Taurine is an abundant free amino acid that interacts with the potent oxidant hypochlorous acid to form the less toxic and more stable oxidant taurine monochloramine (TauNHCl). TauNHCl has diverse cellular effects ranging from inhibiting the production of proinflammatory mediators to inhibiting cell proliferation and inducing cell death. We hypothesized that TauNHCl could activate a cell death pathway involving Bcl-2 members and the activation of caspase proteases. FL5.12 cells are lymphocytic cells that undergo apoptosis following interleukin-3 (IL-3) withdrawal. Therefore, cell death following TauNHCl treatment of FL5.12 cells was compared and contrasted with IL-3 withdrawal. We found that TauNHCl treatment activates a cell death pathway with kinetics very similar to IL-3 withdrawal. TauNHCl-treated cells undergo an annexin V-positive/propidium iodide-negative phase of death consistent with apoptosis. TauNHCl treatment results in a conformational change in BAX that is associated with its activation. Both Bcl-2 and, to a lesser degree, the dominant negative form of caspase-9 inhibit cell death following TauNHCl treatment. In contrast with IL-3 withdrawal, TauNHCl treatment of FL5.12 cells results in a rapid cell cycle arrest that is cell cycle phase-independent. These results demonstrate that TauNHCl treatment induces a rapid, cell cycle-independent proliferative arrest followed by the activation of a cell death pathway involving Bcl-2 family members and caspase activation.

On activation, leukocytes generate large quantities of the strongly oxidizing species hypochlorous acid (HOCl). Although HOCl can directly oxidize a variety of biomolecules, it is believed that the predominant reaction is the formation of chloramines with the amine groups of protein or free amino acids. Of these reactions, the predominant formation is HOCl with taurine, a sulfur-containing amino acid that interacts rapidly and stoichiometrically with HOCl to form taurine monochloramine (TauNHCl), a long-lived and less reactive oxidant (2–4). At higher concentrations, TauNHCl has been shown to induce cell death in Burkitt’s lymphoma cells (5).

TauNHCl is known to play several roles in immunomodulation. TauNHCl significantly decreases the production of many proinflammatory mediators from both adherent and non-adherent leukocytes. TauNHCl inhibits intracellular nitric-oxide synthase transcription and nitric oxide production as well as the levels of tumor necrosis factor α in RAW 264.7 macrophage-like cells (6). In activated NR8383 cells, TauNHCl decreased NFκB migration into the nucleus without directly inhibiting IκB kinase (7). Kanayama et al. (8) found a molecular mechanism for TauNHCl-induced inhibition of NFκB activation. TauNHCl treatment resulted in the oxidation of IκBζ on Met45, preventing its degradation and dissociation from NFκB. TauNHCl also induced an apoptotic or apoptotic-like cell death, which may regulate the inflammatory response. Burkitt’s lymphoma cells treated with HOCl in regular medium showed caspase-3 cleavage suggesting that an apoptotic pathway is involved (5). However, the role of Bcl-2 family members in TauNHCl-induced cell death has not been examined.

The Bcl-2 family contains a number of related genes that are critical regulators of apoptosis (9). The family can be divided into those that inhibit (Bcl-2, Bcl-x, etc.) and those that promote cell death. Those that promote apoptosis can be further characterized as multidomain members (Bax, Bak, and Bok) with more extensive homology to Bcl-2 and BH3-only family members, in which homology to Bcl-2 is limited to this single BH3 domain (10). The Bcl-2 family appears to regulate apoptosis through its effects on mitochondria. Antiapoptotic members prevent the release of mitochondrial proteins such as cytochrome c, Smac/Diablo, and apoptosis-inducing factor (AIF), which are involved in caspase activation and DNA degradation, two biochemical hallmarks of apoptosis (11). The ability of Bcl-2 to act upstream of caspases and preserve mitochondrial function may contribute to its more potent oncogenic activity relative to other antiapoptotic genes that act by the direct inhibition of caspases (12). Current evidence supports a model in which Bcl-2 prevents the formation or opening of a large mitochondrial channel that contains the proapoptotic family members Bax or Bak (13).

Recent studies (13, 14) have demonstrated that the BH3-only Bcl-2 family members act upstream of the multidomain member BAK or BAX to disrupt mitochondrial function and release cytochrome c. Furthermore, the main function of BCL-2 or BCL-x may be to prevent BCL-2 homology domain 3 (BH3)-only family members from “activating” BAX or BAK and releasing proapoptotic mitochondrial components (15). The activated form of BAX can be detected using conformation-specific antib
bodies. No studies have investigated specifically the role of Bcl-2 family members in TauNHCl-induced death. In the present study, we examined the role of Bcl-2 family members and caspase activation in cell death following treatment with TauNHCl.

MATERIALS AND METHODS

Plasmid Constructs—Both the pCDNA3 and the SFFV vector (16, 17) were used in FL5.12 cells. Preliminary studies indicated that the SFFV vector provided a higher expression and was used for these studies. The parental SFFV-Neo plasmid, the SFFV-HAbax plasmid (17), and the SFFV-Human Bcl-2 plasmid have been characterized previously (18) and were kindly provided by Dr. Stan Korsmeyer (Dana-Farber Cancer Institute). The cDNA containing a dominant negative form of caspase-9 (Casp9DN) with a cysteine to serine mutation at the active site (19) was kindly provided by Dr. Scott Lowe (Cold Spring Harbor Laboratory). The entire cDNA for Casp9DN was cloned into pBluescript and amplified using PCR with the Pfu polymerase. The PCR product was cloned into the EcoRI site of the SFFV plasmid and sequenced to rule out mutations and to confirm the cysteine to serine mutation.

Cell Lines and Media—Murine FL5.12 hematopoietic polyphagocytic B cells (20) were maintained in FL5.12 complete medium (RPMI 1640 medium with 10% fetal bovine serum (Invitrogen) supplemented with 10% Me2SO. Cells were maintained in medium without IL-3 withdrawals, FL5.12 cells decrease metabolism, undergo cell cycle arrest, and activate an apoptotic pathway that can be blocked by Bcl-2 family members (21). Cells were frozen in complete media with 10% Me2SO. Cells were maintained with 10% Me2SO to prevent any out phenotypic changes. A prolonged passage of FL5.12 cells was avoided because of the expansion of a tetraploid population. Cells were discarded, and fresh cells were thawed when the tetraploid population exceeded 5% based on DNA content analysis with flow cytometry (22). Cells were maintained at 0.1–1 million cells/ml with passages every other day. All experiments were performed on transfected cells and included the empty SFFV plasmid (FL5-Neo) or SFFV plasmid driving the expression of human Bcl-2 (FL5-Bcl-2) and FL5-Casp9DN. The FL5-Neo cells demonstrated the same cell sensitivity to IL-3 withdrawal as non-transfected parental cells (data not shown). For IL-3 deprivation experiments, cells were pelleted (1200 × g for 7 min) and resuspended in PBS. Cells were pelleted again and resuspended in complete medium without IL-3 or 2-mercaptoethanol. FL5-Neo and FL5-Bcl-2 cells were transferred to FL5.12 complete medium and allowed to recover for 24 h. The cells were then washed and cultured with Genetec/G418 (catalog no. 11811-031, Invitrogen) at 1 mg/ml (activity, 0.6–0.7 mg/ml) for 10–14 days. Bulk transfecteds were then examined for protein expression and for sensitivity to withdrawal of IL-3. Following IL-3 withdrawal, FL5.12 cells decrease metabolism, undergo cell cycle arrest, and activate an apoptotic pathway that can be blocked by Bcl-2 family members (21). Cells were frozen in complete media with 10% Me2SO. Cells were maintained with 10% Me2SO to prevent any out phenotypic changes. A prolonged passage of FL5.12 cells was avoided because of the expansion of a tetraploid population. Cells were discarded, and fresh cells were thawed when the tetraploid population exceeded 5% based on DNA content analysis with flow cytometry (22).}

Reagents—TauNHCl was freshly prepared for each experiment by the addition of 100 mM HOCl to 200 mM taurine in water. The concentration of TauNHCl was determined in each experiment by measuring the absorption at 252 nm and using an extinction coefficient of 415 M M−1 cm−1. Annexin V-FITC was obtained from Santa Cruz Biotechnology (catalog no. SC-4252). Buthionine-sulfoximine (BSO) (catalog no. B2515), taurine, bromodeoxyuridine (BrdUrd) (catalog no. B5002), digoxigenin (catalog no. D-141), and propidium iodide (PI) (catalog no. P4170) were used in FL5.12 cells. Preliminary studies indicated that the SFFV vector provided a higher expression and was used for these studies. The parental SFFV-Neo plasmid, the SFFV-HAbax plasmid (17), and the SFFV-Human Bcl-2 plasmid have been characterized previously (18) and were kindly provided by Dr. Stan Korsmeyer (Dana-Farber Cancer Institute). The cDNA containing a dominant negative form of caspase-9 (Casp9DN) with a cysteine to serine mutation at the active site (19) was kindly provided by Dr. Scott Lowe (Cold Spring Harbor Laboratory). The entire cDNA for Casp9DN was cloned into pBluescript and amplified using PCR with the Pfu polymerase. The PCR product was cloned into the EcoRI site of the SFFV plasmid and sequenced to rule out mutations and to confirm the cysteine to serine mutation.

Cell Cycle Analysis—For DNA content analysis, cells were stained with 5 μg/ml propidium iodide (PI) (catalog no. P4170) and stained with anti-BrdUrd and PI as described previously (25). Viability was determined at the indicated times using the Guava ViaCount reagent as described under “Materials and Methods.”

TauNHCl treatment. At the indicated time, cells were pelleted and fixed with ice-cold 70% EtOH and stored at −20 °C prior to staining with anti-BrdUrd and PI as described previously (25).

TauNHCl Preparation and Cell Treatment—Cells were pelleted and washed in sterile PBS and resuspended in media either with or without IL-3 at between 0.1 and 1 million cells/ml. In some experiments, cells were pretreated with 1 μM BSO for 48 h. Cells were treated to the indicated concentration of TauNHCl by directly adding the cells to the FL5.12 complete medium.

Cell Viability Assays—A Guava personal cell analyzer (Guava Technologies, Inc.) was used to assess cell viability. In brief, 10 μl of cells was added to 40 μl of Guava ViaCount reagent and allowed to stain for 5–20 min. Just prior to placing them in the instrument, the cells were diluted with 50 μl of PBS and mixed, and the data were acquired. Experiments were performed in duplicate or triplicate, and the data represent the mean ± S.D. for each sample. For annexin V staining, cells were pelleted and resuspended in annexin V binding buffer (10 mM HEPES, 150 mM NaCl, 5 mM KCl, 1 mM MgCl2, 1.8 mM CaCl2, pH 7.4) containing annexin V-FITC (1:1000) and PI at 1 μg/ml. Cells were incubated for 5 min at room temperature and analyzed on a FACS Calibur flow cytometer using CellQuest software (BD Biosciences). For mitochondrial membrane staining, cells were incubated with 5 μM DioC6 in complete medium. Cells were incubated for 5 min at 37 °C and analyzed on a FACS Calibur flow cytometer using CellQuest software (BD Biosciences).

Clonogenic Survival Assay—A clonogenic survival assay of FL5-Neo and FL5-Bcl-2 cells was performed by plating cells by limited dilution into 96-well plates. Viable cell concentration was determined in duplicate by either trypan blue counting or the use of the Guava PCA system. Cells were diluted to 2000 cells/ml and then treated with 0.2 or 0.25 mM TauNHCl for 16 h. Cells were then plated out at a limiting dilution to allow for growth of some but not all the wells. Untreated cells were diluted to 2–10 cells/ml, and 100 μl was added to each well. The number of positive wells was determined after 10–14 days of growth. The number of colony-forming units/well was corrected with Poisson statistics using the formula, colony-forming units/well = −ln(% negative wells). The clonogenic survival of untreated cells varied between 60 and 90%. For each experiment, data were normalized to the clonogenic survival of the untreated cells. As stated earlier, the TauNHCl killing activity is dependent on cell concentration, and this accounts for increased activity of TauNHCl in these experiments.

Activated BAX Staining by Flow Cytometry—FL5-Neo and FL5-Bcl-2 were treated as described above with TauNHCl. Activated Bax staining was performed as described previously (26–28). In brief, 1–2 × 10^6 FL5-12 cells were pelleted and fixed in 0.25% paraformaldehyde for 5 min at room temperature. Fixed cells were washed with PBS and resuspended in 100 μl of blocking buffer (PBS with 0.01% digitonin, 5 μg/ml anti-FC, and 10% normal rat serum). The sample was split in half and stained with anti-Bax antibody (clone 6A7, eBioscience) or isotype control (clone P3, eBioscience) at 2.5 μg/sample. Cells were stained for 30 min at room temperature, washed, and then stained with 1 μg/100 μl of FITC-conjugated goat anti-mouse IgG antibody (BD Biosciences catalog no. 554001) for 30 min. The cells were washed with a solution of...
PBS, 0.01% digitonin prior to staining for DNA content with PI and RNase as described previously (27). Cells were acquired and analyzed on a FACSCalibur flow cytometer. Doublets and subdiploid cells were excluded from analysis. Under all conditions, fewer than 1% of the cells stained with the isotype control were positive.

**Caspase Activation Assay by Guava**—For the caspase activation assay, the Guava multiscaspase detection kit protocol was followed. In brief, 50,000 cells were pelleted and resuspended in 100 µl of apoptosis wash buffer (Guava Technologies, Inc.). 2 µl of sulforhodamine-VAD-fluoromethyl ketone (SR-VAD-fmk) (Guava Technologies, Inc.) reagent was added and incubated at 37 °C with 5% CO₂ for 1 h. SR-VAD-fmk is a fluorochrome that covalently binds active caspases 1 and 3–9. After incubation, the samples were washed three times with apoptosis wash buffer and resuspended in 100 µl of the apoptosis wash buffer before adding 5 µl of the 7-amino-actinomycin D reagent. The samples were incubated for 10 min at room temperature and adjusted to 200 µl with

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**FIG. 2. Annexin V staining in TauNHCl-treated FL5-Neo cells.** FL5-Neo cells were treated with 1 mM TauNHCl for the indicated times. Cells were stained with annexin V-FITC and PI and analyzed on a flow cytometer as described under “Materials and Methods.” The percentage of viable cells (annexin V-negative/PI-negative) is indicated at each time point. The arrows indicate the annexin V-positive/PI-negative cells.
the apoptosis wash buffer, and the data were acquired and analyzed with the Guava PCA using the Cytosoft multicaspase assay module.

RESULTS

Cell Viability following TauNHCl Treatment—To determine the effects of TauNHCl on cell survival, control-transfected FL5.12 cells (FL5-Neo) were exposed to various concentrations of TauNHCl, and cell death was determined by dye exclusion (Guava PCA cell analysis). Following treatment, the cells underwent a time- and dose-dependent cell death (Fig. 1). The time course observed was comparable to that seen when the cells were deprived of IL-3. It is important to note that measuring cell death by dye exclusion does not distinguish between necrotic and apoptotic cell death. However, the time course we observed was consistent with an apoptotic pathway. During apoptosis, cells undergo the loss of membrane asymmetry, which exposes phosphatidylserine on the cell surface. The loss of membrane asymmetry can be detected by annexin V binding, and during apoptosis, the loss of asymmetry precedes the loss of membrane integrity. FL5.12 cells treated with TauNHCl demonstrated a time-dependent increase in annexin V staining, which preceded the loss of membrane integrity (Fig. 2). This same pattern was observed following IL-3 withdrawal (data not shown). This result suggested that TauNHCl treatment activates an apoptotic pathway.

Although TauNHCl is a relatively mild oxidative agent, it is likely that its effects on cell viability are caused by its oxidative reactions with cellular components. Specifically, TauNHCl is known to react with thiol such as the tripeptide glutathione (4, 29). Glutathione is a prevalent antioxidant found in all cells and may be protective against TauNHCl treatment. To determine the importance of GSH in the protection from TauNHCl treatment, cells were treated with BSO, an inhibitor of the GSH synthetic pathway. BSO pretreatment alone was not toxic to FL5-Neo cells and only modestly accelerated cell death following IL-3 withdrawal. However, GSH depletion with BSO markedly sensitized FL5-Neo cells to TauNHCl-induced cell death (Fig. 3). These data showed that GSH protected cells from TauNHCl toxicity and...
suggested that the cell killing following TauNHCl treatment was the result of oxidative reactions.

**BAX Activation following TauNHCl Treatment**—Following IL-3 deprivation of FL5.12 cells, BAX undergoes a conformational change that can be detected by a conformation-specific antibody (27). Previous studies (30–32) demonstrate that this conformational change is associated with the activation and mitochondrial localization of BAX. These events result in the release of cytochrome c and the subsequent activation of caspases involved in apoptosis (33). The conformational status of BAX following TauNHCl treatment of FL5-Neo cells was determined by intracellular staining with monoclonal antibody 6A7 followed by analysis by flow cytometry. TauNHCl induces a time-dependent conformational change in BAX in FL5-Neo cells. Untreated cells and cells treated for 4 h with TauNHCl did not stain appreciably with 6A7. However, by 24 h of TauNHCl treatment, a significant fraction of the cells stained positive with 6A7, implying a conformational change in BAX (Fig. 4, A–C). The 6A7 staining observed following TauNHCl treatment was comparable with that seen following IL-3 deprivation (Fig. 4D). TauNHCl-treated cells did not stain at any time with the isotype control antibody (data not shown). Of note, when TauNHCl was added directly to the medium 3 h prior to the addition of the FL5.12 cells, no cell killing activity was observed (data not shown).

BAX is thought to translocate to the mitochondria, and this is associated with the loss of the mitochondrial membrane potential and the release of cytochrome c (34). Therefore, the mitochondrial membrane potential was examined at several times post-TauNHCl treatment. As shown in Fig. 5, TauNHCl induces a time-dependent loss of mitochondrial membrane potential. Histogram plots of FL5-Neo (A) and FL5-Bcl-2 (B) cells are shown. Cells were treated with 1 mM TauNHCl, and samples were acquired for DiOC₆ staining at 0, 12, 24, and 48 h. The percentage of cells with low DiOC₆ staining is indicated for each plot.

**Fig. 5.** TauNHCl-induced loss of mitochondrial membrane potential. Histogram plots of FL5-Neo (A) and FL5-Bcl-2 (B) cells are shown. Cells were treated with 1 mM TauNHCl, and samples were acquired for DiOC₆ staining at 0, 12, 24, and 48 h. The percentage of cells with low DiOC₆ staining is indicated for each plot.

**Fig. 6.** Bcl-2 blocks BAX conformational change following TauNHCl treatment. FL5-Neo (left panel) and FL5-Bcl-2 (right panel) overexpressing cells were cultured for 48 h following treatment with 1.5 mM TauNHCl. 6A7 antibody-specific staining was performed as described under “Materials and Methods,” and the subdiploid cells were excluded by co-staining with PI. The result of staining with isotype control antibody was similar to untreated controls, with less than 1% of cells positive (data not shown). Data are representative of three independent experiments.
time points following TauNHCl treatment of FL5-Neo cells. As expected, TauNHCl induced a time-dependent increase in the percentage of FL5-Neo cells with reduced mitochondrial membrane potential as measured by DiOC₆ staining (Fig. 5 A).

Bcl-2 Protection from TauNHCl-induced Cell Death—The conformational change in BAX in FL5.12 cells following TauNHCl treatment suggested that a mitochondrial cell death pathway was involved. Previous studies (35) demonstrate that Bcl-2 prevents the conformational change and translocation of Bax following drug-induced and Fas-induced cell death pathways (36). In addition, Bcl-2 prevents the oligomerization and mitochondrial localization of Bax following ATP depletion (37). If Bax mediates cell killing following TauNHCl treatment, Bcl-2 expression should suppress cell death following this treatment. Indeed, FL5-Bcl-2 cells were protected from the loss of mitochondrial membrane potential and cell death following TauNHCl treatment (Fig. 5B).

Although the mechanism of Bax activation and translocation are controversial, previous studies (35–37) suggest that Bcl-2 prevents the conformational change in Bax, associated with activation. TauNHCl oxidizes a methionine residue in IκBα and inhibits its degradation following tumor necrosis factor treatment (8). Therefore, it is possible that the oxidation of BAX by TauNHCl leads directly to the conformational change in BAX, associated with activation. If this possibility is correct, Bcl-2 expression is unlikely to effect the conformational change in Bax following TauNHCl treatment. Therefore, the conformational state of Bax was assessed in FL5-Bcl-2 cells following treatment with TauNHCl. We found that the conformational change in BAX following TauNHCl treatment was blocked by the expression of BCL-2 (Fig. 6). In addition, no change in the migration of BAX on SDS-PAGE was observed (data not shown). These results and the absence of 6A7 antibody staining 4 h after TauNHCl treatment (Fig. 4) do not support the direct oxidation and activation of BAX by TauNHCl.

Role of Caspase Activity in Cell Death following TauNHCl Treatment—Following Bax activation, mitochondria release proapoptotic factors (cytochrome c and Smac/Diablo) that can activate a proteolytic cascade involving caspases (38). Therefore, we examined the role of caspase activity in TauNHCl-mediated cell death. Examination of cells using the multicaspase assay (Guava Technologies, Inc.) demonstrated that
one or more caspases were activated following TauNHCl treatment (Fig. 7A). Given the involvement of Bcl-2 family members in TauNHCl-dependent cell killing, we expected caspase-9 might also have been activated following TauNHCl treatment. To determine whether caspase-9 activity contributed to the cell death of the TauNHCl-induced death of FL5-Neo cells, we generated FL5-Casp9DN cells, which overexpress the dominant-negative form of caspase-9 (19). These cells showed transient protection from cell death following IL-3 deprivation (data not shown). FL5-Casp9DN cells were protected from cell death following treatment with TauNHCl (Fig. 7B). These results demonstrate a role for caspase-9 activity in TauNHCl-induced cell death.

**Effect of TauNHCl on Cell Proliferation**—The studies described in Fig. 1 involved measuring cell viability using the Guava PCA analyzer. Unlike flow cytometry, this instrument provides information related to cell concentration in addition to the percentage of viable cells. Despite the fact that cell death rates were similar for both IL-3 withdrawal and TauNHCl treatment (Fig. 1), the total concentration of FL5-Neo cells (live + dead) increased for the IL-3-deprived samples but not for the TauNHCl-treated samples (Fig. 8A). Similar results were found for the FL5-Bcl-2 cells (data not shown). These results suggested that, whereas IL-3-deprived cells were able to progress through the cell cycle and undergo cell division prior to cell death, TauNHCl-treated cells were not. One explanation for this result would be a cell cycle block late in the cell cycle such as in mitosis. To test this possibility, FL5-Bcl-2 cells were stained for DNA content with PI. Surprisingly, cells treated with TauNHCl had cell cycle profiles that were nearly identical to the profiles of untreated cells. As expected, IL-3-deprived cells had a reduction in the percentage of cells in S/G2/M (Fig. 8B). These results do not support a cell phase-specific arrest in cells following TauNHCl treatment.

BrdUrd is a thymidine analog that is incorporated into cells in the S phase and is a useful reagent for examining cell cycle progression in a cell population. Therefore, FL5-Neo cells were pulsed with BrdUrd to label S phase cells. The cells were either left untreated or treated with TauNHCl, and progression through the cell cycle was followed using simultaneous staining with anti-BrdUrd antibodies and PI. As expected, in the untreated control samples, the BrdUrd-positive cells progressed through the S phase and entered G2/M or even G1 phase within 4–6 h of treatment. Likewise, BrdUrd-negative cells progressed into the S phase during this time period. In contrast, the TauNHCl-treated cells showed little or no change in the BrdUrd-PI profile during the time course of this experiment (Fig. 9). These data suggest that TauNHCl treatment of cells leads to an immediate cell cycle arrest that is independent of the cell cycle phase. Similar results were found for FL5-Bcl-2 cells (data not shown).

**Bcl-2 and Clonogenic Survival**—The cell cycle arrest observed following TauNHCl treatment was not affected by Bcl-2. This suggested that Bcl-2 protection from cell death might only be transient and that the cells might not be able to proliferate following TauNHCl treatment. To determine whether Bcl-2 protected cells are able to proliferate, clonogenic survival assays were performed on FL5-Neo and FL5-Bcl-2 cells using limiting dilution in 96-well plates. FL5-Bcl-2 cells demonstrated increased clonogenic survival following TauNHCl treatment compared with FL5-Neo cells (Fig. 10). This suggested that, despite an initial cell cycle arrest, cells expressing BCL-2 retain the long-term capacity to proliferate following TauNHCl treatment.

**FIG. 8.** **TauNHCl effects on cell division and cell DNA content.** A, total cell concentration (live + dead) of FL5-Neo cells left untreated (+IL-3), deprived of IL-3 (−IL-3), or treated with 0.5 and 0.75 mM TauNHCl is shown at 0, 19, and 41 h. B, histogram plots of DNA content analysis are shown for untreated (Control), IL-3-deprived (−IL3), and 1 mM TauNHCl-treated (1 TC) FL5-Bcl-2 cells. Analysis was performed 2 days after IL-3 deprivation or TauNHCl treatment.

**DISCUSSION**

Taurine and TauNHCl have been proposed to be important modulators of the immune response. HOCl produced by neutrophils during an inflammatory immune response interacts with taurine to form TauNHCl (39). TauNHCl has been shown to effect cellular proliferation, cytokine processing, and NFKB signaling (8, 40), and TauNHCl dose-dependently inhibits inflammatory cytokine release from leukocytes (6, 41, 42). Other studies (5, 43) have shown that TauNHCl can induce cell death. In total, previous studies demonstrate that TauNHCl may modulate the immune response during an inflammatory reaction by diverse effects on multiple cell types. FL5.12 is a lym-
phocytic cell line that has been used to examine apoptotic cell death pathways following IL-3 withdrawal. To better understand the direct effects of TauNHCl on lymphocyte survival, we sought to compare and contrast the cell death of FL5.12 cells following IL-3 withdrawal and TauNHCl treatment.

These studies demonstrated that TauNHCl induced a cell death pathway with kinetics consistent with the activation of an apoptotic pathway. TauNHCl treatment resulted in a time-dependent conformational change in BAX that has been associated with its activation. Under these culture conditions, the cell killing activity of TauNHCl was rapid (less than 3 h). If TauNHCl was added to the medium more than 3 h prior to the addition of the cells, no cell killing activity was observed. In contrast, if TauNHCl-treated cells are washed and placed in fresh media 3 h after treatment, cell killing was retained. These results suggested that TauNHCl initiated a cell death pathway that manifested at a much later time. Based on previous results with IxB (8) one possibility is that TauNHCl may directly oxidize BAX, resulting in the conformational change in BAX associated with its activation. However, our studies failed to detect this conformational change in BAX at 4 h, a time point when the TauNHCl cytotoxic activity was absent from the medium. Together, these results suggested that activation of BAX by TauNHCl was indirect. Therefore, as for IL-3 deprivation and other apoptotic signals, the biochemical and/or cellular processes that resulted in the conformational change in BAX remain unknown.

Cytochrome c and other factors are released from mitochondria following the activation of BAX (44). Cytochrome c then interacts with APAF-1 and caspase-9 to form a complex (apoptosome) that results in the activation of caspase-9 and caspase-3 (45). Caspase activity then results in many of the characteristics associated with apoptosis. However, a number of studies (46) suggest that caspase-independent pathways may also result in cell death. TauNHCl treatment of FL5.12 cells resulted in caspase activity, and cells expressing a dominant negative form of caspase-9 were protected from TauNHCl-induced cell death. These data support a model in which TauNHCl activates a classic apoptotic pathway that involves Bax activation and caspase-9 activity. However, cells expressing Casp9DN were not protected as effectively from TauNHCl-induced cell death as the cells expressing Bcl-2. These data suggest that caspase-independent mitochondrial pathways, such as apoptosis-inducing factor (AIF) or endonuclease G, may

![Fig. 9. TauNHCl-induced cell cycle-independent arrest by BrdUrd staining in FL5-Neo cells. FL5-Neo cells were pulsed for 30 min with BrdUrd, washed, and then left untreated (Control) or treated with 1 mM TauNHCl. The anti-BrdUrd FITC/PI contour plots at 0, 4, and 6 h are shown. For the control conditions, both the BrdUrd-positive and -negative cells progressed through the cell cycle, whereas the contour plots for the TauNHCl-treated cells showed little change.](http://www.jbc.org/Downloadedfrom/fig9.jpg)
and Methods.” Clonogenic survival was normalized to the untreated treated with the indicated concentration of TauNHCl and plated by FL5-Neo (HCl treatment.

These studies utilized [3H]thymidine uptake and did not deter-

mitochondria following TauNHCl treat-

also be released from mitochondria following TauNHCl treat-

TauNHCl inhibits cell proliferation of human lymphocytes following activation with phytohemagglutinin (40). However, these studies utilized [3H]thymidine uptake and did not determine the phase of the cell cycle in which the block occurred. In our studies, cells pulsed with BrdUrd just prior to TauNHCl treatment failed to show any cell cycle progression at 4 and 6 h. We found that TauNHCl treatment completely arrested cell division in a cell cycle-independent manner. This is in sharp contrast with the G2/M arrest that is observed following IL-3 withdrawal (47). TauNHCl arrested both FL5-Neo and FL5-Bcl-2 cells equivalently. However, FL5-Bcl-2 cells were protected at the level of clonogenic survival. Therefore, at least a subset of the cells is able to begin cycling and to progress through the multiple cellular divisions required for the detection of colonies observed with this assay. We conclude that cell cycle arrest following TauNHCl treatment is reversible. Eluci-

dation of the pathways involved in the cell cycle independent arrest of cell proliferation by TauNHCl will be of future interest.

Our studies demonstrate that TauNHCl, a compound known to be formed in abundance at sites of localized inflammation, has profound effects on the survival and proliferation of immortalized pre-B FL5.12 cells. Following TauNHCl exposure, cells rapidly underwent cell cycle arrest in a cell cycle-inde-

Also, the expression of Casp9DN may not com-

This is in sharp contrast with the G2/M arrest that is observed following IL-3 withdrawal (47). TauNHCl arrested both FL5-Neo and FL5-

Bcl-2 pathway.

lysophytic cells activates a classic mitochondrial apoptotic pathway.
