Neurodegeneration in Lurcher Mice Occurs via Multiple Cell Death Pathways

Martin L. Doughty,1 Philip L. De Jager,1 Stanley J. Korsmeyer,3 and Nathaniel Heintz1,2

1Laboratory of Molecular Biology and 2Howard Hughes Medical Institute, The Rockefeller University, New York, New York 10021, and 3Harvard Medical School, Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, Massachusetts 02115

Lurcher (Lc) is a gain-of-function mutation in the δ2 glutamate receptor (GRID2) that results in the cell-autonomous death of cerebellar Purkinje cells in heterozygous lurcher (+/Lc) mice. This in turn triggers the massive loss of afferent granule cells during the first few postnatal weeks. Evidence suggests that the death of Purkinje cells as a direct consequence of GRID2Lc activation and the secondary death of granule cells because of target deprivation occur by apoptosis. We have used mice carrying null mutations of both the Bax and p53 genes to examine the roles of these genes in cell loss in lurcher animals. The absence of Bax delayed Purkinje cell death in response to the GRID2Lc mutation and permanently rescued the secondary death of granule cells. In contrast, the p53 deletion had no effect on either cell death pathway. Our results demonstrate that target deprivation induces a Bax-dependent, p53-independent cell death response in cerebellar granule cells in vivo. In contrast, Bax plays a minor role in GRID2Lc-mediated Purkinje cell death.

Key words: lurcher; cerebellum; Bax; p53; caspase-3; apoptosis

Evidence supporting a role for apoptosis in mammalian neurodegenerative diseases has arisen from cell culture models of neuronal cell death [for review, see Lee et al. (1999)] and from descriptive studies documenting the activation of developmental cell death genes in neurological disorders [for review, see Heintz and Zoghbi (2000)]. Several issues have arisen from these studies that require direct investigation in well characterized models of neurodegeneration in vivo. For example, studies of cell death in a variety of in vitro culture systems (Choi, 1994; Deshmukh and Johnson, 1998; Lee et al., 1999), as well as the investigation of developmental cell death in the nervous system in vivo (Pettmann and Henderson, 1998), have established both that different molecules can participate in apoptosis in distinct cell types and that disparate stimuli can activate distinct cell death pathways in a single cell type. Descriptive studies of both human and mouse neurodegeneration in vivo reflect the complex picture that has arisen from these investigations and support the general idea that a wide variety of abnormal stimuli can result in the activation of the effector phase of apoptosis in neurons (Green, 1998; Los et al., 1999; Vaux and Korsmeyer, 1999). These studies have also demonstrated a direct role for specific proteins in neuronal cell death in vitro or in vivo. Thus, analysis of cell death in Bax null mutant mice has established a direct role in neuronal cell death in response to trophic factor withdrawal (Deckwerth et al., 1996) and during the normal development of a variety of brain structures (White et al., 1998). A role for p53 in both radiation-induced neuronal cell death in vivo (Woods and Youle, 1995) and excitotoxic cell death in vitro (Xiang et al., 1998) has also been demonstrated. These studies provide a foundation for the investigation of inherited neurodegenerative disease.

Lurcher (Lc) is a semidominant mouse mutation that results in ataxia because of massive loss of cerebellar neurons during the first 4 postnatal weeks (Caddy and Biscoe, 1979). The lurcher mutation results in the activation of the δ2 glutamate receptor (GRID2) in cerebellar Purkinje cells, leading to a constitutive, inward current (Zuo et al., 1997). Death of Purkinje cells in lurcher animals is cell autonomous (Wetts and Herrup, 1982a,b) and arises as a direct consequence of the increased activity of the receptor, because null mutations of GRID2 do not result in Purkinje cell death (Kashiwabuchi et al., 1995). Descriptive studies of cerebellar degeneration in lurcher mice suggest that Purkinje cell death occurs by apoptosis (Norman et al., 1995; Wullner et al., 1995). This is consistent with the elevated expression of Bax, Bcl-X (De Jager, 1998; Wullner et al., 1998), and procaspase-3 (Selimi et al., 2000) in postnatal lurcher Purkinje cells and with the increased levels of active caspase-3 (Heintz and De Jager, 1999) and DNA nicking (Norman et al., 1995) in the small percentage of these cells that have entered the effector phase of the cell death pathway. The death of granule cells and inferior olivary neurons in lurcher animals is secondary to the death of Purkinje cells and is presumably caused by the loss of Purkinje cells as targets for these two neuronal populations.

Because of the elevated level of Bax expression observed in lurcher animals (Wullner et al., 1995; De Jager, 1998) and the evidence supporting a role for p53 in Bax-mediated neuronal cell death in response to both genotoxic and excitotoxic activity in neurons (Xiang et al., 1998), we have examined the roles of these genes in neurodegeneration in the lurcher cerebellum. We report here that Purkinje cell death in +/Lc:Bax−/− mice is delayed relative to that in Bax-expressing +/Lc littermates and that granule cell death is permanently rescued in these animals. Examina-
tion of +/Lc:p53−/− mice revealed no alteration in cell death. These results demonstrate that granule cell death as a result of target deprivation in vivo occurs via a Bax-dependent, p53-independent pathway and that Bax plays a minor role in cell death induced by the constitutive activation of the 82 glutamate receptor in cerebellar Purkinje cells. Finally, in contrast to Bax-expressing +/Lc littersmates, we observed an absence of active caspase-3 in the Purkinje cells of +/Lc:Bax−/−, indicating the activation of a Bax-independent, caspase-3-dependent death pathway in these cells.

MATERIALS AND METHODS

Breeding and genotyping of mice. Mice (C57BL/6) heterozygous for Bax (Knudson et al., 1995) were mated with Balb/C lurcher (+/Lc) mice to obtain F1 +/Lc:Bax−/− offspring. +/Lc:Bax−/− mice were crossed to generate +/Lc and wild-type (+/+ ) mice that were either Bax−/−, Bax+/+, or Bax+/−. The mice were genotyped for Bax by single PCR from the wild-type Bax allele and from the neo cassette using DNA prepared from tails (Qiagen, Hilden, Germany). A region of the wild-type Bax allele was amplified using an exon 2 forward primer (5′-CTTGGGAGAAAGAACAACACTGC-3′) and an intron 3 reverse primer (5′-CTGACAGACATGAGAAGACGAG-3′). A region of the neo cassette was amplified using the forward 5′-GATGTGACAGGCTTTCGG-3′ and reverse 5′-CTCCTGGGAAACGTGCTTCGG-3′ primers. The mice were identified as +/Lc by their ataxia. The +/Lc genotype was sometimes confirmed by single PCR from the Lc allele of GRID2 (GRID2Lc) using an intron 2 forward primer (5′-GCACGATGTATGACCTCAG-3′) and an exon B reverse primer (5′-GGTGATAGTGAGGAAAGT-3′).

Mice (129S3) heterozygous for p53 (Tpr53+/−/−; The Jackson Laboratory, Bar Harbor, ME) were mated with Balb/C+/Lc/ mice to obtain F1 +/Lc:p53−/− offspring. +/Lc:p53−/− mice were crossed to generate +/Lc and +/+ mice that were either p53−/−, p53+/−, or p53+/+. The mice were genotyped for p53 by single PCR from the wild-type p53 allele and from the neo cassette using DNA prepared from tails (Qiagen). A region of the wild-type p53 allele was amplified using an exon 6 forward primer (5′-CCCGAGATCTGGGACAG-3′) and an exon 7 reverse primer (5′-ATAGGTCCCGGTTCTCAT-3′). The neo cassette was amplified using the same neo primers used for the Bax genotyping. The mice were identified as +/Lc as described above.

Histology and immunocytochemistry. Mice killed for cresyl violet staining and calbindin immunocytochemistry were deeply anesthetized with sodium pentobarbital and perfused intracardially with 0.9% NaCl followed by 95% ethanol. The brains were post-fixed in 75% ethanol and 25% acetic acid, dehydrated, and embedded in paraffin. Mid sagittal cerebellar sections 10 μm thick were cut, mounted on slides, and stained with cresyl violet or incubated overnight in 4°C in a 1:500 dilution of mouse monoclonal anti-calbindin antibodies (Sigma, St. Louis, MO). The immunolabeling was revealed using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and DAB (Sigma).

Mice killed for anti-active caspase-3 immunocytochemistry were deeply anesthetized with sodium pentobarbital and decapitated, and the brain was frozen in ornithine carbamyl transferase compound (Sakura) in an acetonate bath at −20°C. The brains were stored at −80°C until use. Sagittal cerebellar sections 10 μm thick were then cut, mounted on slides, fixed in acetonate at −20°C, washed in 0.035% H2O2 to block endogenous peroxidases, and incubated overnight at 4°C in a 1:500 dilution of polyclonal anti-active caspase-3 antibodies (PharMingen, San Diego, CA). Immunolabeling was revealed as described above.

Cell counts. The number of granule cells per midsagittal cerebellar section was estimated from the total area and granule cell density of the internal granule cell layer (IGL). The area of the IGL was measured from an image captured on a video graphics card using NIH Image software and a CCD video camera attached to a Nikon microscope at 20× magnification. The IGL of the captured image was outlined free-hand, and the area enclosed was measured using NIH Image software. The IGL cell density was estimated from the number of granule cells enclosed in a 3600 μm2 area defined by an ocular grid at 1000× magnification. Granule cell counts were taken from the posterior, mid, and anterior cerebellum and used to calculate an average density.

Purkinje cell counts were performed on calbindin-immunostained midsagittal cerebellar sections. The total number of immunostained cell bodies in the Purkinje cell layer (PCL) and molecular layer (ML) of the section was counted at 200× magnification using Nomarski optics. All percentages of adult wild-type cell numbers quoted in the text are calculated from the +/+ :Bax−/− value.

RESULTS

Bax deletion reduces lurcher cerebellar atrophy

The increased expression of the proapoptotic Bcl-2 family member Bax in the dying cerebellar Purkinje cells of lurcher (+/Lc) mice (Wullner et al., 1995; De Jager, 1998) strongly favors a role for this protein in the putative excitotoxic death of the cell. To determine whether Bax expression is required for +/Lc Purkinje cell death, we generated +/Lc mice deficient for Bax (+/Lc:Bax−/−) by crossing +/Lc heterozygous Bax mice (+/Lc:Bax+/−) with Bax−/−. +/Lc:Bax−/− crosses resulted in the death of on average one in four of the neonates, consistent with the neonatal lethality noted in homozygous lurcher pups (Cheng and Heintz, 1997). The remaining pups developed normally into the third postnatal week when a majority of the litter began to exhibit ataxia characteristic of +/Lc; Bax+/−. Genotyping revealed that these ataxic mice included +/Lc:Bax−/− as well as +/Lc:Bax+/− and +/Lc:Bax+/+. No difference was observed in the timing of the onset of ataxia between the three +/Lc:Bax genotypes. All these mice remained ataxic into adulthood and were killed for morphological analysis at postnatal day 30 (P30; n = 5 mice). After dissection it was evident that the cerebellum of +/Lc:Bax−/− mice were noticeably larger with deeper, more-pronounced fissures than that of their +/Lc:Bax+/− and +/Lc:Bax+/+ littermates (Fig. 1). This observation indicated a reduction of the cerebellar atrophy associated with the +/Lc mutation in mice deficient for Bax. A similar effect was observed in +/Lc:Bax−/− mice analyzed at later ages (P60; n = 2 mice, and P300; n = 3 mice).
Bax deletion rescues granule cells and delays Purkinje cell death in lurcher mice

Granule cells make up the bulk of the cerebellar mass, and cresyl violet staining of cerebellar sections from /+Lc:Bax+/− mice revealed the presence of a large, cell-dense IGL. The numerous sparse, atrophic lobules of the 1littermates (Fig. 2). Despite the abundant presence of granule cells, the examination of /+Lc:Bax+/− sections under high-powered objectives revealed the presence of a few Purkinje cells at the interface of the IGL and ML. The large-scale absence of Purkinje cells in /+Lc:Bax+/− mice was confirmed by immunolabeling P30 cerebellar sections with antibodies to calbindin (Fig. 3). Calbindin immunolabeling also revealed abnormal Purkinje cell morphology in /+Lc:Bax+/− mice; the Purkinje cells were not aligned in a monolayer and had stunted, poorly developed dendritic trees that failed to reach the pial surface (Fig. 3b), a morphology that is characteristic of /+Lc mice.

We estimated the number of granule cells per midsagittal cerebellar section at P30 and P300 using morphometric analysis and cell counts. The data revealed the presence of 60% of the wild-type (+/+:Bax+/−) number of granule cells in /+Lc:Bax+/− mice at both ages (~32,000 granule cells per section), compared with a value of 10% or less in the /+Lc:Bax+/− and /+Lc:Bax+/− mice at P30 (Fig. 4a). The persistence of 60% of the wild-type number of granule cells in the /+Lc:Bax+/− mice indicates that the deletion of Bax permanently rescues these cells from target-related cell death. In spite of the evidence of widespread Purkinje cell death in the calbindin-immunolabeled sections of /+Lc:Bax+/− mice, Purkinje cell counts revealed significantly increased numbers of Purkinje cells in the Bax-deficient /+Lc mice at P30. At this age there was 15% of the wild-type (+/+:Bax+/−) number of Purkinje cells in /+Lc:Bax+/− mice compared with 6% or less in /+Lc mice with either one or both Bax alleles (Fig. 4b). However, the examination /+Lc:Bax+/− mice at P30 demonstrated that /+Lc Purkinje cell death was delayed but not prevented by the deletion of Bax, because the number of Purkinje cells was reduced to 1% of the wild-type value in these older mice (Fig. 4b). The delay but eventual death of Purkinje cells in /+Lc:Bax+/− mice indicates the existence of a Bax-independent cell death pathway in Purkinje cells.

We next examined /+Lc mice deficient for Bax at the younger age of P15, the approximate midpoint in the granule cell death response in lurcher mice (see Doughty et al., 1999). By examining these younger mice, we hoped to establish whether (1) the presence of 60% of the wild-type number of granule cells at P30 and older was the result of the partial rescue of the cell population or the prevention of all target-related granule cell death in the mutant, (2) Purkinje cell death was delayed in all or only a regional subset of cells, and (3) /+Lc Purkinje cell development was ameliorated in the absence of Bax expression. We conducted the same analysis as before using cresyl violet staining and calbindin immunocytochemistry of midsagittal cerebellar sections (n = 5 mice). At P15 there was already a clear increase in the size and density of the IGL of /+Lc:Bax+/− compared with /+Lc:Bax+/− littermates (Fig. 5a). Cell counts and morphometric analysis of the IGL confirmed a significant increase in granule cell numbers in the /+Lc:Bax+/− mice (Fig. 5c). The estimated number of granule cells per section at P15 was very similar to the
value obtained for +/Lc:Bax<sup>−/−</sup> mice at P30 and P300 (33,000 cells per section at P15 compared with on average 32,000 cells per section in the older animals). The presence of equal numbers of granule cells at P15, P30, and P300 in +/Lc:Bax<sup>−/−</sup> mice, in addition to the absence of any pyknotic profiles in the IGL at P15 (in contrast to the numerous profiles observed in +/Lc:Bax<sup>+/−</sup> littermates), suggests that target-related granule cell death in +/Lc mice is prevented by the deletion of Bax. If granule cell death is completely rescued by Bax deletion, then the decrease in total granule cell numbers in +/Lc:Bax<sup>−/−</sup> mice is presumably attributable to reduced production of granule cells in response to local signals from Purkinje cells (Smeyne et al., 1995; Wechsler-Reya and Scott, 1999). Cell counts revealed greater numbers of Purkinje cells in the +/Lc:Bax<sup>−/−</sup> at P15 (62% of the adult wild-type value) compared with P30 (15% of the adult wild-type value) (Fig. 5d). Calbindin immunolabeling of P15 cerebellar sections revealed a similar pattern of Purkinje cell distribution in +/Lc:Bax<sup>−/−</sup> and +/Lc:Bax<sup>+/−</sup> littermates (Fig. 5b), although all of the remaining cells had poorly developed dendrites. The facts that the number of surviving Purkinje cells in early postnatal +/Lc:Bax<sup>−/−</sup> animals is directly proportional to the number of granule cells present in the adult, that Purkinje cell loss in these animals is eventually complete, that no further granule cell loss is noted after P15 in +/Lc:Bax<sup>−/−</sup> mice, and that granule cell production is known to be controlled locally by Purkinje cells (Smeyne et al., 1995; Wechsler-Reya and Scott, 1999) are all consistent with the complete rescue of secondary granule cell death in response to loss of Bax expression. However, we cannot exclude the possibility of a Bax-independent cell death pathway as the cause for the lower number of granule cells in +/Lc:Bax<sup>−/−</sup> mice.

**Bax-mediated neuronal death in lurcher mice is p53 independent**

The expression of the tumor suppressor gene p53 has been shown to be required for many Bax-mediated cell death pathways (Aloyz et al., 1998; Xiang et al., 1998; Cregan et al., 1999). To examine the possible requirement of p53 expression for the Bax-mediated neuronal cell death in +/Lc mice, we generated +/Lc mice deficient for p53 (+/Lc:p53<sup>−/−</sup>) by crossing +/Lc mice heterozygous for p53 (+/Lc:p53<sup>+/−</sup>). We hypothesized that a rescue of cerebellar cell death in +/Lc:p53<sup>−/−</sup> mice comparable with that observed in +/Lc:Bax<sup>−/−</sup> mice would indicate the need for p53 expression to activate the Bax cell death pathway in the +/Lc cerebellum. As is the case for Bax, the deletion of p53 failed to prevent the development of ataxia in the third postnatal week in +/Lc:p53<sup>−/−</sup> mice. However in contrast to the +/Lc:Bax<sup>−/−</sup> mice, +/Lc:p53<sup>−/−</sup> mice showed no sign of amelioration in the cerebellar atrophy associated with the +/Lc mutation when killed at P30 (n = 4 mice). Histological analysis confirmed that the cerebella of +/Lc:p53<sup>−/−</sup> mice were indistinguishable from that of their +/Lc:p53<sup>+/−</sup> littermates (data not shown). The meager presence of Purkinje cells and granule cells in the cerebella of +/Lc:p53<sup>−/−</sup> mice demonstrates that the Bax-mediated death of these cells is independent of p53 expression.

**Caspase-3 activation in lurcher mice is Bax dependent**

Caspase-3, a widespread effector protease in apoptotic cell death, has been shown to be activated in the dying Purkinje cells and granule cells of +/Lc mice (Selimi et al., 2000). Interestingly, the activation of caspase-3 in granule cell cultures switched from medium containing high to low concentrations of potassium requires the expression of Bax (Miller et al., 1997). We therefore examined the expression of active caspase-3 in +/Lc:Bax<sup>−/−</sup> and +/Lc:Bax<sup>+/−</sup> mice during the period of Purkinje cell death (P15–P20) using an antibody that only recognizes the active subunit of the protease. The immunocytochemical labeling of cerebellar sections from +/Lc:Bax<sup>−/−</sup> mice confirmed the expression of active caspase-3 in dying Purkinje cells and granule cells (data not shown). Consistent with the results of Selimi et al. (2000), few Purkinje cells were immunolabeled with the antiserum (on aver-
independent cell death pathway in the cell.

is not activated in the dying Purkinje cells of
animal). These contrasting observations suggest that caspase-3

Aged
postnatal day 30 and postnatal day 300 (Aged) mice from +/Lc:Bax1/− crosses. a, Bax deletion permanently rescues granule cells from target-
related cell death in +/Lc mice. The number of granule cells per midsagittal cerebellar section was estimated from the area and cell density of the
internal granule cell layer. b, Bax deletion delays but does not prevent
cell-autonomous Purkinje cell death in +/Lc mice. The number of Purkinje
cells per midsagittal cerebellar section was counted from sections
processed for calbindin immunocytochemistry. The data are presented as the
mean value ± SEM (n = 3–5 mice). Asterisks denote statistically
significant differences: a, *p < 0.000001; b, *p < 0.05 (unpaired two-tailed
Student’s t test).

age, three per sagittal section of the cerebellar vermis; n = 2 mice
from 2 litters, 5 sections per animal), indicating the rapid death of
the cell after cleavage and activation of the caspase. In contrast,
the simultaneous labeling of cerebellar sections from +/Lc:Bax−/− mice failed to detect any active caspase-3 expression in Purkinje
cells or granule cells (n = 3 mice from 3 litters, 5 sections per
animal). These contrasting observations suggest that caspase-3
is not activated in the dying Purkinje cells of +/Lc:Bax−/− mice and suggest the existence of a caspase-3-independent, Bax-

independent, cell death pathway in the cell.

**DISCUSSION**

Previous studies of +/Lc mice suggest an apoptotic mechanism of
Purkinje cell death involving Bax expression and induction of
caspase-3 activity (Norman et al., 1995; De Jager, 1998; Wullner
et al., 1998; Selimi et al., 2000). By applying a genetic approach,
we have demonstrated the involvement of Bax in both the pri-
mary, cell-autonomous death of Purkinje cells and the secondary
loss of granule cells in +/Lc mice. The deletion of Bax expression
in +/Lc mice permanently rescued granule cells from target-
related cell death and delayed but did not prevent Purkinje cell
decay. From these loss-of-function experiments, we conclude
there is an absolute requirement for Bax expression in target-
related granule cell death, despite the presence of only 60% of the
+/+ number in adult and aged +/Lc:Bax−/− mice. This conclu-
sion is based on the strong evidence of a positive mitogenic role
of Purkinje cells in granule cell production (Smeyne et al., 1995;
Wechsler-Reya and Scott, 1999). Thus the retarded development of Purkinje cells in +/Lc:Bax−/− mice is likely to reduce granule
cell production in these mice. The delay but failure to prevent
+/Lc Purkinje cell death by the deletion of Bax expression indicates
the presence of both Bax-dependent and Bax-independent
death pathways in these cells. Next, we examined the role of p53,
a molecule widely implicated in the regulation of Bax-mediated
neuronal cell death (Aloyz et al., 1998; Xiang et al., 1998; Cregan
et al., 1999). Our analysis showed that the deletion of p53 expres-
sion in +/Lc mice did not prevent target-related granule cell
death, demonstrating that Bax is expressed independently of p53
in these cells. Finally, we suggest that the activation of caspase-3
in +/Lc cerebella (Selimi et al., 2000) requires the expression of
Bax. Because Purkinje cells continue to die in +/Lc:Bax−/− mice,
this observation suggests the activation of a Bax-independent,
caspase-3-independent death pathway in the cell.

Bax activity is essential for many programmed cell death
(PCD) events in CNS development (Deckwerth et al., 1996;
White et al., 1998), and the protein has been shown to regulate
neuronal sensitivity to both excitotoxic and genotoxic injury
(Xiang et al., 1998; Cregan et al., 1999). Therefore the observa-
tion that Bax expression is increased in +/Lc Purkinje cells (De
Jager, 1998; Wullner et al., 1998) suggested a role for this protein
in the cell-autonomous death of this cell. Interestingly, increased
Bax expression was not reported in the cerebellar granule cells of
+/Lc mice by these authors, despite the large-scale death of these
neurons after the loss of target Purkinje cells. Contrary to these
observations, our loss-of-function experiments clearly demon-
strate that the target-related death of granule cells is dependent
on Bax expression, whereas Bax deletion does not prevent +/Lc
Purkinje cell death. Nevertheless, the rate of +/Lc Purkinje cell
death was slowed in the absence of Bax. This indicates the
involvement of Bax in Purkinje cell death, but it also reveals the
activation of a Bax-independent pathway in the cell.

The presence of a Bax-independent cell death pathway in
Purkinje cells is not surprising considering the strong activation
of the α2 glutamate receptor in response to the *lurcher* mutation.
Thus, induction of both Bax and Bel-X has been demonstrated in
+/Lc Purkinje cells (De Jager, 1998; Wullner et al., 1998), sug-
gestig a possible role for the proapoptotic isoform of Bel-X in a
parallel pathway for activation of the effector phase of apoptotic
cell death in these neurons. This is consistent with the demon-
stration that granule cells from Bax−/− mice will undergo exci-
toxic death in response to NMDA application in culture (Miller
et al., 1997). Alternatively, the removal of Bax as a rate-limiting
component of the cell death pathway active in +/Lc Purkinje cells
may provoke a less well defined pathway. The observation that
active caspase-3 is not detected in +/Lc:Bax−/− Purkinje cells
demonstrates that this alternative cell death pathway does not
involve the activation of this protease.

The permanent rescue of secondary granule cell loss in +/Lc:
Bax−/− mice is consistent with previous studies of apoptotic cell
death in response to target deprivation. Thus, a requirement for Bax in cell death attributable to trophic factor withdrawal for sympathetic and motor neurons has been demonstrated using Bax−/− mice (Deckwerth et al., 1996). Furthermore, partial rescue of inferior olivary neurons from target-related cell death by the overexpression of Bcl-2 in +/Lc mice has been reported (Zanjani et al., 1998) (it should be noted that Bcl-2 was not overexpressed in granule cells in these mice). These examples support the activation of a common, Bcl-2 family-mediated cell death response to the loss of target support. In spite of experiments indicating a role for p53 in cell death models induced by DNA damage (Woods and Youle, 1995), excitotoxicity (Xiang et
al., 1998), PCD, and trophic factor withdrawal (Aloyz et al., 1998) and the expression of p53 in developing cerebellar granule cells (van Lookeren Campagne and Gill, 1998), our results failed to reveal a role for p53 in granule cell death in response to target deprivation. This is interesting considering the reported induction of a Bax-dependent cell death pathway in response to adenovirus-mediated delivery of p53 in granule cells in vitro (Cregan et al., 1999). We conclude that the activation of Bax in response to target deprivation in vivo occurs by an alternative p53-independent mechanism, indicating the presence of multiple pathways for the activation of Bax-dependent cell death in cerebellar granule neurons. Although we have not tested directly the requirement for caspase-3 in granule cell death in +/Lc:Bax−/− mice, the fact that active caspase-3 was not observed in these cells strongly supports an important role for this protease.

The data presented here suggest that the constitutive activation of GRID2 knockout mice induces multiple death pathways in the Purkinje cell that involve apoptotic (Bax expression and caspase-3 activation) and unknown, possibly necrotic, mechanisms. The complete rescue of granule cells in +/Lc:Bax−/− mice, on the other hand, confirms that these cells die by a purely apoptotic mechanism. Recently, a number of studies (Martinou et al., 1994; Ankarcrona et al., 1995; Krajewski et al., 1995; Chen et al., 1998; Namura et al., 1998) have indicated that apoptosis is a key component of ischemic brain injury (for a review of current perspectives, see Lee et al. (1999)). Thus, the features of +/Lc cell death in many ways mirror the events of brain injury caused by transient cerebral ischemia (Heintz and Zoghbi, 2000). In both cases, the primary insult is the activation of glutamate receptors, and this leads first to the expression of neuroprotective Bcl-2 family members that, once overwhelmed, results in the activation of cell death pathways that include Bax expression and caspase activation, as well as unknown molecules. In turn, the primary lesion leads to the secondary loss of adherent neurons by apoptosis. However, unlike models of cerebral ischemia, the site of the primary lesion is well characterized in +/Lc mice, and this advantage should prove invaluable in the use of this mutant as a model to study cell death mechanisms in ischemic brain injury.

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