

Commentary

Mechanisms of tumor necrosis factor α action on neurodegeneration: Interaction with insulin-like growth factor-1

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The role of cytokines and growth factors in diverse forms of neurodegeneration is now widely recognized. Several proinflammatory cytokines, notably tumor necrosis factor (TNF) α and IL-1, have been shown to mediate diverse forms of experimental neurodegeneration (1, 2), although in several cases both neurotoxic and neuroprotective actions have been reported. Similarly numerous growth factors, including insulin-like growth factor (IGF), fibroblast growth factor, transforming growth factor β , ciliary neurotrophic factor, vascular endothelial growth factor, glia-derived neurotrophic factor, brain-derived neurotrophic factor, nerve growth factor, and several neurotrophins have neuroprotective and/or neurotrophic actions *in vivo* and *in vitro* (e.g., refs. 3 and 4). The mechanisms of action of such cytokines and growth factors and their interactions between these molecules are largely unknown but are major questions in neurobiology and are of direct relevance in the search for potential treatments for neurodegenerative disease.

In this issue of the *Proceedings*, Venters *et al.* (5) describe the mechanism of interaction between TNF α and IGF-1 in the death of cerebellar granule cells *in vitro*, which may explain the observed contribution of TNF α to neurodegeneration. They first showed that cell death, induced in primary cultures of rodent cerebellar granule cells by serum withdrawal and low potassium, is markedly inhibited by addition of recombinant IGF-1 in a dose-dependent manner. This protection by IGF-1 was paralleled by an increase in activity of the enzyme phosphatidylinositol 3'-kinase (PI3-kinase), which is known to be essential for neuroprotective effects of IGF-1 (6). As shown previously (7), TNF α alone did not influence survival of cerebellar granule neurons, even at high concentrations (5). However, very low concentrations (less than 1 pM) of TNF α inhibited the protective effects of IGF-1. TNF α suppressed IGF-1-induced phosphorylation of the insulin receptor substrate-2 (IRS-2) docking molecule and IRS-2-precipitable PI3-kinase activity, thus causing cell death. Crosstalk between TNF α and IGF-1 and insulin signaling has been reported previously in the periphery (e.g., ref. 8) but not in the brain, so this work suggests that TNF α may contribute to cell death in cerebellar granule cells by inducing resistance to the protective effects of IGF-1 through blocking its signaling pathway.

The major questions arising from this work are whether the same or similar mechanisms of interaction between intracellular signaling pathways can explain cell death in other neurons in response to other insults, whether these mechanisms apply *in vivo*, and whether they are applicable to the actions of other cytokines and growth factors.

The evidence that TNF α contributes to neurodegeneration is considerable but controversial. Expression of TNF α is rapidly increased in response to experimental injury induced by cerebral ischemic, excitotoxic, and traumatic injury (see ref. 9). TNF α immunoreactivity has been reported on neurones, astrocytes, microglia, and endothelial cells, and is present within perivascular spaces (10). Although TNF α does not

cause cell death when infused into a normal rodent brain, there is considerable evidence that TNF α mediates neuronal death in an injured brain (e.g., ref. 9). Administration of *exogenous* TNF α markedly exacerbates ischemic injury *in vivo* (7) and, most importantly, acute inhibition of TNF α action, by administration of TNF soluble receptor (which prevents its action) (7, 11) or a neutralizing antibody to TNF α (e.g., refs. 7 and 12) markedly attenuates ischemic brain damage in rat and mouse.

In contrast, studies in mice lacking TNF α receptor suggest that TNF α may also have neuroprotective actions, mediated by the p55 receptor (13, 14). Ischemic and excitotoxic injury is greater in animals that lack either the p55 receptor or both TNF receptors, compared with wild-type animals or those lacking the p75 receptor (13, 14). This apparent anomaly between the studies described above, in which protection was observed when TNF α was inhibited acutely, and mice lacking TNF α receptors that show greater neuronal injury remains to be explained. The discrepancy could reflect differences between acute and chronic modification of TNF α , changes and adaptations associated with gene knockout—for example, developmental alterations and/or variations in growth factor expression, or the presence of other ligands for the TNF receptors.

IGFs are multifunctional peptides essential for normal growth and development (15). There is now considerable evidence to suggest that IGFs are also important in adulthood, particularly during neurodegenerative conditions. Injury to the brain results in increased expression of IGFs, their receptors, and binding proteins (e.g., ref. 16). This response is presumably protective, because exogenous IGF-1 protects the brain from hypoxic (e.g., ref. 17) and ischemic (e.g., ref. 18) injury *in vivo*. *In vitro*, IGF-1 protects hypothalamic, hippocampal, septal, and cerebellar granule neurones from diverse forms of injury (19–22).

Venters *et al.* (5) suggest that TNF α and IGF-1 are expressed in close proximity after insults *in vivo*, suggesting a potential relationship. However, although both peptides have been described in perivascular spaces (10, 16), no studies have determined whether they are coexpressed by the same cells. Venters *et al.* (5) report data on primary culture cerebellar granule cells. Although these cells represent a very useful experimental system of relatively pure neurones, they do not normally degenerate in clinical conditions and are slightly unusual in that they require depolarizing conditions for survival. Given that IGF-1 and TNF α are known to influence survival of other neuronal cell types *in vitro* and *in vivo*, it seems reasonable to suppose that the relationship described by Venters *et al.* (5) may also be relevant to other cell types and may occur *in vivo*, but this remains to be tested.

Abbreviations: TNF α , tumor necrosis factor α ; IGF, insulin-like growth factor.

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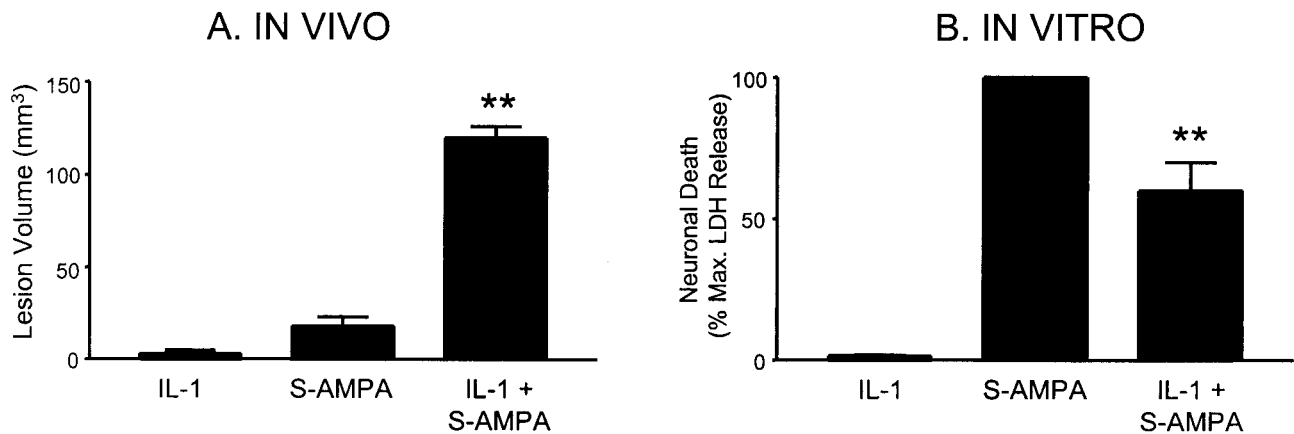


FIG. 1. Effect of IL-1 on excitotoxic damage. (A) Coinfusion of recombinant IL-1 β (10 ng) with the excitotoxin *S*- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (*S*-AMPA) markedly exacerbates brain damage (adapted from ref. 30) determined histologically 48 h later. (B) In contrast, in primary cultures of cortical rat neurones cell death (assessed by lactic dehydrogenase release) induced by *S*-AMPA is significantly inhibited by IL-1 β (500 ng/ml) (adapted from ref. 29). **, $P < 0.01$ vs. respective *S*-AMPA group.

The mechanisms described by Venters *et al.* (5) may also be relevant to the actions of other cytokines in neurodegeneration. IL-1, like TNF α , is induced by experimental and clinical brain insults and exacerbates ischemic brain damage. Inhibiting exogenous IL-1 action, by administration of IL-1 receptor antagonist, significantly limits damage induced by focal or global ischemia, excitotoxins, or traumatic brain injury in rodents (see refs. 1 and 23). Indeed, there is some evidence that IL-1 can influence expression of the IGF family (e.g., ref. 24) and inhibit IGF-1 activity, possibly by interfering with IGF-1 receptor phosphorylation (e.g., ref. 25).

The work of Venters *et al.* (5) may also help to resolve a number of major anomalies in observations on the involvement of cytokines in neurodegeneration. For example, TNF α and IL-1 clearly contribute to and enhance experimental neurodegeneration, yet even at very high doses fail to *cause* cell death in healthy brain. This failure to induce death directly may be because these molecules are *not neurotoxic per se*, but influence survival by inhibiting the protective effect of an endogenous growth factor that is produced in the injured brain. The fact that TNF α and IL-1 contribute to but do not cause cell death is an important issue because both of these and other proinflammatory cytokines are produced in the central nervous system in response to systemic insults such as infection or inflammation (26) and act as mediators of an array of host defense responses, including fever, appetite suppression, and neuroendocrine changes (27). Clearly such cytokine production does not lead to overt neurodegeneration. Interestingly, though, there is evidence that systemic infections worsen clinical neurological conditions such as stroke and multiple sclerosis. Thus, in otherwise healthy brain, cytokine production may have no deleterious effect, but when neuronal damage is present, its expression may worsen neurodegeneration.

Secondly, there are significant discrepancies between *in vitro* studies, particularly on primary neuronal cultures, and *in vivo* experiments on cytokines. There are several reports that, in contrast to animal studies, in neuronal cultures, TNF α (28) and IL-1 (29) not only fail to exacerbate injury but actually *reduce* cell death (see Fig. 1).

It is possible that these effects in cell culture reflect the fact that endogenous growth factors (many of which are produced by glia) are not present. Interestingly, IL-1 and TNF α have been reported to exacerbate neuronal death on mixed cultures of neurones and glia (31, 32). It has been assumed that this may reflect release of neurotoxins from glia, but instead it may be caused by cytokine inhibition of endogenous growth factors such as IGF-1 produced by glia in the culture, which are

inhibiting neuronal cell death. Further elucidation of the role of endogenous cytokines and growth factors and their mechanisms of interaction may require studies on mixed populations of neurones and glia. The primary source of TNF α and IL-1 after injury is microglia, but astrocytes, oligodendrocytes, neurones, and vascular endothelial cells may also produce cytokines (26), further adding to the potential complexity of this cascade of interactions.

Venters *et al.* (5) report that serum withdrawal combined with low KCl in cerebellar granule cells leads to cell death associated with features of apoptosis. There is considerable debate about the functional importance of apoptosis in neurodegeneration in the adult nervous system (33). Thus, it will be important to determine whether similar crosstalk between TNF α and IGF-1 contributes to cell death induced by stimuli that induce cell death, which appears to be largely necrotic, and whether similar mechanisms apply to other neuronal cell types.

The major questions that remain to be addressed are whether cytokine inhibition of growth factor protection is a common mechanism of neurodegeneration, and what are the downstream signaling pathways by which growth factors and cytokines modify cell death.

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