The Overexpression of Bax Produces Cell Death upon Induction of the Mitochondrial Permeability Transition*

(Received for publication, November 21, 1997, and in revised form, December 16, 1997)

John G. Pastorino, Sing-Tsung Chen, Marco Tafani, Jack W. Snyder, and John L. Farber‡

From the Department of Pathology and Cell Biology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

† To whom correspondence should be addressed: Rm. 251, Jefferson Alumni Hall, Thomas Jefferson University, Philadelphia, Pennsylvania 19107.

‡ The abbreviations and other systematic and trivial names used are: MPT, mitochondrial permeability transition; PARP, poly(ADP-ribose)-polymerase; CyA, cyclosporin A; ArA, aristolochic acid; DiOC6(3), 3,3′-dihexyloxacarbocyanine; Z-DEVD-FMK, Z-Asp-Glu-Val aspartic acid fluoromethylketone (Z-DEVD-FMK); MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; PARP, poly(ADP-ribose)-polymerase; Bax, a member of the Bel-2 family of proteins that has been associated with apoptotic cell death both in cell culture (1) and in intact animals (2). Alterations in mitochondrial function in general and induction of the mitochondrial permeability transition (MPT) in particular are proposed to play a critical role in apoptosis (3–5). Bax is localized to mitochondria (6, 7), and the cell death that accompanied the overexpression of Bax was associated with loss of the mitochondrial membrane potential and an increased production of reactive oxygen species (8). However, neither the nature of the responsible mitochondrial alterations nor their relationship to the loss of cell viability and to other features of apoptosis have been defined. Here we show that the induction of the overexpression of Bax in stably transfected Jurkat cells induces the MPT, an event that is accompanied by typical features of apoptosis, namely cytosolic accumulation of cytochrome c, caspase activation, cleavage of poly(ADP-ribose)-polymerase (PARP), DNA fragmentation, and cell death. Inhibition of the MPT prevents all manifestations of apoptosis, whereas caspase inhibition prevents PARP cleavage and DNA fragmentation but not cytochrome c release or cell death.

**EXPERIMENTAL PROCEDURES**

Generation of Stable Transfectants with Inducible Bax Expression—Jurkat cells were stably transfected with an inducible expression system encoding mouse Bax. Total RNA was isolated from mouse fibrosarcoma cells (L929). A 5′–primer (5′-CTTTGAGGTGATGCTGATC-3′) and a 3′-primer (5′-GATATGGCCAGTGATTCTC-3′) were designed and utilized for reverse transcription and polymerase chain reaction amplification of the cDNA for Bax from the isolated total RNA. The polymerase chain reaction products were electrophoresed, and the 579-base pair mouse Bax cDNA was identified. The fragment was then cloned into pIND downstream of the ecdysone response element to generate pINDBax. The insert was sequenced and found to be 100% identical to the published sequence of mouse Bax (GenBank accession number 1.22472). To generate inducible clones, wild-type Jurkat cells were first transfected with pVgRXX, which encodes for a heterodimer of the ecdysone receptor, and the retinoid X receptor, which binds the ecdysone response element (encoded on pINDBax) in the presence of muristerone A. Stable transfectants were obtained (JvGReX) and in turn transfected with the pIND(Bax) construct. Stable transfectants (JtBax1 and 2) were then selected. JtLacZ clones were generated as above with the exception that cDNA for β-galactosidase was cloned into pIND instead of Bax.

**Determination of Bax Expression and PARP Cleavage—**Cells (5 × 10⁶) were pelleted at 700 × g, resuspended in 20 μL of SDS-sample buffer, and boiled for 10 min. Protein content was determined by the bicinchoninic acid assay with bovine serum albumin as a standard. Samples were then run on an 8 or 12% SDS-polyacrylamide electrophoresis gel for determination of PARP cleavage or Bax expression, respectively. Kaleidoscope prestained standards (Bio-Rad) were used to determine molecular weights. The gels were electroblotted onto nitrocellulose membranes. For the determination of PARP cleavage, the blots were probed with anti-human PARP monoclonal antibody (C2–10; Enzyme Systems Products, Dublin, CA) at 1:5,000 dilution. For Bax expression, a rabbit polyclonal anti-Bax antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was used at a 1:2,000 dilution. A secondary hors eradish peroxidase-labeled goat-antimouse or goat-antirabbit antibody at 1:2,000 was detected using enhanced chemiluminescence for PARP or Bax, respectively.

**Measurements of Cell Viability—**Cell viability was determined by trypan blue exclusion and the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS). For trypan blue exclusion, 10 μL of a 0.5% solution of the dye was added to 100 μL of treated cells (1.0 × 10⁶ cells/mL). The suspension was then applied to a hemocytometer. Both viable and nonviable cells were counted. A minimum of 200 cells were counted for each data point in a total of eight microscopic fields. For the MTS assay, a 100-μL aliquot of cells (1.0 × 10⁶ cells/mL) was placed in the well of a 96-well plate. The reaction was started by the addition of MTS and phenazine methosulfate (PMS). The absorbance change obtained upon reduction of MTS was read 90 min later with a 96-well plate reader at 490 nm. 100% cell killing was determined by the addition of Triton X-100.
X-100 to a final concentration of 0.5%, 30 min prior to MTS and PMS addition. The MTS assay and trypan blue exclusion gave identical results.

**Measurement of Mitochondrial Energization—**Mitochondrial energization was determined as the retention of the dye 3,3′-dihexyloxacarboanilide (DiOC₆(3); Molecular Probes Inc, Eugene, OR). Cells (5 × 10⁵ in 500 μl of complete RPMI 1640 medium) were loaded with 100 nm DiOC₆(3) during the last 30 min of treatment. The cells were then pelleted at 700 × g for 10 min. The supernatant was removed, and the pellet was resuspended and washed in PBS twice. The pellet was then lysed by the addition of 600 μl of deionized water followed by homogenization. The concentration of retained DiOC₆(3) was read on a Perkin-Elmer LS-5 fluorescence spectrophotometer at 488 nm excitation and 500 nm emission.

**Determination of DNA Fragmentation—**Cells (1.0 × 10⁶) were collected by centrifugation at 2,000 × g for 10 min. The cell pellet was washed in PBS and then lysed in 200 μl of 10 mM Tris, pH 8.0, 10 mM EDTA, 0.5% Triton X-100. The lysate was centrifuged at 13,000 × g for 10 min at 4 °C. RNase (0.2 mg/ml) was added, and the lysate was incubated for 30 min at 37 °C. Proteinase K (0.1 mg/ml) and SDS (final concentration 1%) were added, followed by incubation at 50 °C for 16 h. DNA was extracted with phenol/chloroform and then chloroform, precipitated with ethanol and sodium acetate, and electrophoresed on 1.2% agarose gels.

**Detection of Caspase-3 Activity—**The assay is based on the ability of the active enzyme to cleave the chromophore pNA from the enzyme substrate DEVD-pNA. Cytosolic fractions isolated as above were diluted 1:1 with 2× reaction buffer (10 mM Tris, pH 7.4, 1 mM dithiothreitol, 2 mM EDTA, 0.1% CHAPS, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml pepstatin, 10 μg/ml leupeptin). DEVD-pNA was added to a final concentration of 50 μM, and the reaction was incubated for 1 h at 37 °C. The samples were then transferred to a 96-well plate, and absorbance measurements were made with a 96-well plate reader at 405 nm.

**Isolation of Cytosol and Mitochondrial Fractions and Determination of Cytochrome c Content—**Cells (1.0 × 10⁶) were harvested by centrifugation at 600 × g for 10 min at 4 °C. The cell pellets were washed once in PBS and then resuspended in 3 volumes of isolation buffer (20 mM Hepes, pH 7.4, 10 mM KCl, 1.5 mM MgCl₂, 1 mM sodium EDTA, 1 mM sodium EGTA, 0.5% Triton X-100). The lysate was centrifuged at 13,000 × g for 10 min. The supernatant was removed, and the cell pellet was resuspended and washed in PBS two times. The pellet was washed in PBS and then lysed in 200 μl of 500 mM Tris, pH 8.0, 10 mM EDTA, 0.5% Triton X-100 to a final concentration of 0.5%, 30 min prior to MTS and PMS addition. The MPTS assay and trypan blue exclusion gave identical results.

**RESULTS**

To study the mechanism of action of Bax, we produced clones (JtBax1 and JtBax2) of stably transfected Jurkat T cells in which Bax expression is inducible by muristerone A. Bax was not detected in uninduced JtBax1 cells (Fig. 1a). In the presence of muristerone A, however, Bax expression was detected within 30 min and increased for 4 h (Fig. 1a). Similar results were obtained with JtBax2 cells (data not shown). Bax expression was accompanied by cell death that was detectable within 2 h (Fig. 2a). By 16 h, 75% of the cells were dead. As a control, the cDNA for β-galactosidase was cloned downstream of the muristerone A-inducible promoter. Stable transfectants (JtLacZ1) were produced that, upon induction by muristerone A, exhibited an increase in β-galactosidase (1000 microunits/10⁶ cells at 6 h). However, induced JtLacZ1 cells showed no loss of viability over the same time course that JtBax1 cells were killed (Fig. 2a).

The MPT refers to the regulated opening of a large, nonspecific pore in the inner mitochondrial membrane (9). The MPT is inhibited by cyclosporin A (CyA) (10–12), an effect enhanced and prolonged by phospholipase A₂ inhibitors, both in isolated mitochondria (9) and in the intact cell (14). CyA in combination with the phospholipase inhibitor aristolochic acid (ArA) completely prevented the killing of JtBax1 cells upon induction of Bax expression (Fig. 2b). By contrast, the caspase-3 inhibitor Z-DEVD-FMK (15) had no effect on the loss of viability (Fig. 2b). As a control, the same concentration of Z-DEVD-FMK completely prevented the killing of JtBax1 cells upon activation of the Fas receptor with an anti-Fas antibody (data not shown). Importantly, CyA plus ArA did not alter the time course or level of Bax expression induced by muristerone A (Fig. 1b).

In addition to its ability to inhibit the MPT, cyclosporin A binds to cytosolic cyclophilin A, and the resulting complex inhibits the Ca²⁺-regulated protein phosphatase calcineurin (16, 17). The ability of CyA to prevent the cell killing by Bax...
Calcineurin inhibitors do not prevent the cell killing by Bax

JtBax1 cells were pretreated for 30 min with the indicated agents. Afterward, muristerone A (1 μm) was added to induce Bax expression. At 8 or 16 h cell viability was determined by both trypan blue exclusion and the MTS assay. Results are the mean ± S.D. of three independent experiments.

| Treatment                        | Percent Dead Cells |
|----------------------------------|--------------------|
|                                 | 8 h    | 16 h    |
| JtBax1                          | 44.0 ± 9.5 | 73.1 ± 6.3 |
| JtBax1 + Cypermethrin (10 μM)    | 47.5 ± 8.3 | 71.3 ± 8.2 |
| JtBax1 + FK506 (5 μM)            | 53.9 ± 2.3 | 79.8 ± 4.7 |
| JtBax1 + Cypermethrin and ArA    | 48.0 ± 4.9 | 79.4 ± 9.5 |
| JtBax1 + FK506 and ArA           | 46.8 ± 5.1 | 75.9 ± 4.9 |

Table I

Inability of CyA plus ArA to prevent the loss of ΔΨm produced by CCCP

| Treatment                        | ΔΨm (3) retention (percent control) |
|----------------------------------|------------------------------------|
|                                 | 1 h      | 2 h      |
| CCCP (10 μM)                     | 37 ± 7   | 17 ± 7   |
| CCCP (10 μM) + CyA and ArA       | 33 ± 5   | 15 ± 3   |

JtBax1 cells were either left untreated or treated with CyA and ArA for 30 min. CCCP was then added to all cultures, and the ability of cells to take up and retain DiOC6(3) was determined as outlined under “Experimental Procedures.”

was not a consequence of the inhibition by CyA of calcineurin. Two other calcineurin inhibitors, cypermethrin A and FK506, alone or in combination with ArA, did not prevent cell killing produced by Bax expression (Table I). FK506 and cypermethrin are inactive against the MPT (18).

The MPT causes the loss of the mitochondrial membrane potential (ΔΨm) (19). We have used the CyA-inhibitable loss of ΔΨm to document the MPT in intact cells independently of the effect of the transition on cell viability (18). The fluorescent dye DiOC6(3) localizes to mitochondria as a consequence of ΔΨm, and the MPT reduces the accumulation of DiOC6(3) as a consequence of the loss of ΔΨm (20, 21). In JtBax1 cells, CCCP, a proton ionophore that dissipates ΔΨm, produced a time-dependent loss of DiOC6(3) (Table II), a result indicating the mitochondrial localization of the majority of the dye. Importantly, CyA plus ArA had no effect on the rate or extent of the loss of DiOC6(3) caused by CCCP (Table II), a result demonstrating the specificity of CyA plus ArA in preventing the loss of ΔΨm as a consequence of the MPT.

Treatment of JtBax1 cells with muristerone A produced a steady decline in ΔΨm (Fig. 3a), and the time course of the loss of ΔΨm upon induction of Bax overexpression paralleled that of the loss of viability. Within 4 h, more than 30% of the dye was lost from the cells (Fig. 3a), and 25% of the cells had died (Fig. 2a). Within 8 h, retention of DiOC6(3) was reduced by 65%, and 40% of the cells were dead.

The time-dependent loss of DiOC6(3) fluorescence that resulted from the induction of Bax expression was completely inhibited by CyA plus ArA (Fig. 3, a and b). Treatment of JtLacz1 cells with muristerone A had no effect on the retention of the dye over the same time course (Fig. 3a). Consistent with its inability to prevent the loss of viability (Fig. 2b), the caspase inhibitor Z-DEVD-FMK had no effect on Bax-induced mitochondrial depolarization (Fig. 3b), a result that confirms a previous report (28).

Degradation of DNA into oligonucleosomal fragments (180-base pair multiples) is a hallmark of apoptosis (22, 23). Induction of Bax expression produced extensive DNA fragmentation, detectable within 2 h and complete by 4 h (Fig. 4, lanes 1 and 2). There was no DNA fragmentation in JtLacz1 cells treated with muristerone A (Fig. 4, lanes 7 and 8). DNA fragmentation induced by Bax expression was a consequence of the MPT, as shown by its prevention by CyA plus ArA (Fig. 4, lanes 3 and 4). DNA fragmentation also depends on caspase-3 activity, as shown by the ability of Z-DEVD-FMK to prevent the appearance of the characteristic ladder of fragmented DNA (Fig. 4, lanes 5 and 6).
lanes 5 and 6). Cleavage of the nuclear enzyme PARP by caspase-3 is another prominent indicator of apoptosis (24). Induction of Bax expression resulted in PARP cleavage that was evident within 2 h and complete by 6 h (Fig. 5). CyA plus ArA, as well as Z-DEVD-FMK, prevented this cleavage of PARP (Fig. 5).

Induction of Bax expression produced a steady increase in the caspase-3 activity of cytosolic extracts of JtBax1 cells, an effect completely prevented by CyA and ArA (Fig. 6a). As a control, addition of CyA plus ArA to the cytosolic extracts obtained from JtBax1 cells induced with muristerone A had no effect on caspase-3 activity (Fig. 6b). CyA plus ArA do not inhibit this enzyme directly or any other component necessary for its activation. Z-DEVD-FMK, added to the JtBax1 cells at the time of induction by muristerone A, pre-vented the increase in caspase-3 activity (Fig. 6b).

The MPT in Bax-induced Cell Death

The data presented above document that the overexpression of Bax induces the MPT, an event that is accompanied by typical features of apoptosis, namely the release of cytochrome c to the cytosol, cleavage of poly(ADP-ribose)-polymerase (PARP), DNA fragmentation, and cell death (Fig. 8). Inhibition of the MPT prevents all manifestations of apoptosis, whereas caspase inhibition prevents PARP cleavage and DNA fragmentation but not cytochrome c release or cell death.

Participation of the MPT in our model of apoptosis was shown by the observation that CyA, a known inhibitor of the MPT, in combination with a phospholipase A2 inhibitor, prevents the cell death, as well as the loss of the mitochondrial membrane potential and cytochrome c release. It might be argued that the effect of CyA plus ArA is not necessarily the consequence of an inhibition of the MPT. According to such a
The MPT in Bax-induced Cell Death

**Fig. 8. Mechanism of Bax-induced apoptosis.**

In addition to the loss of viability, Bax expression produced the other typical manifestations of apoptosis, namely caspase activation with DNA fragmentation and PARP cleavage. All of these changes are a likely consequence of the MPT, as they were prevented by CyA plus ArA. PARP is cleaved by caspase-3, and DNA fragmentation has recently been linked to the caspases through activation of DNA fragmentation factor (DFF) (32). In our model, the activation of caspases is clearly the upstream event since Z-DEVD-CMK prevented PARP cleavage and the fragmentation of DNA. The caspase inhibitor, however, did not prevent induction of the MPT and, thus, the loss of cell viability. These results indicate that caspase activation is downstream of the MPT.

The release of cytochrome c from the mitochondria readily accounts for the activation of caspases upon Bax-mediated induction of the MPT. As cytochrome c was retained in the cytosol, it decreased in mitochondria. This redistribution of cytochrome c was prevented by CyA plus ArA, but not by Z-DEVD-CMK. The time course of the release of cytochrome c also paralleled that of the loss of mitochondrial energization. However, the consequences of the release of cytochrome c, namely caspase activation (Fig. 6), PARP cleavage (Fig. 5), and DNA fragmentation (Fig. 4), evolved over a time course that might appear inconsistent with that of the full evolution of the MPT. PARP cleavage was complete within 6 h, a time at which ∆Ψm was reduced by slightly greater than 50% (Fig. 3). We would argue that the release of cytochrome c during the first 6 h following induction of Bax expression and consequent MPT activates caspase-3 to an extent that can account for the degree of PARP cleavage and DNA fragmentation occurring during this period.

Previously, the MPT was discounted as a mechanism of cytochrome c release upon induction of apoptosis by staurosporine in HL-60 cells (27) because the accumulation of cytochrome c in the cytosol preceded a detectable decline in ∆Ψm. It deserves, emphasis that the absence of mitochondrial depolarization, as assessed by the redistribution of fluorescent dyes, does not necessarily imply that the MPT has not occurred. The mitochondrial population reacts heterogeneously to induction of the MPT, with some mitochondria undergoing the MPT very early or very late and some not at all (33). As a consequence there can be a redistribution of membrane-sensitive dyes from depolarized to still polarized mitochondria at earlier time points (34).

The present report has not defined how the MPT is coupled to the loss of cell viability. Importantly, cell death following the MPT is not necessarily the consequence of the loss of an energy-dependent function. Cell killing with inhibition of electron transport is prevented by CyA without restoration of ∆Ψm or ATP levels (35). The link between the MPT and the loss of plasma membrane integrity may involve alterations in the cytoskeleton. The microtubule-associated protein MAP2 binds to porin, thereby linking the mitochondria to the cytoskeleton (36). Although disruption of microtubule structure enhances the MPT (37), it is possible that the opposite is true, that is, the MPT may disrupt the cytoskeleton, a structure that is, in turn, in intimate association with the plasma membrane. Alternatively, the MPT may release mitochondrial matrix proteins, such as mitochondrial phospholipase A2, that may directly damage the plasma membrane (38, 39).

**REFERENCES**

1. Hassouna, I., Wickert, H., Zimmerman, M., and Gillardon, F. (1996) Neuronsci. Lett. 204, 85–89
2. Yin, C., Knudson, C. M., Korsmeyer, S. J., and Van Dyke, T. (1997) Nature 385, 637–640
3. Reed, J. C. (1997) Nature 387, 773–776
4. Orrenius, S., Burgess, D. H., Hampton, M. B., and Zhivotovsky, B. (1997) Cell Death Differ. 4, 427–428
5. Zamzami, N., Hirsch, T., Dallaporta, B., Petit, P. X., and Kroemer, G. (1997) J. Bioenerg. Biomembr. 29, 185–193
6. Olivi, Z. N., Milliman, C. L., and Korsmeyer, S. J. (1997) Cell 74, 609–619
7. Zha, H., Aimé-Sempé, C., Takaaki, S., and Reed, J. C. (1996) J. Biol. Chem. 271, 7440–7444
8. Xiang, J., Gao, D. T., and Korsmeyer, S. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14559–14563
9. Bernardi, P., Broekemeier, K. M., and Pfeiffer, D. R. (1994) J. Bioenerg. Biomembr. 26, 509–517
10. Fournier, N., Ducet, G., and Crevet, A. (1987) J. Bioenerg. Biomembr. 19, 297–303
11. Crompton, M., Ellinger, H., and Costi, A. (1988) Biochem. J. 255, 357–360
12. Broekemeier, K. M., Dempsey, M. E., and Pfeiffer, D. R. (1988) J. Biol. Chem. 263, 7826–7830
13. Broekemeier, K. M., and Pfeiffer, D. R. (1995) Biochemistry 34, 16440–16449
14. Imberti, R., Nieminen, A. L., Herman, B., and Lemasters, J. J. (1992) Rev. Commum. Chem. Pathol. Pharmacol. 78, 27–38
15. Nicholson, D. W., Ali, A., Thornerby, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffin, P. R., Labelle, M., Lachouli, V. A., Munday, N. A., Raji, S. M., Smulson, M. E., Yamin, T.-T., Yu, V. L., and Miller, D. K. (1995) Nature 376, 37–43
16. Liu, J., Farmer, J. R., Jr., Lanne, W. S., Friedman, J., Weissman, I., and Schreiber, S. L. (1991) Cell 66, 807–815
17. Rao, A., Luo, C., and Hogan, P. G. (1997) Annu. Rev. Immunol. 15, 707–747
18. Pastorino, J. G., Simbula, G., Yamamoto, K., Glascott, P. A., Jr., Rothman, R. J., and Farber, J. L. (1996) J. Biol. Chem. 271, 29792–29798
19. Nieminen, A. L., Saylor, A. K., Tesfai, S. A., Herman, B., and Lemasters, J. J. (1995) Biochem. J. 307, 99–106
20. Vayssieres, J.-L., Petit, P. X., Risler, Y., and Mignotte, B. (1994) Proc. Natl.
The MPT in Bax-induced Cell Death

21. Krippner, A., Matsuno-Yagi, A., Gottlieb, R. A., and Babior, B. M. (1996) J. Biol. Chem. 271, 21629–21636
22. Wyllie, A. H. (1980) Nature 284, 555–556
23. Wyllie, A. H. (1985) Curr. Opin. Genet. Dev. 5, 97–104
24. Dubrez, L., Savoy, I., Hamman, A., and Solary, E. (1996) EMBO J. 15, 5594–5602
25. Kantrow, S. P., and Piantadosi, C. A. (1997) Biochem. Biophys. Res. Commun. 232, 669–671
26. Igbavboa, U., Zwizinski, C. W., and Pfeiffer, D. R. (1989) Biochem. Biophys. Res. Commun. 161, 619–625
27. Yang, J., Liu, X., Bhalla, K., Kim, C. N., Brado, A. M., Cai, J., Peng, T. I., Jones, D. P., and Wang, X. (1997) Science 275, 1129–1132
28. Zou, H., Hengel, W. J., Liu, X., Lutschg, A., and Wang, X. (1997) Cell 90, 405–413
29. Deleted in proof
30. Schendel, S. L., Xie, Z., MONTAL, M. O., Matsuyama, S., and MONTAL, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5113–5118
31. Muchmore, S. W., Sattler, M., Liang, H., Meadows, R. P., Harlan, J. E., Yoon, H.S., Nettesheim, D., Chang, B. S., Thompson, C. B., Wong, S. L., Ng, S. C., and Fesik, S. W. (1996) Nature 381, 335–341
32. Liu, X., Zou, H., and Wang, X. (1997) Cell 89, 175–184
33. Petronilli, V., Cola, C., Massari, S., Colonna, R., and Bernardi, P. (1993) J. Biol. Chem. 268, 21839–21845
34. Gunter, T. E., Gunter, K. K., Sheu, S. S., and Gavin, C. E. (1994) Am. J. Physiol. 267, C313–C339
35. Pastorino, J. G., Simbula, G., Gilfor, E., Hoek, J. B., and Farber, J. L. (1994) J. Biol. Chem. 269, 31041–31046
36. Lindem, M., and Karlsson, G. (1996) Biochem. Biophys. Res. Commun. 218, 823–836
37. Evtodienko, Y. V., Teplova, V. V., Sidash, S. S., Ichas, F., and Mazat, J. P. (1996) FEBS Lett. 333, 86–88
38. Schalkwijk, C. G., Marki, F., Wiesenber, I., and van den Bosch, H. (1991) J. Lipid Mediators 4, 83–96
39. Aarsman, A. J., de Jong, J. G. N., Arnoldussen, E., Neys, F. W., van Wasenaar, P. D., and Van den Bosch, H. (1989) J. Biol. Chem. 264, 10008–10014.
The Overexpression of Bax Produces Cell Death upon Induction of the Mitochondrial Permeability Transition

John G. Pastorino, Sing-Tsung Chen, Marco Tafani, Jack W. Snyder and John L. Farber

J. Biol. Chem. 1998, 273:7770-7775.
doi: 10.1074/jbc.273.13.7770

Access the most updated version of this article at http://www.jbc.org/content/273/13/7770

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 38 references, 12 of which can be accessed free at http://www.jbc.org/content/273/13/7770.full.html#ref-list-1