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Contribution of the ICE Family to Neurodegeneration

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ABSTRACT: The ICE family of cysteine proteases mediates necrotic or apoptotic events in the nervous system as well as in other tissues. This suggests that inhibitors may be of therapeutic value in acute and, perhaps, chronic neurodegenerative disease. In addition, some members of this family may respond to intercellular signals controling proliferation or differentiation. This possibility should be kept in mind as therapeutics are pursued.

FAMILY TIES

The available evidence is now considerable that members of the interleukin-1β-converting enzyme (ICE) family participate in at least two biological processes: one member is responsible for the maturation of a proinflammatory cytokine and others appear to represent part of the machinery for cell death.[1] Either of these two processes, when dysregulated, can lead to disease.

It has been proposed that ICE family nomenclature be rationalized by referring to these cysteine proteases collectively as caspases.[2] The "c" derives from the utilization of cysteine by this enzyme's active site, and the "aspase" derives from the cleavage of the enzyme's substrate at an aspartic acid. Until this nomenclature is widely used, we will endeavor to supply the name originally proposed as well as the caspase designation.

NECROSIS

ICE (caspase-l) is the signature member of this family. This enzyme cleaves the proinflammatory cytokine proIL-1β to its bioactive 17-kDa form. Studies on this

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cytokine point to a role for IL-1β in mediating loss of brain function in ischemia, Alzheimer's disease,[3] and traumatic brain injury. The evidence in ischemia is supported by results from animal studies showing that antagonists to the IL-lβ receptor (antibodies and an intrinsic antagonist) are neuroprotective.[4] The evidence in Alzheimer's disease and traumatic brain injury is largely circumstantial.

To increase our sense of security that targeting ICE for inhibition in stroke will prove to be neuroprotective, we have used the ICE-deficient mouse[5] to examine the effect of middle cerebral artery occulsion on the size of the infarct produced by this surgery. We have found the volume of lost tissue in mice without ICE to be about half that lost in mice in which ICE was functional (Schielke, Yang, Shivers and Betz, manuscript in preparation). Indeed, in the absence of ICE to process it (Fig.1), we have found that proIL-1β is increased threefold (Fig. 2).

REGULATION OF ICE

We and others have found that ICE is constitutively expressed in macrophages[6] and microglia. The immunostimulant LPS increases the mRNA encoding the proenzyme about twofold (Fig. 1). With our ICE antiserum to the

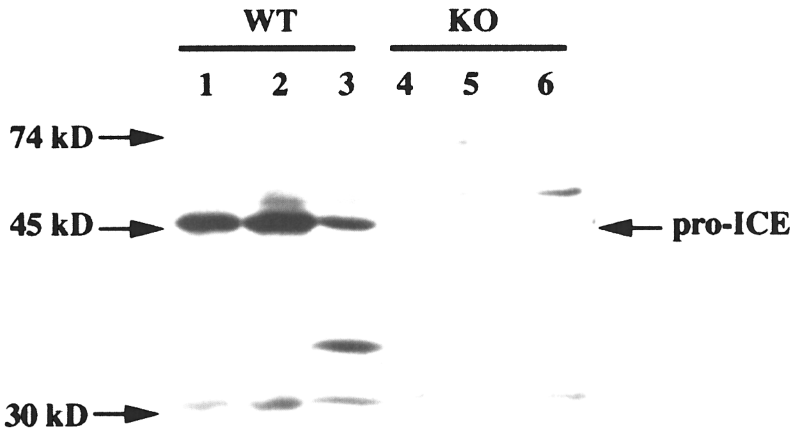

FIGURE l. Immunoblot analysis of spleens from wild-type (WT, lanes 1–3) and ICE-deficient (KO, lanes 4–6) mice. The 45-kDa, proenzyme was readily seen in the wild-type mice but was, as expected, absent in the knock-out mice spleens. A nonspecific protein of around 30 kDa was routinely observed in all tissues in both wild-type and ICE-deficient mice.

Methods: The tissue was homogenized in HNTG buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 1% Triton X-l00, 10% glycerol, 1.5 mM $MgC1_2$, 1 mM EDTA, 10 mM sodium pyrophosphate, 10 mM NaF), and the protein quantified with a BCA assay. Lysates (100 µg/lane) were electrophoresed on a 12% Tris-glycine gel before transfer onto nitrocellulose. This membrane was first incubated in a rabbit ICE antiserum made against an NH_2-terminal peptide sequence conserved in rodent and man and used at a dilution of 1:1000. The blot was then washed and probed with a secondary anti-rabbit-HRP solution diluted 1:3000. The labeled proteins were detected by ECL and autoradiography and the film quantified with a phosphorimager.

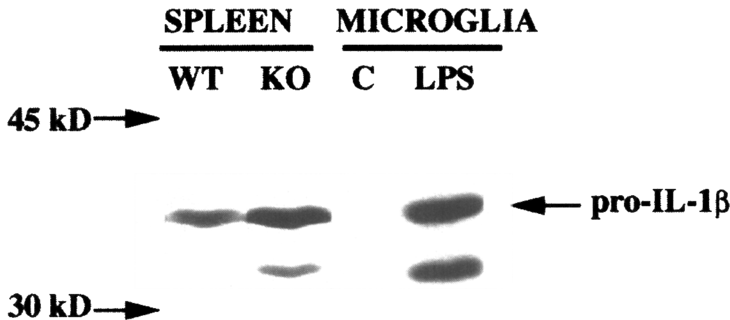

FIGURE 2. Immunoblot of ICE knock-out (KO) and wild-type (WT) mice spleen lysates as well as untreated (C) and LPS-stimulated lysates from mouse transformed microglia probed with an anti-interleukin-1β. There was a threefold increase in the unprocessed 33-kDa form of interleukin-1β in the ICE-deficient mice. ProIL-1β was easily detected in the LPS-stimulated microglia but not in the untreated cell lysates. The processed form of IL-1β (17 kDa) was not detected. A breakdown product can be seen in the KO and LPS lanes.

Methods: To treat cells, the immunostimulant LPS (4 μg/ml) was added for 4 hr to the transformed mouse microglial cell line (BV2). Proteins from spleen or cell lysates (50 μg) were separated on a 12% Tris gel, transferred to nitrocellulose, probed with mouse IL-lβ antibody (Genzyme, 10 μg/ml), and detected as described.

NH_2 terminus, which is cleaved during processing, we have been unable to observe processed (p20 or pl0) ICE. Moreover, using a fluorogenic substrate for ICE, we have been unable to demonstrate large changes in ICE activity following LPS in a transformed microglia cell line. However, LPS dramatically increases IL-β mRNA (Fig. 2) as well as bioactive IL-lβ[7] in the supernatant from these microglia (in the presence of ATP) but not in the absence of LPS (Fig. 2). This suggests that the most dramatic positive regulation is at the level of the substrate, not the enzyme.

Others have found that glucocorticoids negatively regulate this enzyme.[8] The factors regulating the formation of active ICE heterodimers remain to be elucidated. Furthermore, preliminary efforts to examine the regulation of ICH-1 (Nedd 2 or caspase-2) and CPP32 (caspase 3) suggest that LPS does not influence the mRNA content of these homologues. (Keane and Shivers, unpublished observations).

BRAIN SITE OF ICE EXPRESSION

ICE was not found by *in situ* hybridization in neurons by ourselves or others.[9] Rather, it was found in blood vessels. In a primary culture of granule neurons, neither ICE mRNA or protein was found (Lynch *et al.*[10]).

APOPTOSIS

We have previously shown that ICE inhibitors fairly specific to ICE over the other members of this family fail to block cell suicide induced by growth factor deprivation.[7] And, whereas a virally produced inhibitor of ICE, crmA, can block neuronal apoptosis, the lack of specificity of this inhibitor and our results with a peptidic, irreversible inhibitor of cysteine protease activity, boc-D-fmk, leads us to think that another homologue is responsible.[10] In any event, lack of expression of ICE in neurons suggests that neurons are not producing IL-1β. Thus, specific ICE inhibitors would not be expected to impede neuronal function directly. It is not known, however, whether supporting cell function (e.g. astrocytes) would be impaired.

Using the perinatal rat pup model of NMDA-induced neurotoxicity,[11] we have found that inhibitors of this family block the deleterious effects of NMDA injected intracerebrally (Fig. 3). The contribution of ICE compared to other family members has not yet been determined. Hence, some neuroprotection conceivably

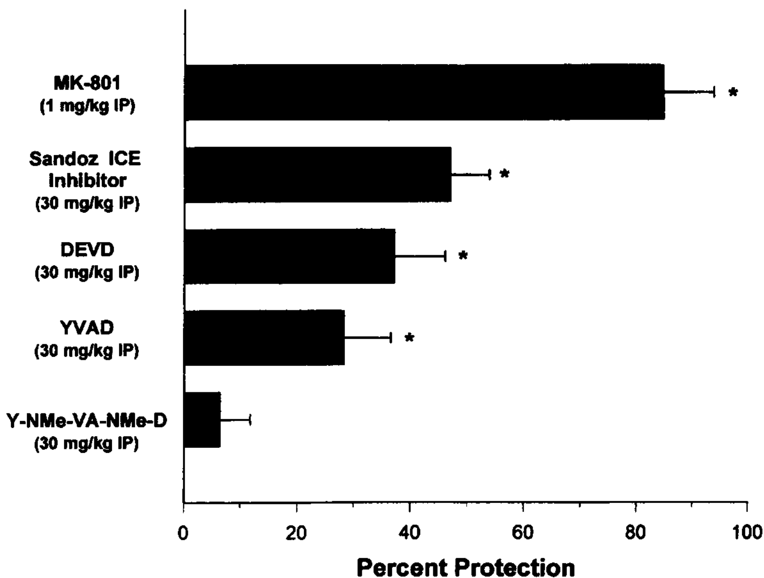

FIGURE 3. Effect of ICE family cysteine protease inhibitors on the neuroprotection achieved in the rat pup injected intracerebrally with NMDA. The best neuroprotection was seen with the NMDA receptor antagonist MK-801. Lesser, though significant, protection was observed with all three ICE family inhibitors: the irreversible ICE inhibitor from Sandoz (Z-VAD-CMK); a peptide aldehyde DEVD that inhibits CPP32 somewhat better than ICE; and YVAD-aldehyde, a reversible peptide inhibitor of ICE. No effect of a control peptide inhibitor, Y-NMe-VA-NMe-D, was measured.

Methods: Rat pups (7 days old) were anesthetized with ether and injected with NMDA (15 nmoles/0.5 μl) unilaterally into the caudate-putamen. The test compounds were injected 15 min later, i.p. The pups were housed for the next 2 hr in a temperature-controlled environment. After 5 days, each hemisphere was removed and weighed to assess brain mass as a measure of neuroprotection. $^{*}p < 0.05$ versus vehicle control.

could be achieved in this model as well as in focal ischemia[12] from blocking an apoptotic event.

PROSPECTS

The evidence from both *in vivo* and *in vitro* studies suggest that ICE inhibitors in stroke may be efficacious. Because anti-apoptotic proteins in the *bcl-2* family have proven to cause tumors and some ICE family members may regulate cell death, some caution should be given to the thought of giving cysteine protease inhibitors chronically to prevent apoptosis. Because several members of this family are expressed in neurons (CPP32 and Nedd-2) as well as in cells from other tissues, it is crucial to understand the contributions of these other homologues to acute and chronic neurodegenerative diseases. And finally, we should give serious experimental consideration to other processes in which these proteases may participate.

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