Interleukin-1 and inflammatory neurodegeneration

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Abstract

Inflammation occurs rapidly in response to acute brain insults such as stroke, haemorrhage or trauma, and can be sustained for long periods of time, for example in Alzheimer’s or Parkinson’s diseases and multiple sclerosis. Experimental evidence indicates that inflammation plays a major role in neurodegeneration under these conditions, and that the cytokine IL-1 (interleukin-1) is a pivotal mediator. IL-1 is expressed rapidly in response to neuronal injury, predominantly by microglia, and elevated levels of endogenous or exogenous IL-1 markedly exacerbate injury. The naturally occurring IL-1RA (IL-1 receptor antagonist) markedly inhibits ischaemia, excitotoxic and traumatic brain injury in rodents, and has shown promise in a Phase II clinical trial in stroke patients. The mechanisms of IL-1 expression, release and action in neurodegeneration are not fully elucidated and appear multiple. Systemic IL-1 markedly enhances ischaemic brain injury via release of neutrophils into circulation, neutrophil adhesion to injured cerebrovasculature and CNS (central nervous system) invasion, and cell death via activation of matrix metalloproteinase-9. IL-1 also influences the release of toxins from glial and endothelial cells. Neuronal responses to excitotoxins and physiological factors may have an impact on neuronal survival. IL-1RA, delivered peripherally, can enter the CNS in animals and humans and has no adverse effects in stroke or subarachnoid haemorrhage patients, but shows potential benefit in acute stroke patients.

Introduction: the importance of inflammation in neurodegeneration

Inflammation is generally a beneficial response of an organism to infection but, when prolonged or inappropriate, it can be detrimental. Neuronal loss in acute (e.g. stroke and head injury) and chronic [e.g. multiple sclerosis and AD (Alzheimer’s disease)] CNS (central nervous system) diseases has been associated with inflammatory processes systemically and in the brain.

Brain inflammation is characterized by activation of microglia and astrocytes, expression of key inflammatory mediators, but limited invasion of circulating immune cells. Inflammation induces rapid expression of key inflammatory mediators – cytokines, chemokines and prostaglandins – which in turn up-regulate adhesion molecules, increase permeability of the BBB (blood–brain barrier), facilitating invasion of peripheral immune cells, induce release of potentially toxic molecules and compromise brain cells.

Cytokines are primary mediators of the inflammatory response. The most extensively studied is the pro-inflammatory cytokine IL-1 (interleukin-1), which contributes to acute neuronal loss in experimental studies and has been implicated in chronic neurodegenerative disorders [1,2].

Evidence for IL-1 involvement in neurodegeneration

Members of the IL-1 family are expressed at low or undetectable levels in healthy brain but their expression is rapidly up-regulated by various experimental brain insults including ischaemia, trauma, hypoxia and neurotoxic or inflammatory stimuli [1]. Central or peripheral administration of IL-1 dramatically increases neuronal death following acute brain injury [3–5]. Exogenous administration or overexpression of the endogenous IL-1RA (IL-1 receptor antagonist) is neuroprotective in diverse rodent models of cerebral ischaemia [6,7], excitotoxicity [4,8] and trauma [9], whereas immunoneutralization or deletion of IL-1RA markedly enhances ischaemic damage [10,11]. Deletion of genes encoding for both agonists IL-1α and IL-1β in mice reduces ischaemic brain damage by ~80% [12].

Pro-IL-1β must be cleaved by the enzyme caspase 1, to produce the active form and to allow cellular release [13]. Deletion, inhibition or inactivation of caspase 1 inhibits experimentally induced neuronal cell death [14–16].

Increasing evidence suggests that IL-1 might also be involved in chronic neurodegenerative diseases. In experimental models of AD in mice, β-amyloid-activated microglia produce IL-1, which in turn promotes production and deposition...
of neurotoxic β-amyloid peptides [17,18]. Chronic expression of IL-1 in rat brain results in extensive demyelinating lesions, mimicking multiple sclerosis, and IL-1RA slows disease progression in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis [19,20].

Therefore extensive evidence suggests that IL-1 is involved in neurodegeneration and thus poses the key question: how can we target IL-1 in CNS disease?

**Regulation of IL-1 production and bioactivity**

Early expression of IL-1 occurs predominantly in activated microglia of the brain parenchyma and perivascular macrophages, whereas later expression occurs in astrocytes, although all resident brain cells including neurons and vascular endothelial cells can express IL-1. Both IL-1α and IL-1β are synthesized as precursor proteins that lack a leader sequence. As a precursor, pro-IL-1α is fully active, whereas pro-IL-1β is inactive and needs to be cleaved to mature IL-1β by the cysteine-aspartate protease caspase 1 [13]. Mature IL-1β is the major form released from cells by an as yet unidentified mechanism, which seems to require purinergic P2X7 receptor activation and leads to caspase-1-dependent cell death [21]. Many intracellular events have been linked to IL-1β release, such as potassium efflux, influx of calcium and activation of PLC (phospholipase C) and PLA2 (phospholipase A2) [22]. However, it is still debated how leaderless IL-1β exits the cell [23].

The biological actions of IL-1α and IL-1β are exerted by the membrane-bound IL-1RI (IL-1 receptor I), which then associates with the IL-1RACP (IL-1R accessory protein) and activates intracellular signalling pathways including the NF-kB (nuclear factor kB) and the MAPKs (mitogen-activated protein kinases) [24]. IL-1RI signals through a conserved intracellular region termed the TIR (Toll/interleukin-1 receptor) domain, which recruits adaptors such as MyD88 (myeloid differentiation factor 88) and TRAF-6 [TNF (tumour necrosis factor)-receptor-associated factor-6]. IL-1 bioactivity is also spatially and temporally controlled by the highly selective IL-1RI antagonist, IL-1RA, and the IL-1RII decoy receptor, which sequesters both IL-1R ligands and the IL-1RACP from the active signalling complex.

IL-1RI gene deletion abolishes all classical pro-inflammatory effects of IL-1, such as activation of MAPKs and NF-kB and subsequent induction of the major IL-1 response genes IL-6, TNFα and PGE2 (prostaglandin E2) [25]. IL-1RI-null mice fail to exhibit normal inflammatory and host-defence responses to IL-1 [26], indicating that these actions of IL-1 depend on IL-1RI, although IL-1β exacerbates damage to the same extent in the IL-1RII-null and wild-type mice [27]. In mixed glial cultures isolated from IL-1RII-null mice, IL-1 affects the expression of a substantial number of genes independently of the classical NF-kB and MAPK pathways, and many of these genes are regulated by IL-1 in the IL-1RACP- and in the MyD88-null mice [28]. This argues against IL-1RI being the sole functional receptor for IL-1, and suggests that the mechanism(s) by which IL-1 contributes to brain injury may be distinct or at least additional to its classical pro-inflammatory function.

**Mechanisms of IL-1 action: cellular actions**

IL-1 injected into a healthy brain does not cause any overt damage. Likewise, IL-1 added directly to pure neurons in culture does not cause death, although it displays several neuronal actions, including depolarization or hyperpolarization, increases in spike frequency, changes in ionic conductance and intracellular calcium concentrations [29–31]. Most IL-1 effects have been described in astrocytes. IL-1 promotes astrocyte proliferation and activation, which leads to astrogliosis [32,33], a typical reaction to brain injury. Microarray analyses confirm the data of numerous studies reporting that IL-1 up-regulates a vast array of genes in astrocytes, which encode neurotoxic mediators including chemokines, MMPs (matrix metalloproteinases), pro-inflammatory cytokines such as IL-6 and TNFα, but also survival-promoting factors such as NGF (nerve growth factor) [34]. IL-1 also acts on the endothelial cells of the brain vasculature to up-regulate the expression of adhesion and chemotactic molecules such as ICAM-1 (intercellular adhesion molecule-1), CCL2 (CC chemokine ligand-2) and E- and P-selectin, and even promotes BBB breakdown, events that are involved in leucocyte recruitment [35]. IL-1 actions on specific brain cells are summarized in Figure 1.

IL-1 is not toxic to pure neurons in culture and can even promote survival through enhancement of synaptic GABA (γ-aminobutyric acid)ergic inhibition or production of NGF [36,37]. However, IL-1 can act directly on neurons through an alternative signalling mechanism involving ceramide production and activation of Src kinase that phosphorylates the NMDAR [NMDA (N-methyl-D-aspartate receptor) subunit 2B, leading to enhanced calcium entry and increased vulnerability to additional insults [31]. IL-1 may also induce neuronal death indirectly by actions on other cells. IL-1 acting on the IL-1RI astrocytic receptor caused caspase-dependent neuronal apoptosis in neuron–glia co-cultures [38]. Neurotoxicity was partially inhibited by the NMDAR blocker MK-801 (also known as dizocilpine), indicating an interaction between the IL-1 system and glutamate receptors in neurons. Notably, IL-1 neurotoxicity required cell contact interactions between astrocytes and neurons, which facilitated activation of astrocytic MMP-9 by the neuronal uPA (urokinase-type plasminogen activator) [38a]. IL-1 may also indirectly, through actions on the vascular endothelium, induce neurotoxicity by promoting leucocyte recruitment [35], particularly neutrophils that damage the neurovascular unit by releasing ROS (reactive oxygen species) and MMPs (Figure 2).

In summary, most in vitro studies have demonstrated that IL-1, acting on different brain cells, induces both toxic and protective factors, and induces neuronal cell death indirectly, by actions on other cells (summarized in Figure 2).
**Figure 1** Effects of IL-1 in the different cell types involved in CNS injury

CCL2, CC chemokine ligand-2; GABA, γ-aminobutyric acid; ICAM-1, intercellular adhesion molecule-1; MIP-2, macrophage inflammatory protein-2; PGE₂, prostaglandin E₂.

**Figure 2** Suggested mechanisms by which IL-1 contributes to CNS injury

ROS, reactive oxygen species; uPA, urokinase-type plasminogen activator.
Mechanisms of IL-1 action: in vivo actions

IL-1 is established as an important mediator of experimentally induced neuronal injury, but the mechanisms by which it contributes to neuronal death in vivo have been difficult to elucidate. IL-1 is a potent pyrogen and increased body temperature exacerbates neuronal loss after experimental injury and worsens prognosis in acute stroke patients [39]. In experimental injury, however, blocking fever by cyclooxygenase inhibitors or glucocorticoids does not significantly affect ischaemic damage [40], and IL-1 injected away from the site of injury can cause fever without exacerbating excitotoxic damage [41]. In addition, a dose of IL-1RA that affords neuroprotection has no effect on core body temperature [42] and systemic administration of IL-1 may even reduce ischaemic brain temperature in rats (A. Parry-Jones, personal communication), further reinforcing the hypothesis that IL-1 mediates fever and neuronal loss via distinct mechanisms.

IL-1 may affect neuronal death via neutrophil mobilization (Figure 2), which can also be coupled with the detrimental effects of systemic inflammation on ischaemic brain damage. Systemic administration of LPS (lipopolysaccharide) or IL-1 exacerbates brain damage induced by cerebral ischaemia in mice, and IL-1RA abolishes the effects of both LPS and IL-1 [5]. IL-1 given systemically also causes elevation of circulating levels of acute-phase proteins, IL-6 and the neutrophil-selective chemokines CXCL1 (CXC chemokine ligand-1) and MIP-2 (macrophage inflammatory protein-2). Cortical infiltration of neutrophils precedes changes in ischaemic damage, and depleting neutrophils abolishes the damaging effects of systemic IL-1 [5]. Clinical studies have also highlighted the correlation between systemic inflammatory mediators and poor prognosis in stroke patients, and peripheral infection is considered a major risk factor for complication after stroke [43].

Clinical relevance

There is extensive evidence implicating inflammation in general in CNS disease. The expression of multiple inflammatory mediators, including various cytokines, is elevated in the CSF (cerebrospinal fluid) and post-mortem brain tissue of patients that have suffered stroke, head injury as well as AD, Parkinson’s disease, multiple sclerosis, CNS infections and tumours [2]. Inflammatory cells, such as neutrophils and monocytes, also migrate into the injured or diseased brain. There are few clinical studies directly implicating IL-1 in CNS injury. Increased IL-1β mRNA in blood mononuclear cells was associated with worse neurological outcome in ischaemic stroke patients [44], and a small study in acute stroke patients showed a positive correlation of plasma IL-1β levels with poor clinical outcome at 3 months [45]. It is difficult to correlate circulating IL-1 levels with severity of disease because IL-1 expression is confined locally within the injured tissue, and only small amounts are needed to propagate an inflammatory response that will determine clinical outcome. Indeed, changes in several downstream mediators of IL-1 indirectly implicate IL-1 as a key driver of CNS inflammation in humans. For example, increased circulating levels of IL-6 and acute-phase proteins, which are downstream effectors of IL-1, have been linked to stroke severity and poor outcome in patients with ischaemic stroke [43].

Several targets have been identified for modifying the IL-1 system in disease, but the most effective blockade, at present successfully used for the treatment of rheumatoid arthritis, is IL-1RA [46]. A randomized Phase II study of IL-1RA in acute stroke patients suggested safety and some benefit, particularly for patients with cortical infarcts [47]. As IL-1RA may act both peripherally and within the CNS to affect brain injury, ongoing studies address both pharmacokinetic properties and IL-1RA transport across the BBB as important issues determining efficacy. A pilot study where IL-1RA was injected intravenously in subarachnoid haemorrhage patients showed no adverse effects and showed that it can at least cross the blood–CSF barrier with favourable kinetics [48].

Accumulating evidence implicates inflammation and particularly IL-1 in many forms of acute and chronic neurodegeneration, and most recently in epilepsy and psychiatric disorders. Chronic CNS diseases represent a greater therapeutic challenge than acute injury, and for many of them it is still not clear whether inflammation is merely an epiphenomenon or rather has a disease-promoting function. There is still much to be done in order to translate interesting findings to therapies, but undoubtedly studying the IL-1 system may not only improve our understanding of inflammatory mechanisms in neurodegeneration, but also serve as a basis for designing effective therapies.

References
