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Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated Ca^{2+} fluxes

(programmed cell death/glucocorticosteroid/mouse lymphoma cell/calcium homeostasis/thapsigargin)

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ABSTRACT **BCL-2 is a 26-kDa integral membrane protein that represses apoptosis by an unknown mechanism. Recent findings indicate that Ca^{2+} release from the endoplasmic reticulum (ER) mediates apoptosis in mouse lymphoma cells. In view of growing evidence that BCL-2 localizes to the ER, as well as mitochondria and the perinuclear membrane, we investigated the possibility that BCL-2 represses apoptosis by regulating Ca^{2+} fluxes through the ER membrane. A cDNA encoding BCL-2 was introduced into WEHI7.2 cells and two subclones, W.Hb12 and W.Hb13, which express high and low levels of BCL-2 mRNA and protein, respectively, were isolated. WEHI7.2 cells underwent apoptosis in response to treatment with the glucocorticoid hormone dexamethasone, whereas W.Hb12 and W.Hb13 cells were protected from apoptosis, revealing a direct relationship between the level of BCL-2 expression and the degree of protection. Significantly, BCL-2 also blocked induction of apoptosis by thapsigargin (TG), a highly specific inhibitor of the ER-associated Ca^{2+} pump. TG completely inhibited ER Ca^{2+} pumping in both WEHI7.2 and W.Hb12 cells, but the release of Ca^{2+} into the cytosol after inhibition of ER Ca^{2+} pumping was significantly less in W.Hb12 cells than in WEHI7.2 cells, indicating that BCL-2 reduces Ca^{2+} efflux through the ER membrane. By reducing ER Ca^{2+} efflux, BCL-2 interfered with a signal for "capacitative" entry of extracellular Ca^{2+}, preventing a sustained increase of cytosolic Ca^{2+} in TG-treated cells. These findings suggest that BCL-2 either directly or indirectly regulates the flux of Ca^{2+} across the ER membrane, thereby abrogating Ca^{2+} signaling of apoptosis.**

Apoptosis, or programmed cell death, is a naturally occurring form of cell death important for the proper development and homeostasis of many tissues (1, 2). An emerging concept of considerable importance is that apoptosis is a physiologically regulated process that, along with cell proliferation, controls tissue homeostasis (2–4). In the nematode *Caenorhabditis elegans*, induction of cell death by the *ced-3* and *ced-4* gene products can be inhibited by the product of the *ced-9* gene (5), while in mammals apoptosis can often be inhibited by expression of the *Bcl-2* gene (6–9).

The *Bcl-2* gene encodes a 26-kDa integral membrane protein (10) that has been localized both to mitochondria (11) and to the endoplasmic reticulum (ER) and continuous perinuclear membrane (12–16). The mechanism of action of BCL-2 is not known, but neither apoptosis nor the protective effect of BCL-2 appears to depend on mitochondrial respiration, raising the possibility that the BCL-2 protein might exert its protective effect through an association with the ER or perinuclear membrane (15).

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In the present report, we have investigated the possibility that the BCL-2 protein might block a critical step in the signal transduction pathway of apoptosis by interfering with efflux of "second messenger" Ca^{2+} from a mobilizable pool located within the ER lumen. The concept that ER Ca^{2+} release mediates apoptosis was recently suggested by two findings: (*i*) Glucocorticoid treatment mobilizes ER Ca^{2+} in mouse lymphoma cells, resulting in a sustained depletion of the ER Ca^{2+} pool that is detected before the onset of DNA fragmentation and apoptotic cell death (17). (*ii*) In an interleukin 3 (IL-3)-dependent hematopoietic cell line, withdrawal of IL-3 induces apoptosis associated with a diminution of the ER Ca^{2+} pool, while the ER Ca^{2+} pool is preserved in cells protected from apoptosis by BCL-2 (18).

We have used thapsigargin (TG) to directly assess the role of ER Ca^{2+} release in apoptosis (17). TG, a sesqueterpene lactone derived from the plant *Thapsia garganica*, is a highly specific and essentially irreversible inhibitor of the ER-associated Ca^{2+}-ATPase that pumps Ca^{2+} across a steep concentration gradient from the cytosol into the ER lumen (19–23). Because of a leak of Ca^{2+} through an unidentified channel in the ER membrane, inhibition of Ca^{2+} pumping by TG induces an efflux of Ca^{2+} from the ER lumen into the cytosol, producing an increase in cytosolic free Ca^{2+} concentration and a sustained depletion of the ER Ca^{2+} pool (19–22). We have found that treating mouse lymphoma cells with TG induces apoptosis, providing direct evidence that Ca^{2+} mobilization from the ER triggers the apoptotic process (17). In experiments described in this report, we show that overexpression of BCL-2 in mouse lymphoma cells reduces the TG-induced efflux of ER Ca^{2+}, thereby interfering with Ca^{2+} signaling and preventing apoptosis.

MATERIALS AND METHODS

Materials. Fura-2 AM was from Molecular Probes. Steroid hormones and other chemicals were from Sigma. TG was from LC Services (Woburn, MA). $^{45}Ca^{2+}$ was from ICN.

Stable Transfections. WEHI7.2 cells were transfected with the human *BCL2*-containing expression vector SFFV-neo (24) by electroporation, followed by selection and soft agar cloning in the presence of G418 (Geneticin, GIBCO) (25). Ten separate G418-resistant cell lines were analyzed for *BCL2* expression by Northern blot analysis (25) and Western blots (24) as described. Two representative cell lines, W.Hb12 and W.Hb13, were chosen for further analysis based on their relatively high and low levels of BCL-2, respectively. As a

Abbreviations: ER, endoplasmic reticulum; TG, thapsigargin; Dex, dexamethasone.

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control, WEHI7.2 cells were transfected with the pMAMneo vector (Clontech) followed by selection and subcloning in the presence of G418.

Cell Culture and Viability Assays. Cells were cultured at a density of 0.2–1.5 × 10^6 cells per ml as described (17). Stock solutions of dexamethasone (Dex) in absolute ethanol were diluted into tissue culture medium and then added to cell suspensions; untreated cells received an equivalent dilution of ethanol. Stock solutions of 10 μM TG in dimethyl sulfoxide (DMSO) were diluted into tissue culture medium and then added to cell suspensions; untreated cells received an equivalent dilution of DMSO. Cells were counted on a hemocytometer after suspension in trypan blue dye. Cell viability was defined by the ability of cells to exclude dye.

Intracellular Ca^{2+} Measurements. Cytosolic Ca^{2+} concentration was measured by fluorometry of fura-2-loaded cells as described (17).

Ca^{2+} Flux Measurements. The rate of $^{45}Ca^{2+}$ uptake into the ER of digitonin-permeabilized cells was measured at 37°C in the presence of 120 mM KCl/30 mM imidazole, pH 6.8/5 mM $MgCl_2$/5 mM ATP/10 mM NaN_3/4 mM oxalic acid/$^{45}CaCl_2$ buffered to 150 nM free $^{45}Ca^{2+}$ (8.9 mCi/mg; 1 Ci = 37 GBq) with EGTA as described (26, 27). Uptake was stopped at periodic intervals by placing aliquots of the cell suspension in buffer containing 140 mM KCl, 10 mM NaCl, 2.5 mM $MgCl_2$, 10 mM Hepes·KOH (pH 7.0), and 1 mM $LaCl_3$, followed by collection on glass fiber filters. To measure the uptake of Ca^{2+} into the non-ER, predominantly mitochondrial pool, the preceding methods were followed except that azide was deleted from buffers, the concentration of ATP was lowered to 1 mM, and free Ca^{2+} concentration was 12 μM, unbuffered by EGTA.

RESULTS

A human *BCL2* cDNA was introduced into the WEHI7.2 mouse lymphoma line and a series of clones stably expressing the BCL-2 mRNA and protein were isolated. The endogenous level of BCL-2 message in WEHI7.2 cells is <10% of that in stably transfected cells. The clones used in this study, W.Hb12 and W.Hb13, express high and low levels of human BCL2 mRNA and protein, respectively (Fig. 1).

Overexpression of BCL-2 interfered with Dex-induced growth inhibition and cell death in WEHI7.2 cells (Fig. 2), consistent with earlier evidence that BCL-2 blocks glucocorticoid-induced apoptosis in lymphoid cells (28–30). Significantly, BCL-2 overexpression also interfered with TG-induced growth inhibition and cell death (Fig. 2). The high level of BCL-2 in W.Hb12 cells afforded greater protection against loss of viability after treatment with either Dex or TG than the lower level of BCL-2 in W.Hb13 cells, indicating a

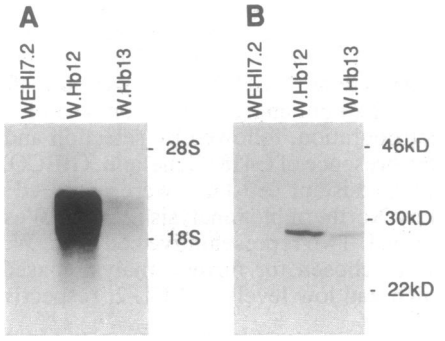

FIG. 1. Analysis of BCL-2 expression levels in WEHI7.2 cells and stable transfectants. (*A*) Northern blot analysis of total RNA probed with *BCL-2* cDNA sequences. (*B*) Western blot analysis of protein extracts using a monoclonal antibody against human BCL2.

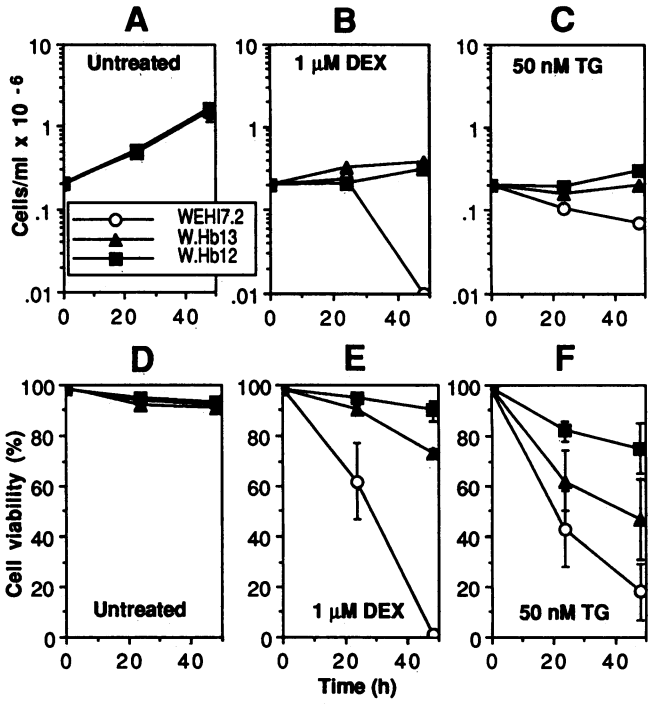

FIG. 2. Effect of BCL-2 on Dex- and TG-induced suppression of cell growth and viability. Exponentially growing cells were incubated with or without Dex or TG for 48 h. Viable cell concentrations (*A–C*) and percentage viable cells (*D–F*) were determined at periodic intervals. Symbols represent means ± SE of four separate experiments (error bars are hidden by symbols in *B* and *C*).

direct dose–response relationship between the level of BCL-2 and protection from apoptosis. Interestingly, the high level of BCL-2 in W.Hb12 cells permitted significant repression of cell proliferation in response to Dex and TG treatment with little loss of cell viability. This observation is consistent with earlier evidence that BCL-2 confers greater protection against Dex-induced cell death than Dex-induced suppression of cell proliferation (30).

The data in Fig. 2 reveal a striking parallel in terms of the effect of BCL-2 on Dex- and TG-induced growth inhibition and cell death, suggesting that BCL-2 may block a signal transduction pathway that is used by both Dex and TG to induce apoptosis. To directly test whether BCL-2 interferes with mobilization of ER Ca^{2+}, we compared the ability of TG to induce Ca^{2+} release from the ER in WEHI7.2 and W.Hb12 cells (Fig. 3). To specifically measure ER Ca^{2+} release, without interference from extracellular Ca^{2+}, cells loaded with the intracellular Ca^{2+} indicator fura-2 were suspended in buffer containing EGTA immediately before fluorescence measurements. Based on fura-2 fluorescence, the basal cytosolic Ca^{2+} level before addition of TG was the same in WEHI7.2 and W.Hb12 cells, but the increase in cytosolic Ca^{2+} after addition of TG was significantly less in W.Hb12 cells compared to WEHI7.2 cells, indicating that BCL-2 overexpression reduces the efflux of Ca^{2+} through the ER membrane after ER Ca^{2+} pump inhibition. In W.Hb13 cells, the TG-induced increase in cytosolic Ca^{2+} was intermediate between that observed in WEHI7.2 and W.Hb12 cells (data not shown). TG-induced Ca^{2+} release was the same in control transfectants and untransfected WEHI7.2 cells, indicating that the transfection and selection procedures do not alter ER-associated Ca^{2+} fluxes (Fig. 3*A*).

The level of Ca^{2+} within the ER lumen governs plasma membrane Ca^{2+} permeability by producing a retrograde signal that increases uptake of extracellular Ca^{2+} in response to ER pool depletion (31). Accordingly, when TG was added

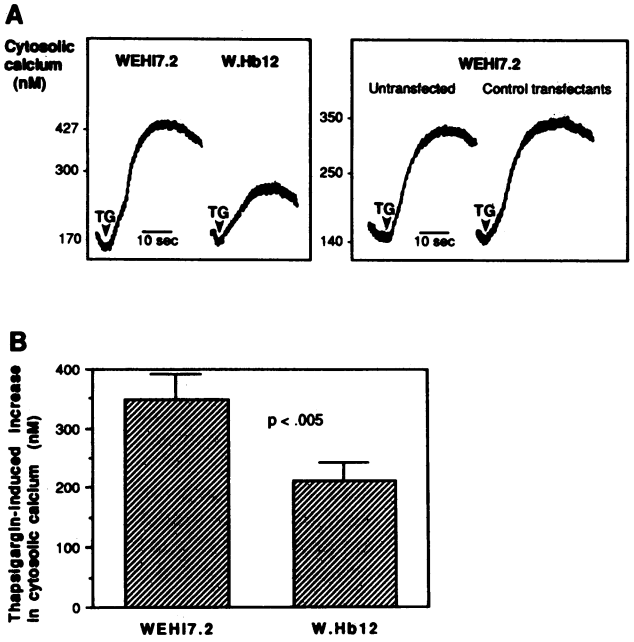

FIG. 3. Influence of BCL-2 on TG-induced Ca^{2+} mobilization. Free cytosolic Ca^{2+} concentration was measured by fura-2 fluorescence in exponentially growing cells before and immediately after addition of 100 nM TG. Note that EGTA was added to cell suspensions immediately before TG to prevent Ca^{2+} influx from the extracellular pool. (*A*) Fluorometer tracing showing that the immediate increase in cytosolic Ca^{2+} after addition of TG is reduced in W.Hb12 cells compared to WEHI7.2 cells. As a control, untransfected WEHI7.2 cells are compared to control cells that were stably transfected with the pMAMneo vector and subjected to the same selection and cloning procedures as W.Hb12 cells. (*B*) Simultaneous comparison of TG-induced increase in cytosolic Ca^{2+} in WEHI7.2 cells versus W.Hb12 cells. Bars represent means ± SE for 11 separate experiments, with *P* value calculated by Student's *t* test.

to WEHI7.2 cells in the absence of extracellular Ca^{2+}, only a transient increase in cytosolic Ca^{2+} was detected; but when TG was added to WEHI7.2 cells in the presence of 1.5 mM extracellular Ca^{2+}, a larger and more sustained increase in cytosolic Ca^{2+} was detected due to influx of extracellular Ca^{2+} (Fig. 4). In the case of W.Hb12 cells, however, only a transient increase in cytosolic Ca^{2+} was detected whether cells were suspended in buffer containing 0 or 1.5 mM Ca^{2+} (Fig. 4). These findings suggest that BCL-2, by reducing efflux of Ca^{2+} across the ER membrane, prevents ER Ca^{2+} pool depletion and thereby interferes with generation of the retrograde signal for "capacitative" Ca^{2+} entry.

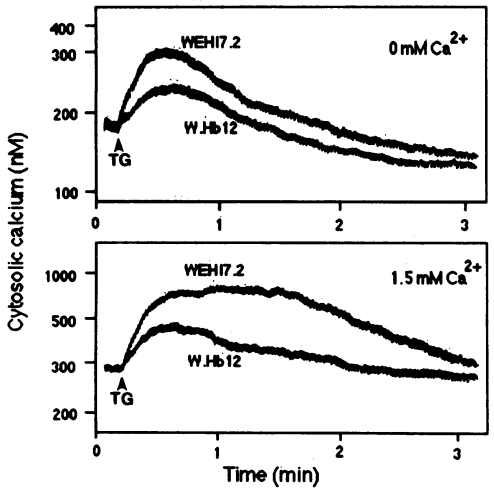

FIG. 4. Effect of BCL-2 on capacitative Ca^{2+} entry. Fura-2-loaded cells were suspended in buffer containing either 0 (*Upper*) or 1.5 (*Lower*) mM Ca^{2+} immediately before adding 100 nM TG. Shown are fluorometer tracings from a single experiment, representative of three separate experiments. Note that EGTA was not included in buffers.

An alternative explanation is that BCL-2 regulates ER-associated Ca^{2+} pumping and prevents inhibition of Ca^{2+} pumping by TG. To test this hypothesis, we measured the rate of $^{45}Ca^{2+}$ uptake into the ER of digitonin-permeabilized cells in the presence and absence of TG (Fig. 5). Note that in these experiments $^{45}Ca^{2+}$ uptake was measured in the presence of azide at a free Ca^{2+} concentration buffered to 150 nM by EGTA, essentially eliminating the uptake of Ca^{2+} into low-affinity mitochondrial stores. TG completely inhibited $^{45}Ca^{2+}$ uptake, confirming that uptake was into a TG-sensitive, ER Ca^{2+} pool. While the rate of Ca^{2+} uptake was slightly greater in W.Hb12 cells than in WEHI7.2 cells (Fig. 5*A*), the sensitivity of ER-associated Ca^{2+} pump activity to inhibition by TG was not significantly different (Fig. 5*B*). We conclude that the effect of BCL-2 overexpression on TG-induced ER Ca^{2+} release described in preceding experiments (Figs. 3 and 4) is due to reduced Ca^{2+} efflux through the ER membrane after inhibition of Ca^{2+} pumping, rather than altered sensitivity of the ER-associated Ca^{2+} pump to inhibition by TG.

Under high Ca^{2+} conditions mitochondria sequester Ca^{2+}, functioning as a low-affinity, high-capacity storage pool (32). To determine whether BCL-2 affects the rate of Ca^{2+} uptake by mitochondria, $^{45}Ca^{2+}$ flux experiments were modified by increasing the free Ca^{2+} concentration to 16 μM while reducing the ATP concentration and eliminating azide in buffers. Under these conditions, uptake of Ca^{2+} into permeabilized cells was inhibited by azide, an inhibitor of mitochondrial respiration, but was not inhibited by TG, confirming that Ca^{2+} uptake was mainly into mitochondria (Fig. 6). Significantly, Ca^{2+} uptake under these conditions was the same in WEHI7.2 and W.Hb12 cells, indicating that the rate of Ca^{2+} uptake into mitochondria was unaffected by BCL-2 overexpression.

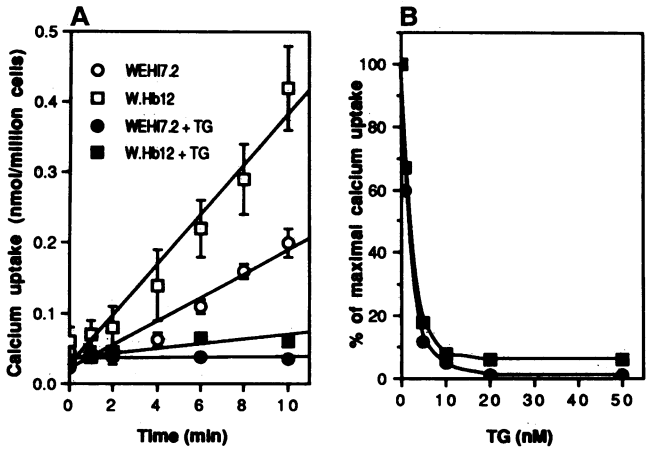

FIG. 5. Effect of BCL-2 on Ca^{2+} uptake by the ER at low (150 nM) free Ca^{2+}. (*A*) $^{45}Ca^{2+}$ uptake was measured in digitonin-permeabilized cells in the presence or absence of 100 nM TG. Symbols represent means ± SE of four separate experiments. (*B*) $^{45}Ca^{2+}$ uptake was measured in the presence or absence of a range of concentrations of TG. The rate of $^{45}Ca^{2+}$ uptake (nmol per 10^6 cells per min) at each TG concentration was calculated from the slopes of $^{45}Ca^{2+}$ uptake curves during the first 10 min of uptake and is expressed as a percentage of the rate of uptake in the absence of TG. Symbols represent means of three separate experiments.

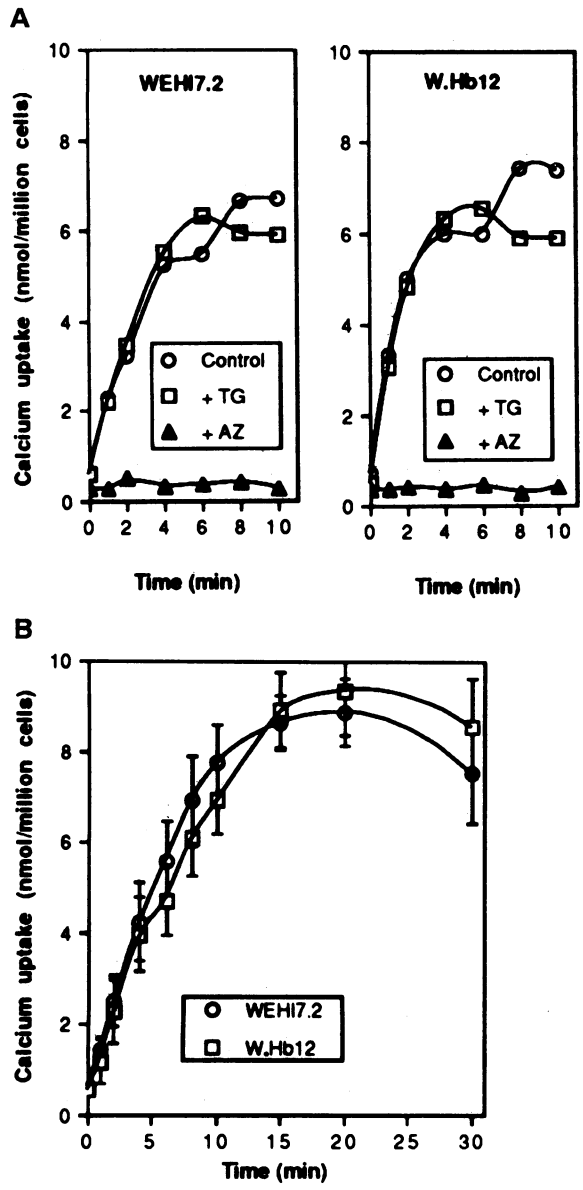

FIG. 6. Effect of BCL-2 on Ca^{2+} uptake by mitochondria measured at high (16 μM) free Ca^{2+}. (*A*) $^{45}Ca^{2+}$ uptake was measured into digitonin-permeabilized cells, either untreated or pretreated with 50 nM TG or 10 mM sodium azide. (*B*) $^{45}Ca^{2+}$ uptake was simultaneously measured in digitonin-permeabilized WEHI7.2 and W.Hb12 cells in the absence of TG or azide. Symbols represent means ± SE of four separate experiments.

DISCUSSION

The findings described in this report provide insight into the mechanism of action of the BCL-2 oncoprotein. The simplest and most direct finding is that overexpression of BCL-2 in WEHI7.2 cells blocks TG-induced cell death and TG-induced mobilization of ER Ca^{2+} without preventing TG-induced inhibition of ER Ca^{2+} pumping. Since the only known effect of TG at the cellular level is a highly specific inhibition of ER-associated Ca^{2+} pump activity (20, 21), this finding indicates that the action of BCL-2 in preventing apoptosis may be directly or indirectly related to its ability to reduce the efflux of Ca^{2+} through the ER membrane.

The ER plays a central role in intracellular signaling by sequestering and releasing Ca^{2+} in response to a variety of agonists (32, 33). Ca^{2+} signal generation by the ER appears to be a biphasic process in which the first phase is a transient increase in cytosolic Ca^{2+} due to a flow of Ca^{2+} from the ER lumen into the cytoplasm. The second phase is a more sustained increase in cytosolic Ca^{2+} due to an influx of extracellular Ca^{2+}, or capacitative Ca^{2+} entry, which amplifies the duration of the Ca^{2+} signal (31). Capacitative Ca^{2+} entry is produced in response to a retrograde signal mediated by a recently identified Ca^{2+} influx factor released from the ER in response to TG-induced depletion of the ER Ca^{2+} pool (34). Our findings suggest that BCL-2, by inhibiting efflux of Ca^{2+} from the ER, interferes with both phases of Ca^{2+} signal generation.

Although an increase in cytosolic Ca^{2+} has been implicated in the signal transduction pathway that mediates apoptosis (35–39), it is possible that ER Ca^{2+} pool depletion may also contribute to cell death. The high Ca^{2+} content of the ER lumen appears necessary for maintaining the structural and functional integrity of the ER (40, 41) as well as for a variety of other cellular functions, including translation (42) and cell division (43). Hence, depletion of the ER Ca^{2+} pool after TG treatment has been associated with altered protein processing (41), translational repression (42), and induction of genes encoding resident ER stress proteins (44). Furthermore, the ER Ca^{2+} pool appears to be necessary for cell cycle progression, as depletion of the ER Ca^{2+} pool in response to TG treatment causes cells to arrest in G_0 (23, 43). Interestingly, it has been reported that the endogenous deoxyribonuclease involved in nuclear DNA degradation is confined to the ER until it gains access to the nucleus during programmed cell death (45). Perhaps ER Ca^{2+} pool depletion triggers release of the endonuclease from the ER, allowing its entry into the nucleus.

It has recently been reported that expression of BCL-2 in neural cells does not interfere with mobilization of Ca^{2+} from intracellular stores by A23187 or ionomycin (46). Indeed, we have made similar observations in mouse lymphoma cells (M.L. and C.W.D., unpublished data). However, these agents are nonspecific ionophores that release Ca^{2+} from a variety of intracellular stores, including the ER and mitochondria (47). In mouse lymphoma cells, for example, the TG-sensitive Ca^{2+} pool is only a small fraction of the total intracellular storage pool that is mobilized in response to ionomycin (17). Our findings indicate that BCL-2 acts by selectively regulating Ca^{2+} efflux from a TG-sensitive ER pool rather than from intracellular Ca^{2+} stores in general.

The mechanism by which BCL-2 regulates ER Ca^{2+} efflux is presently unknown, but BCL-2 does not appear to bind Ca^{2+} (T. McCormick and C.W.D., unpublished data). Although it is possible that BCL-2 might regulate ER-associated Ca^{2+} fluxes by an indirect mechanism, the concept that the BCL-2 protein may interact directly with a Ca^{2+} channel in the ER membrane is more appealing in light of growing evidence that the BCL-2 protein is located in the ER membrane (12–15). The ER membrane channel through which Ca^{2+} flows after TG treatment has not been identified but appears to be distinct from the Ca^{2+} channel associated with the inositol 1,4,5-trisphosphate receptor (33, 48, 49). Identification of ion channels associated with the ER membrane may be a requisite for fully understanding the mechanism of action of BCL-2.

Recently, it has been suggested that BCL-2 may either interfere with the generation of oxygen radicals or prevent membrane damage by oxygen radicals (50, 51). There is a complex interrelationship between oxygen radical damage and intracellular Ca^{2+} fluxes, including evidence that oxidative damage can mobilize Ca^{2+} from intracellular pools located in the ER and mitochondria (see refs. 52 and 53 for review). Therefore, it is interesting to speculate that the effects of BCL-2 on intracellular Ca^{2+} homeostasis and oxygen radical damage may be related.

In summary, the findings reported here indicate that the BCL-2 oncoprotein inhibits the apoptotic pathway of mouse

lymphoma cells by selectively interfering with release of Ca^{2+} from a TG-sensitive ER pool. Mobilization of Ca^{2+} from the ER is likely to be only one step in a complex cascade of events that constitute the apoptotic pathway; whether BCL-2 acts solely to interfere with ER Ca^{2+} release or also regulates other steps in the apoptotic process remains to be determined.

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1. Ellis, R. E., Yuan, J. & Horvitz, H. R. (1991) *Annu. Rev. Cell Biol.* **7**, 663–698.
2. Vaux, D. L. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 786–789.
3. Williams, G. T. (1991) *Cell* **65**, 1097–1098.
4. Raff, M. C. (1992) *Nature (London)* **356**, 397–400.
5. Hengartner, M. O., Ellis, R. E. & Horvitz, H. R. (1992) *Nature (London)* **356**, 494–499.
6. Vaux, D. L., Cory, S. & Adams, J. (1988) *Nature (London)* **335**, 440–442.
7. McDonnell, T. J., Deane, N., Platt, F. M., Nunez, G., Jaeger, U., McKearn, J. P. & Korsmeyer, S. J. (1989) *Cell* **57**, 79–88.
8. Tsujimoto, Y. (1989) *Oncogene* **4**, 1331–1336.
9. Nunez, G., London, L., Hockenbery, D., Alexander, M., McKearn, J. P. & Korsmeyer, S. J. (1990) *J. Immunol.* **144**, 3602–3610.
10. Chen-Levy, Z. & Cleary, M. L. (1990) *J. Biol. Chem.* **265**, 4929–4933.
11. Hockenbery, D., Nunez, G., Milliman, C., Schreiber, R. D. & Korsmeyer, S. J. (1990) *Nature (London)* **348**, 334–336.
12. Chen-Levy, Z., Nourse, J. & Cleary, M. L. (1989) *Mol. Cell. Biol.* **9**, 701–710.
13. Alnemri, E. S., Robertson, N. M., Fernandes, T. F., Croce, C. M. & Litwack, G. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7295–7299.
14. Monaghan, P., Robertson, D., Amos, T. A. S., Dyer, M. J. S., Mason, D. Y. & Greaves, M. F. (1992) *J. Histochem. Cytochem.* **40**, 1819–1825.
15. Jacobson, M. D., Burne, J. F., King, M. P., Miyashita, T., Reed, J. C. & Raff, M. C. (1993) *Nature (London)* **361**, 365–368.
16. Krajewski, S., Tanaka, S., Takayama, S., Schibler, M. J., Fenton, W. & Reed, J. C. (1993) *Cancer Res.* **53**, 4701–4714.
17. Lam, M., Dubyak, G. & Distelhorst, C. W. (1993) *Mol. Endocrinol.* **7**, 686–693.
18. Baffy, G., Miyashita, T., Williamson, J. R. & Reed, J. C. (1993) *J. Biol. Chem.* **268**, 6511–6519.
19. Thastrup, O., Cullen, P. J., Drobak, B. K., Hanley, M. R. & Dawson, A. P. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2466–2470.
20. Lytton, J. & MacLennan, D. H. (1988) *J. Biol. Chem.* **263**, 15024–15031.
21. Lytton, J., Westlin, M. & Hanley, M. R. (1991) *J. Biol. Chem.* **266**, 17067–17071.
22. Mason, M. J., Garcia-Rodriguez, C. & Grinstein, S. (1991) *J. Biol. Chem.* **266**, 20856–20862.
23. Ghosh, T. K., Bian, J., Short, A. D., Rybak, S. L. & Gill, D. L. (1989) *J. Biol. Chem.* **266**, 24690–24697.
24. Cuende, E., Ales-Martinez, J. E., Ding, L., Gonzalez-Garcia, M., Martinez-A, C. & Nunez, G. (1993) *EMBO J.* **12**, 1555–1560.
25. Dowd, D. R. & Miesfeld, R. L. (1992) *Mol. Cell. Biol.* **12**, 3600–3608.
26. Chueh, S.-H., Mullaney, J. M., Ghosh, T. K., Zachary, A. L. & Gill, D. L. (1987) *J. Biol. Chem.* **262**, 13857–13864.
27. Missiaen, L., De Smedt, H., Droogmans, G. & Casteels, R. (1992) *J. Biol. Chem.* **267**, 22961–22966.
28. Sentman, C. L., Shutter, J. R., Hockenbery, D., Kanagawa, O. & Korsmeyer, S. J. (1991) *Cell* **67**, 879–888.
29. Alnemri, E. S., Fernandes, T. F., Haldar, S., Croce, C. M. & Litwack, G. (1992) *Cancer Res.* **52**, 491–495.
30. Miyashita, T. & Reed, J. C. (1992) *Cancer Res.* **52**, 5407–5411.
31. Putney, J. W. & Bird, G. S. J. (1993) *Cell* **75**, 199–201.
32. Carafoli, E. (1987) *Annu. Rev. Biochem.* **56**, 395–433.
33. Sambrook, J. F. (1990) *Cell* **61**, 197–199.
34. Randriamampita, C. & Tsien, R. Y. (1993) *Nature (London)* **364**, 809–814.
35. Kaiser, N. & Edelman, I. S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 632–642.
36. Caron-Leslie, L.-A. M. & Cidlowski, J. A. (1991) *Mol. Endocrinol.* **5**, 1169–1179.
37. Dowd, D. R., MacDonald, P. N., Komm, B. S., Haussler, M. R. & Miesfeld, R. (1991) *J. Biol. Chem.* **266**, 18423–18426.
38. McConkey, D. J., Nicotera, P., Hartzell, P., Bellomo, G., Wyllie, A. H. & Orrenius, S. (1989) *Arch. Biochem. Biophys.* **269**, 365–370.
39. Dowd, D. R., MacDonald, P. N., Komm, B. S., Haussler, M. R. & Miesfeld, R. L. (1992) *Mol. Endocrinol.* **6**, 1843–1848.
40. Booth, C. & Kock, G. L. E. (1989) *Cell* **59**, 729–737.
41. Lodish, H. F., Kong, N. & Wikstrom, L. (1992) *J. Biol. Chem.* **267**, 12753–12760.
42. Brostrom, C. O. & Brostrom, M. A. (1990) *Annu. Rev. Physiol.* **52**, 577–590.
43. Short, A. D., Bian, J., Ghosh, T. K., Waldron, R. T. & Rybak, S. L. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 4986–4990.
44. Li, W. W., Alexandre, S., Cao, X. & Lee, A. S. (1993) *J. Biol. Chem.* **268**, 12003–12009.
45. Peitsch, M. C., Polzar, B., Stephan, H., Crompton, T., MacDonald, H. R., Mannherz, H. G. & Tschopp, J. (1993) *EMBO J.* **12**, 371–377.
46. Zhong, L.-T., Sarafian, T., Kane, D. J., Charles, A. C., Mah, S. P., Edwards, R. H. & Bredesen, D. E. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 4533–4537.
47. Albert, P. R. & Tashjian, A. H. (1986) *Am. J. Physiol.* **251**, C887–C891.
48. Berridge, M. J. & Irvine, R. F. (1989) *Nature (London)* **341**, 197–204.
49. Bansal, V. S. & Majerus, P. W. (1990) *Annu. Rev. Cell Biol.* **6**, 41–67.
50. Hockenbery, D. M., Oltvai, Z. N., Yin, X.-M., Milliman, C. L. & Korsmeyer, S. J. (1993) *Cell* **75**, 241–251.
51. Kane, D. J., Sarafian, T. A., Anton, R., Hahn, H., Gralla, E. B., Valentine, J. S., Örd, T. & Bredesen, D. E. (1993) *Science* **262**, 1274–1277.
52. Reed, D. J. (1990) *Chem. Res. Toxicol.* **3**, 495–502.
53. Orrenius, S., Burkitt, M. J., Kass, G. E. N., Dypbukt, J. M. & Nicotera, P. (1992) *Ann. Neurol.* **32**, S33–S42.