Interleukin-1 and Stroke: Biomarker, Harbinger of Damage, and Therapeutic Target

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Key Words

Interleukin-1 • IL-1Ra

Abstract

Inflammation is established as a contributor to cerebrovascular disease. Risk factors for stroke include many conditions associated with chronic or acute inflammation, and inflammatory changes in the brain after cerebrovascular events contribute to outcome in experimental studies, with growing evidence from clinical research. The brain is extremely susceptible to inflammatory challenge, but resident glia, endothelial cells and neurones can all mount a pronounced inflammatory response to infection or injury. Recent discoveries highlight the importance of peripherally-derived immune cells and inflammatory molecules in various central nervous system disorders, including stroke. The inflammatory cytokine, interleukin-1 (IL-1), plays a pivotal role in both local and systemic inflammation, and is a key driver of peripheral and central immune responses to infection or injury. Inhibition of IL-1 has beneficial effects in a variety of experimental paradigms of acute brain injury and is a promising clinical target in stroke. We propose that blockade of IL-1 could be therapeutically useful in several diseases which are

risk factors for stroke, and there is already considerable pre-clinical and clinical evidence that inhibition of IL-1 by IL-1 receptor antagonist may be valuable in the management of acute stroke.

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Introduction

Stroke is the leading cause of neurological disability worldwide and is one of the major causes of death. Despite increased use of thrombolysis (recombinant tissue plasminogen activator, rtPA) in acute ischaemic stroke, there is still a large unmet medical need since rtPA is applicable only to a limited range of patients. The absence of new treatments is not due to lack of effort; more than a hundred potential therapies have been tested in clinical trials, without success to date. Possible reasons for such poor translation were discussed by the Stroke Therapy Academic and Industry Roundtable (STAIR) in 1999 [1]. Several STAIR recommendations followed, mostly clinical. The most recent guidelines, published in 2009 [2], were set up in response to the failure of the free radical trapping agent NXY-059. This promising treatment was

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believed widely to have fulfilled all the initial STAIR criteria, yet it still failed to show efficacy in the clinic. The outcome of this was STAIR VI and several other publications highlighted the need for greater rigour in preclinical stroke studies. This has been borne out by systematic review and meta-analysis of preclinical interventions, where increased experimental rigour reveals lower efficacy.

One exception is interleukin-1 receptor antagonist (IL-1Ra), as reviewed by Banwell et al. [3], in 2009. Despite concerns about certain interpretations made in this article [4], as the quality of experiment was deemed to have increased, so did the efficacy of IL-1Ra. Therefore, IL-1 appears to be a very promising target for stroke.

IL-1 and Acute Brain Injury

IL-1 is an established mediator of inflammation and damage in central nervous system (CNS) diseases in experimental studies. The IL-1 family consists of three main ligands, the agonists IL-1 α and IL-1 β , and the endogenous antagonist, IL-1Ra. Most studies to date on CNS injury have focused on IL-1 β . Recent data have begun to highlight the importance of IL-1 α , including its role as a key mediator of sterile inflammation [5], but this relates mainly to the periphery. Therefore, unless specifically stated otherwise, the term IL-1 in this review is used to refer to IL-1 β .

Early reports showed that IL-1 is upregulated rapidly in the brain after diverse forms of experimental brain injury, which is associated with multiple inflammatory changes [6, 7]. Intracerebroventricular [8] or intraparenchymal [9] injection of IL-1 in rodents induces neutrophil infiltration, blood-brain barrier (BBB) damage, astrogliosis and neovascularisation. Blockade of IL-1 action using recombinant IL-1Ra reduces brain damage in focal cerebral ischaemia, traumatic and excitotoxic injury in rodents, identifying endogenous IL-1 as an important mediator of experimental brain damage [10]. The role of IL-1 was confirmed by many subsequent studies, using transgenic animals (knock-out mice for most IL-1 family members exist) or by blocking IL-1 production and/or actions. For example, ischaemic brain injury is markedly reduced in IL-1 α / β -deficient ($-/-$) mice [11, 12], a selective IL-1 β antibody reduces ischaemic damage after transient middle cerebral artery occlusion (MCAo) in rat [13], and caspase-1 inhibitors (which prevent IL-1 processing and release) reduce brain damage in focal cerebral is-

chaemia [14–16]. Similarly, caspase-1 inhibitors are protective in experimental models of subarachnoid haemorrhage (SAH), and prevent neurogenic pulmonary oedema after SAH [17, 18]. Inhibition of the neuronal NLRP1 inflammasome (which is involved in the processing and release of IL-1 through the activation of caspase-1) improves outcome after stroke, traumatic or spinal cord injury [19–21]. Further support for a role for IL-1 in stroke comes from the observations that IL-1 or IL-1Ra gene polymorphisms are associated with altered susceptibility to stroke, carotid atherosclerosis and intracranial haemorrhage in humans [22–27].

Mechanisms of IL-1 Action in CNS Inflammation after Stroke

The mechanisms of IL-1 actions on neuroinflammation in response to stroke are complex. In vivo and in vitro studies in animals show that all resident brain cells and invading immune cells that have been implicated in stroke can produce and/or respond to IL-1 (see [28] for review). Some of these studies suggest new mechanisms of IL-1 actions that can possibly occur independently of classical IL-1 receptors and signaling pathways, although this has to be further investigated.

IL-1 acts via its functional type 1 receptor, IL-1R1, which is expressed by all brain cells with the apparent exception of microglia [29], and recruits an accessory protein (IL-1RAcP) for signalling (fig. 1). IL-1RAcP increases the binding affinity of IL-1 to IL-1R1 [30, 31] and recruits downstream adaptor proteins such as the myeloid differentiation factor 88 (MyD88) and IL-1R-associated kinase(s) (IRAK) [32, 33]. IL-1RAcP is expressed constitutively throughout the brain by both neurones and astrocytes [34]. A recently identified isoform, IL-1RAcPb, seems to be expressed exclusively by neurones [35]. IL-1RAcPb does not mediate canonical IL-1 responses, but modulates neuronal gene expression and IL-1-induced neuroinflammatory responses [35]. In neurones, IL-1 induces fast electrophysiological and febrile responses (within minutes) in an IL-1R1- and MyD88-dependent manner, which is mediated through activation of ceramide and Src independently of gene expression [36–38]. The neuromodulatory effect of IL-1 seems to be highly dose-dependent; lower concentrations induce depolarization, and higher concentrations induce hyperpolarization and synaptic transmission inhibition [39]. IL-1 also regulates N-methyl-D-aspartic acid (NMDA) receptor phosphorylation, calcium influx and mediates excitotox-

Fig. 1. Mechanisms and blockade of IL-1 actions. IL-1 exerts multiple effects on different cell types through IL-1R1, both in the periphery and the CNS. As key mediators of inflammation, both IL-1α and IL-1β induce expression of various inflammatory cytokines, adhesion molecules and a diverse array of other inflammatory substances. In the brain in response to injury or infection, these processes lead to altered BBB permeability, glial proliferation and recruitment of resident and blood-borne inflammatory cells. Via its neuronal actions, IL-1 influences several physiologi-

cal processes such as synaptic plasticity, neuronal activity or memory formation, but can also mediate pathological actions such as excitotoxicity. In disease, IL-1-mediated inflammatory and neuronal responses can lead to neuronal injury. Interventions against IL-1 binding to its receptor (by antibody neutralization, IL-1 Trap or IL-1RA) or IL-1 signalling cascades have been shown to confer neuroprotection in multiple brain injury models. See detailed information in the text.

icity [40–42]. A second IL-1 type 2 receptor, IL-1R2, has no intracellular domain and is believed to act primarily as a soluble decoy receptor.

IL-1 is not directly toxic to healthy neurones *in vitro* or *in vivo*, but appears to cause neuronal death indirectly through actions on astrocytes and brain endothelial cells. IL-1 activates astrocytes via IL-1R1 and the classic mitogen-activated kinase (MAPK)/nuclear factor-kappa B (NF- κ B) signalling pathways [43] leading to production of neurotoxic, neuroprotective and inflammatory mediators including IL-6, tumour necrosis factor- α (TNF- α) and various chemokines that influence CNS inflammation. IL-1 also induces astrogliosis, an important cellular response for the formation of glial scarring [44]. However, IL-1-induced astrogliosis is also accompanied by increased astrocytic matrix-metalloproteinase-9 (MMP-9) activity that can induce or contribute to neuronal death [45].

The brain endothelium is a primary target of IL-1. IL-1 activates brain endothelial cells via binding to IL-1R1 and activation of MAPK/NF- κ B pathways, leading to endothelial expression of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 and release of various chemokines, including CXCL1, which in turn leads to neutrophil adhesion/infiltration [46]. Intracerebroventricular administration of IL-1 in mice induces activation of the cerebrovascular endothelium, leading to leucocyte infiltration, which is inhibited by selective deletion of IL-1R1 in endothelia [47]. IL-1 also activates the brain endothelium to cause breakdown of endothelial tight junctions, leading to loss of BBB integrity [41] that may influence neutrophil recruitment into the brain tissue and subsequent neuronal injury. IL-1 is also a potent inducer of neuronal chemokines which may influence local microglia responses and the acute neuroinflammatory response [48]. Thus, IL-1 can mediate the central inflammatory response through actions on all brain cells. However, the contribution of each cellular response will vary temporally and spatially depending on the nature/severity of the insult and underlying inflammatory status of the individual.

Role of IL-1 in Systemic Inflammatory Disease and Co-Morbidities for Stroke

In humans, several seemingly unrelated ‘auto-inflammatory’ diseases that manifest in the periphery can be effectively treated with neutralization of IL-1 β or IL-1R1

blockade. These include gout, osteoarthritis, type-2 diabetes and post-myocardial infarction heart failure [49–51]. Systemic inflammatory diseases, characterized by recurrent fevers, leucocytosis, anaemia, and elevated acute-phase proteins are linked to IL-1 activity [52]. IL-1 is an important endogenous pyrogen, and induces multiple autonomic responses including activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, release of vasopressin, and increases in blood pressure and heart rate [53–56].

Growing evidence also implicates IL-1 as an important contributor to several conditions that are common risk factors for cerebrovascular diseases (fig. 2). Major causative factors in stroke include atherosclerosis, hypertension, diabetes, obesity and infection, all known to involve chronic or acute inflammation [57, 58]. A key role for the NLRP3 inflammasome, which mediates caspase-1 activation and IL-1 β processing and release, has been confirmed recently in experimental models of obesity and atherosclerosis [59, 60]. Ingestion of a high-fat diet has multiple proinflammatory effects and is a key driver of atherosclerosis and obesity. Palmitate (a saturated fatty acid) induces activation of the NLRP3 inflammasome and impairs insulin signalling in several target tissues to reduce glucose tolerance and insulin sensitivity [61]. Similarly, IL-1 regulates multiple atherogenic processes induced by feeding high-fat diets in mice. Selective loss of IL-1 signalling reduces plaque burden and blood pressure in high-fat-fed ApoE $^{-/-}$ $\times$ IL-1R1 $^{-/-}$ (double knock out) mice [62].

Atherosclerosis and obesity are independent risk factors for stroke, but until recently it was not clear whether these chronic peripheral conditions alone may induce inflammatory changes in the brain. We have confirmed that an atherogenic diet induces microglial and vascular activation in the brain parenchyma, resulting in infiltration of leucocytes into the choroid plexus in ApoE $^{-/-}$ mice (which develop advanced atherosclerosis in response to high-fat feeding), in the absence of any experimental brain injury [63]. Neuroinflammatory changes have also been observed in aged, obese and atherosclerotic corpulent rats (which have a natural mutation of the leptin receptor). Furthermore, we have found increased inflammation in the brain by using positron emission tomography in a small group of patients at risk of stroke (multiple risk factors for stroke and chronically elevated C-reactive protein; CRP), but without any neurological symptoms or brain injury detectable by magnetic resonance imaging (MRI) [63].

Fig. 2. The role of IL-1 in systemic inflammatory diseases and stroke. Injury, infection, high-fat containing diet and other metabolic alterations can induce the expression of IL-1 α , IL-1 β and can drive inflammasome activation and IL-1 β processing/release. Non-resolving inflammation can lead to chronic inflammatory diseases, several of which – such as atherosclerosis, obesity or diabetes – involve systemic inflammatory changes and contribute to stroke. Chronic inflammatory diseases are associated with in-

duction of inflammatory mediators, vascular inflammation, activation of different leucocyte subsets and platelets in the periphery. Systemic inflammation is associated with vascular and glial activation which primes and renders the brain susceptible to a subsequent cerebrovascular event. After stroke, central and peripheral IL-1 and other proinflammatory cytokines contribute to neuronal injury and overall outcome. See detailed information in the text.

The contribution of IL-1 to an atherogenic diet-induced brain inflammation in mice is supported by a markedly reduced leucocyte infiltration, microglial and vascular activation in high-fat-fed ApoE^{-/-} × IL-1R1^{-/-} mice compared to IL-1 responsive ApoE^{-/-} animals [unpubl. data].

As well as contributing to chronic inflammatory disease that contributes to stroke risk, IL-1 may also have a key role in acute systemic inflammatory challenges, such as infection. Experimentally this is supported by data showing peripheral administration of lipopolysaccharide (LPS) or IL-1 exacerbates neuronal death and BBB breakdown in response to experimental stroke in mice, which is prevented by IL-1Ra [64]. This exacerbation of the ischaemic brain damage is prevented by neutrophil depletion or MMP-9 blockade [64, 65]. IL-1 is a potent inducer of vascular activation, and neutrophils are also highly responsive to IL-1. The systemic effects of IL-1 on increased BBB breakdown after stroke may be explained by increased neutrophil recruitment to the ischaemic hemisphere, where breakdown of tight junction proteins is promoted by neutrophil-derived MMP-9 [64, 65]. IL-1 also potentiates acute-phase responses and expression of CXC chemokines after experimental stroke in mice [64]. IL-1 produced locally by perivascular macrophages influences the cerebrovascular endothelium [66], and our recent data indicate that platelets are an important source of IL-1 α , which promotes vascular activation and neutrophil recruitment to the brain [67].

The results presented above indicate that peripherally-derived IL-1 is a key driver of several common risk factors of stroke, and also contributes directly to acute brain injury that is exacerbated by systemic inflammatory conditions.

Infection and Stroke: A Role for IL-1 and Other Proinflammatory Mediators?

Pre- or post-stroke infections are associated with poor outcome in patients [57, 58]. There are several potential mechanisms whereby infections may affect stroke outcome, such as increased circulating inflammatory factors, coagulation or microvascular injury in the brain. Infections are associated with elevated platelet activation and increased platelet-leucocyte aggregation in stroke patients [68]. IL-1 and other pro-inflammatory factors such as IL-6 and TNF- α derived from activated leukocytes, are known to promote coagulation [69–73]. In an experimen-

tal model of chronic systemic infection, increased brain damage was associated with increased platelet aggregation and microvascular injury [74]. Similarly, influenza is a significant risk factor for poor outcome in human stroke and a recent experimental study demonstrated increased ischaemic brain damage and microvascular MMP-9 expression in mice with influenza infection [75–77]. Both these experimental studies of infection identified a pro-inflammatory chemokine, CCL5 (which can be induced by IL-1 or interferon- γ), as a candidate mediator of the systemic effects of infection on brain injury [74, 77].

Patients with pneumonia develop a systemic cytokine response and show elevated circulating IL-1 concentrations [78–80], whilst sterile lung injury, pneumonia induced lung injury, or resistance against pneumococcal infection appear to be mediated by IL-1 in experimental rodent models [81–83]. Elevated circulating IL-1Ra concentrations are also associated with increased levels of infection in patients with respiratory disease [79, 84, 85] and with post-stroke infection [86]. This may indicate that increased IL-1 activity and reduced host defence against the infectious agents occur.

The examples above indicate that IL-1 and other systemic inflammatory factors may contribute to the impact of infection on ischaemic brain injury. Further research is required to determine the specific role of IL-1 crosstalk mechanisms between acute and chronic inflammatory conditions and cerebrovascular disease.

IL-1 and Stroke Induced Immunosuppression

Acute IL-1 treatment can lead to suppression of certain peripheral immune responses. Patients respond to IL-1 administration with multiple endocrine changes, including cortisol secretion [87]. Intracerebroventricular infusion of femtomolar quantities of IL-1 in rodents and primates or increases of endogenous IL-1 in the brain activates the HPA axis, increases the secretion of cortisol, and decreases cellular immune responses, NK cell activity, the response to mitogen, as well as IL-2 production of splenic and blood lymphocytes. These effects are partly dependent on sympathetic nervous system activity and the release of corticotropin-releasing hormone (CRH) [88, 89]. Corticosteroids may be important to counterbalance the systemic effects of IL-1 as adrenalectomised mice show increased anorexia and mortality in response to IL-1 [90]. Exposure to LPS (acts on Toll-like receptor 4, which shares several common signalling cascades with IL-1) induces a transient unresponsive state of cells to

subsequent LPS re-stimulation, a phenomenon known as endotoxin tolerance. This occurs via dysregulation of LPS-induced Toll-like receptor 4-MyD88 complex formation and IRAK-1 activation in monocytes [91]. Acute administration of endotoxin in various species stimulates adrenocorticotrophic hormone (ACTH) and cortisol secretion and the release of CRH and vasopressin (AVP) in the hypophysial portal blood [92]. It is not clear whether immunosuppression and infections after stroke are linked directly to altered IL-1 activity. Nevertheless, IL-1Ra reverses stroke-induced peripheral immunosuppression in patients [93] and also reduces stroke-induced elevation of cortisol in the blood [Smith and Hopkins, pers. commun.].

Current Interventions and New Approaches to Inhibit IL-1 in Inflammatory Disease

Numerous pre-clinical studies have proposed the IL-1 system as a valid therapeutic target in acute and chronic inflammatory diseases and clinical stroke. Current strategies target IL-1 expression using caspase-1/NALP3 inhibitors, release of IL-1 using Rilonacept (also known as IL-1 Trap, dimeric recombinant extracellular domain of the IL-1R1/IL-1RAcP fused to human IgG1), canakinumab and XOMA052 (human IL-1 β -specific blocking antibodies), and Anakinra (recombinant non-glycosylated human IL-1Ra) (see [94] for review).

IL-1Ra is the most advanced therapeutic approach to date, since it is in clinical use for rheumatoid arthritis and other conditions and has been studied in clinical trials of ischaemic stroke and SAH. Nevertheless, there may be a need for developing further approaches to block IL-1 effects in acute and chronic diseases. Although our pharmacokinetic studies indicated sustained IL-1Ra levels for several hours after peripheral (subcutaneous or intravenous) injection, the brain penetration of IL-1Ra may be limited and repeated administration for prolonged actions may be clinically very expensive. Furthermore, IL-1Ra does not block all actions of IL-1 on brain cells, including glia and neurones [43, 95]. In addition, IL-1-induced brain damage in IL-1R1 $^{-/-}$ mice is not blocked by central IL-1Ra administration [11], suggesting the possible expression of additional brain-specific IL-1 receptors which are not affected by IL-1Ra. A recent study found that *IL-1R1* gene expression is regulated by 7 putative novel promoters, one of which could lead to the expression of a truncated IL-1R1 which could bind IL-1 for signalling [96]. These

new insights into the regulation of IL-1R1 expression could explain earlier observations of IL-1R1-independent IL-1 actions in stroke.

A number of additional approaches to inhibiting IL-1 are in development. A small-molecular-weight inhibitor of IL-1 receptor complex (RYTVELA), designed from the IL-1RAcP sequence to block IL-1R1, has been developed [97]. Its specificity for IL-1R1, has been demonstrated [98] and its potential application in acute neuroinflammation after stroke remains to be determined. The current aim now is to fully understand the mechanism of receptor complex assembly by NMR analysis for the development of new small inhibitors. Indeed, the 3D structure of IL-1 bound to IL-1R2 and IL-1RAcP has recently been unravelled [99], but resolving the mechanism of IL-1R1 and IL-1RAcP assembly will be critical for the development of future therapeutic strategies.

IL-1Ra: Meeting the STAIR Criteria

The quality of experimental stroke studies and the interpretation of their results have been suggested as a major factor in the failure of several stroke trials [100]. The STAIR criteria address several important issues and recommends guidelines regarding preclinical neuroprotective and restorative drug development [1, 2]. In light of these guidelines, IL-1Ra seems a promising therapeutic target in stroke.

IL-1Ra has the advantages of specificity, absence of safety concerns in patients and extensive pre-clinical studies [4, 101, 102]. In a small phase 2 clinical study of stroke, IL-1Ra was safe. The study was not powered to detect efficacy, but clinical outcomes were promising [103]. IL-1Ra is well established as a treatment for rheumatoid arthritis, where it appears to be safe and well tolerated [104]. Peripheral administration (single bolus followed by infusion) of IL-1Ra is neuroprotective in rats after MCAo, and IL-1Ra plasma and CSF concentration reach therapeutic concentrations that are similar to those obtained in SAH patients treated with IL-1Ra [105]. Indeed, the pharmacokinetic properties of IL-1Ra in SAH patients is now well established [106]. IL-1Ra administered subcutaneously in rat reaches the brain tissue and is neuroprotective after MCAo [107], and intravenous administration of IL-1Ra leads to concentrations in the CSF of SAH patients that are neuroprotective in rodents [108].

These translational studies indicate that IL-1Ra can be used safely in clinical stroke settings with the prediction

of good efficacy. Apart from intravenous and subcutaneous administration (see above), the feasibility of efficient aerosol delivery of the human IL-1Ra for reduction of acute lung inflammation has been demonstrated recently in mice [109].

A recent meta-analysis of IL-1Ra in pre-clinical studies indicated an average of 38.2% reduction in infarct volume through 16 published and one unpublished data sources [3]. It was stated that no studies to date have used animals with hypertension or diabetes or tested efficacy of administration beyond 3 h [3]. We have now performed a series of experiments to investigate the effi-

cacy of IL-1Ra in co-morbid animals. IL-1Ra administered subcutaneously at reperfusion to aged corpulent rats and aged lean controls results in an approximately 50% reduction in infarct volume and BBB breakdown after transient MCAo [110]. Subcutaneous injection of IL-1Ra also leads to a 31% reduction of infarct volume in aged lean rats with 3 h delayed administration [Pardillo, unpubl. data].

In conclusion, IL-1RA appears to be a promising treatment regimen in stroke and on-going research aims to strengthen existing evidence about its therapeutic potential.

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