FEBS Letters 485 (2000) 104–108 FEBS 24298

Protection against oxidant-mediated lysosomal rupture: a new anti-apoptotic activity of Bcl-2?

Ming Zhao[a], John W. Eaton[a,b], Ulf T. Brunk[a,*]

[a] *Division of Pathology II, Faculty of Health Sciences, Linköping University, SE-581 85 Linköping, Sweden*
[b] *James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202, USA*

Received 13 October 2000; accepted 16 October 2000

First published online 3 November 2000

Edited by Barry Halliwell

Abstract Bcl-2 antagonizes apoptosis through mechanisms which are not completely understood. We have proposed that apoptosis is initiated by minor lysosomal destabilization followed some time later by secondary massive lysosomal rupture. In J774 cells over-expressing Bcl-2, early oxidant-induced lysosomal destabilization is unaffected but secondary lysosomal rupture and apoptosis are suppressed, despite the fact that wild-type and Bcl-2 over-expressing cells degrade hydrogen peroxide at similar rates. It may be that Bcl-2 directly blocks the effects of released lysosomal enzymes and/or prevents downstream activation of unknown cytosolic pro-enzymes by released lysosomal hydrolases, suggesting a new and heretofore unknown activity of Bcl-2.

Key words: Lysosome stability; Bcl-2; Oxidative stress; Apoptosis

1. Introduction

Bcl-2 suppresses cellular apoptosis engendered by oxidants and other agonists but the mechanisms of Bcl-2 action are not firmly established. Bcl-2 is associated with mitochondrial and other intracellular membranes [1–4] and belongs to a superfamily of mammalian protein homologs of the *Caenorhabditis elegans* 'Ced-9' proteins which also are involved in the control of programmed cell death [5]. Some earlier work suggests that, under oxidative stress, iron-catalyzed oxidation within the lysosomal compartment leads to lysosomal leak and rupture [6–9]. Minor lysosomal destabilization of this sort will trigger reparative autophagocytosis and restoration of normal cellular function. However, more marked lysosomal leakage and rupture is associated with apoptotic and, in extremis, necrotic cell death [7,10–17].

Interestingly, early and minor lysosomal leak caused by oxidative stress will cause further lysosomal destabilization and, ultimately, apoptosis which occurs long after removal of the oxidant stress [15,17–19]. The intimate involvement of lysosomal leakage in apoptosis is emphasized by the finding that the release of mitochondrial cytochrome *c*, generally considered to be an important distal event in the occurrence of apoptosis, occurs secondary to this lysosomal destabilization [20,21]. Therefore, the release of lysosomal hydrolytic enzymes into the cytosol may be a key event, leading to proteolytic activation of pro-caspases [13,17] and/or other cytosolic lytic pro-enzymes. These early events then might lead to further lysosomal destabilization and direct attack on mitochondria, with consequent cytochrome *c* release. This scenario would be consistent with the aforementioned progressive lysosomal rupture occurring long after removal of the oxidant stress.

We have therefore sought to determine whether Bcl-2 might somehow act to interrupt this sequence of events. Surprisingly, in J774 cells over-expressing Bcl-2, the early iron-catalyzed lysosomal destabilization caused by oxidants is unaffected. However, Bcl-2 over-expression effectively prevents the later lysosomal destabilization and, expectably, apoptosis. These findings raise the possibility that Bcl-2 somehow blocks downstream events that result in the later phase of lysosomal rupture, and that this activity may be involved in the still mysterious mode of action of this anti-apoptotic protein.

2. Materials and methods

2.1. Materials

Chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise indicated. RPMI 1640 medium, fetal bovine serum, glutamine, penicillin and streptomycin were obtained from Gibco BRL (Grand Island, NY, USA). The polyclonal antibodies against Bcl-2 (sc-783) and monoclonal antibody against β-actin (sc-8432) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA), while the enhanced chemiluminescence Western blotting reagents were obtained from Amersham Pharmacia Biotech (Stockholm, Sweden). The CaspACE FITC-VAD-FMK In Situ Marker® was purchased from Promega Biosciences (San Luis Obispo, CA, USA). DOTAP was from Boehringer Mannheim (Indianapolis, IN, USA).

2.2. Cell cultures

J774 cells (a murine histiocytic lymphoma cell line) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin and 1% non-essential amino acids. Cells were maintained in plastic flasks and subcultivated at confluence twice a week. Medium was changed daily. For experiments, cells were transferred into 35 mm six -well plates (5×10^5 cells/well) 24 h before use.

2.3. Transfection assays

J774 cells were subcultivated and grown in six-well plates (5×10^5 cells/well) as described above. The cells were transfected with mBCL-2α/pMKITNeo expression plasmids [22], which contain a neomycin resistance element, using liposomal delivery by DOTAP according to the manufacturer's directions. Ten clones of stably transfected cells were selected by neomycin resistance, and their expression levels of Bcl-2 were assayed by Western blot analysis (vide infra). Compared to the wild-type J774 cells, three clones of the transfected cells had a five-fold increase of immunoreactive Bcl-2 protein. These clones were expanded, mixed and used for experiments. The Bcl-2 over-expressing J774 cell line hereafter is termed 'J774-Bcl-2'.

*Corresponding author. Fax: (46)-13-22 15 29.
E-mail: ulf.brunk@pat.liu.se

PII: S0014-5793(00)02195-5

2.4. Western blot analysis

Approximately 10^6 J774 and J774-Bcl-2 were lysed in a buffer containing 20 mM Tris–HCl, 120 mM NaCl, 10% glycerol, 1 mM Na_3VO_4, 2 mM EDTA, 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride, pH 7.5. Whole cell lysates were separated on 12% SDS–PAGE gels and transferred onto an Immobilon® membrane for 2 h at 200 mA and 4°C. Filters then were incubated at room temperature for 1 h in blocking buffer (5% low-fat milk powder in Tris-buffered saline (TBS)) and for another 2 h in dilution buffer (0.5% low-fat milk powder in TBS) containing a 1:500 dilution of the polyclonal anti-Bcl-2 antibodies, or a 1:1000 dilution of the monoclonal antibody against β-actin. After washing in TBS with 0.06% Tween 20, Immobilon membranes were incubated for 1 h at room temperature in a buffer containing a 1:1500 dilution of the peroxidase-conjugated secondary antibody. After washing, peroxidase-dependent chemiluminescence was detected employing enhanced chemiluminescence Western blotting reagents and hyperfilm according to the manufacturer's instructions.

2.5. Hydrogen peroxide assay

Hydrogen peroxide (H_2O_2) concentration was assayed as described previously [23]. Briefly, 50 μl of sample was reacted with 3 ml α-hydroxylphenylacetic acid (40 μM) and horseradish peroxidase (5.0 U/ml) in buffer (0.1 M KPO_4, pH 7.4) for 5 min at 37°C. Then the system was incubated at room temperature for an additional 5 min. The fluorescent product was measured using a Shimadzu RF-540 spectrofluorophotometer with excitation at 315 nm and emission at 410 nm.

2.6. Pretreatment of cells with desferrioxamine (Des)

In some experiments, J774 cells were pre-treated with Des for 1 h under otherwise normal culture conditions by exposure to pre-warmed (37°C) complete medium containing 1 mM Des (final concentration). The cells then were incubated under standard culture conditions (without Des) for another 30 min and, finally, exposed to H_2O_2 as described below.

2.7. Exposure to H_2O_2

Wild-type J774 cells and J774-Bcl-2 cells (5×10^5 in 2 ml of complete medium), cultured in six-well plates as described above, were exposed for 1 h at 37°C to a steady-state concentration (40 μM) of H_2O_2 as described previously [24]. To abruptly obtain this steady-state concentration of H_2O_2, 1.5 μg/ml glucose oxidase was added together with H_2O_2 in an amount to give 45 μM. Following 1 h incubation, under otherwise standard culture conditions, catalase was added to clear the H_2O_2. The cells were then quickly rinsed with sterile phosphate-buffered saline (PBS), and fresh complete medium was added.

2.8. Lysosomal stability assessment

Cells were assessed for lysosomal stability using the acridine orange (AO) uptake and relocation methods [25]. AO is a lysosomotropic base and a metachromatic fluorochrome exhibiting red fluorescence when highly concentrated (as is the case in intact lysosomes where AO is retained in its charged, AOH^+, form) and green fluorescence at low concentration (as is the case when some lysosomes have ruptured and AO relocates to the cytosol where it occurs predominantly in the deprotonated form). The number of remaining intact lysosomes may be evaluated by assaying red fluorescence (AO uptake method) following AO staining of the cells after exposure to oxidative stress. Because of the much higher sensitivity of the photomultiplier to green than to red photons, this method is far less sensitive than following AO relocation within cells stained with AO before oxidant exposure (AO relocation method). In order to detect early and minor lysosomal destabilization, we therefore used the AO relocation method, while the AO uptake method was used to assay later and more pronounced lysosomal rupture.

In all AO experiments, AO fluorescence was measured by flow cytometry using a Becton-Dickinson FACScan (Becton-Dickinson, Mountain View, CA, USA) equipped with a 488 nm argon laser. In AO uptake experiments, following oxidant exposure cells within individual wells (5×10^5) were briefly stained with 5 μg/ml AO for 15 min under otherwise standard culture conditions and then rinsed in complete medium (×2). The cells then were detached by scraping with a rubber policeman and collected for flow cytometric assessment of red (uptake, FL3 channel) or green (relocation, FL1 channel) AO fluorescence. In the case of relocation studies, AO staining was done before exposure (5–60 min) to oxidative stress, whereas uptake studies were done on cells which first were exposed to 60 min of oxidative stress, returned to standard culture conditions for up to 12 h and then briefly stained with AO as described above. CellQuest software was used to analyze the data.

2.9. Apoptosis assessment assay

The DNA fragmentation (Nicoletti) assay is based on propidium iodide staining of nuclear DNA and was performed essentially as described elsewhere [26]. Briefly, the cell pellets from individual wells, prepared as described above, were gently resuspended in 1.5 ml of a hypotonic and membrane-disrupting solution of propidium iodide (50 μg/ml in 0.1% sodium citrate with 0.1% Triton X-100) in 12×75 mm polypropylene tubes. The tubes were kept at 4°C in the dark overnight before the flow-cytometric analyses. The propidium iodide-induced red fluorescence of suspended individual nuclei was measured using the FL3 channel for red fluorescence.

Apoptosis also was evaluated by microscopical analysis of living cells, initially stained according to the manufacturer's directions with a FITC-conjugated broad spectrum inhibitor of caspases, CaspACE™ FITC-VAD-FMK In Situ Marker®, that irreversibly binds to activated caspases. Briefly, J774 cells were seeded at 5×10^5 cells/ml, exposed to H_2O_2 as described above, and then incubated under standard culture conditions for another 4 h. The marker was added to the medium at a final concentration of 10 μM. The cells then were incubated in the dark for 20 min, rinsed three times in PBS (5 min in total), and observed, counted and photographed using a Nikon fluorescence microscope.

2.10. Statistical analysis

All experiments were repeated three times. The values are given as arithmetic means ± S.D. Statistical significance was determined using

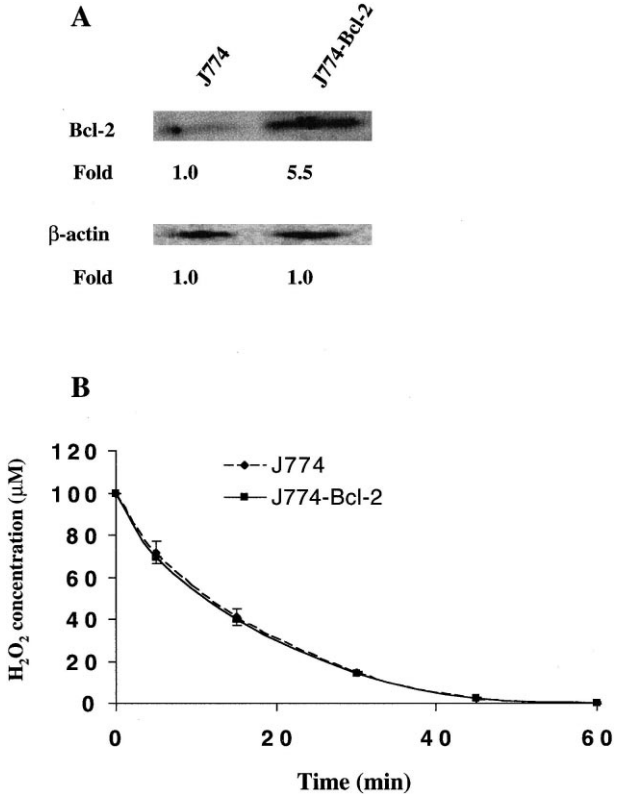

Fig. 1. Bcl-2 over-expression in J774 cells and lack of effect of that over-expression on hydrogen peroxide degradation. A: Western blots for Bcl-2 reflecting a five-fold increase for the transfected cells. β-actin was used as a loading control. B: Wild-type and Bcl-2 over-expressing J774 (10^6) cells were exposed to a bolus dose of H_2O_2 and residual H_2O_2 was measured at the indicated times. Each point represents the mean ± S.D. of triplicate determinations.

Student's two-tailed *t*-test. A *P*-value ≤ 0.05 was considered significant.

3. Results

The relative levels of Bcl-2 expression by J774 and J774-Bcl-2 cells are shown in Fig. 1A, with actin used as a loading control. To ensure that any differences between wild-type and Bcl-2 over-expressing cells observed in response to H_2O_2 were not due to changes in H_2O_2 catabolism, we measured H_2O_2 clearance following the addition of a bolus dose of 100 μM H_2O_2 to 10^6 cells. As shown in Fig. 1B, clearance of H_2O_2 by J774 cells was unaffected by Bcl-2 over expression. This excludes the possibility that the Bcl-2 transfected cells had somehow gained an enhanced capacity to degrade H_2O_2 (for example by up-regulating catalase and/or glutathione peroxidase).

During the initial 5–15 min of exposure of the cells to steady-state 40 μM H_2O_2, there was no significant difference between wild-type and Bcl-2 transfected cells with respect to early lysosomal rupture (as assayed by an increase in cytosolic green fluorescence secondary to the leak of AO from damaged lysosomes). However, distinct differences were noted with respect to later lysosomal rupture at 30 and 60 min (Fig. 2) and at 8–12 h when lysosomal rupture was assayed by the numbers of 'pale' cells (having reduced numbers of intact lysosomes) (Fig. 3A,B). The lysosomes of cells pre-exposed to the potent iron chelator, Des, were completely stabilized (Fig. 2). Des appears to enter cells largely by endocytosis and, thus, ends up almost exclusively in the lysosomal compartment [27–30], suggesting that intra-lysosomal iron-catalyzed oxidative reactions may be an important factor in oxidative lysosomal destabilization. The similarity between wild-type and J774-Bcl-2 during the early phases of oxidative stress suggests that Bcl-2 – unlike Des – is not protecting against early iron-catalyzed intra-lysosomal oxidation, but rather against some secondary event(s) which leads to later lysosomal destabilization and rupture.

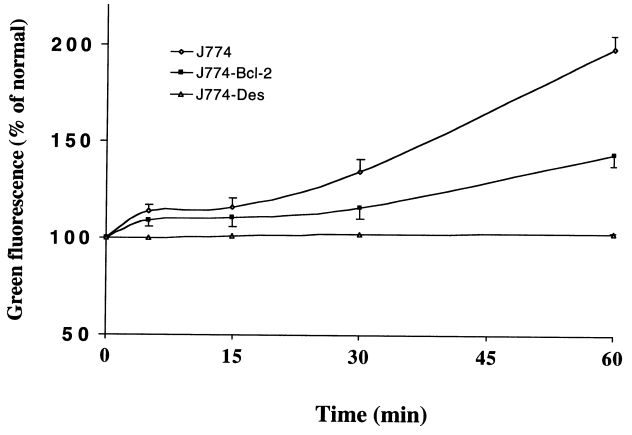

Fig. 2. Lysosomal rupture is lessened at 30–60 min of hydrogen peroxide exposure in J774 cells over-expressing Bcl-2. Wild-type and Bcl-2 over-expressing cells were pre-stained with AO as described in Section 2 and then exposed to a steady-state concentration of H_2O_2 for the indicated periods of time. In some experiments, J774 cells were pre-treated with 1 mM Des. Green AO-induced fluorescence was analyzed as described in Section 2. The results are presented as percentage of values before initiation of oxidative stress. Each point represents the mean ± S.D. of triplicate determinations. All points for the Des-curve are statistically different ($P < 0.01$) from each of those for the J774 and J774-Bcl-2, while only the time points beyond 15 min differ between J774 and J774-Bcl-2 ($P < 0.01$).

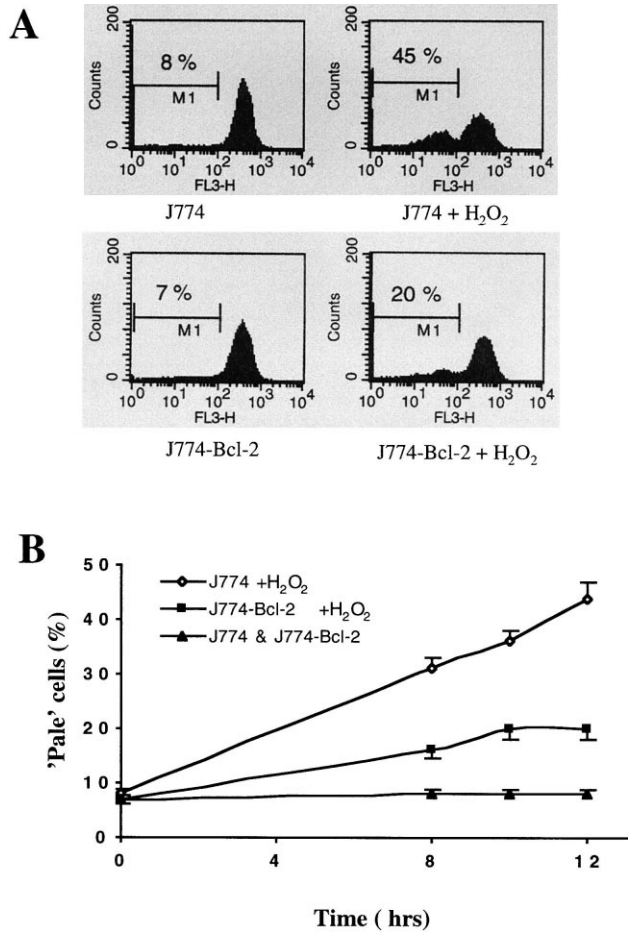

Fig. 3. Bcl-2 over-expressing J774 cells exhibit enhanced lysosomal stability 12 h after oxidant exposure. Wild-type and Bcl-2 over-expressing cells were exposed to a steady-state concentration of H_2O_2 for 1 h. Cells then were returned to normal culture conditions for the indicated periods of time, AO stained and assayed for red fluorescence as described in Section 2. A: Flow cytofluorometric analysis of the percentage of 'pale' cells (i.e. deficient in intact lysosomes) following 1 h of exposure to oxidative stress and another 12 h under normal culture conditions. B: Quantitation of the results shown in A. Each point represents the mean ± S.D. of triplicate determinations. All values shown in A and B for oxidative stress-exposed J774 vs J774-Bcl-2 (except '0' time) are significantly different ($P < 0.001$), while there are no differences between the unexposed cells.

When the frequency of apoptosis was assessed 12 h after exposure to oxidative stress (Fig. 4), the number of apoptotic cells corresponded closely to the number of 'pale' cells. It should be pointed out (see Fig. 3A) that some 'pale' cells have lost few lysosomes, while others are almost devoid of these organelles. The latter cells probably are post-apoptotic necrotic. Roughly twice as many wild-type J774 cells were apoptotic/post apoptotic compared to the J774-Bcl-2.

4. Discussion

Oxidative stress-induced apoptosis is suppressed by over-expression of the anti-apoptotic protein, Bcl-2, but the mechanisms involved are not well understood [1,31,32]. The Bcl-2

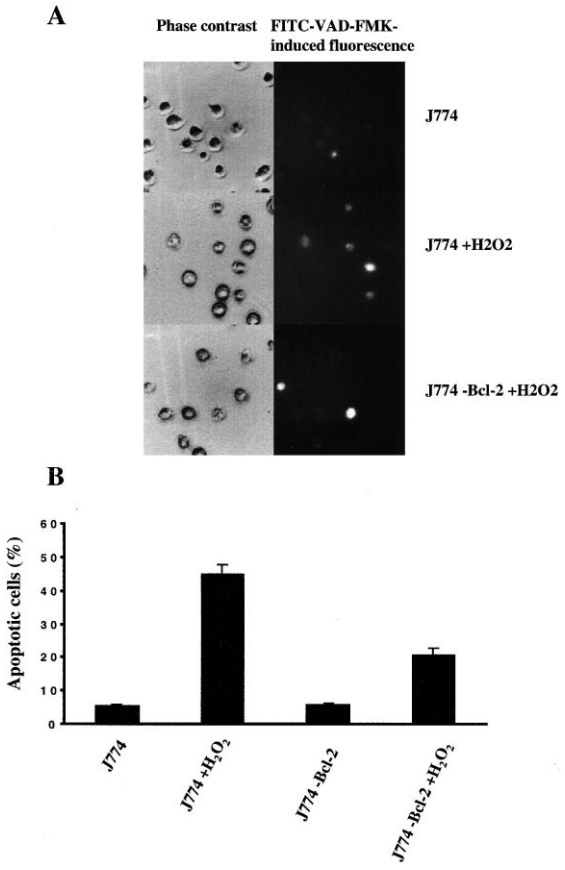

Fig. 4. Bcl-2 over-expressing J774 cells are less susceptible than wild-type cells to oxidative stress-induced apoptosis. Wild-type and Bcl-2 over-expressing cells were exposed to a steady-state concentration of H_2O_2 for 1 h and then returned to normal culture conditions for another 12 h. The cells were then (A) stained with CaspACE™ FITC-VAD-FMK In Situ Marker® or (B) assayed for apoptosis by the Nicoletti assay as described in Section 2. Results shown in (B) represent the mean ± S.D. of triplicate determinations. The difference between J774+H_2O_2 and J774-Bcl-2+H_2O_2 is significant at $P < 0.001$. ←

family presently consists of the anti-apoptotic proteins Bcl-2 and Bcl-X_L, but also contains pro-apoptotic proteins such as Bax, Bik and Bak [33,34]. Bcl-2 is active as an anti-apoptotic protein only in its phosphorylated form and when dimerized or complexed with Bcl-X_L. Thus, it is activated by protein kinase C and inactivated by protein phosphatase 2 or when complexed with Bax, Bik or Bad [22,35–40]. It has been suggested that Bcl-2 might act either as an anti-oxidant [41] and/or a regulator of so-called mitochondrial permeability transition pores [5,42–44]. In the latter case, Bcl-2 might suppress the release of apoptosis-inducing cytochrome *c*, perhaps by controlling ion fluxes between cellular compartments.

The findings of the present study may suggest a completely new function for Bcl-2, namely prevention of the labilization of lysosomes that occurs secondary to oxidant interactions with intra-lysosomal iron. As indicated by the present results, and the hypothetical scheme shown in Fig. 5, early oxidative lysosomal rupture appears to lead to the eventual release of lysosomal hydrolytic enzymes. These destructive enzymes may then be sufficient to induce apoptosis, either by direct activation of pro-caspases or by attack on mitochondrial and lysosomal membranes, resulting in release of pro-apoptotic cytochrome *c* and more lysosomal enzymes. Alternatively, it may be that these lysosomal hydrolytic enzymes activate as-yet undefined cytosolic factors which, in turn, attack both lysosomes and mitochondria, causing apoptosis by further enhancement of lysosomal and/or mitochondrial permeability.

Of possible relevance to our observations are scattered earlier reports suggesting a link between the activation of phospholipase A2 and apoptosis [45,46]. Furthermore, inhibitors of cytosolic phospholipases (A2 and C) have been shown to prevent apoptotic cell death [46,47], raising the intriguing possibility that Bcl-2 might act, directly or indirectly, to prevent

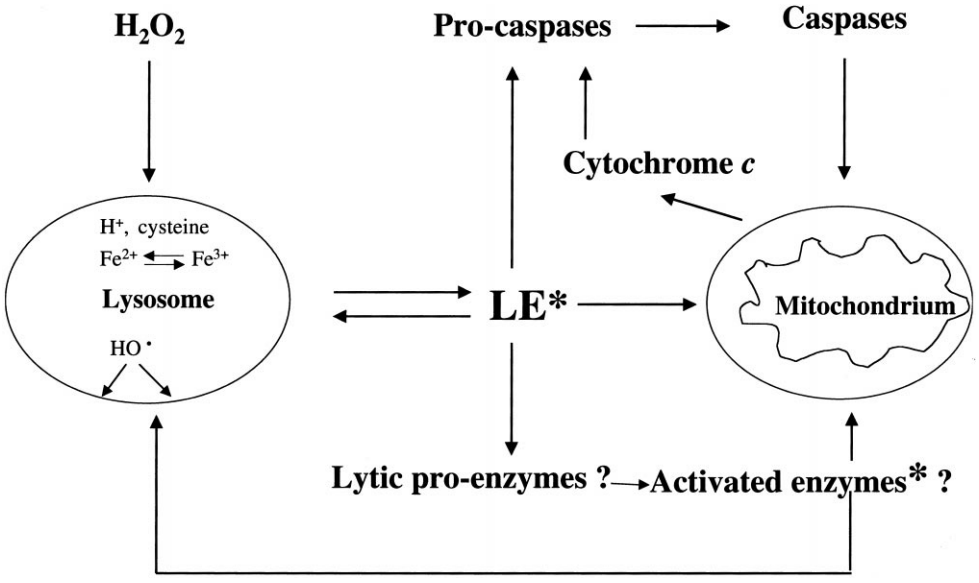

Fig. 5. The lysosomal-mitochondrial axis theory of oxidative stress-induced apoptosis. Intralysosomal, iron-catalyzed oxidation leads to oxidative lysosomal rupture secondary to production of hydroxyl radicals. Some released lysosomal enzymes (LE) may activate pro-caspases as well other cytosolic pro-enzymes. Together, these may attack mitochondrial and lysosomal membranes, causing release to the cytosol of cytochrome *c* and of additional LE. Bcl-2 may block the later stages, perhaps by acting at indicated (*) steps, although not the first phase of LE release.

phospholipase-mediated destabilization of lysosomal and mitochondrial membranes. Regardless of whether this speculation is correct, it is clear from the present results that Bcl-2 suppresses delayed lysosomal rupture and that this is associated with protection of cells against apoptotic cell death following exposure to low dose oxidant stress. Inasmuch as Bcl-2 over expression does not affect the ability of cells to catabolize H_2O_2, these results suggest a novel, but presently undefined, anti-apoptotic function of Bcl-2.

Acknowledgements: This study was supported by Grants from the Swedish Cancer Society (4296) and the Swedish Medical Research Council (4481). J.W.E. was the recipient of a visiting professorship from the Linköping University Hospital.

References

[1] Hockenbery, D., Nunez, G., Milliman, C., Schreiber, R.D. and Korsmeyer, S.J. (1990) Nature 348, 334–336.
[2] Monaghan, P., Robertson, D., Amos, T.A., Dyer, M.J., Mason, D.Y. and Greaves, M.F. (1992) J. Histochem. Cytochem. 40, 1819–1825.
[3] Monney, L., Olivier, R., Otter, I., Jansen, B., Poirier, G.G. and Borner, C. (1998) Eur. J. Biochem. 251, 295–303.
[4] Zhu, W., Cowie, A., Wasfy, G.W., Penn, L.Z., Leber, B. and Andrews, D.W. (1996) EMBO J. 15, 4130–4141.
[5] Shimizu, S., Narita, M. and Tsujimoto, Y. (1999) Nature 399, 483–487.
[6] Ollinger, K. and Brunk, U.T. (1995) Free Radic. Biol. Med. 19, 565–574.
[7] Brunk, U.T., Zang, H., Roberg, K. and Öllinger, K. (1995) Redox Rep. 1, 267–277.
[8] Garner, B., Li, W., Roberg, K. and Brunk, U.T. (1997) Free Radic. Res. 27, 487–500.
[9] Garner, B., Roberg, K. and Brunk, U.T. (1998) Free Radic. Res. 29, 103–114.
[10] Zdolsek, J., Zhang, H., Roberg, K. and Brunk, U. (1993) Free Radic. Res. Commun. 18, 71–85.
[11] Brunk, U.T., Zhang, H., Dalen, H. and Öllinger, K. (1995) Free Radic. Biol. Med. 19, 813–822.
[12] Hellquist, H.B., Svensson, I. and Brunk, U.T. (1997) Redox Rep. 3, 65–70.
[13] Brunk, U.T., Dalen, H., Roberg, K. and Hellquist, H.B. (1997) Free Radic. Biol. Med. 23, 616–626.
[14] Li, W., Yuan, X.M., Olsson, A.G. and Brunk, U.T. (1998) Arterioscler. Thromb. Vasc. Biol. 18, 177–184.
[15] Brunk, U.T. and Svensson, I. (1999) Redox Rep. 4, 3–11.
[16] Neuzil, J., Svensson, I., Weber, T., Weber, C. and Brunk, U.T. (1999) FEBS Lett. 445, 295–300.
[17] Li, W., Yuan, X., Nordgren, G., Dalen, H., Dubowchik, G.M., Firestone, R.A. and Brunk, U.T. (2000) FEBS Lett. 470, 35–39.
[18] Olejnicka, B.T., Dalen, H. and Brunk, U.T. (1999) APMIS 107, 747–761.
[19] Kessel, D., Luo, Y., Mathieu, P. and Reiners Jr., J.J. (2000) Photochem. Photobiol. 71, 196–200.
[20] Roberg, K., Johansson, U. and Öllinger, K. (1999) Free Radic. Biol. Med. 27, 1228–1237.
[21] Roberg, K. and Öllinger, K. (1998) Am. J. Pathol. 152, 1151–1156.
[22] Ito, T., Deng, X., Carr, B. and May, W.S. (1997) J. Biol. Chem. 272, 11671–11673.
[23] Hinsberg, W.D., Milby, K.H. and Zare, R.N. (1981) Anal. Chem. 53, 1509–1512.
[24] Antunes, F. and Cadenas, E. (2000) FEBS Lett. 475, 121–126.
[25] Zdolsek, J.M., Olsson, G.M. and Brunk, U.T. (1990) Photochem. Photobiol. 51, 67–76.
[26] Nicoletti, I., Migliorati, G., Pagliacci, M.C., Grignani, F. and Riccardi, C. (1991) J. Immunol. Methods 139, 271–279.
[27] Marzabadi, M.R., Sohal, R.S. and Brunk, U.T. (1988) Mech. Ageing Dev. 46, 145–157.
[28] Lloyd, J.B., Cable, H. and Rice-Evans, C. (1991) Biochem. Pharmacol. 41, 1361–1363.
[29] Olejnicka, B.T., Ollinger, K. and Brunk, U.T. (1997) APMIS 105, 689–698.
[30] Cable, H. and Lloyd, J.B. (1999) J. Pharm. Pharmacol. 51, 131–134.
[31] Yin, X.M., Oltvai, Z.N., Veis-Novack, D.J., Linette, G.P. and Korsmeyer, S.J. (1994) Cold Spring Harb. Symp. Quant. Biol. 59, 387–393.
[32] Desagher, S. and Martinou, J.C. (2000) Trends Cell Biol. 10, 369–377.
[33] Tao, W., Kurschner, C. and Morgan, J.I. (1997) J. Biol. Chem. 272, 15547–15552.
[34] McDonnell, T.J., Beham, A., Sarkiss, M., Andersen, M.M. and Lo, P. (1996) Experientia 52, 1008–1017.
[35] Xie, Z., Schendel, S., Matsuyama, S. and Reed, J.C. (1998) Biochemistry USA 37, 6410–6418.
[36] Ruvolo, P.P., Deng, X., Ito, T., Carr, B.K. and May, W.S. (1999) J. Biol. Chem. 274, 20296–20300.
[37] Yin, X.M., Oltvai, Z.N. and Korsmeyer, S.J. (1994) Nature 369, 321–323.
[38] Hanada, M., Aime-Sempe, C., Sato, T. and Reed, J.C. (1995) J. Biol. Chem. 270, 11962–11969.
[39] Boyd, J.M., Gallo, G.J., Elangovan, B., Houghton, A.B., Malstrom, S., Avery, B.J., Ebb, R.G., Subramanian, T., Chittenden, T. and Lutz, R.J. (1995) Oncogene 11, 1921–1928.
[40] Yang, E., Zha, J., Jockel, J., Boise, L.H., Thompson, C.B. and Korsmeyer, S.J. (1995) Cell 80, 285–291.
[41] Hockenbery, D.M., Oltvai, Z.N., Yin, X.M., Milliman, C.L. and Korsmeyer, S.J. (1993) Cell 75, 241–251.
[42] Jacotot, E., Costantini, P., Laboureau, E., Zamzami, N., Susin, S.A. and Kroemer, G. (1999) Ann. N. Y. Acad. Sci. 887, 18–30.
[43] Schlesinger, P.H., Gross, A., Yin, X.M., Yamamoto, K., Saito, M., Waksman, G. and Korsmeyer, S.J. (1997) Proc. Natl. Acad. Sci. USA 94, 11357–11362.
[44] Narita, M., Shimizu, S., Ito, T., Chittenden, T., Lutz, R.J., Matsuda, H. and Tsujimoto, Y. (1998) Proc. Natl. Acad. Sci. USA 95, 14681–14686.
[45] Jaattela, M., Benedict, M., Tewari, M., Shayman, J.A. and Dixit, V.M. (1995) Oncogene 10, 2297–2305.
[46] Wu, Y.L., Jiang, X.R., Lillington, D.M., Allen, P.D., Newland, A.C. and Kelsey, S.M. (1998) Cancer Res. 58, 633–640.
[47] Kishida, E., Yano, M., Kasahara, M. and Masuzawa, Y. (1998) Biochim. Biophys. Acta 1391, 401–408.