Inhibition of Apoptosome Activation Protects Injured Motor Neurons from Cell Death*

Received for publication, February 6, 2008, and in revised form, May 29, 2008 Published, JBC Papers in Press, June 10, 2008, DOI 10.1074/jbc.M800988200

Anish Kumar Kanungo‡, Zhenyue Hao‡, Andrew James Elia‡, Tak Wah Mak‡, and Jeffrey Theodore Henderson†,§

From the ‡Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario M5S 3M2, Canada and the §Campbell Family Institute for Breast Cancer Research, University of Health Network, Toronto, Ontario MSG 2C1, Canada

Within the mammalian central nervous system many forms of neurodegenerative injury are regulated via programmed cell death, a highly conserved program of cellular suicide. Programmed cell death is regulated by multiple signaling pathways, which have been identified within mammalian cells, although several lines of evidence suggest that the intrinsic pathway predominately regulates the death of motor neurons following acute injury in vivo. We have tested this hypothesis by performing axonotomies on cytochrome c knock-in mice containing a point mutation in the genomic locus of cytochrome c resulting in a lysine to alanine conversion at position 72 of the protein. The introduced mutation inhibits the ability of cytochrome c to induce the formation of the apoptosome, a protein complex that is principally required for the activation of the intrinsic pathway, but does not alter its function in oxidative phosphorylation. Homozygous cytochrome c knock-in mutants displayed a significant enhancement in motor neuron survival following injury when compared with littermate controls, thus establishing the apoptosome as a viable target for protecting motor neurons from neural injury. However, protection of facial motor neurons differs from that previously reported in mice either overexpressing anti-apoptotic or lacking pro-apoptotic members of the Bcl-2 family, which are thought to regulate several aspects of mitochondrial dys-function including the release of cytochrome c from the mitochondria to the cytoplasm. Therefore, these results directly demonstrate for the first time the influence of the apoptosome on injury-induced neuronal programmed cell death in vivo isolated from upstream Bcl-2 family-mediated effects.

Neurons within the mammalian central nervous system are exquisitely sensitive to traumatic injury (1–4). Programmed cell death (PCD)‡ is a significant contributor to a variety of central nervous system insults, including the loss of motor neurons following acute (5) or chronic injury (6–8). A substantial body of literature on the signal transduction of PCD over the past 25 years has demonstrated the central integrative role of the mitochondrial in regulating this process. Principle among these mitochondrial events is the interaction of pro-apoptotic and anti-apoptotic Bcl-2 family members (such as Bax and Bcl-2, respectively), which integrate a wide array of stimuli including the loss of trophic support, genotoxic DNA damage, excitotoxic glutamate injury, and death receptor-mediated signaling (9). The balance of these interactions dictates the activation of the intrinsic pathway through the release of cytochrome c from the mitochondria into the cytosol. Binding of holo-cytochrome c to Apaf-1 in the presence of ATP promotes Apaf-1 oligomerization into a tripartite structure known as the apoptosome (10, 11). The apoptosome acts as a signaling platform upon which procaspase-9 becomes an active dimer (12–17). This event triggers an irreversible proteolytic cascade whereby caspase-9 activates downstream executioner caspases, such as caspase-3, caspase-6, and caspase-7, which are responsible for the destructive ultra-structure changes that are distinctive of this form of cell death (18, 19).

With respect to the central nervous system, previous work has demonstrated that genomic modifications such as the loss of Bax (20) or the overexpression of Bcl-2 (21) can dramatically alter the response of central nervous system neurons to acute and chronic injury. Cytochrome c, Smac (second mitochondrial activator of caspases), and apoptosis-inducing factor (AIF) have all been shown to be released following mitochondrial outer membrane permeabilization (MOMP), and each has the ability to alter the progression of PCD (22). However, because of the embryonic lethality of mice deficient in cytochrome c, AIF, Apaf-1, or caspase-9 (23–31), the specific role of each of these factors remains to be clearly elucidated with respect to injury-induced neuronal PCD in vivo. To analyze the contributions made by the intrinsic pathway in primary motor neurons, we examined PCD in mice containing a point mutation in cytochrome c following axotomy of the facial nerve (cranial nerve VII). The introduced point mutation (lysine to alanine at position 72, denoted KA) does not inhibit oxidative phosphorylation but rather prevents the binding of cytochrome c to Apaf-1 (32), thus preventing apo-opotome formation and activation of the intrinsic pathway downstream of the mitochondria. These results thus directly identify the component of injury-induced apoptosis that is regulated through apoptosome activation alone.

**EXPERIMENTAL PROCEDURES**

Animals—cyt c [+/-], [KA/+] or [KA/CA] mice were examined on both inbred (C57BL/6j) and outbred (mixed 129 Ola and C57BL/6j) genetic backgrounds and genotyped by PCR.
as previously described (32). Although cyt c [KA/KA] mutants were recovered at a higher frequency on the outbred background, no other significant differences in the effects described herein were observed between these genetic backgrounds. The results reported were obtained from the outbred genetic background. In addition, no significant differences between cyt c [+/+ ] and [KA/+ ] mice were observed for any of the analyses performed. The number of outbred cyt c [KA/KA] animals that survived to P3.5 were recovered at less than half of the expected Mendelian frequency (11%) with approximately half of these displaying hydrocephalus and exencephaly as previously described (32); animals that did not display overt cranial defects were able to survive past P10.5 into adulthood. All of the procedures were in accordance with the Canadian Council on Animal Care and approved by the University of Toronto Faculty Advisory Committee on Animal Services.

Facial Nerve Axotomy—Axotomy of the left facial nerve was performed on P3.5 cyt c [+/+ ], [KA/+ ], and [KA/KA] mouse pups. Briefly, a 5-mm incision was made caudal to the left ear under hypothermia anesthesia, and the musculature was separated to expose the facial nerve. A 1-mm segment of the left facial nerve distal to the stylomastoid foramen was resected, and the incision was sutured closed (Ethicon, 6.0; 0.7 metric; C1). The animals were allowed to recover for a period of 24 h, 48 h, or 7 days, at which time they were sacrificed, and the brainstems were removed and fixed at 4 °C in 4% paraformaldehyde in phosphate-buffered saline. Tissue samples were collected prior to perfusion for genotyping, and the brainstems were processed for paraffin embedding.

Motor Neuron Counts—For quantification of survival, brainstems containing the facial nucleus were processed as serial sets of 7-μm paraffin sections. Following dewaxing, the sections were stained with 0.1% thionin, dehydrated, and mounted according to standard procedures. Stereologic counts were performed on every 9th section, and total motor neuron numbers were assessed by the method of physical dissector (33) (frame interval, 63 μm). Only motor neurons with intact nucleus and distinct nucleoli were counted. For quantification of motor neuron numbers within the cervical (C6-T1) and lumbar (T13-L1) spinal cord, stereologic counts were performed as described above every 11th section. A statistical analysis of variance (one-way ANOVA) was performed on the counts, and the p values <0.001 were assessed as significant.

Immunohistochemical Analysis—Cytochrome c (1:20, Promega, G7421), SM132 (1:200, Sternberg Biochemicals), islet-1 (1:1000, K5, kindly provided by Dr. Thomas Jessell, Columbia University), p75 (1:1000, kindly provided by Dr. Louis Reichardt, University of California, San Francisco), activated caspase-3 (1:200, NEB, 9661), phosphorylated c-Jun (1:200, serine 73, Upstate, 06-659), and AIF (1:200, Chemicon, AB16501) were utilized to examine cytochrome c mutant and control littermate tissues. The tissues were cryoprotected in sucrose overnight at 4 °C and then embedded in OCT the next day. 15-micron frozen sections were collected every 5th section and incubated in permeabilization solution (0.3% Triton X-100) for 30 min at room temperature with agitation. The sections were then washed three times for 5 min and blocked in 5% goat sera, 0.2% Tween 20 (in phosphate-buffered saline) for 1 h prior to overnight incubation in primary antibody at 4 °C with agitation. The sections were then incubated in secondary antibody for 2 h at room temperature with agitation, after which they were either counterstained with 4,6-diamidino-2-phenylindole (DAPI) and directly visualized on a Nikon E1000 fluorescent microscope or post-processed for diamino benzidine-based horseradish peroxidase detection (Vector Labs). For phospho-c-Jun, the sections were co-incubated with neuronal marker NeuN (1:100, Chemicon, MAB377). Secondary antibodies used were anti-mouse 488 (Molecular Probes, A11001, 1:200) anti-rabbit Alexa 488 (Molecular Probes, A11008), and anti-rabbit biotin (1:200, Vector labs, BA-1000).

Imaging and Analysis—The images were captured by SimplePCI (version 5.3, Compix Inc.) on a Nikon Eclipse E1000 fluorescent microscope equipped with a Hamamatsu C4742-95 camera for fluorescence microscopy and a Nikon DS-Fi1 color camera for brightfield analysis. The images were assembled and adjusted for brightness and contrast using Photoshop 7.0 (Adobe Systems). Statistical ANOVA was performed using GraphPad PRISM (version 3.0).

RESULTS

Analysis of Facial and Somatic Motor Neurons in cyt c [KA/KA] Mice following the Period of Developmental PCD—To first examine the role of cytochrome c-mediated apoptosis in develop-
Apoptosome Inhibition Enhances Neuronal Survival in Vivo

Analysis of Facial Motor Neurons in cyt c [KA/KA] Mice following Neonatal Facial Axotomy—To determine whether apoptosis activation is involved in regulating PCD of motor neurons following injury, motor neurons were counted in facial nuclei at 1 week post-lesion (P10.5). In comparison with control littermates, cyt c [KA/KA] mice demonstrated a considerable enhancement of motor neuron survival in the injured nucleus (Fig. 2, B and D, respectively). By 1 week post-injury nearly all cyt c [KA/+] facial motor neurons had degenerated completely (Fig. 2F), with subsequent gliosis, relative to the contralateral uninjured side (Fig. 2E). In contrast, the surviving cyt c [KA/KA] motor neurons demonstrated a relatively healthy cytostructure (Fig. 2G). The percentage of surviving facial motor neurons increased from 17.5% in cyt c [+/+] (n = 5; data not shown) and [KA/+] (n = 11) mice to 56% in cyt c [KA/KA] (n = 9) animals (summarized in Fig. 2H), indicating that impairment of apoptosome function downstream of the mitochondria leads to the protection of approximately half of those motor neurons normally fated to die following axotomy.

As shown in Fig. 3, cyt c [KA/KA] neonatal facial motor neurons were delineated by the local expression of p75 (Fig. 3A, red) and islet-1 (Fig. 3B, red). Within this neural population, the cellular distribution of cytochrome was examined 24 h following facial axotomy. In uninjured motor neurons, cytochrome exhibited a punctate distribution, consistent with a mitochondrial localization in both cyt c [KA/+] and [KA/KA] mice (Fig. 3, C and E, respectively). Following axotomy, the majority of cytochrome c was redistributed to the cell cytoplasm in lesioned motor neurons of both cyt c [KA/+] and [KA/KA] mice (Fig. 3, D and F, respectively). Furthermore, no significant difference was observed in the relative proportion of facial motor neurons displaying cytosolic cytochrome c localization 24 h post-axotomy in cyt c [KA/KA] mice in comparison with littermate controls (Fig. 3G). These data demonstrate that introduction of the lysine to alanine point mutation (at position 72) to cytochrome c does not significantly alter its release from the mitochondria following the initiation of programmed cell death.

Because the level of motor neuron rescue following apoptosis inhibition was not complete, we examined the pattern of caspase-3 activation in cyt c [KA/KA] and control (cyt c [KA/+]) mice 48 h post-axotomy. A significant elevation in caspase-3 activation was observed in the lesioned (ipsilateral) facial nuclei of cyt c [KA/+] mice at 48 h following axotomy (Fig. 4B). However, no significant increase in caspase-3 activation was seen in cyt c [KA/KA] mice following axotomy (Fig. 4D; summarized in Fig. 4E). The number of motor neurons positive for activated caspase-3 in the injured facial nucleus of cyt c [KA/KA] mice was comparable with the level in the unlesioned (contralateral) facial nuclei of cyt c [KA/+] and [KA/KA] mice (Fig. 4, A and C, respectively), which occurs as a result of developmental PCD that continues into the early postnatal period within lesioned and unlesioned facial nuclei. Thus, despite substantially reduced but still significant levels of motor neuron loss in lesioned cyt c [KA/KA] facial nuclei, caspase-3 activation does not appear to be elevated at 48 h post-injury. These data suggest that those motor neurons that do die follow-
Apoptosome Inhibition Enhances Neuronal Survival in Vivo

FIGURE 3. Release of cytochrome c at P4.5 following facial axotomy at P3.5. A, expression of p75 (red) is shown in facial motor neurons of cyt c [KA/KA] mice. The motor neurons are also stained for the pan-neuronal marker NeuN (green) and with the general nuclear stain DAPI (blue) to highlight surrounding glial cells. The scale bar denotes a distance of 30 microns. B, expression of islet-1 (red) and SMI32 (green) in facial motor neurons of cyt c [KA/KA] mice. The cells have also been stained with the nuclear stain DAPI (blue) to highlight proximal glial cells. The scale bar denotes a distance of 30 microns. C–F, staining of cyt c [KA/+] and [KA/KA] facial motor neuron using islet-1 (red) and cytochrome c (green). The scale bar denotes a distance of 30 microns. The sections shown in C and E demonstrate the pattern of cytochrome c distribution in uninjured facial motor neurons within the nonaxotomized facial nucleus from cyt c [KA/+] and [KA/KA] mice, respectively. The sections shown in D and F demonstrate the pattern of cytochrome c distribution in injured axotomized facial motor neurons at 24 h following facial nerve transection in cyt c [KA/+] and [KA/KA] mice, respectively. G, analysis of the distribution of facial motor neurons demonstrating cytosolic localization of cytochrome c as a function of injury status and genotype. The data are plotted as percentage ± S.E. (n = 3 and 3 facial nuclei for cyt c [KA/+] and [KA/KA] groups, respectively). Statistical differences between groups were analyzed through one-way ANOVA; using pairwise multiple comparison procedures. Tukey test; p < 0.001; p < 0.05. contra, contralateral facial nucleus; ipsi, ipsilateral facial nucleus.

DISCUSSION

Apoptosome Inhibition Prevents PCD in Motor Neurons following Injury but Not during Development—Early post-natal axotomy of cranial nerve VII in mice has been shown to result in a well characterized pattern of PCD in facial motor neurons because of the removal of target-derived trophic support (44, 45). Depending upon the strain of mice utilized, 80–90% of facial motor neurons die via PCD by 7 days following axotomy when performed at P2–3 (37, 46, 47). Local application of neurotrophic factors, such as glial-derived neurotrophic factor, brain-derived neurotrophic factor, and ciliary neurotrophic factor, to the resected nerve stump results in an almost complete protection of motor neurons following facial axotomy (46, 48–55). Although the therapeutic use of neurotrophic factors systemically is limited because of their extra-neural side effects
these early studies identified a mechanism whereby motor neurons could be rescued following injury. A similar level of motor neuron protection following facial axotomy was observed in mice deficient in the proapoptotic Bcl-2 family member Bax, as well as mice overexpressing anti-apoptotic Bcl-2 (86% and 95%, respectively) (20, 21, 56, 57). Although the precise mechanism remains unresolved, it is widely agreed that the interaction between proteins such as Bax and Bcl-2 regulates the release of cytochrome c from the mitochondrial intermembranous space to the cell cytoplasm (9, 22). These data, combined with knock-out studies on mice lacking Apaf-1 or caspase-9 (26), strongly implicate the apoptosome as a key regulator of neuronal PCD.

Conversion of a lysine to alanine at position 72 of cytochrome c has been previously shown to inhibit the interaction with the WD-40 repeat motifs of Apaf-1 while preserving its activity with respect to oxidative phosphorylation (32, 58). We have examined homozygous cyt c [KA/KA] mutants to directly test the role of apoptosome activation in the regulation of neuronal PCD separate from the effects of mitochondrial outer membrane permeabilization (MOMP). The results from cyt c [KA/KA] mice have therefore provided us with the first glimpse of the true role that apoptosome-mediated events play in regulating injury-induced PCD post-natally. The results demonstrate that cytochrome c-dependent apoptosome formation plays a substantial but not unitary role in regulating motor neuron PCD following facial axotomy.

During embryonic development, motor neurons are generated in excess and compete with each other for a limited supply of target-derived trophic support, with ~50% of these postmitotic neurons lost through a naturally occurring form of PCD (59, 60). Overexpression of either Bcl-2 or neurotrophic factors such as glial cell line-derived neurotrophic factor has been shown to prevent both developmental and injury-induced PCD in motor neurons in vivo (21, 61). By contrast, genetic inhibition of cytochrome c, caspase-9, or Apaf-1 (34) are each ineffective in preventing the loss of motor neurons during development. The present demonstration that the inhibition of apoptosome activation results in the protection of a substantial portion (47%) of motor neurons normally fated to die following injury reveals a critical branch point that exists with respect to developmental versus injury-induced PCD signaling in motor neurons.

Apoptosome Inhibition Enhances Neuronal Survival in Vivo

![Image of Figure 4]

**FIGURE 4. Analysis of caspase-3 activation in facial motor neurons at P5.5 following facial axotomy at P3.5.** A and B, expression of activated caspase-3 within the contralateral (A) and ipsilateral (B) facial nucleus of cyt c [KA/+] mice at P5.5 following axotomy at P3.5. C and D, expression of activated caspase-3 within the contralateral (C) and ipsilateral (D) facial nucleus of cyt c [KA/KA] mice at P5.5 following axotomy at P3.5. The scale bar represents a distance of 100 μm. The red arrowheads denote motor neurons exhibiting activated caspase-3 staining. E, stereologic counts of motor neurons positive for activated caspase-3 through the full extent of the facial nucleus were performed (interval distance, 75 μm). The data are plotted as the percentages of control ± S.E. (n = 4 and 4 for cyt c [KA/+] and [KA/KA] groups, respectively). The numbers above the columns represent the proportion of motor neurons per nucleus observed to be positive for activated caspase-3 at P5.5 for that group with respect to the ipsilateral facial nucleus of cyt c [KA/+] mice. The difference between mutant samples and their wild-type counterparts was statistically analyzed by an analysis of variance (one-way ANOVA; all pairwise multiple comparison procedures: Tukey test; p < 0.001; p < 0.05).
extrinsic pathway such as Fas and FADD (62), and mice expressing hypomorphic Fas (lpr/lpr) or a dominant negative form of FADD (FADD-DN) demonstrate a small but significant (11% and 16.5%, respectively) increase in motor neuron survival following axotomy (47). However, this is an unlikely source for the motor neuron loss seen in cyt c [KA/KA] mutants following axotomy, because Fas-dependent PCD results in an increased level of downstream caspase-3 activation (62), which is disrupted within the motor neurons of these mice following injury.

MOMP is associated with the release of other mitochondrial proteins that are thought to promote PCD, such as Smac, HtrA2/Omi, and AIF (22). Smac and HtrA2/Omi are unlikely candidates, because they act by removing the inhibition of activated caspases by IAPs (inhibitor of apoptosis proteins) (22).

AIF, a mitochondrial flavoprotein with NADH oxidase activity (63), has been shown to translocate to the nucleus upon acute PCD injury in several neuronal populations (64). However, there is considerable debate as to whether the release of AIF from the mitochondria requires the prior activation of caspases, because contradictory results have been previously reported in several cell types including mouse embryonic fibroblasts (38, 64–69). Nuclear translocation of AIF occurred shortly following injury in control cyt c [KA/+] but not homozygous mutant [KA/KA] motor neurons, indicating that AIF release requires apoptosis activation and is therefore caspase-dependent in the context of injury-induced motor neuron PCD. The blockade of AIF release seen in cyt c [KA/KA] mice removes this as a potential contributor to the caspase-independent PCD observed in these mice.
Apoptosome Inhibition Enhances Neuronal Survival in Vivo

Rather, it appears that in addition to controlling cytochrome c release, regulation of injury-induced PCD may involve other features of MOMP and/or Bcl-2 effects. As such, control of ionic gradients and calcium homeostasis both at the mitochondria and the endoplasmic reticulum may be critical for maintaining the viability of injured motor neurons (70, 71). The corresponding involvement of calcium-dependent proteases, such as calpains, may hold the key to understanding this caspase-independent feature of PCD.

REFERENCES

1. Mattson, M. P. (2000) Nat. Rev. Mol. Cell Biol. 1, 120–129
2. Blomgren, K., Leist, M., and Groc, L. (2007) Apoptosis 12, 993–1010
3. Liou, A. K., Clark, R. S., Henshall, D. C., Yin, X. M., and Chen, J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 103–142
4. Pettmann, B., and Henderson, C. E. (1998) Neuron 20, 633–647
5. Goldberg, J. L., and Barres, B. A. (2000) Annu. Rev. Neurosci. 23, 579–612
6. Guegan, C., and Przedborski, S. (2003) Annu. Rev. Neurosci. 26, 579–612
7. Acehan, D., Jiang, X., Morgan, D. G., Heuser, J. E., Wang, X., and Akey, C. W. (2002) J. Biol. Chem. 277, 6570–6577
8. Hao, Z., Duncan, G. S., Chang, C. C., Elia, A., Fang, M., Wakeham, A., Okada, H., Calzascia, T., Jang, Y., You-Ten, A., Yeh, W. C., Ohashi, P., Wang, X., and Mak, T. W. (2005) Cell 121, 579–591
9. Coggeshall, R. E., and Lekan, H. A. (1996) J. Comp. Neurol. 364, 6–15
10. Oppenheim, R. W., Flavell, R. A., Vinsant, S., Prevette, D., Kuan, C. Y., and Mak, T. W. (1996) J. Neurosci. 21, 4752–4760
11. Houde, C., Danks, K. G., Coulombe, N., Rasper, D., Grim, E., Roy, S., Simpson, E. M., and Nicholson, D. W. (2004) J. Neurosci. 24, 9977–9984
12. Davis, R. J. (2000) Cell 103, 239–252
13. Keramaris, E., Vanderluit, J. L., Bahadori, M., Mousavi, K., Davis, R. J., Flavell, R., Slack, R. S., and Park, D. S. (2005) J. Biol. Chem. 280, 1132–1141
14. Susin, S. A., Lorenzo, H. K., Zamzami, N., Marzo, I., Snow, B. E., Brothers, G., Mangan, J., Jacobot, E., Costantini, P., Loeffler, M., Larochette, N., Goodlett, D. R., Aebersold, R., Siderovski, D. P., Penninger, J. M., and Kroemer, G. (1999) Nature 397, 411–446
15. Tournois, C., Hess, P., Yang, D. D., Xu, J., Turner, T. K., Nimnual, A., Bar-Sagi, D., Jones, S. N., Flavell, R. A., and Davis, R. J. (2000) Science 288, 870–874
16. Papadakis, E. S., Finegan, K. G., Wang, X., Robinson, A. C., Guo, C., Kayahara, M., and Tournois, C. (2002) FEBS Lett. 580, 1320–1326
17. Donovan, N., Becker, E. B., Konishi, Y., and Bonni, A. (2002) J. Biol. Chem. 277, 40944–40949
18. Putcha, G. V., Le, S. F., Frank, S., Besiri, C. G., Clark, K., Chu, B., Alix, S., Youle, R. J., LaMarache, A., Maroney, A. C., and Johnson, E. M., Jr. (2003) Neurotoxicology 24, 889–914
19. Whitfield, J., Neame, S. J., Paquet, L., Bernard, O., and Ham, J. (2001) Neuro 29, 629–643
20. Snider, W. D., Elliott, J. L., and Yan, Q. (1992) J. Neurosci. 12, 1231–1246
21. Morin, L. B., and Graeber, M. B. (2004) Brain Res. Brain Res. Rev. 44, 154–178
22. Zhao, Z., Alam, S., Oppenheim, R. W., Prevette, D. M., Evenson, A., and Parsadanian, A. (2004) Exp. Neurol. 190, 356–372
23. Uoglini, G., Raoul, C., Ferri, A., Haenggeli, C., Yamamoto, Y., Saha, D., Henderson, C. E., Kato, A. C., Pettmann, B., and Hueber, A. O. (2003) J. Neurosci. 23, 8526–8531
24. Sendtner, M., Kreutzberg, G. W., and Tohen, H. (1990) Nature 345, 440–441
25. Henderson, C. E., Phillips, H. S., Pollock, R. A., Davies, A. M., Lemenelle, C., Armanini, M., Simmons, L., Moffet, B., Vanden, R. A., Koliatsos, V. E., and Rosenthal, A. (1994) Science 266, 1062–1064
26. Clatterbuck, R. E., Price, D. L., and Koliatsos, V. E. (1994) J. Comp. Neurol. 342, 45–56
27. Henderson, J. T., Mullen, B. J., and Roder, J. C. (1996) Cytokine 8, 784–793
28. Houenou, L. I., Oppenheim, R. W., Li, L., Lo, A. C., and Prevette, D. (1996) Cell Tissue Res. 286, 219–223
29. Sendtner, M., Gotz, R., Holtmann, B., and Thoenen, H. (1997) J. Neurosci. 17, 6999–7006
30. Yan, Q., Elliott, I., and Snider, W. D. (1992) Nature 360, 753–755
31. Yan, Q., Elliott, J. L., Matheson, C., Sun, J., Zhang, L., Mu, X., Rex, K. L., and Snider, W. D. (1993) J. Neurobiol. 24, 1555–1577
32. Alberi, S., Raggenbass, M., de Bilbao, F., and Dubois-Dauphin, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 3978–3983
33. de Bilbao, F., and Dubois-Dauphin, M. (1996) Neuroscience 71, 111–1119
34. Yu, T., Wang, X., Purring-Koch, C., Wei, Y., and McLendon, G. L. (2001) J. Biol. Chem. 276, 13034–13038
35. Oppenheim, R. W. (1991) Annu. Rev. Neurosci. 14, 453–501
36. Oppenheim, R. W. (1989) Trends Neurosci. 12, 252–255
37. Oppenheim, R. W., Houenou, L. I., Johnson, J. E., Lin, L. F., Li, L., Lo, A. C., Newsome, A. L., Prevette, D. M., and Wang, S. (1995) Nature 373, 344–346
38. Raoul, C., Henderson, C. E., and Pettmann, B. (1999) J. Cell Biol. 147, 1049–1062
39. Miramar, M. D., Costantini, P., Ravagnan, L., Saraiva, L. M., Haozoi, D., Brothers, G., Penninger, J. M., Pelato, M. L., Kroemer, G., and Sisin, S. A. (2001) J. Biol. Chem. 276, 16391–16398
40. Cande, C., Vahsen, N., Garrido, C., and Kroemer, G. (2004) Cell Death Differ. 11, 591–595
Apoptosome Inhibition Enhances Neuronal Survival in Vivo

65. Arnoult, D., Gaume, B., Karbowski, M., Sharpe, J. C., Cecconi, F., and Youle, R. J. (2003) *EMBO J.* **22**, 4385–4399

66. Lorenzo, H. K., Susin, S. A., Penninger, J., and Kroemer, G. (1999) *Cell Death Differ.* **6**, 516–524

67. Susin, S. A., Daugas, E., Ravagnan, L., Samejima, K., Zamzami, N., Loeffler, M., Costantini, P., Ferri, K. F., Irinopoulou, T., Prevost, M. C., Brothers, G., Mak, T. W., Penninger, J., Earnshaw, W. C., and Kroemer, G. (2000) *J. Exp. Med.* **192**, 571–580

68. Arnoult, D., Parone, P., Martinou, J. C., Antonsson, B., Estaquier, J., and Ameisen, J. C. (2002) *J. Cell Biol.* **159**, 923–929

69. Daugas, E., Susin, S. A., Zamzami, N., Ferri, K. F., Irinopoulou, T., Laroche, N., Prevost, M. C., Leber, B., Andrews, D., Penninger, J., and Kroemer, G. (2000) *FASEB J.* **14**, 729–739

70. Pinton, P., and Rizzuto, R. (2006) *Cell Death Differ.* **13**, 1409–1418

71. Morishima, N., Nakanishi, K., Takenouchi, H., Shibata, T., and Yasuhiko, Y. (2002) *J. Biol. Chem.* **277**, 34287–34294