

The Role of Interleukin 1 in Acute Neurodegeneration and Stroke: Pathophysiological and Therapeutic Implications

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The last 5 yr have witnessed significant changes in research direction and new discoveries about the mechanisms of neurodegeneration. Earlier studies on neuronal death focused on neurons, neuronal pathways, and neurotransmitters. Now similar interest is directed toward glia and vascular cells, peptides, and inflammatory processes in the brain. This research has already revealed several potential therapeutic targets for the treatment of acute brain damage such as stroke and brain injury.

The brain fails to exhibit a classical "inflammatory response," generally characterized by early invasion of macrophages and leukocytes. Nevertheless, the brain can exhibit many of the hallmarks of inflammation when subjected to ischemia, injury, or infection. Such responses include edema, activation of resident phagocytic cells (microglia), invasion of circulating immune cells, and activation or induction of numerous inflammatory molecules including cyclooxygenase products, kinins, complement, acute phase proteins, and proinflammatory cytokines. Of the cytokines, we have identified IL-1 as a mediator of diverse forms of acute neurodegeneration. IL-1 has also been implicated in chronic neurological conditions.

IL-1

The IL-1 family currently comprises at least two known agonists, IL-1 α and IL-1 β . Most studies to date suggest that IL-1 β plays the major role in the brain and in neurodegeneration. Rapid induction of IL-1 β (mRNA transcripts within 15 min and protein within 1 h) has been reported in the brains of rodents subjected to experimental insults that lead to neuronal damage and death such as cerebral ischemia, brain trauma, or administration of excitotoxins (1). These observations are supported by clinical studies in which increased IL-1 expression has been observed in postmortem brain tissue or cerebrospinal fluid of adult patients with various neurodegenerative conditions, including stroke, brain injury, movement disorders such as Parkinson's disease, Alzheimer's disease, epilepsy, multiple sclerosis, and central nervous system (CNS)¹ infections, inflammation, and tumors (1).

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Received for publication 4 November 1997.

1. Abbreviations used in this paper: CNS, central nervous system; ICE, IL-1 β converting enzyme; IL-1ra, IL-1 receptor antagonist.

J. Clin. Invest.

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0021-9738/97/12/2648/05 \$2.00

Volume 100, Number 11, December 1997, 2648–2652

<http://www.jci.org>

IL-1 β is formed as an inactive precursor that requires cleavage by a specific enzyme, IL-1 β converting enzyme (ICE or caspase 1). ICE shares homology with *ced-3*, a death gene in the worm *Caenorhabditis elegans*, and was the first identified member of the caspase family, a group of cysteine proteases that execute apoptosis (2). ICE is also present in the brain, where apoptosis may contribute to various forms of neurodegeneration.

The third member of the IL-1 family, IL-1 receptor antagonist (IL-1ra), is probably the only current example of a naturally occurring molecule that functions solely as a selective, competitive receptor antagonist, with no significant agonist actions. IL-1ra blocks all known actions of IL-1, and has proved an invaluable experimental tool. IL-1ra is also a potentially effective therapeutic agent that appears to have no side effects or toxicity, at least to date.

IL-1 mediates diverse forms of neurodegeneration

Constitutive expression of IL-1 in the brain of normal healthy animals is extremely low. However, this cytokine plays a major role in neuroimmune interactions, and mediates diverse responses to systemic and cerebral disease and injury (3).

IL-1 itself is not toxic to healthy brain tissue or normal neurons, but does markedly enhance ischemic, excitotoxic and traumatic brain injury at low (picomolar) concentrations (4). This suggests that it may interact with other molecules released or induced by damage, or that it affects only compromised neurons.

We reported that blocking the actions of IL-1 by intracerebroventricular administration of IL-1ra reduces ischemic and excitotoxic brain damage in the rat (5), and therefore we proposed that IL-1 mediates these forms of neurodegeneration. These initially controversial findings have now been verified and extended by numerous studies (Fig. 1). IL-1ra reduces damage caused by focal or global, permanent or reversible ischemia, excitotoxic, traumatic, or inflammatory brain damage. It also inhibits detrimental effects of heat stroke and epilepsy (6).

Unusually, IL-1ra protects striatal as well as cortical tissue in focal cerebral ischemia (middle cerebral artery occlusion) (6). It also reduces edema, inhibits glial activation, and improves neurological function of the animals (7). It is effective when administered peripherally and when treatment is delayed up to 1 h after focal ischemia and at least 4 h after brain trauma (3, 4).

The findings that IL-1ra inhibits neuronal loss caused by various types of damage suggest that endogenous IL-1 acts at a late stage in the pathway of neuronal death, which is common to a range of insults.

IL-1ra blocks all known actions of IL-1 α and IL-1 β , and therefore does not distinguish the importance of either form of IL-1. However, additional studies using anti-IL-1 β criteria to neutralize IL-1 β , or inhibitors of ICE to prevent cleavage (8),

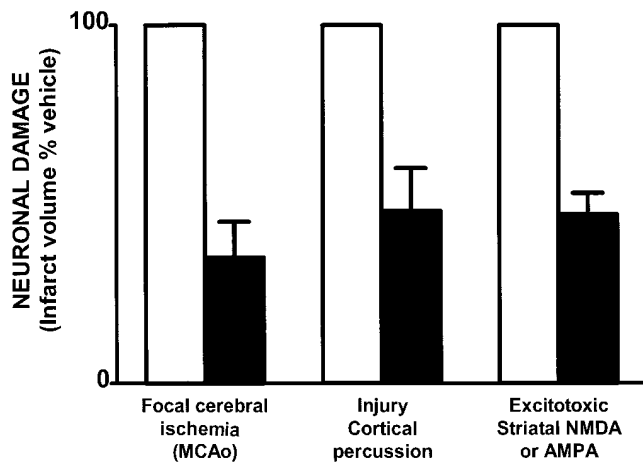


Figure 1. Summary of neuroprotective effects of recombinant IL-1ra in rodents in focal cerebral ischemic (middle cerebral artery action, IL-1ra, 10 μ g, intracerebroventricularly), lateral fluid perfusion injury (IL-1ra, 10 μ g, intracerebroventricularly), and striatal injection of NMDA or AMPA receptor agonists (IL-1ra, 5 μ g, intrastrially). Open bars, vehicle; filled bars, IL-1ra.

lend support to the notion that IL-1 β is the major player. Nevertheless, contributions of IL-1 α , and a putative third member of the family, IL-18/IL-1 γ , cannot be excluded.

IL-1, ICE, and apoptosis

ICE and related caspases are the subject of intensive research efforts because of their involvement in apoptosis. In the CNS, their biological and pathological importance and the contribution of apoptosis to neuronal death remain somewhat controversial. Numerous reports now provide evidence that apoptotic death contributes to neurodegeneration in the adult brain largely based on measurements of DNA scission. In contrast, the number of cells showing classical apoptotic morphology and ultrastructural changes (e.g., as detected by electron microscopy) is usually very low. However, the use of morphological criteria derived from studies on apoptosis in immune cells may be inappropriate for adult neurons that are postmitotic, are probably not phagocytosed rapidly, and may enter secondary necrosis. Indeed, functional data suggest that apoptotic mechanisms do operate in the brain, since several pathways and molecules associated with apoptosis are induced by brain damage. For example, caspases, members of the bcl-2 family, and p53 are upregulated in the brain in response to the insults leading to neuronal death. Overexpression of molecules that inhibit apoptosis (such as bcl-2) reduces neuronal death, and inhibition of caspase activity also inhibits neurodegeneration in vitro and in vivo. The nonselective caspase inhibitor, zVAD, reduces ischemic brain damage in the rat in a similar pattern to the protection offered by IL-1ra (8). Furthermore, mice in which the ICE (caspase 1) gene is deleted or disabled (by overexpression of a dominant negative recombinant ICE gene) also exhibit reduced ischemic damage (9). All of these findings indicate that known mechanisms and mediators of apoptosis are important in neurodegeneration.

Thus, a major question is whether there is a direct functional relationship between the actions of ICE on cleavage of pro-IL-1 β (and pro-IL-18/IL-1 γ) to release active cytokine and its role in apoptosis. Given that this enzyme is highly con-

served (for example between *C. elegans* and humans), it appears unlikely that such functions have evolved by chance and are functionally unrelated. Earlier studies suggested that cleavage of IL-1 β is not essential for apoptosis, and that IL-1 itself does not contribute to apoptotic cell death. However, recent findings question this view. Although IL-1 β does not cause apoptosis directly, it does appear to be required for apoptosis in several cell types in vitro (10).

Mechanisms of IL-1 action in neurodegeneration

At this time, the mechanisms and mediators of IL-1 actions in neurodegeneration are unknown, though several hypotheses have been proposed. Two factors have hindered studies to investigate the mechanisms of IL-1 action. First, IL-1 itself is not neurotoxic in the absence of other insults, so most studies have focused on exacerbation of damage by IL-1 or inhibition (by blocking release or actions of endogenous IL-1). Second, in vitro experiments have frequently failed to mimic observed effects of IL-1 or IL-1ra on neuronal death in vivo. In primary cultured neurons, IL-1 inhibits excitotoxic death, and this protection is reversed by blocking nerve growth factor activity (11). Unlike its effects in vivo, IL-1ra fails to protect against excitotoxicity in cultured neurons (11), although IL-1 is toxic to neurons cocultured with glia even in the absence of other challenges. Therefore, IL-1 may influence neuronal survival or death via complex actions involving glia. Further studies indicate that IL-1 can also influence neuronal survival by actions on neuronal pathways and on vascular or endothelial cells in the brain (3, 4).

We have observed highly site-specific effects of IL-1 and IL-1ra in the rat brain that can influence neuronal death at distant sites. IL-1 injected into the cerebral ventricles exacerbates ischemic brain damage, and this effect is mimicked by local injection of IL-1 into the striatum, which markedly increases both striatal and cortical damage (4). In contrast, injection of IL-1 (even at much higher doses) into the cortex has no effect on damage in either region. Similarly, IL-1ra is protective when injected into the striatum, but not the cortex of rats exposed to cerebral ischemia (4). In excitotoxic damage, IL-1ra is again protective when infused into the striatum, but not in the cortex. Coinfusion of IL-1 with S-AMPA into the striatum leads to very extensive damage in the cortex (Fig. 2), but no exacerbation is seen when IL-1 is administered directly into the cortex (12). Therefore, we have proposed that IL-1 and IL-1ra must activate receptors and mediators present in the striatum, but not the cortex, and that these can affect neuronal survival locally (i.e., the striatum) and at distant sites, (e.g., in the cortex) probably by activating specific neuronal pathways. The nature of these receptors, mediators, and pathways are as yet unknown.

Experiments based largely on in vitro approaches have identified various factors that may be induced or activated by IL-1 and could subsequently cause, or contribute to, neuronal damage. These "factors" include phospholipase A₂, cyclooxygenase, and their products, nitric oxide, superoxides, complement, adhesion molecules, β -amyloid precursor protein (β APP), corticotrophin-releasing factor, and, undoubtedly, numerous others (3, 4).

Endogenous regulators of IL-1

Important regulators of IL-1 have been identified, largely from studies on peripheral tissues or cells. Several of these are

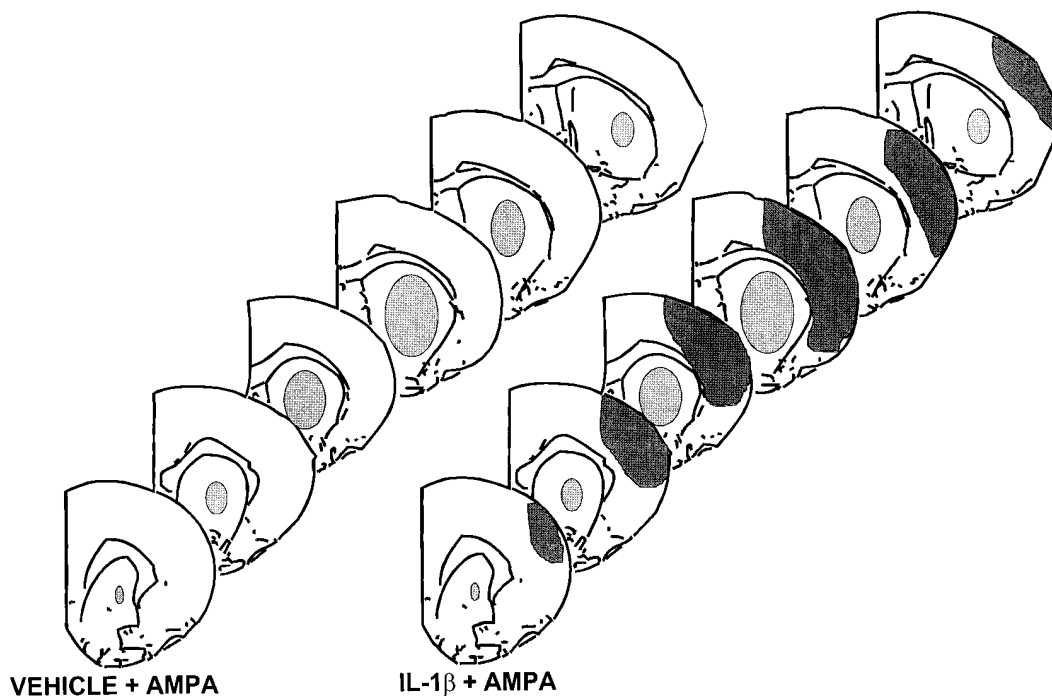


Figure 2. IL-1 exacerbates excitotoxic damage caused by S-AMPA, leading to extensive cortical neuronal loss. Cartoon depicting damage (shaded) caused by local infusion of S-AMPA (left, 7.5 nmol) into the striatum of rats. Infusion of IL-1 alone (10 ng) causes no damage (data not shown). Coinfusion of IL-1 and S-AMPA into the striatum (right) causes extensive cortical damage.

known to function in the brain. Glucocorticoids inhibit the expression and actions of IL-1 but have complex effects on neuronal survival. In contrast, lipocortin-1 (annexin-1), a mediator of glucocorticoid actions, is a potent inhibitor of ischemic and excitotoxic brain damage and is upregulated in the CNS in response to these insults (13).

Perhaps the most potent and selective inhibitor of IL-1 actions is its naturally occurring antagonist, IL-1ra. Immunoneutralization of endogenous IL-1ra exacerbates ischemic brain damage (14), suggesting that IL-1ra is a functional IL-1 antagonist that may limit neuronal death. Using an affinity-purified rabbit anti-rat IL-1ra antiserum, we have assessed (by immunohistochemistry) IL-1ra expression in rat brain after lateral fluid percussion trauma, which causes localized cortical damage. IL-1ra distribution in the brain of control (nonoperated) animals (Fig. 3 A) is comparable to that seen in the brain of sham-operated rats (Fig. 3 B). In both cases, IL-1ra protein is expressed at low level in the hippocampus and cortex. Fluid percussion trauma causes a dramatic increase in IL-1ra expression, particularly in the CA₂-CA₃ layers and dentate gyrus of the hippocampus, and in neurons in the injured cortex, in the area adjacent to the impact site (Fig. 3 C). IL-1ra expression is most evident in areas adjacent to the lesion in which neuronal death does not occur, which is consistent with the proposed endogenous protective action of this molecule. Identifying and understanding the factors which differentially regulate IL-1 and IL-1ra expression may lead to effective therapeutic targets. Antiinflammatory cytokines such as IL-4, IL-10, and TGF, which can induce IL-1ra and suppress IL-1 expression, are potential candidates, and have already been shown to be neuroprotective.

IL-1 and chronic neurodegenerative conditions

The data discussed above indicate that IL-1 contributes directly to acute, experimental neuronal death such as stroke

and brain injury. Further evidence exists to implicate IL-1 in chronic disorders. The expression of IL-1 is reportedly increased in the brains of patients with various neurological disorders including epilepsy, multiple sclerosis, Parkinson's disease, motor neuron disease, Alzheimer's disease, and Down's syndrome. In most cases, measurements have been reported in patients with advanced disease, so IL-1 expression may result from, rather than cause, neuronal damage. Nevertheless, cerebral ischemia and excitotoxicity may contribute to many of the chronic disorders noted above, and head injury is a significant risk factor for Alzheimer's disease.

IL-1ra markedly inhibits the clinical symptoms of experimental allergic encephalomyelitis (an animal model of multiple sclerosis), and also inhibits induction of β APP after acute brain damage in rodents (15). Indeed IL-1 can induce many of the responses that characterize Alzheimer's disease (e.g., expression of β APP and acute phase proteins, induction of complement, and activation of glia), thus providing further circumstantial evidence that IL-1 may contribute to the development or progression of this and related dementias (15).

Therapeutic implications

Very few treatments have shown significant benefit in neurodegenerative diseases to date. Indeed, failures of major clinical trials for new treatments in stroke and head injury are being reported with alarming regularity. There are numerous potential explanations for these failures, including insufficient preclinical data in varied animal models, the poor clinical relevance of rodent models to clinical conditions, and design faults or limitations of clinical trials. A significant issue in such trials has been the balance between safety and efficacy, and it is notable that most therapeutic interventions to date have targeted processes that are overactivated in stroke or injury, but are also important for normal brain function (e.g., modification of glutamatergic pathways or ion channel activity).

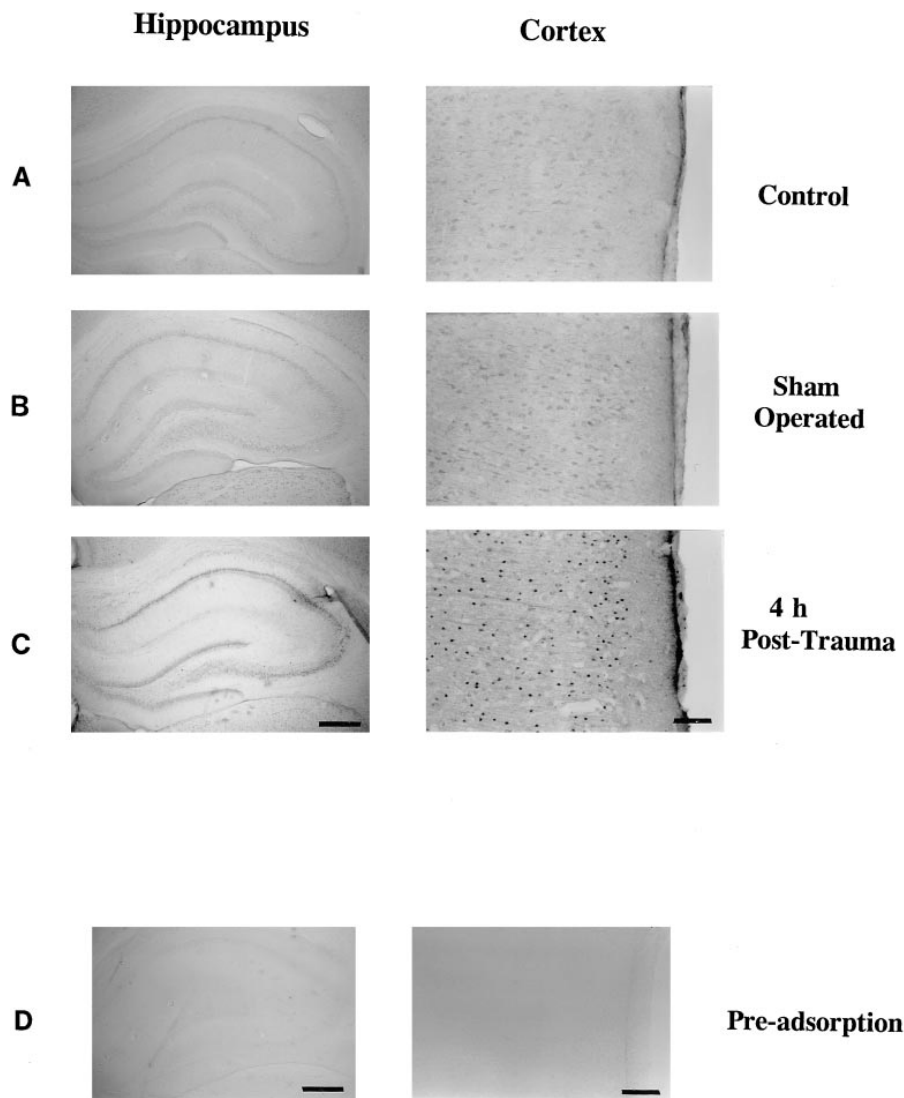


Figure 3. Expression of IL-1ra protein in normal, sham-operated, and injured rat brain. Immunocytochemistry on fixed brain sections using rabbit anti-rat IL-1ra antibody. Hippocampal and cortical neurons were faintly IL-1ra immunopositive in the brains of control (A) and sham-operated animals (B). 4 h after lateral fluid percussion trauma, intensification of IL-1ra immunostaining was visible in pyramidal cells of the hippocampus, particularly in the CA₂ and CA₃ layers, and in the cortex (C). Positive staining was abolished by preadsorption of IL-1ra observed with recombinant rat IL-1ra (D). Scale bar, 500 μ m (hippocampus), 100 μ m (cortex). Photographs representative of results observed in three rats per group in two independent experiments.

IL-1ra has shown quite remarkable protection in diverse experimental models of neurodegeneration and is effective (albeit at high doses) in rodents when administered peripherally, even sometime after the insult. Furthermore, IL-1ra appears to have few if any side effects or toxicity in animals or humans. Some of the other actions of IL-1ra, such as inhibition of fever and sickness behavior (e.g., loss of appetite) and analgesia, may also be useful in treating neurodegenerative diseases. In spite of these considerable attractions of IL-1ra, future therapeutic strategies are likely to focus in small, nonpeptide inhibitors of the synthesis, cleavage, or actions of IL-1. This goal is already the subject of intense investigation.

Acknowledgments

This work was supported by MRC, The Wellcome Trust, and the Royal Society.

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