# Regulation of Apoptosis in Myeloid Cells by Interferon Consensus Sequence–Binding Protein

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## Summary

Mice with a null mutation of the gene encoding interferon consensus sequence–binding protein (ICSBP) develop a disease with marked expansion of granulocytes and macrophages that frequently progresses to a fatal blast crisis, thus resembling human chronic myelogenous leukemia (CML). One important feature of CML is decreased responsiveness of myeloid cells to apoptotic stimuli. Here we show that myeloid cells from mice deficient in ICSBP exhibit reduced spontaneous apoptosis and a significant decrease in sensitivity to apoptosis induced by DNA damage. In contrast, apoptosis in thymocytes from ICSBP-deficient mice is unaffected. We also show that overexpression of ICSBP in the human U937 monocytic cell line enhances the rate of spontaneous apoptosis and the sensitivity to apoptosis induced by etoposide, lipopolysaccharide plus ATP, or rapamycin. Programmed cell death induced by etoposide was specifically blocked by peptides inhibitory for the caspase-1 or caspase-3 subfamilies of caspases. Studies of proapoptotic genes showed that cells overexpressing ICSBP have enhanced expression of caspase-3 precursor protein. In addition, analyses of antiapoptotic genes showed that overexpression of ICSBP results in decreased expression of Bcl-$X_L$. These data suggest that ICSBP modulates survival of myeloid cells by regulating expression of apoptosis-related genes.

Key words: apoptosis • caspase • chronic myelogenous leukemia • interferon • interferon consensus sequence–binding protein

Interferon consensus sequence–binding protein (ICSBP)[1] is a transcription factor of the IFN regulatory factor (IRF) family (1). Members of the family—IRF-1, -2, -3, -4, -6, -7, IFN-stimulated gene factor (ISGF)3γ, v-IRF, and ICSBP—are structurally related, bind to the IFN-stimulated response element (ISRE), and regulate expression of genes stimulated by type I IFN (IFN-α/β) (2–5). Type II IFN (IFN-γ), on the other hand, stimulates transcription of genes through the IFN-γ activation site (GAS) element that binds the signal transducer and activator of transcription (STAT)1, a member of the STAT transcription factor family (5, 6). A number of IFN-responsive genes are stimulated by both types of IFN, as there is extensive overlap of the two transcription pathways (7).

ICSBP was originally identified as a transcription factor that, similar to IRF-2, acts as a repressor and inhibits IFN-inducible promoter activities (8). Many attempts have been made to establish its contributions to IFN signaling, with recent studies revealing complex roles for this factor in immunity, cell cycle regulation, and hematopoiesis (9, 10). Evidence that IRF family proteins play important roles in the growth of hematopoietic cells is seen in mice with null mutations of IRF-1 and IRF-2 (11), which are generally expressed, as well as IRF-4 (also called PIP or LSIRF) and ICSBP, which are almost exclusively expressed in hematopoietic cells (12). IRF-$1^{-/-}$ mice have developmental defects in thymocytes and $CD8^+$ T cell differentiation, whereas IRF-$2^{-/-}$ mice exhibit abnormalities of bone marrow hematopoiesis and B cell development (11). IRF-$4^{-/-}$ mice exhibit profound alterations of the function and homeostasis of both mature B and T cells (12). ICSBP$^{-/-}$ mice are characterized by altered hematopoiesis that manifests as a syndrome similar to human chronic myelogenous leukemia (CML; reference 10). The most prominent early features of this disorder are marked expansions of the granulocytic, monocytic, and, to a lesser extent, lymphoid lin-

[1]*Abbreviations used in this paper:* CHX, cycloheximide; CML, chronic myelogenous leukemia; FMK, fluoromethyl ketone; ICSBP, interferon consensus sequence–binding protein; IRF, interferon regulatory factor; ISGF, IFN-stimulated gene factor; ISRE, interferon-stimulated response element; RT, reverse transcriptase; STAT, signal transducer and activator of transcription; TUNEL, TdT-mediated dUTP-biotin nick-end labeling.

eages. Older mice experience a transition from this chronic phase of disease to a clonal, malignant blast crisis (10). A striking clinical counterpart to myeloid malignancies of ICSBP$^{-/-}$ mice comes from the observation that ICSBP transcripts are greatly decreased in cells of patients with CML (13).

Human CML is a complex disorder, with enhanced proliferation of granulocyte precursors and reduced sensitivity of myeloid cells to apoptosis suggested as contributing factors. A role for IRF family members in regulating cell death has precedent in the demonstration that DNA damage–induced apoptosis of peripheral T cells is dependent on IRF-1 (14). Here we show that myeloid cells of ICSBP$^{-/-}$ mice have increased resistance to apoptosis, and transfected cells overexpressing ICSBP have increased sensitivity.

## Materials and Methods

*Mice.* ICSBP mutant mice were generated as described (10). Homozygous mutant (−/−) and wild-type (+/+) mice on a (C57BL/6 × 129/Sv) $F_2$ background were bred and maintained under specific pathogen-free conditions.

*Cell Cultures.* Single-cell suspensions from spleens, bone marrow, and thymi of wild-type and knockout mice were prepared and resuspended in RPMI 1640 medium (Quality Biological, Inc.) containing 10% FCS, 15 mM glutamine, 100 U/ml penicillin/streptomycin, nonessential amino acids (GIBCO BRL or Biofluid, Inc.), and 50 μM 2-ME. For studies of apoptosis, cells at a concentration of $10^6$ cells/ml were incubated as 1-ml triplicate aliquots in 24-well plates. U937 human monocytic cells were stably transfected by electroporation with full length ICSBP (U937$^+$) or empty vector (pcxn2; U937$^-$) as previously described (15). Transfectants were maintained in RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin/streptomycin, and 200 μg/ml G418 (all from GIBCO BRL). Cells were harvested during exponential growth.

For proliferative responses, single-cell preparations from spleen, lymph node, and bone marrow were cultured in 96-well plates at $2 \times 10^5$ cells/ml for 24–72 h. Cells were pulsed with [$^3$H]thymidine for the last 18 h of culture and assayed for incorporation.

*Induction of Apoptosis.* Single-cell suspensions from spleens, bone marrow, and thymi of ICSBP$^{-/-}$ mice and ICSBP$^{+/+}$ littermates were isolated and cultured with 10 μg/ml etoposide (Sigma Chemical Co.) or 30 ng/ml TNF-α (R & D Systems, Inc.) plus 10 μg/ml cycloheximide (CHX; Sigma Chemical Co.). After 18 h, cells were stained and TUNEL (TdT-mediated dUTP-biotin nick-end labeling) assay and flow cytometric analyses were performed.

U937$^+$ and U937$^-$ transfectants were washed and cultured for 24 h at a concentration of $3 \times 10^5$ cells/ml in serum-free RPMI 1640 medium supplemented with 500 U/liter insulin (Eli Lilly and Co.) and 5 mg/liter transferrin (GIBCO BRL). Cells were treated with 20 μg/ml etoposide or 30 ng/ml TNF-α plus 10 μg/ml CHX, incubated for 18 h, and then harvested and assayed for apoptosis. In some experiments, transfectants were treated with 1 μg/ml LPS (Sigma Chemical Co.) for 4 h and then with 2 mg/ml ATP (Sigma Chemical Co.) for 30 min, incubated for 6 h, and then harvested and assayed for apoptosis. For protease inhibition experiments, cysteine protease inhibitors ZVAD-fluoromethyl ketone (FMK), BD-FMK, and the control ZFA-FMK reagent (provided by Dr. P.A. Henkart, National Cancer Institute, Bethesda, MD) were added at a concentration of 50 μM 1 h before induction of apoptosis.

*TUNEL Assay.* The presence of DNA nicking in apoptotic cells was assayed, with minor modifications, according to Gorczyca et al. (16). In brief, cell samples were collected and fixed in 1% formaldehyde in PBS on ice, washed once with PBS, resus-

**Table I.** *Sequences of PCR Primers*

| Gene | | Primers | Probe |
|---|---|---|---|
| *Caspase-2* | Sense | 5′-GTTACCTGCACACCGAGTCACG-3′ | 5′-TGACAACGCCAACTGCCCAAGCCT-3′ |
| | Antisense | 5′-AGGGGATCCTGCGCGTGGTTCTTATC-3′ | |
| *Caspase-3* | Sense | 5′-CTGCCTCTTCCCCCATTCTC-3′ | 5′-GGAAGCGAATCAATGGACTC-3′ |
| | Antisense | 5′-CTTGTCGGCATACTGTTTCA-3′ | |
| *Caspase-1* | Sense | 5′-TGATTGACTCCGTTATTCC-3′ | 5′-ACAATGCTGCTACAAAATC-3′ |
| | Antisense | 5′-CTTCTCTATGTGGGCTTTC-3′ | |
| *Caspase-7* | Sense | 5′-CGAAACGGAACAGACAAAGAT-3′ | 5′-TTATGGGAAAGATGGTGTCACA-3′ |
| | Antisense | 5′-TGGAATAGGCGAAGAGGAGGAAGT-3′ | |
| *Bcl-$X_L$* | Sense | 5′-CAGTGAGTGAGCAGGTGTTTTG-3′ | 5′-GGCGGCTGGGATACTTTTGTGG-3′ |
| | Antisense | 5′-AGGGGGTGGGAGGGTAGAGTGG-3′ | |
| *MCL-1* | Sense | 5′-CCGCTTGAGGAGATGGAA-3′ | 5′-CGACGCCGCCGCCAGCAG-3′ |
| | Antisense | 5′-ACGCCGTCGCTGAAAACA-3′ | |
| *cIAP2* | Sense | 5′-TACTCCAGCCTTCCTCCAAACC-3′ | 5′-GCCTGCTTTGCCTGTGGTGGGA-3′ |
| | Antisense | 5′-CTCCAGATTCCCAACACCTCAA-3′ | |
| $\beta_2$m | Sense | 5′-AGCGTACTCCAAAGATTCA-3′ | 5′-GAGTATGCCTGCCGTGTGA-3′ |
| | Antisense | 5′-CTACCTGTGGAGCAACCTG-3′ | |

MCL-1, myeloid cell leukemia sequence 1; IAP, inhibitor of apoptosis protein.

pended in cold 70% ethanol in PBS, and stored at −20°C. After rehydration, cells were resuspended in cacodylate buffer, 25 mM $CoCl_2$, and 0.5 nM biotin-dUTP in the presence or absence of 10 U TdT enzyme (Boehringer Mannheim Biochemicals) and incubated at 37°C. After washing in PBS, cells were resuspended in saline citrate buffer containing 4× SSC, 2.5 μg/ml FITC–avidin (Boehringer Mannheim Biochemicals), 0.1% Triton X-100 (Sigma Chemical Co.), and 5% nonfat dry milk. After a 30-min incubation in the dark, cells were washed in PBS with 0.1% Triton X-100 and 0.5% BSA and resuspended in Sorter medium (Quality Biological, Inc.). dUTP incorporation by individual cells was measured by green fluorescence on a FACScan™ flow cytometer using Cellquest™ data software (Becton Dickinson).

*Flow Cytometric Analysis and DNA Labeling.* Cells were stained with FITC-labeled Abs, including those to CD4, CD8, B220, Mac-1, and 8C5 (GR-1; all from PharMingen). In brief, cells were resuspended in 100 μl PBS containing 2% FCS and incubated with various mAbs (1 μg/ml) for 20 min. After one wash with PBS, cells were stained for TUNEL assay. Analysis was performed on a FACScan™ flow cytometer.

*Reverse Transcriptase PCR.* mRNA was isolated using RNAzol (Tel-Test) followed by extraction with chloroform and isopropanol. 1 μg of RNA was transcribed using MMLV-H(−) reverse transcriptase (RT; Promega Corp.) and then amplified by PCR using primers described in Table I under the following conditions: 1× reaction buffer (Promega Corp.), 2.5 mM $MgCl_2$, 2.5 mM dNTP, 5 μM of each primer, and 1 U of Taq DNA polymerase (Promega Corp.) in a total volume of 40 μl. Amplification was carried out through 30 cycles. PCR products were separated on 1.2% agarose gels and analyzed by Southern blot hybridization with FITC-labeled probes using an ECL-3′ oligolabeling and detection system and Hyperfilm-ECL (Amersham International).

*Western Blot Analysis.* Whole cell extracts were prepared by lysing cells for 30 min at 4°C with lysis buffer (50 mM Tris, pH 7.5, 2 mM EDTA, 100 mM NaCl, 1 mM $Na_3VO_4$, 1% NP-40, 10 μg/ml leupeptin, 5 μg/ml aprotinin, and 10 μg/ml PMSF) at a concentration of $10^6$ cells/50 μl. After centrifugation at 4°C for 15 min, aliquots of the extracts containing 100 μg of protein were subjected to 10% SDS-PAGE. The proteins were then electrophoretically transferred to PVDF (polyvinylidine difluoride) membrane filters (Bio-Rad Labs.). Filters were probed with sc-515, a rabbit polyclonal Ab to human caspase-1; sc-625, a rabbit polyclonal Ab to caspase-2 of human origin; sc-1224, a goat polyclonal Ab to human caspase-3; sc-1041, a rabbit polyclonal Ab to human Bcl-$X_{S/L}$; or an Ab to p53 (all from Santa Cruz Biotechnology, Inc.) at a concentration of 1 μg/ml in PBS with 5% dried milk and 0.2% Tween 20. Detection was carried out with secondary horseradish peroxidase–linked anti–rabbit or anti–goat Abs (Amersham International) and the Amersham ECL system.

*Transient Transfection and Luciferase Assay.* Primers corresponding to the human Bcl-$X_L$ promoter at positions 77–101 (AAGTCAGATTGCAGATCTGAGGCAG) and positions 745–769 (GAATTCACTTCATAGAACCTTGGAT) as previously reported (17; sequence available from EMBL/GenBank/DDBJ under accession number D30746) were used in a genomic PCR reaction to obtain the Bcl-$X_L$ promoter region. The PCR product was cloned into a basic pGL3 luciferase vector (Promega Corp.) cut with SacI and HindIII. The resulting plasmid was prepared by the CsCl gradient method as previously reported (18) and used for transient transfection assays. RAW cells, a mouse macrophage tumor line, were transfected by electroporation using the Cell-Porator (GIBCO BRL). In brief, up to 30 μg of plasmid DNA was mixed with $1.0 \times 10^7$ cells/ml and resuspended in 0.4 ml of fresh complete RPMI 1640 medium containing 10% FCS. The cell/DNA mixture was then incubated on ice for 10 min before electrical shock at 300 mV and 800 μF. Cells were then transferred into 10 ml of fresh medium and left at room temperature for 10 min before incubation at 37°C. 24 h after transfection, luciferase assays were performed as reported (19).

## Results

*Apoptosis Is Decreased in Myeloid Cells from ICSBP$^{-/-}$ Mice.* Although it was initially assumed that CML is caused by uncontrolled cell proliferation resulting in the clonal expansion evident in this disease, some studies have indicated that the rates of proliferation are not significantly increased (20, 21). Other studies have shown that myeloid cells of CML patients have increased resistance to apoptosis, indicating that this disease may result from suppression of cell death rather than ungoverned proliferation (22, 23). To investigate the possible relatedness of the disease of ICSBP$^{-/-}$ mice to CML, we first studied the levels of spontaneous proliferation and apoptosis of cells from nor-

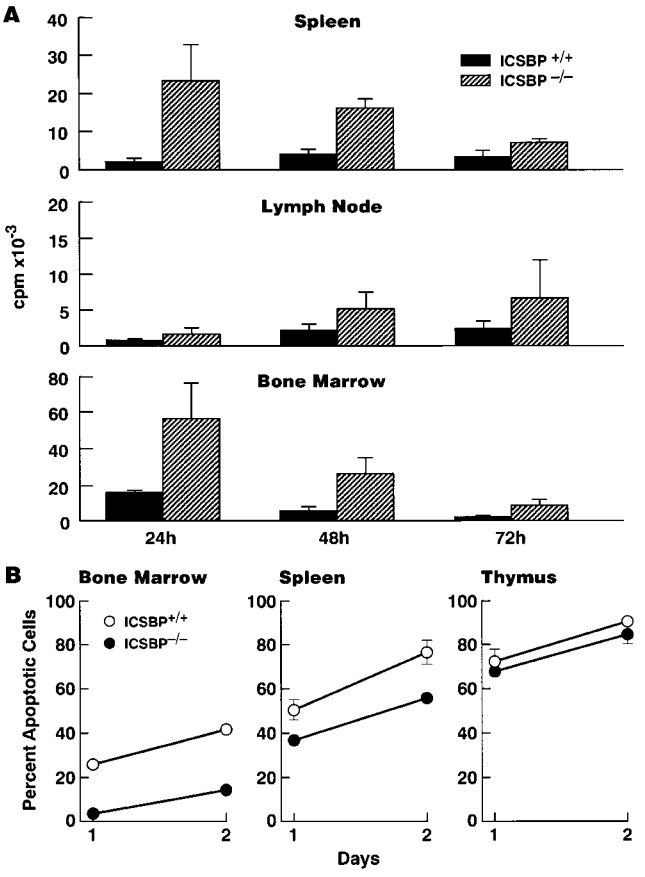


**Figure 1.** Spontaneous proliferation and apoptosis in cells of ICSBP$^{-/-}$ mice. (A) Cells were cultured for 24–72 h and pulsed with [$^3$H]thymidine for the last 18 h of culture. (B) Cells from −/− mice and +/+ littermates were cultured for 1 and 2 d and analyzed for apoptosis by TUNEL assay. Data are representative of three experiments using 10–16-wk-old mice.

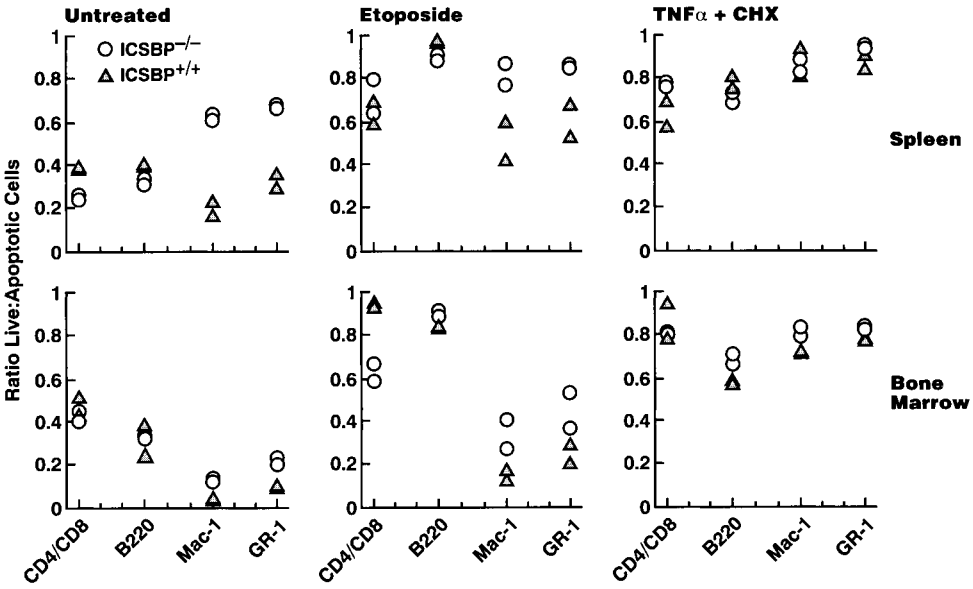


**Figure 2.** Macrophages and granulocytes account for reduced levels of apoptosis in spleen and bone marrow cells of ICSBP$^{-/-}$ mice. Cells from ICSBP$^{-/-}$ mice and ICSBP$^{+/+}$ littermates were cultured for 18 h alone, with etoposide (10 μg/ml), or with TNF-α (30 ng/ml) plus CHX (10 μg/ml), stained with the indicated Ab, and fixed for TUNEL assays. Data are representative of three experiments using 10–16-wk-old mice.

mal and mutant mice. As shown in Fig. 1 A, the proliferation of cultured spleen, lymph node, and bone marrow cells of mutant mice was increased over normal when assayed at 24–72 h. To examine whether reduced programmed cell death might contribute to the expansion of myeloid cells in ICSBP$^{-/-}$ mice, we examined spontaneous apoptosis of cultured cells from spleen, thymus, and bone marrow by TUNEL assay (Fig. 1 B). These studies revealed a significant reduction in the level of apoptotic death exhibited by spleen and bone marrow cells from ICSBP$^{-/-}$ mice. In contrast, thymocytes from ICSBP$^{-/-}$ and ICSBP$^{+/+}$ mice behaved similarly. To determine whether specific cell populations accounted for the reduced rate of apoptosis in spleen and bone marrow, cells were assayed simultaneously for apoptosis and expression of cell lineage markers. Mac-1$^+$ and, to a lesser extent, GR-1$^+$ cells from spleen and bone marrow of ICSBP$^{-/-}$ mice exhibited a significant reduction in the rate of apoptosis (Fig. 2, untreated cells). In contrast, the rates of apoptosis were comparable among CD4$^+$/CD8$^+$ and B220$^+$ cells from mice of either genotype.

To further investigate the possible role of ICSBP in apoptosis of myeloid cells, we examined programmed cell death induced by etoposide, an agent that induces DNA breaks by inhibiting the religation activity of topoisomerase II. As shown in Fig. 2, apoptosis induced by etoposide was significantly reduced in GR-1$^+$ and Mac-1$^+$ cells from spleens and bone marrow of ICSBP$^{-/-}$ mice. In contrast, apoptosis of splenic B and T cells was not affected. Interestingly, bone marrow T cells from ICSBP$^{-/-}$ mice were significantly more sensitive to etoposide than were cells from heterozygotes. Finally, we tested cells from ICSBP$^{-/-}$ and ICSBP$^{+/+}$ mice for their sensitivity to apoptosis induced by TNF-α plus CHX. No differences in responsiveness were noted for cells of any phenotype for either genotype (Fig. 2). These findings indicate that ICSBP selectively affects distinct pathways leading to apoptosis of myeloid cells: induction by etoposide is clearly modulated by ICSBP, whereas the pathway activated by TNF-α plus CHX is not.

*Apoptosis Is Enhanced in U937 Cells Overexpressing ICSBP.* A full length ICSBP construct was stably transfected into U937 human monocytic cells (U937$^+$), resulting in levels of ICSBP proteins that were ~10–30-fold higher than endogenous ICSBP (15). Transfectants containing the vector without insert (U937$^-$) were used as controls. For these studies, three clones of each transfectant were selected and pooled. Using the TUNEL assay for detection of DNA strand breaks, we examined apoptosis of both cell populations cultured in serum-free medium supplemented with insulin and transferrin. During the first 18 h of culture, neither U937$^+$ nor U937$^-$ cells showed clear evidence of apoptosis, but prominent differences were seen at 38 and 68 h (Fig. 3), indicating a proapoptotic role for ICSBP in myeloid cells.

Apoptosis is controlled by many distinct signals (24, 25), but it appears that different signaling pathways ultimately converge to activate a common program (26). It has been shown that U937 cells undergo apoptosis in response to multiple agents (27, 28). To further investigate the function of ICSBP in the regulation of distinct apoptotic pathways, we treated U937$^+$ and U937$^-$ cells with different apoptosis-inducing agents. U937$^+$ cells exhibited greater susceptibility to DNA damage–induced death than did control cells. After 10 h of treatment with etoposide, almost 70% of U937$^+$ cells were TUNEL positive, in contrast to 30% of control cells (Fig. 4). Interestingly, apoptosis was induced by TNF-α plus CHX at the same levels in U937$^-$ and U937$^+$ cells (data not shown), suggesting again that ICSBP is involved only in specific pathways of programmed cell death. A moderately enhanced sensitivity to apoptosis was also observed in U937$^+$ cells after treatment with LPS plus ATP. It was shown previously that ATP

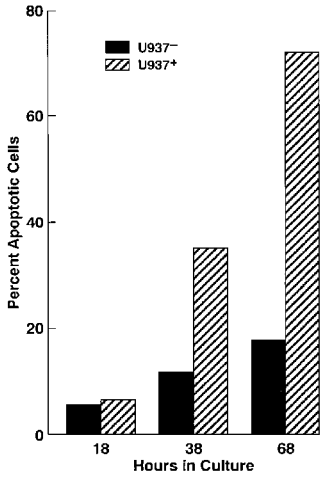


**Figure 3.** U937 cells overexpressing ICSBP show increased spontaneous apoptosis. U937$^+$ and U937$^-$ cells were cultured for the indicated times and assayed for apoptosis by TUNEL. Data are representative of three independent experiments.

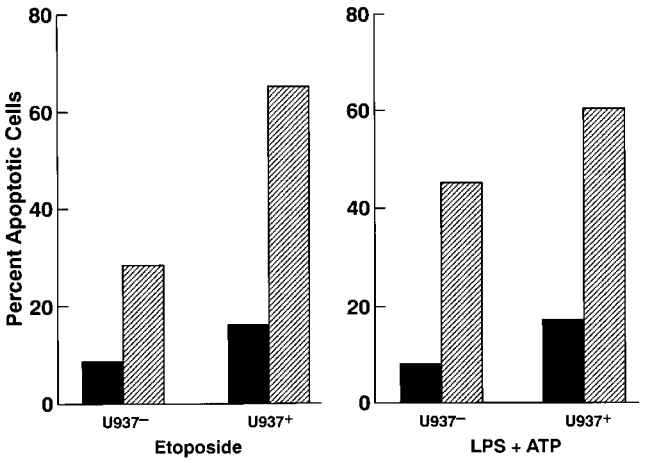


**Figure 4.** Apoptosis induced by etoposide or LPS plus ATP is enhanced in U937 cells overexpressing ICSBP. Cells were treated with reagents as indicated in Materials and Methods and assayed for apoptosis by TUNEL. Data are representative of three or more experiments. Solid bars, untreated cells; hatched bars, treated cells.

treatment leads to apoptosis via a caspase-1–independent pathway (29). We found that treatment with LPS for 4 h and then with ATP for 30 min induced apoptosis in 60% of U937$^+$ cells but only 45% of U937$^-$ cells (Fig. 4). Moreover, U937$^+$ cells were significantly more sensitive to rapamycin, a potent immunosuppressive drug that has been shown to interfere with growth factor–induced cell proliferation and induce apoptosis in a murine B cell line (30) (data not shown). Although to date there is no direct evidence for the involvement of ICSBP in cell growth pathways, our results clearly show that ICSBP plays a role in the apoptotic response of U937 cells to specific death-inducing signals and confirm that ICSBP itself might contribute to spontaneous and DNA damage–induced apoptosis in this system.

*Inhibition of Etoposide-induced Apoptosis by ZVAD-FMK and BD-FMK in U937$^+$ Cells.* We next examined the role of caspases in programmed cell death of U937$^+$ cells. It is evident that caspases are important effector molecules of apoptosis (26, 31). To determine whether specific caspase subfamilies contribute to apoptosis in U937$^+$ cells treated with etoposide, we tested the activity of two cell-permeable peptide FMK inhibitors of caspase-1 (IL-1β converting enzyme-family) proteases (32). ZVAD-FMK specifically blocks the caspase-1–like subfamily, and BD-FMK preferentially inhibits caspase-3–like proteases. ZFA-FMK, which lacks inhibitory activity for caspases, was used as a negative control. Both ZVAD-FMK and BD-FMK inhibited apoptotic death induced by etoposide in U937$^+$ (Fig. 5) and U937$^-$ cells (data not shown). These results suggest that ICSBP enhances the sensitivity of U937 monocytic cells to apoptosis induced by etoposide and that the apoptotic process involves the activity of members of the caspase-1 and caspase-3 subfamilies.

*Control of Caspase Gene Expression by ICSBP.* To address the possibility that ICSBP may interfere with the activation of caspases, we examined the expression of caspase-1, -2, -3, and -7 (33–36). Semiquantitative RT-PCR was performed on each cell population before and after treatment with etoposide (Fig. 6, left). Transcripts for caspase-2 and -3 were present at similar levels before and after treatment of U937$^+$ and control cells. In contrast, caspase-7 and, to a lesser extent, caspase-1 transcripts were increased in U937$^+$ before and after induction of apoptosis with etoposide. β2 microglobulin transcripts, run as a control, were comparable in both clones. These results suggest

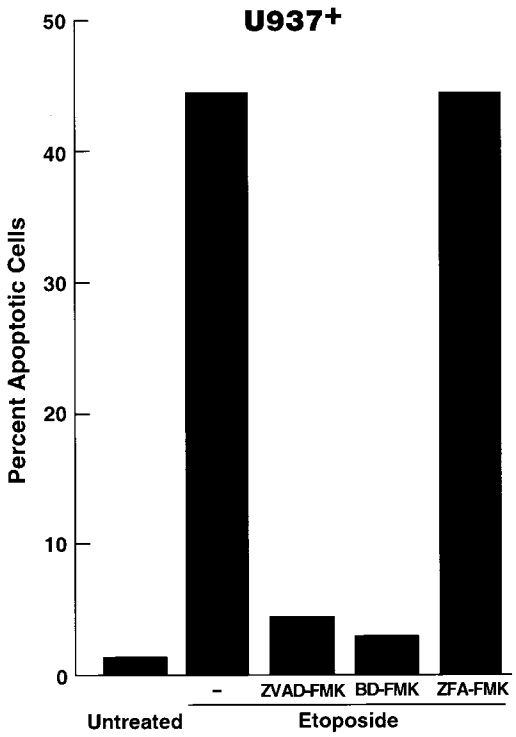


**Figure 5.** Caspase inhibitors block apoptosis in cells overexpressing ICSBP. Cells were incubated with caspase inhibitors ZVAD-FMK and BD-FMK and the inactive reagent ZFA-FMK for 1 h before addition of etoposide. TUNEL assays were performed 16 h later. Data are representative of three experiments.

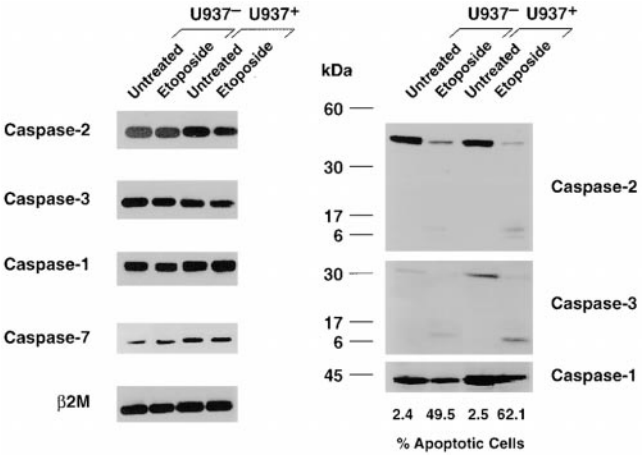


**Figure 6.** Caspase expression is modulated in cells overexpressing ICSBP. U937⁻ and U937⁺ cells were treated to induce apoptosis with the indicated reagents. Left, RT-PCR analyses of transcripts for the indicated genes. Right, Western blot analysis of caspase expression. Data are representative of three experiments.

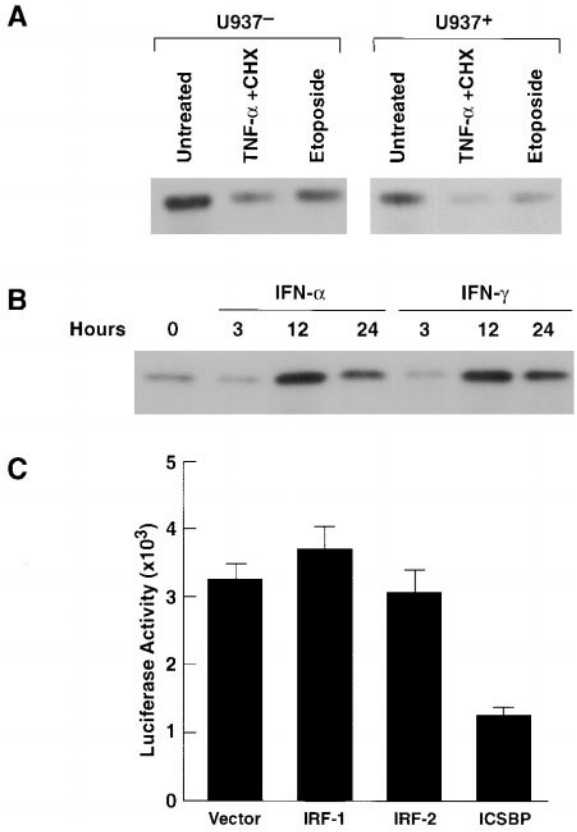


**Figure 7.** Regulation of Bcl-$X_L$ expression. (A) RT-PCR analyses of Bcl-$X_L$ transcripts in untreated cells and cells treated for 18 h with TNF-α plus CHX or with etoposide. (B) Western blot analyses of Bcl-$X_L$ levels in U937⁻ cells stimulated for the indicated times with IFN-α or IFN-γ. (C) Luciferase assays of Bcl-$X_L$ promoter activity in RAW cells. Cells were transfected with vector either alone or with IRF-1, IRF-2, or ICSBP. Data are representative of two experiments.

that overexpression of ICSBP in U937 cells may affect apoptosis by enhancing expression of the proapoptotic genes of caspase-1 and -7.

This study was extended by Western blot analysis. Whole cell lysates were prepared from U937⁺ and U937⁻ cells before and after treatment with etoposide, and immunoblot analysis was performed. The 48-kD precursor protein of caspase-2 was detectable before treatment in both cell types (Fig. 6, right), whereas two smaller fragments were generated from the cleavage of the caspase-2 precursor after treatment with etoposide. Notably, the strength of the low-molecular-mass fragments correlated with the increased sensitivity of U937⁺ cells to etoposide.

We next examined the expression of caspase-3 precursor protein. The most striking finding was markedly increased levels of the precursor in U937⁺ cells. After treatment with etoposide, the precursor was processed to generate a lower-molecular-mass fragment, reflecting induction of apoptosis. Although our previous results showed that expression of caspase-3 mRNA was not altered in ICSBP clones, Western blot analysis revealed a markedly enhanced expression of caspase-3 protein. These data suggest that ICSBP might control caspase-3 activity through modulation of translation or protein stability rather than transcription. In light of enhanced caspase-1 mRNA expression in U937⁺ cells, it was of interest to determine whether caspase-1 protein expression was also affected. We found the 45-kD precursor of caspase-1 was slightly overexpressed in U937⁺ cells. Because U937⁺ cells show enhanced sensitivity to apoptosis induced by ATP via a caspase-1–independent pathway (29), these new findings suggest that increased expression of caspase-1 is unlikely to be the basis for this phenotype. It thus seems that caspase-3 plays a major role in cell death signaling pathways controlled by ICSBP.

*Bcl-$X_L$ Expression Is Reduced in U937⁺ Cells.* We next asked whether ISRE-binding activities could control expression of antiapoptotic genes. Using semiquantitative RT-PCR analysis, we examined expression of members of the Bcl-2 family, including Bcl-$X_L$, Bcl-2, and Mcl-1 (37), as well as the proapoptotic gene, Bax (38). Bcl-$X_L$ transcripts were decreased by ~50% in U937⁺ cells (Fig. 7 A), whereas the levels of Bcl-2, Mcl-1, and Bax transcripts were comparable (data not shown). Treatment with etoposide resulted in downregulation of Bcl-$X_L$ expression in both cell types. Because U937⁺ and U937⁻ cells were equally sensitive to TNF plus CHX and U937⁺ cells were much more sensitive to etoposide, the importance of changes in Bcl-$X_L$ expression induced by ICSBP may contribute to changes in only some pathways of programmed cell death. The reduced levels of Bcl-$X_L$ transcripts observed in U937⁺ cells suggested that IFN might have a previously unappreciated role in regulating expression of this gene. We therefore examined levels of Bcl-$X_L$ protein in U937⁻ cells stimulated with IFN-α or IFN-γ. Both cytokines induced significant increases in Bcl-$X_L$ expression that were maximal at 12 h of treatment (Fig. 7 B).

To further examine ICSBP regulation of Bcl-$X_L$, we tested Bcl-$X_L$ promoter activity in RAW cells, a mouse

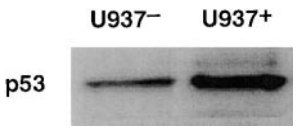


**Figure 8.** Western blot analyses of p53 expression in U937$^-$ and U937$^+$ cells.

macrophage tumor line, using a transient transfection assay. As shown in Fig. 7 C, luciferase activity driven by a 645-bp Bcl-$X_L$ promoter was significantly reduced by ICSBP but was not significantly affected by cotransfection of IRF-1 or IRF-2. As a control, RSV (Rous sarcoma virus) promoter activity was also unaffected, indicating specificity of the effect and suggesting that ICSBP represses transcription of Bcl-$X_L$.

Finally, we examined the expression of the antioncogenic transcription factor p53, which has been shown to be required to induce cell death in oncogene-expressing cells that have suffered DNA damage (39). p53 protein levels were significantly increased in U937$^+$ cells (Fig. 8), suggesting another mechanism by which ICSBP might be involved in the induction of spontaneous apoptosis and the DNA damage response pathway.

## Discussion

It is widely recognized that myeloid cells die via apoptosis (40), but the mechanisms involved in determining apoptotic death in this lineage have only recently come to be partially understood (41, 42). ICSBP knockout mice exhibit dysregulated hematopoiesis, defining a role for ICSBP in the proliferation and differentiation of hematopoietic cells. Here we report that myeloid cells from ICSBP-deficient mice exhibited reduced spontaneous apoptosis and a significant decrease in sensitivity to apoptosis induced by DNA damage. Moreover, overexpression of ICSBP in U937 cells was sufficient to enhance their sensitivity to spontaneous and induced apoptosis. Our results thus define ICSBP as a regulator of myeloid cell survival. We also point out that ICSBP is likely to normally function in regulation of the cell cycle in hematopoietic cells, because the spontaneous proliferation of bone marrow, spleen, and lymph node cells from mutant mice was significantly increased.

ICSBP has been identified as a negative transcription factor that binds to ISREs found in the promoters of type I IFNs and IFN-inducible genes (43, 44). Unlike IRF-1, IRF-3, and ISGF3γ, ICSBP exhibits a tissue-restricted pattern of expression largely limited to cells of the immune system, particularly the monocytic and lymphoid lineages. Expression of ICSBP is constitutive and can be dramatically enhanced by IFN-γ. The intrinsically weak DNA binding affinity of ICSBP (45) is dramatically increased after heterodimerization with IRF-1 or IRF-2 (3, 46). ICSBP/IRF-2 binding to DNA is constitutive, whereas ICSBP/IRF-1 DNA binding is only induced by IFN-γ. Several studies implicate IRF-1 as a critical tumor suppressor, regulating oncogene-induced cell transformation or apoptosis (39, 47). It has been reported that IRF-1 regulates DNA damage–induced apoptosis in mitogen-activated peripheral T cells (14). IRF-1 is also required for the induction of apoptosis in fibroblasts carrying an activated c-Ha-ras gene after DNA damage or culture in low serum (39). Moreover, IRF-1 has been implicated in IFN-γ–induced apoptosis of hematopoietic progenitor cells (48). Our data provide evidence that ICSBP, another member of the IRF family, can regulate apoptosis of myeloid cells.

ICSBP$^{-/-}$ mice exhibit expanded populations of granulocytic, monocytic, lymphoid, and perhaps megakaryocytic lineages (10), suggesting that the decisive alteration occurs in an early common progenitor cell. Our studies revealed that the sensitivity to apoptosis of Mac-1$^+$ and GR-1$^+$ cells from spleen and bone marrow of ICSBP$^{-/-}$ mice was significantly reduced. Expression of these markers is different in myeloid subpopulations; granulocytes are GR-1$^+$Mac-1$^+$ or GR-1$^+$Mac-1$^-$, and macrophages are GR-1$^-$Mac-1$^+$ (49), indicating that both macrophages and granulocytes from ICSBP$^{-/-}$ mice exhibited impaired programmed cell death. In contrast, the rate of apoptosis was comparable in T and B cells from +/+ or −/− mice, suggesting a specific role for ICSBP in apoptosis in the myeloid lineage. Analysis of apoptosis induced by etoposide confirmed the specific proapoptotic role of ICSBP in myeloid cells. GR-1$^+$ and Mac-1$^+$ cells from spleen and bone marrow of ICSBP$^{-/-}$ mice showed a significantly reduced sensitivity to apoptosis induced by this DNA-damaging agent. In contrast, etoposide-induced apoptosis of B and T cells from ICSBP$^{-/-}$ and wild-type mice was similar.

We also found that the proliferation index of bone marrow, spleen, and lymph node cells of ICSBP$^{-/-}$ mice was increased over normal. This suggests that the early accumulation of hematopoietic cells that characterizes these animals is mediated by enhanced proliferation as well as by reduced apoptosis. Studies of U937 cells overexpressing ICSBP provided strong support for the proposed role of ICSBP in regulating apoptosis in myeloid cells. ICSBP expressed at levels 10–30-fold higher than endogenous levels sensitized cells to spontaneous apoptosis and selective pathways of response to exogenous stimuli. U937$^+$ cells exhibited greater sensitivity to apoptosis induced by DNA damage, treatment with LPS plus ATP, and inhibition of TOR/FRAP kinase by rapamycin (reference 50; data not shown), but not to treatment with TNF-α plus CHX. This suggests that apoptotic pathways initiated by engagement of death receptors leading to rapid activation of caspase cascades are insensitive to effects mediated by ICSBP, whereas other pathways are sensitive. This pattern of sensitivity and resistance to the effects of ICSBP is similar to that suggested for Bcl-2 and Bcl-$X_L$: little or no effect on apoptosis induced by death receptor engagement but prominent regulation of apoptosis induced by growth factor withdrawal or DNA damage (51–53). It is unclear whether these selective effects on apoptosis are mediated by ICSBP alone or in combination with other IRF family members. IRF-1 is an important requirement of DNA damage–induced apoptosis of peripheral T cells (14) but not myeloid cells, at least on its own, because it is expressed at normal levels in ICSBP$^{-/-}$ mice.

Inhibition of TOR/FRAP by rapamycin (53) leads to G1 arrest in some systems (54) and acceleration of apoptosis in others (55). The results of TOR activation—either growth arrest or apoptosis—resemble the pattern of responses to p53 activation (56). Because p53 expression is enhanced in $U937^+$ cells, ICSBP may regulate apoptosis by acting as a rheostat to balance the activities of these bifunctional genes. Induction of apoptosis by p53 in some systems is thought to involve transcriptional induction of Bax (57). This activity is unlikely to be relevant to our system, because Bax transcripts were similar in control and $U937^+$ cells. The importance of TOR to apoptosis could reflect its regulation by Akt, which functions in the phosphatidylinositol-3 kinase pathway to inhibit apoptosis (58). Inhibition of TOR may induce apoptosis by inactivating downstream signaling pathways that involve $p70^{S6K}$ kinase or 4E-BP-1 (59).

Further analysis of $U937^+$ cells revealed that ICSBP may affect expression of caspases involved in the central control and execution stages of cell death (60–63). Our results revealed that transcripts for caspase-2 and -3 were expressed at the same level in $U937^-$ and $U937^+$ cells before and after induction of apoptosis with etoposide. In contrast, caspase-7 and, to a lesser extent, caspase-1 transcripts were increased before and after induction of apoptosis of $U937^+$ cells with etoposide. These data show that expression of caspases belonging to distinct subfamilies is differentially regulated by ICSBP.

Our studies also revealed that caspase protein expression is specifically modulated in $U937^+$ cells. First, activation of caspase-2 was enhanced in $U937^+$ cells. Second, although caspase-3 transcripts were not increased in cells overexpressing ICSBP, levels of the caspase-3 precursor protein were significantly increased. Finally, caspase-1 precursor levels were higher in $U937^+$ than in control cells before and after induction of apoptosis with etoposide. These results clearly show that increased expression of caspase-1, -3, and -7 occurred in $U937^+$ cells in association with enhanced sensitivity to spontaneous and induced apoptosis. Although we have no direct evidence at present for the transcriptional or translational control of caspases by ICSBP, the enhanced levels of one or more could account for the priming of a cascade that leads to the effector phases of apoptosis.

Cell death is regulated by a number of genes that influence cell survival in either a positive or negative fashion. The Bcl-2 family consists of molecules that can either promote survival or augment programmed cell death (64, 65). The capability of type I and type II IFNs to modulate the expression of genes belonging to the Bcl-2 family is a controversial issue (66, 67). Recent reports revealed that IFN-γ significantly inhibits eosinophil apoptosis but does not upregulate Bcl-2 expression (68). In addition, IFN-α2 is reported to inhibit the growth of myeloma cells in a manner independent of Bcl-2 and Bcl-$X_L$ expression (69). On the other hand, a recent report has shown that leukemia inhibitory factor induces Bcl-$X_L$ mRNA via a STAT1-binding cis-element in cardiac myocytes, preventing a cytoprotective effect (70). We found that $U937^+$ cells exhibited a specific decrease in Bcl-$X_L$ transcripts. Moreover, our data show that Bcl-$X_L$ can be strongly induced by IFN-α and IFN-γ. Finally, our results clearly demonstrate that a consensus ISRE present in the promoter region of Bcl-$X_L$ is sufficient to confer efficient IFN-α inducibility. Surprisingly, Bcl-2 expression was not affected in $U937^+$ cells. These results argue for a Bcl-2–independent but Bcl-$X_L$–dependent mechanism of apoptosis regulation by ICSBP. In our model, downregulation of Bcl-$X_L$ could promote caspase-1–dependent disruption of the mitochondrial inner transmembrane potential and the subsequent activation of caspase-3 and -7. Recently, posttranscriptional modifications of some Bcl-2 family members have been shown to regulate cell death (71, 72). Because Bcl-$X_L$ by itself is able to form ion channels in membranes (73) and is present in both soluble and membrane-bound forms, it would be interesting to determine whether mRNA expression, posttranscriptional modifications, and/or subcellular localization of this antiapoptotic protein represent important steps in the pathway by which IRF family members regulate cell death. Furthermore, because the functional relationship between the Bcl-2 family of apoptotic modulators and the caspases remains unresolved, our model might be useful for elucidating specific interactions between these families of proteins.

Although the precise mechanism by which ICSBP modulates expression of genes involved in apoptosis remains to be elucidated, our data argue for a specific proapoptotic activity elicited in U937 cells overexpressing ICSBP. In addition, studies of ICSBP-deficient mice strongly support the involvement of this factor as a regulator in the apoptotic pathway of myeloid cells whose leukemic transformation might follow escape from normal apoptotic controls; however, the possible relations between the myeloid disease of ICSBP mice and human CML remain to be clearly defined. We also found that the proliferative rate of bone marrow cells from $ICSBP^{-/-}$ mice was increased, suggesting that enhanced proliferation as well as prolonged survival may contribute to this myeloid disorder.

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## References


1. Nguyen, H., J. Hiscott, and P.M. Pitha. 1997. The growing family of interferon regulatory factors. *Cytokine Growth Factor Rev*. 8:293–312.
2. Moore, P.S., C. Boshoff, R.A. Weiss, and Y. Chang. 1996. Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. *Science*. 274:1739–1744.
3. Sharf, R., D. Meraro, A. Azriel, A.M. Thornton, K. Ozato, E.F. Petricoin, A.C. Larner, F. Schaper, H. Hauser, and B.Z. Levi. 1997. Phosphorylation events modulate the ability of interferon consensus sequence binding protein to interact with interferon regulatory factors and to bind DNA. *J. Biol. Chem*. 272:9785–9792.
4. Zhang, L., and J.S. Pagano. 1997. IRF-7, a new interferon regulatory factor associated with Epstein-Barr virus latency. *Mol. Cell. Biol*. 17:5748–5757.
5. Darnell, J.E., Jr., I.M. Kerr, and G.R. Stark. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science*. 264:1415–1421.
6. Shuai, K., C. Schindler, V.R. Prezioso, and J.E. Darnell, Jr. 1992. Activation of transcription by IFN-γ: tyrosine phosphorylation of a 91-kD DNA binding protein. *Science*. 258: 1808–1812.
7. Kalvakolanu, D.V., and E.C. Borden. 1996. An overview of the interferon system: signal transduction and mechanisms of action. *Cancer Invest*. 14:25–53.
8. Nelson, N., M.S. Marks, P.H. Driggers, and K. Ozato. 1993. Interferon consensus sequence-binding protein, a member of the interferon regulatory factor family, suppresses interferon-induced gene transcription. *Mol. Cell. Biol*. 13:588–599.
9. Bovolenta, C., J. Lou, Y. Kanno, B.K. Park, A.M. Thornton, J.E. Coligan, M. Scubert, and K. Ozato. 1995. Vesicular stomatitis virus infection induces a nuclear DNA binding factor specific for the interferon-stimulated response element. *J. Virol*. 69:4173–4181.
10. Holtschke, T., J. Lohler, Y. Kanno, T. Fehr, N. Giese, F. Rosenbauer, J. Lou, K.P. Knobeloch, L. Gabriele, J.F. Waring, et al. 1996. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. *Cell*. 87:307–317.
11. Matsuyama, T., T. Kimura, M. Kitagawa, K. Pfeffer, T. Kawakami, N. Watanabe, T.M. Kundig, R. Amakawa, K. Kishihara, A. Wakeham, et al. 1993. Targeted disruption of IRF-1 or IRF-2 results in abnormal type I IFN gene induction and aberrant lymphocyte development. *Cell*. 75:83–97.
12. Mittrucker, H.W., T. Matsuyama, A. Grossman, T.M. Kundig, J. Potter, A. Shahinian, A. Wakeham, B. Patterson, P.S. Ohashi, and T.W. Mak. 1997. Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. *Science*. 275:540–543.
13. Schmidt, M., S. Nagel, J. Proba, C. Thiede, M. Ritter, J.F. Waring, F. Rosenbauer, D. Huhn, B. Wittig, I. Horak, et al. 1998. Lack of interferon consensus sequence binding protein (ICSBP) transcripts in human myeloid leukemias. *Blood*. 91: 22–29.
14. Tamura, T., M. Ishihara, M.S. Lamphier, N. Tanaka, I. Oishi, S. Aizawa, T. Matsuyama, T.W. Mak, S. Taki, and T. Taniguchi. 1995. An IRF-1-dependent pathway of DNA damage-induced apoptosis in mitogen-activated T lymphocytes. *Nature*. 376:596–599.
15. Thornton, A.M., V.V. Ogryzko, A. Dent, R. Sharf, B.Z. Levi, Y. Kanno, L.M. Staudt, B.H. Howard, and K. Ozato. 1996. A dominant negative mutant of an IFN regulatory factor family protein inhibits both type I and type II IFN-stimulated gene expression and antiproliferative activity of IFNs. *J. Immunol*. 157:5145–5154.
16. Gorczyca, W., J. Gong, and Z. Darzynkiewicz. 1993. Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assays. *Cancer Res*. 53:1945–1951.
17. Grillot, D.A., M. Gonzalez-Garcia, D. Ekhterae, L. Duan, N. Inohara, S. Ohta, M.F. Seldin, and G. Nunez. 1997. Genomic organization, promoter region analysis, and chromosome localization of the mouse bcl-x gene. *J. Immunol*. 158: 4750–4757.
18. Sambrook, J., E.F. Frisch, and T. Maniatis. 1989. Molecular Cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
19. Wang, I.M., J.C. Blanco, S.Y. Tsai, M.J. Tsai, and K. Ozato. 1996. Interferon regulatory factors and TFIIB cooperatively regulate interferon-responsive promoter activity in vivo and in vitro. *Mol. Cell. Biol*. 16:6313–6324.
20. Bedi, A., B.A. Zehnbauer, M.I. Collector, J.P. Barber, M.S. Zicha, S.J. Sharkis, and R.J. Jones. 1993. BCR-ABL gene rearrangement and expression of primitive hematopoietic progenitors in chronic myeloid leukemia. *Blood*. 81:2898–2902.
21. Emanuel, P.D., L.J. Bates, R.P. Castlebery, R.J. Gualtieri, and K.S. Zukerman. 1991. Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. *Blood*. 77:925–929.
22. Bedi, A., B.A. Zehnbauer, J.P. Barber, S.J. Sharkis, and R.J. Jones. 1994. Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. *Blood*. 83:2038–2044.
23. Fernandes, R.S., A.M. Gorman, A. McGahon, M. Lawler, S. McCann, and T.G. Cotter. 1996. The repression of apoptosis by activated abl oncogenes in chronic myelogenous leukemia. *Leukemia*. 2:S17–S21.
24. Rowan, S., and D.E. Fisher. 1997. Mechanisms of apoptotic cell death. *Leukemia*. 11:457–465.
25. Yuan, J. 1997. Transducing signals of life and death. *Curr. Opin. Cell Biol*. 9:247–251.
26. Henkart, P.A. 1996. ICE family proteases: mediators of all apoptotic cell death? *Immunity*. 4:195–201.
27. Noguchi, K., M. Naito, S. Kataoka, S. Yonehara, and T. Tsuruo. 1995. A recessive mutant of the U937 cell line acquired resistance to anti-Fas and anti-p55 tumor necrosis factor receptor antibody-induced apoptosis. *Cell Growth Differ*. 6:1271–1277.
28. Kikuchi, H., R. Iizuka, S. Sugiyama, G. Gon, H. Mori, M.

Arai, K. Mizumoto, and S. Imajoh-Ohmi. 1996. Monocytic differentiation modulates apoptotic response to cytotoxic anti-Fas antibody and tumor necrosis factor α in human monoblast U937 cells. *J. Leukoc. Biol.* 60:778–783.
29. Li, P., H. Allen, S. Banerjee, S. Franklin, L. Herzog, C. Johnston, J. McDowell, M. Paskind, L. Rodman, J. Salfeld, et al. 1995. Mice deficient in IL-1 β-converting enzyme are defective in production of mature IL-1 β and resistant to endotoxic shock. *Cell.* 80:401–411.
30. Muthukkumar, S., T.M. Ramesh, and S. Bondada. 1995. Rapamycin, a potent immunosuppressive drug, causes programmed cell death in B lymphoma cells. *Transplantation.* 60: 264–270.
31. Green, D.R. 1998. Apoptotic pathways: the roads to ruin. *Cell.* 94:695–698.
32. Thornberry, N.A., and Y. Lazebnik. 1998. Caspases: enemies within. *Science.* 281:1312–1316.
33. Sarin, A., M.L. Wu, and P.A. Henkart. 1996. Different interleukin-1β converting enzyme (ICE) family protease requirements for the apoptotic death of T lymphocytes triggered by diverse stimuli. *J. Exp. Med.* 184:2445–2450.
34. Cerretti, D.P., C.J. Kozlosky, B. Mosley, N. Nelson, K. Van Ness, T.A. Greenstreet, C.J. March, S.R. Kronheim, T. Druck, L.A. Cannizzaro, et al. 1992. Molecular cloning of the interleukin-1β converting enzyme. *Science.* 256:97–100.
35. Duan, H., A.M. Chinnaiyan, P.L. Hudson, J.P. Wing, W.W. He, and V.M. Dixit. 1996. ICE-LAP3, a novel mammalian homologue of the *Caenorhabditis elegans* cell death protein Ced-3, is activated during Fas- and tumor necrosis factor-induced apoptosis. *J. Biol. Chem.* 271:1621–1625.
36. Wang, L., M. Miura, L. Bergeron, H. Zhu, and J. Yuan. 1994. Ich-1, an Ice/ced-3-related gene, encodes both positive and negative regulators of programmed cell death. *Cell.* 78:739–750.
37. Adams, J.M., and S. Cory. 1998. The Bcl-2 protein family: arbiters of cell survival. *Science.* 281:1322–1326.
38. Oltvai, Z.N., C.L. Milliman, and S.J. Korsmeyer. 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell.* 74:609–619.
39. Tanaka, N., M. Ishihara, M. Kitagawa, H. Harada, T. Kimura, T. Matsuyama, M.S. Lamphier, S. Aizawa, T.W. Mak, and T. Taniguchi. 1994. Cellular commitment to oncogene-induced transformation or apoptosis is dependent on the transcription factor IRF-1. *Cell.* 77:829–839.
40. Cotter, T.G., R.S. Fernandes, S. Verhaegen, and J.V. McCarthy. 1994. Cell death in the myeloid lineage. *Immunol. Rev.* 142:93–112.
41. Lotem, J., and L. Sachs. 1996. Control of apoptosis in hematopoiesis and leukemia by cytokines, tumor suppressor and oncogenes. *Leukemia.* 10:925–931.
42. Ruchaud, S., and M. Lanotte. 1997. cAMP and 'death signals' in a myeloid leukaemia cell: from membrane receptors to nuclear responses: a review. *Biochem. Soc. Trans.* 25:410–415.
43. Weisz, A., P. Marx, R. Sharf, E. Appella, P.H. Driggers, K. Ozato, and B.Z. Levi. 1992. Human interferon consensus sequence binding protein is a negative regulator of enhancer elements common to interferon-inducible genes. *J. Biol. Chem.* 267:25589–25596.
44. Weisz, A., S. Kirchhoff, and B.Z. Levi. 1994. IFN consensus sequence binding protein (ICSBP) is a conditional repressor of IFN inducible promoters. *Int. Immunol.* 6:1125–1131.
45. Driggers, P.H., D.L. Ennist, S.L. Gleason, W.H. Mak, M.S. Marks, B.Z. Levi, J.R. Flanagan, E. Appella, and K. Ozato. 1990. An interferon γ-regulated protein that binds the interferon-inducible enhancer element of major histocompatibility complex class I genes. *Proc. Natl. Acad. Sci. USA.* 87: 3743–3747.
46. Bovolenta, C., P.H. Driggers, M.S. Marks, J.A. Medin, A.D. Politis, S.N. Vogel, D.E. Levy, K. Sakaguchi, E. Appella, J.E. Coligan, et al. 1994. Molecular interactions between interferon consensus sequence binding protein and members of the interferon regulatory factor family. *Proc. Natl. Acad. Sci. USA.* 91:5046–5050.
47. Harada, H., M. Kitagawa, N. Tanaka, H. Yamamoto, K. Harada, M. Ishihara, and T. Taniguchi. 1993. Anti-oncogenic and oncogenic potentials of interferon regulatory factors-1 and -2. *Science.* 259:971–974.
48. Sato, T., C. Selleri, N.S. Young, and J.P. Maciejewski. 1997. Inhibition of interferon regulatory factor-1 expression results in predominance of cell growth stimulatory effects of interferon-γ due to phosphorylation of Stat1 and Stat3. *Blood.* 90: 4749–4758.
49. Hestdal, K., F.W. Ruscetti, J.N. Ihle, S.E. Jacobsen, C.M. Dubois, W.C. Kopp, D.L. Longo, and J.R. Keller. 1991. Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cells. *J. Immunol.* 147:22–28.
50. Brown, E.J., M.W. Albers, T.B. Shin, K. Ichikawa, C.T. Keith, W.S. Lane, and S.L. Schreiber. 1994. A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature.* 369:756–758.
51. Strasser, A., A.W. Harris, D.C. Huang, P.H. Krammer, and S. Cory. 1995. Bcl-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apoptosis. *EMBO (Eur. Mol. Biol. Organ.) J.* 14:6136–6147.
52. Newton, K., A.W. Harris, M.L. Bath, K.G. Smith, and A. Strasser. 1998. A dominant interfering mutant of FADD/MORT1 enhances deletion of autoreactive thymocytes and inhibits proliferation of mature T lymphocytes. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:706–718.
53. Smith, K.G., A. Strasser, and D.L. Vaux. 1996. Crm A expression in T lymphocytes of transgenic mice inhibits CD95(Fas/APO-1)-transduced apoptosis, but does not cause lymphadenopathy or autoimmune disease. *EMBO (Eur. Mol. Biol. Organ.) J.* 15:5167–5176.
54. Morice, W.G., G.J. Brunn, G. Wiederrecht, J.J. Siekierka, and R.T. Abraham. 1993. Rapamycin-induced inhibition of p34cdc2 kinase activation is associated with G1/S-phase growth arrest in T lymphocytes. *J. Biol. Chem.* 268:3734–3738.
55. Shi, Y., A. Frankel, L.G. Radvanyi, L.Z. Penn, R.G. Miller, and G.B. Mills. 1995. Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro. *Cancer Res.* 55:1982–1988.
56. Levine, A. 1997. p53, the cellular gatekeeper for growth and division. *Cell.* 88:323–331.
57. Miyashita, T., and J.C. Reed. 1995. Tumor-suppressor p53 is a direct transcriptional activator of the human BAX gene. *Cell.* 80:293–299.
58. Kauffmann-Zeh, A., P. Rodriguez-Viciana, E. Ulrich, C. Gilbert, P. Coffer, J. Downward, and G. Evan. 1997. Suppression of c-Myc-induced apoptosis by ras signalling through PI(3)K and PKB. *Nature.* 385:544–548.
59. Brown, E.J., and S.L. Schrieber. 1996. A signaling pathway to translational control. *Cell.* 86:517–520.
60. Rao, L., and E. White. 1997. Bcl-2 and the ICE family of apoptotic regulators: making a connection. *Curr. Opin.*

*Genet. Dev.* 7:52–58.
61. Thornberry, N.A., T.A. Rano, E.P. Peterson, D.M. Rasper, T. Timkey, M. Garcia-Calvo, V.M. Houtzager, P.A. Nordstrom, S. Roy, J.P. Vaillancourt, et al. 1997. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. *J. Biol. Chem.* 272:17907–17911.
62. Fraser, A., and G. Evan. 1996. A license to kill. *Cell.* 85: 781–784.
63. Enari, M., R.V. Talanian, W.W. Wong, and S. Nagata. 1996. Sequential activation of ICE-like and CPP32-like proteases during Fas-mediated apoptosis. *Nature.* 380:723–726.
64. Jacobson, M.D. 1997. Apoptosis: Bcl-2-related proteins get connected. *Curr. Biol.* 7:R277–R281.
65. Reed, J.C. 1997. Double identity for proteins of the Bcl-2 family. *Nature.* 387:773–776.
66. Chaouchi, N., C. Wallon, J. Taieb, M.T. Auffredou, G. Tertian, F.M. Lemoine, J.F. Delfraissy, and A. Vazquez. 1994. Interferon-α-mediated prevention of in vitro apoptosis of chronic lymphocytic leukemia B cells: role of bcl-2 and c-myc. *Clin. Immunol. Immunopathol.* 73:197–204.
67. Jewell, A.P., C.P. Worman, P.M. Lydyard, K.L. Yong, F.J. Giles, and A.H. Goldstone. 1994. Interferon-α up-regulates bcl-2 expression and protects B-CLL cells from apoptosis in vitro and in vivo. *Br. J. Haematol.* 88:268–274.
68. Ochiai, K., M. Kagami, R. Matsumura, and H. Tomioka. 1997. IL-5 but not interferon-γ (IFN-γ) inhibits eosinophil apoptosis by up-regulation of bcl-2 expression. *Clin. Exp. Immunol.* 107:198–204.
69. Egle, A., A. Villunger, M. Kos, G. Bock, J. Gruber, B. Auer, and R. Greil. 1996. Modulation of Apo-1/Fas (CD95)-induced programmed cell death in myeloma cells by interferon-α2. *Eur. J. Immunol.* 26:3119–3126.
70. Fujio, Y., K. Kunisada, H. Hirota, K. Yamauchi-Takihara, and T. Kishimoto. 1997. Signals through gp130 upregulate bcl-x gene expression via STAT1-binding cis-element in cardiac myocytes. *J. Clin. Invest.* 99:2898–2905.
71. Wang, H.G., U.R. Rapp, and J.C. Reed. 1996. Bcl-2 targets the protein kinase Raf-1 to mitochondria. *Cell.* 87:629–638.
72. Golstein, P. 1997. Controlling cell death. *Science.* 275:1081–1082.
73. Hsu, Y.T., K.G. Wolter, and R.J. Youle. 1997. Cytosol-to-membrane redistribution of Bax and Bcl-$X_{(L)}$ during apoptosis. *Proc. Natl. Acad. Sci. USA.* 94:3668–3672.