result in the conversion of highly soluble proteins into insoluble polymers, rich in β-plated sheet structures that accumulate in the central nervous system in disease-specific and protein-specific ways. A second common feature of these diseases is neuroinflammation, defined as the presence of activated microglia, reactive astrocytes and inflammatory mediators.

AD is the most common cause of dementia in elderly persons and is one of the best characterized chronic neurodegenerative disorders. Two hallmarks of the disease are senile plaques, which are extracellular deposits of β-amyloid, and neurofibrillary tangles, which consist of intracellular aggregates of aberrantly phosphorylated *tau* protein. Activated microglia surrounding senile plaques and increased levels of elements of the complement system, cytokines, chemokines and free radicals have been observed in AD [4]. These observations resulted in the concept that neuroinflammation may drive a self-propagating toxic cycle in which several factors – protein aggregates, abnormal cellular components, injured neurones and abnormal synapses – activate microglia to release inflammatory mediators, which in turn exacerbate β-amyloid deposition and neuronal injury [5•]. Evidence for detrimental effects of neuroinflammation in other neurodegenerative diseases of ageing has been reported [6].

Among the proinflammatory molecules found in association with plaques, cytokines are thought to play a central role in the self-propagation of neuroinflammation, with a prominent function for interleukin (IL)-1 [5•]. Polymorphisms in the regulatory regions of IL-1α and IL-1β, IL-6 and tumour necrosis factor (TNF)-α are associated with a higher risk for developing AD [4,6], although there are some exceptions [7]. Recently, the IL-1β + 3953 T/T polymorphism was found to be associated with delayed onset of disease but shorter cumulative survival in AD patients [8]. The −889 C/T polymorphism of the IL-1α gene, when combined with the apolipoprotein E ε_4 allele, influences the degree of microglial activation, evaluated as the percentage area of tissue occupied by ferritin-immunostained material in persons with necropsy confirmed AD [9].

Less well understood is the role played in IL-1 or other inflammatory mediators in neurodegenerative diseases with only *tau* inclusions, collectively called 'taupathologies'. Recently, Bellucci *et al.* [10•] reported increased immunoreactivity for IL-1β and cyclo-oxygenase (COX)-2, the inducible key enzyme in prostaglandin synthesis, in brains of transgenic mice bearing the mutant human P301S *tau* protein. This *tau* mutation in frontotemporal dementia and parkinsonism linked to chromosome 17 has strong functional effects and is associated with early age at disease onset [11]. In P301S transgenic mice, IL-1β and COX-2 staining was detected in neurones with hyperphosphorylated *tau* but not in microglial cells, in spite of their activated morphology [10•].

IL-1 has been implicated in the transformation of diffuse β-amyloid aggregates into β-amyloid plaques, as well as in the spread of plaques and neuronal degeneration, but many other inflammation-related mechanisms, involving generation of free radicals, secretion of soluble factors and membrane receptor-mediated processes, could exacerbate neuronal loss [12]. An unexpected chemical link between sporadic AD and inflammation has been proposed by Zhang *et al.* [13•]. Hypercholesterolaemia is a risk factor for AD, and observational studies have suggested that the statins (cholesterol-lowering drugs) decrease the incidence of disease [14]. Zhang *et al.* [13•] reported that cholesterol can be modified by ozone, which is produced during inflammation. Two of these abnormal cholesterol metabolites were detected in AD brain specimens and were shown to accelerate in-vitro β-amyloid aggregation and amyloid formation, supporting the hypothesis that such mechanisms might contribute to plaque formation *in vivo*.

Functional properties of activated microglia in neurodegenerative diseases

In spite of the large body of evidence indicating that chronic inflammation might influence the pathogenesis of degenerative diseases, there is considerable debate regarding whether microglial activation is beneficial or harmful. Several important aspects of microglia remain incompletely understood. What activates microglia and when do they become activated during disease progression? What molecules are synthesized by activated microglia, and is the secretory pattern different during the evolution of the disease?

Stimulation of cultured microglia with lipopolysaccharide (LPS) or fibrillogenic peptides triggers the synthesis of a wide array of mediators, including proinflammatory molecules [12,15]. However, the relatively short period of stimulation and the abrupt addition of activating agents bear little resemblance to the slow build-up of amyloid deposits that occurs *in vivo*. It is well known that macrophages may become refractory to inflammatory stimulation and activated to an anti-inflammatory state [16,17]. Emerging evidence indicates that the same phenomenon can occur in microglia. Persistent activation of cultured rat microglia with LPS induces significant alterations in the signalling network downstream from the LPS receptor, and progressive downregulation of TNF-α and nitric

oxide (NO) production [18]. This suggests that protracted exposure to inflammatory agents triggers significant rearrangement in the functioning of microglia. A similar process could occur in the presence of β-amyloid because β-amyloid filbrils and LPS can share receptors and intracellular signalling cascades [19•]. In addition, Ferrari *et al*. [20•] reported that chronic expression of IL-1 in rat striatum leads to neutrophil infiltration, activation of astrocytes and microglia, blood–brain barrier disruption and demyelination, but not overt neurodegeneration. The effects were reversible and were largely resolved after few weeks, although IL-1 tissue levels were still significantly elevated, suggesting that, *in vivo*, astrocytes and microglia can become refractory to chronic exposure to IL-1.

The ability of microglia to prevent or worsen neuronal damage is highly dependent on balanced cross-talk between microglia and neurones [21]. Healthy neurones control the amplitude and duration of microglial activation, and ageing-related impairment in neuronal functions are likely to contribute to the progressive activation of microglia in aged brain [5•]. The addition of apoptotic neuronal cells to microglial cultures decreases the secretion of proinflammatory cytokines and promotes the release of other molecules such as nerve growth factor, transforming growth factor-β, prostaglandin-E_2 and IL-10, suggesting that phagocytosis of apoptotic neurones specifically stimulates microglial cells to acquire an anti-inflammatory phenotype [22,23]. This process appears to be dependent on interaction between the aminophospholipid phosphatidylserine, which is externalized during the apoptotic process [24], and its receptor on microglial cells [25••]. That this receptor plays a role in controlling microglial activation is further supported by the increased number of morphologically activated microglia found in brain tissues from phosphatidylserine receptor-deficient mice [26].

Transgenic mouse models of AD are widely used to study neuroinflammation. Most of these mice develop plaques, especially when amyloid precursor protein and presenilin-1 transgenes are combined, and exhibit microglial activation [27]. However, they fail to develop neurofibrillary tangles and neuronal loss, although some neuritic atrophy and abnormal behaviour phenotypes may reflect neuronal impairment [28•,29••]. In these models, the postulated influence of damage neurones on the acquisition by microglia of an anti-inflammatory phenotype would be absent.

An anti-inflammatory profile reminiscent of that observed in microglial cells cocultured with apoptotic neurones has been observed in an animal model of prion disease [1]. In these mice, the number of microglia with activated morphology increases following the progressive spongiform degeneration, but the levels of IL-1, IL-6, TNF-α and interferon-γ remain very low throughout the various stages of disease. In contrast, transforming growth factor-β_1 and prostaglandin-E_2 are substantially increased, and microglial cells exhibit strong immunoreactivity for COX-2 but not for COX-1, the constitutive form of the enzyme [1,30••]. Morphological activation of microglial cells in the absence of proinflammatory cytokine expression was also reported in an animal model of PD – a major neurodegenerative disease characterized by progressive loss of dopamine-containing neurones in the substantia nigra [31].

Endogenous and exogenous factors that affect microglial activation and neuroinflammation

Increasing evidence indicates that there are several states of microglial activation, but what is the relation between proinflammatory and anti-inflammatory states, and what directs microglia toward a specific phenotype?

It has been demonstrated that atypical activated microglia expressing an anti-inflammatory profile can be turned into aggressive proinflammatory cells when mice with prion disease are challenged with an intraperitoneal dose of LPS to mimic a systemic infection [32]. This suggests that systemic inflammation may trigger proinflammatory functions in atypical activated microglia, which have been 'primed' by the degenerative process. According to this hypothesis, the increased levels of proinflammatory molecules in brains of persons affected by AD could be related to systemic infections, which frequently occur in these patients, thus contributing to disease progression [2••]. Systemic infection and increased serum levels of IL-1β also have an impact on cognitive decline in patients with AD [33].

On the other hand, the nervous system tightly controls peripheral inflammation through the activity of the autonomic nervous system. Inflammatory stimuli activate the sensory pathways that relay information to the hypothalamus; the inflammatory input generates 'the inflammatory reflex' and evokes anti-inflammatory responses [34]. Electrical stimulation of the vagus nerve attenuates inflammation during endotoxaemia in rats [35], and acetylcholine effectively inhibits the release of proinflammatory mediators, including TNF-α, by peripheral macrophages. This cholinergic anti-inflammatory pathway is mediated by the α7 nicotinic acetylcholine receptor subunit (α7 nAChR), which is not exclusive to neurones but is expressed in peripheral macrophages and other non-neuronal cell types [36]. Recently, the expression of α7 nAChR was reported in microglial cells purified from mouse and rat brain, supporting the existence of a brain cholinergic anti-inflammatory pathway. Acetylcholine and nicotine inhibited the release of

TNF-α and NO by LPS-activated microglial cells [37•,38•]. In contrast, nicotine treatment significantly increased COX-2 expression and prostaglandin-E_2 synthesis [38•]. Loss of α7 nAChR has been reported in AD, PD and dementia with Lewy bodies [39,40], and cholinesterase inhibitors used for symptomatic treatment of AD appear to exert additional benefit by increasing nicotinic receptor subunits, including α7 subunit [41]. In addition, administration of agonists targeting nicotinic receptors reduces β-amyloid deposition in a transgenic mouse model of AD [42•], suggesting that α7 nAChR is a potential target for controlling brain chronic inflammation.

Noradrenaline (norepinephrine) is another important neurotransmitter that may protect neurones by influencing many important processes, including neuroinflammation [42•]. Noradrenergic depletion enhances the inflammatory response in rat brain [44], and β-adrenergic agonists inhibit the release of proinflammatory molecules in cultured microglia and astrocytes [45].

Anti-inflammatory strategies

The use of anti-inflammatory drugs to treat AD was proposed in early 1990s [46]. Since then several studies have been undertaken to test this hypothesis. Retrospective epidemiological studies have shown that lengthy treatments with nonsteroidal anti-inflammatory drugs (NSAIDs) protect against AD [47,48], although recent clinical trials have not confirmed this positive effect [49,50•]. The presumed mechanism of protection by NSAIDs is inhibition of COX and especially of its inducible isoform COX-2. Several studies have shown neuronal COX-2 upregulation in AD brains and it has been suggested that this enzyme contributes to neurodegeneration. However, the involvement of COX-2 in AD remains controversial [30••], and recent clinical trials have failed to demonstrate a beneficial effect of the selective COX-2 inhibitor rofecoxib on AD progression [49,50•]. A number of experimental studies have shown that a subset of NSAIDs (indomethacin, ibuprofen, flurbiprofen and sulindac) affects β-amyloid metabolism and reduces amyloid deposition [51••]. These effects are unrelated to their COX-inhibiting activity and appear to be mediated by activation of peroxisome proliferator-activated receptor-γ [52,53], inhibition of the Ras signalling pathway [54] and interaction with presenilin-1 [55•].

Recently, chronic NSAID use was reported to decrease the risk for developing PD [56]. Conflicting results on the ability of various NSAIDs to reduce neurodegeneration in cellular and animal models of PD argue against the hypothesis that the protective effects are based on the shared COX-inhibiting property [57••]. Free radical scavenging activity or peroxisome proliferator-activated receptor-γ activation by some nonselective NSAIDs are likely to contribute to the protective effects [57••]. However, COX-2 was found to be induced in SN dopaminergic neurones in postmortem PD specimens, and its expression and enzymatic activity appear crucial for dopaminergic degeneration in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model [58,59•]. In dopaminergic neurones the peroxidase activity of COX-2 could generate, in a reaction mediated by H_2O_2, the oxidant species dopamine-quinone, which is implicated in the pathogenesis of PD [60]. This mechanism could explain the protective effect of rofecoxib in the MPTP model [58]. However, in a recent study [61•] rofecoxib was not protective when administered shortly after MPTP.

Given the strong evidence of cardiovascular risks of COX-2 selective inhibitors (rofecoxib, celecoxib and valdecoxib; see the report by Psaty and Furberg [62••] and references therein), and the little effects of these drugs in clinical trials [49,50•], additional prospective clinical trials with nonselective NSAIDs may be warranted.

Other agents with anti-inflammatory activities such as minocycline [63••], dapsone and clioquinol are presently being tested in clinical trials (http//clinicaltrials.gov).

Enhancing microglial activation

One alternative approach to treating neurodegenerative diseases is to stimulate the protective activities of microglia.

Despite the experimental evidence indicating that microglia can efficiently phagocytose and partly degrade amyloid fibrils, the persistence of amyloid plaques *in vivo* suggests that microglial phagocytic activity may be hampered in neurodegenerative diseases. In an elegant study, Ciesielski-Treska *et al.* [64••] showed that limited amounts of pathogenic prion protein can be internalized by microglia and processed into lysosome compartments, but this process results in severe impairment of their phagocytic activity. Thus, treatments that could stimulate or restore microglial phagocytic activity might be beneficial [65,66].

Both active vaccination and passive immunization in transgenic mouse models result in reduction in β-amyloid deposits and ameliorate cognitive deficits. Microglial activation appears instrumental in enhancing β-amyloid clearance [67]. The first vaccination trial was halted in January 2002 because two patients developed aseptic meningoencephalitis, possibly caused by brain infiltration of autoreactive T lymphocytes [68]. Nonetheless, immunotherapy in AD may still hold promise. Novel strategies aimed at reducing undesired side effects are under development. For example, development of conformation-specific antibodies could reduce the risk for

exacerbation of cerebral amyloid angiopathy [69], a deleterious consequence of β-amyloid immunization [70••].

Interestingly, some drugs that have potential benefit in the treatment of neurodegenerative diseases promote microglial activation. It was found that NCX2216, a NO-releasing derivative of the nonselective NSAID flurbiprofen [51••], reduced the cerebral amyloid load in a transgenic mouse model of AD to a greater extent than did ibuprofen. This effect was accompanied by increased activation of microglia in the peri-plaque area, an effect not observed with other NSAIDs [71]. As mentioned above, the use of statins has been associated with reduced incidence of AD [14], and a member of this family of drugs is currently being tested in clinical trials (http//clinicaltrials.gov). In organotypic hippocampal slice cultures, statins have been shown to induce microglial activation and proliferation [72•]. However, the relevance of this acute model to chronic pathologies remains to be determined.

Conclusion

Despite significant advances in our understanding of degenerative diseases, the debate regarding the role played by neuroinflammation continues. A dynamic relationship between beneficial and detrimental effects of neuroinflammation is likely to exist during the progression of disease. A deeper knowledge of microglia biology and the development of in-vitro and in-vivo models that replicate the relevant features of chronic degenerative diseases will be crucial in developing long-awaited effective treatments for these neurological disorders. Other important tools will result from the identification of biomarkers that are disease or inflammation related; these will help in assessing the specific state of disease and monitoring the clinical efficacy of drug treatment.

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