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Abstract
We have analyzed the interleukin-4 (IL-4)-triggered mechanisms implicated in cell survival and show here that IL-4 deprivation induces apoptotic cell death but does not modulate Bcl-2 or Bcl-x expression. Since Bcl-x expression is insufficient to ensure cell survival in the absence of IL-4, we speculate that additional molecules replace the antiapoptotic role of Bcl-2 and Bcl-x in an alternative IL-4-triggered pathway. Cell death is associated with Bcl-3 downregulation and Bcl-3 expression blocks IL-4 deprivation-induced apoptosis, suggesting that Bcl-3 acts as a survival factor in the absence of growth factor. To characterize the IL-4-induced regulation of murine Bcl-3 expression, we cloned the promoter of this gene. Sequencing of the promoter showed no TATA box element but did reveal binding sites for AP1, AP1-like, and SP1 transcription factors. Retardation gels showed that IL-4 specifically induces AP1 and AP1-like binding activity and that mutation of these binding sites abolish...

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Bcl-3 Expression Promotes Cell Survival following Interleukin-4 Deprivation and Is Controlled by AP1 and AP1-Like Transcription Factors

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We have analyzed the interleukin-4 (IL-4)-triggered mechanisms implicated in cell survival and show here that IL-4 deprivation induces apoptotic cell death but does not modulate Bcl-2 or Bcl-x expression. Since Bcl-x expression is insufficient to ensure cell survival in the absence of IL-4, we speculate that additional molecules replace the antiapoptotic role of Bcl-2 and Bcl-x in an alternative IL-4-triggered pathway. Cell death is associated with Bcl-3 downregulation and Bcl-3 expression blocks IL-4 deprivation-induced apoptosis, suggesting that Bcl-3 acts as a survival factor in the absence of growth factor. To characterize the IL-4-induced regulation of murine Bcl-3 expression, we cloned the promoter of this gene. Sequencing of the promoter showed no TATA box element but did reveal binding sites for AP1, AP1-like, and SP1 transcription factors. Retardation gels showed that IL-4 specifically induces AP1 and AP1-like binding activity and that mutation of these binding sites abolishes the IL-4-induced Bcl-3 promoter activity, suggesting that these transcription factors are important in Bcl-3 promoter transactivation. IL-4 deprivation induces downregulation of Jun expression and upregulation of Fos expression, both of which are proteins involved in the formation of AP1 and AP1-like transcription factors. Overexpression of Jun family proteins transactivates the promoter and restores Bcl-3 expression in the absence of IL-4 stimulation. Taken together, these data describe a new biological role for Bcl-3 and define the regulatory pathway implicated in Bcl-3 expression.

Bcl-3 was originally identified as a putative oncogene and cloned from a chromosomal breakpoint in the (14;19) translocation, which is found in some cases of chronic B-cell lymphocytic leukemias (31). Bcl-3 is a member of the IκB multigene family, which modulates the activities of NF-κB/Rel transcription factors (2, 3, 41, 43). The Bcl-3 protein contains a proline-rich amino terminus, a series of seven tandem ankyrin repeats, and a proline- and serine-rich carboxyl terminus (22). Bcl-3 can increase transcription from NF-κB responsive promoters, although contradictory data exist concerning this role for Bcl-3 (35). On the other hand, Bcl-3 can dissociate p50-p52 homodimers from DNA (6, 14, 15). The picture is further complicated by the finding that Bcl-3 is phosphorylated and that its phosphorylation status affects its interaction with both p50 and p52 (8, 30).

Physiological functions of Bcl-3 have been revealed by the generation of Bcl-3-deficient mice (13, 38). Bcl-3 is required for T-cell-dependent immunity. Bcl-3-deficient mice are defective in antigen-specific antibody production and germinal-center formation and fail to resist infection (13, 38). Bcl-3 may also contribute to B-cell survival, which may explain its oncogenic potential when expressed at high levels as result of chromosomal translocation.

Bcl-3 overexpression is proposed to contribute to the development of chronic lymphocytic leukemia through dysregulation of genes important in cell proliferation and differentiation (31, 47). Sequence analysis of the human Bcl-3 gene predicted a protein with identity to a number of products of genes involved in cell cycle control and in cell lineage determination. Bcl-3 is the first known oncprotein containing the SWI6/cdc10 motif, suggesting that this protein may be involved in cellular proliferation. Nonetheless, such motifs have also been observed in membrane-associated proteins that are not necessarily involved in cell cycle (27).

Bcl-3 is detected in different tissues, especially the spleen and other lymphoid organs (30). The regulation of its expression has been inadequately investigated. This gene was shown to be induced by mitogenic stimuli in B and T cells (5, 31). The induction of Bcl-3 by both granulocyte-macrophage colony-stimulating factor (GM-CSF) and erythropoietin (Epo) in proliferating human erythroid precursors involves enhanced expression of Bcl-3 mRNA, as well as an increase in the level of Bcl-3 protein (46). After Epo or GM-CSF stimulation, a gradual translacation of Bcl-3 to the nucleus is observed. In addition, Bcl-3 expression was recently shown to be induced by interleukin-9 (IL-9) (35).

IL-4 is a cytokine produced predominantly by T cells, mast cells, and basophils. It stimulates the proliferation of T and B cells as well as of mast cells and exerts distinctive biologic effects on a variety of cells (32). The biological functions of IL-4 are mediated via its binding to a specific cell surface receptor. This receptor is composed of two chains that are members of the type I cytokine receptor superfamily (10), a ligand-binding chain and the common γ chain, which is shared with the IL-2, IL-7, IL-9, and IL-15 receptors (16, 22-24, 29, 36, 37). IL-4 treatment of cells elicits many distinct biological responses, including an increase in cell proliferation and the transcription of a series of genes (32). Some of these responses are unique to IL-4, whereas others are also elicited by different cytokines. Although the receptors for IL-2 and IL-4 have several features in common, including their use of the γ chain as...
a receptor component. IL-4 evokes responses that IL-2 does not (9, 18, 26, 34). Many factor-dependent cell lines respond to IL-4 with increased thymidine incorporation into DNA, but only a few lines have been successfully adapted for growth in IL-4 alone. Of these, TS1β and LD8 can be propagated indefinitely in IL-4.

We present here the cloning and characterization of the murine Bcl-3 gene promoter. We have delineated a positive regulatory region important for IL-4-inducible promoter activity of the Bcl-3 gene. The API and API-like binding sites were deleted in the Bcl-3 promoter and tested and their mutation abrogates promoter activity. We show that Jun proteins, which are involved in the formation of API and API-like transcription factors, play an important role in IL-4-induced Bcl-3 expression. Finally, we demonstrate that Bcl-x expression is not sufficient to ensure cell survival in the absence of IL-4 and that Bcl-3 expression is able to block apoptosis in IL-4-deprived cells. This is the first description of the role of API and API-like transcription factors in the control of Bcl-3 promoter activation and protein expression, as well as the antiapoptotic role of Bcl-3 in our cellular model.

MATERIALS AND METHODS

Cells and cultures. TS1β is a murine T-cell line stably transfected with the human IL-2 receptor α and β chains (33). This cell line responds independently to IL-2, IL-4, or IL-9. Cells were cultured in RPMI 1640 (BioWhittaker, Walkersville, Md.) supplemented with 5% heat-inactivated fetal calf serum (Gibco-BRL, Gaithersburg, Md.), 2 mM glutamine, 10 mM HEPES, 0.5 mM arginine, 0.24 mM asparagine, 50 μM 2-mercaptoethanol and 60 μL of IL-4 per ml or 5 ng of recombinant IL-2 (RBI) per ml.

Lymphokines, antibodies, reagents, plasmids, and probes. Murine rIL-4 or supernatant of a HeLa subline transfected with pCKRI-L-4-neo was used as a source of murine IL-4. Anti-Bcl-3 antibody was from Santa Cruz (Santa Cruz, Calif.) or UBI (Lake Placid, N.Y.). Anti-Jun antibodies were from Oncogene Science (Cambridge, Mass.) or Transduction Laboratories (Lexington, Ky.), and anti-Fos antibody was from Santa Cruz. Bcl-2 and Bcl-x antibodies were from Transduction Laboratories. Anti-histone antibodies were from Chemicon International (Temecula, Calif.). Peroxidase-conjugated goat anti-rabbit or anti-mouse immunoglobulin antibody was from Dako (Glostrup, Denmark). Enhanced chemiluminescence (ECL) and 32P-labeled reagents were from Amersham (Little Chalfont, United Kingdom). NP-40 was from Boehringer, Mannheim (Mannheim, Germany), DEAE-dextran was from Pharmacia (Uppsala, Sweden), and the Capture-Tec pHook 3 kit was from Invitrogen (San Diego, Calif.). The QuikChange site-directed mutagenesis kit was from Stratagene (La Jolla, Calif.). pAd10SacBII vector was from Genome Systems (St. Louis, Mo.), pGL3 basic vector was from Promega (Madison, Wis.), and the CAPFINDER PCR cDNA synthesis kit was from Clontech, Palo Alto, Calif., and the CAPFINDER PCR cDNA synthesis kit was from Clontech, Madison, Wis., Expression vectors for c-Jun, JunB, and JunD proteins were provided by M. Yaniv, Pasteur Institute, Paris, France.

Analysis of RNA expression. Total RNA was isolated using the Trizol reagent from Gibco-BRL. For Northern blot analysis, RNA samples (15 μg) were electrophoresed in a 1% agarose gel in the presence of formaldehyde and then transferred to a nitrocellulose filter. After hybridization using a Bcl-3 probe spanning the first five exons of the Bcl-3 cDNA, the filter was washed and exposed to X-ray film at −70°C with intensifying screens.

Cloning, sequencing, and mutagenesis of the Bcl-3 promoter. A mouse genomic library constructed in the pAd10SacBII vector was screened by PCR using oligonucleotides specific for the Bcl-3 5′-untranslated region. DNA was extracted from one positive clone and digested with EcoRI, and the resulting fragments were subcloned into pTZ19R. Subclones were screened by colony hybridization using a Bcl-3 probe spanning the first five exons of the Bcl-3 cDNA. Positive clones were isolated and sequenced using plasmid-derived primers. One clone contained a 7-kb fragment showing identity to the 5′-end of the Bcl-3 gene. A 1.5-kb NcoI-BglII DNA fragment containing the Bcl-3 promoter and part of exon 1 was subcloned into the pGL3 basic vector at the Smal site. A shorter DNA fragment containing the promoter was generated by PCR amplification from the latter clone, in which the initiation codon of Bcl-3 gene has been deleted and cloned in the pGL3 vector in front of the reporter luciferase gene. The Bcl-3 promoter was sequenced on both strands with an automatic sequencer (Applied Biosystem, Foster City, Calif.).

Bcl-3 promoter 5′ deletion constructs were generated from pGL3 containing the full-length promoter. The plasmid was linearized, and deletions were generated by NotI, SfiI, BamHI, and XhoI digestions, the last two generated by partial digestions. The deleted ends were treated with Klenow fragment and then ligated.

Site-directed mutagenesis was performed using the QuikChange site-directed mutagenesis kit. The primers used for mutation were as follows: API 5′- AGA GGG CTG GTA AGGTACGAG ATCAA GAA TAA GAG 3′ and for API-like: 5′- GAGA ATC TCG AAG GGG GGG GGG GAC GAC GAC GAG 3′. Putative binding sites are underlined, and point mutations are shown in bold type.

Transcription start site mapping. Transcription start sites were mapped by PCR using the CAPFINDER PCR cDNA synthesis kit (Clontech), in which only cDNA derived from capped mRNA is exponentially amplified by PCR. The oligonucleotide primers used in defining the transcription start site were 5′- SSCCGGGAGGGGACGACGAC GAC GAC GAC GAC GAG and the internal primer from the Bcl-3 gene, 5′- GTCGGCGGACAGCTGGCGACGACGAC 3′. Putative binding sites are underlined, and point mutations are shown in bold type.

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RESULTS

IL-4 induces Bcl-3 expression in TS1β cells. We found that Bcl-2 is differentially regulated by IL-2 and IL-4 (19) and asked whether other genes might be differentially regulated by IL-2 and IL-4 (25). To examine this possibility, we selected the Bcl-3 gene for study. Bcl-3 is a member of the bcl-2 family of proteins that play a role in regulating cell death. The role of Bcl-3 in our cellular model.
regulated by IL-2 and IL-4 in TS1αβ cells. Bcl-3 was recently shown to be induced by IL-9 and IL-4 in mouse T helper cells (35). We identified Bcl-3 as a gene expressed in IL-4- but not IL-2-stimulated TS1αβ cells (Fig. 1A). When IL-4-maintained cells were deprived of lymphokine, Bcl-3 expression was downregulated. The amount of Bcl-3 decreased throughout the period of IL-4 deprivation, reaching undetectable levels at 24 h of deprivation. Cells maintained in IL-2 (5 ng/ml) were used as a negative control for Bcl-3 expression. Protein bands were detected using the ECL system. The blot was stripped and reprobed with pan-Ras antibody as an internal control of protein loading. Similar results were obtained in two independent experiments. Molecular weights of the corresponding proteins are shown. (B) IL-2-stimulated TS1αβ cells were switched to IL-4 for the times indicated and then lysed. Protein extracts were separated by SDS-PAGE, transferred to nitrocellulose, and probed with anti-Bcl-3 antibody. Cells maintained in IL-2 (5 ng/ml) were used as a negative control for Bcl-3 expression. Protein bands were detected using the ECL system. The blot was probed with pan-Ras antibody as an internal control of protein loading. The molecular masses of the corresponding proteins are shown. (C) Nuclear proteins were isolated from IL-4-stimulated (60 U/ml) or -deprived cells and from IL-2-stimulated (5 ng/ml) cells. After quantification, proteins were resolved by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with anti-Bcl-3 antibody. As a nuclear marker of protein loading, the blot was probed with anti-histone H1, H2a, H2b, H3, and H4 antibodies. Protein bands were detected using the ECL system. Similar results were obtained in two independent experiments. As an internal control of protein fractionation, nuclear or cytoplasmic proteins were blotted and probed with pan-Ras antibody (cytoplasmic marker). (D) Total RNA was isolated from 2 × 10⁶ IL-2-stimulated (5 ng/ml), IL-4-stimulated (60 U/ml), and IL-4-deprived cells (24 h). RNA (15 μg) was electrophoresed in 1% agarose with formaldehyde, blotted, and hybridized under high-stringency conditions to a ³²P-labeled Bcl-3 probe. The DNA probe was labeled by random priming. Both 28S and 18S are shown to estimate RNA levels. The size of the mRNA for Bcl-3 is indicated. Data are representative of two independent experiments.

Given that Bcl-3 is predominantly a nuclear protein, we analyzed its expression in nuclear extracts under IL-4 stimulation or deprivation conditions (Fig. 1C). High Bcl-3 levels were detected in nuclear extracts of IL-4-stimulated cells by Western blot analysis. The amount of Bcl-3 decreased at 12 h in IL-4-deprived cells, with minimum Bcl-3 levels detected after 24 h of lymphokine deprivation, suggesting an IL-4-induced modulation of nuclear Bcl-3 protein.

Since IL-4 regulates Bcl-3 expression, it was of interest to determine whether IL-4 could modulate Bcl-3 mRNA levels. Total mRNA was isolated from IL-4- or IL-2-stimulated and IL-4-deprived cells, electrophoresed, and hybridized with a Bcl-3 probe. The result shows that the absence of IL-4 downmodulates the Bcl-3 mRNA level (Fig. 1D). In the presence of IL-2, as well as in the absence of IL-4, we were unable to detect mRNA for Bcl-3.
Structure of the Bcl-3 promoter. To characterize the regulation of the gene encoding Bcl-3, we have isolated, sequenced, and characterized the promoter of this gene. To isolate the Bcl-3 promoter region, we screened by PCR a mouse genomic library using oligonucleotides specific for the 5′ untranslated region. Two clones containing sequences located upstream of the transcribed Bcl-3 gene were isolated. One of these contained a 1.5-kb fragment of the Bcl-3 promoter, which were identified in the sequence. We cloned the 1.4-kb fragment in front of the luciferase reporter gene into the pGL3 basic vector. Figure 3A shows the restriction map of the full length Bcl-3 promoter and the nested 5′ deletion promoter-luciferase constructs, showing binding sites for some transcription factors such as AP1, AP1-like, and SP1. The full-length Bcl-3 promoter (Fig. 3D) showed luciferase activity. The result suggests that the cloned Bcl-3 promoter responds to stimulation by IL-4.

To delineate the cis-acting elements and trans-acting factors that regulate Bcl-3 expression, we examined nested 5′ deletion promoter-luciferase constructs (Fig. 3C) in IL-4-stimulated or -deprived TS1β cells by using transient-transfection assays. Control full-length Bcl-3 promoter (NotI-Luc) showed luciferase activity. StuI-Luc deletion does not significantly modify the IL-4-induced luciferase activity, compared to the level observed in control transfected cells. Promoter activity was strongly reduced in the BamHI-Luc deletion and was almost undetectable in the XcmI-Luc deletion. Finally, no luciferase activity was observed with any of the constructs when cells were IL-4 deprived after transfection (Fig. 3C). This result allowed us to delineate the minimum Bcl-3 region with promoter activity, the Stu-I-Luc construct. IL-2 was not able to transactivate the full-length Bcl-3 promoter at any time after transfection (Fig. 3D). IL-4-stimulated cells after transfection with the full-length Bcl-3 promoter at any time after transfection did not exhibit luciferase activity. The result suggests that the cloned Bcl-3 promoter responds to stimulation by IL-4.

Characterization of IL-4-induced proteins binding to the Bcl-3 promoter. Since the StuI-Luc deletion appears to be the shortest fragment with promoter activity and since additional deletion of this fragment (BamHI-Luc) shows a strong reduction in luciferase activity, we focused our attention on the identification of putative binding sites for transcription factors in the StuI-BamHI promoter fragment. We performed bandshift assays and competition using double-stranded oligonucleotides corresponding to the AP1, AP1-like, and SP1 sites, which were identified in the StuI-BamHI promoter fragment, and nuclear proteins derived from IL-4-stimulated or -deprived TS1β cells.

We detected protein binding activity to AP1, AP1-like, and SP1 oligonucleotides when using nuclear extracts from IL-4-stimulated (Fig. 4A). When cells were IL-4 deprived for 12 to 24 h after transfection, cells deprived of IL-4 for 12 to 24 h after transfection...
24 h, protein binding to the AP1 and AP1-like sites was markedly reduced while binding to SP1 site was not affected. Specific DNA–nuclear-protein interaction was confirmed by competition with unlabeled oligonucleotides (data not shown). This suggests that IL-4 specifically induces AP1 and AP1-like activation and that these transcription factors may be responsible for the IL-4 inducibility of the Bcl-3 promoter. Supershift assays were performed to determine whether the nuclear binding activities contained AP1 and AP1-like proteins. Preincubation of the IL-4-stimulated TS1<sup>ab</sup>n nuclear extracts with c-Jun and JunB antibodies resulted in a supershift of the AP1 and AP1-like complexes (Fig. 4B). No supershift was observed when the antibodies were preincubated with IL-4-deprived nuclear extracts. This result suggests that the complexes contain Jun proteins.

Since AP1 and AP1-like transcription factors are composed of Jun and of Fos protein family members, we asked whether IL-4 deprivation could modulate Jun and Fos expression. TS1<sup>ab</sup>n cells were stimulated or deprived of IL-4 for different periods and total Jun and Fos protein expression was analyzed by Western blotting. Jun and Fos expression was detected in control IL-4-stimulated cells. Progressive increase of Fos expression was detected throughout the starvation period, reaching maximum at 24 h after IL-4 deprivation (Fig. 5). In contrast, Jun expression decreased following IL-4 deprivation, reaching almost undetectable levels at 24 h after lymphokine deprivation (Fig. 5). This deprivation period corresponds to the period of maximum Fos level detected. The result suggests that absence of AP1 and AP1-like activity in IL-4-deprived cells may be due to the lack of expression of Jun, one of the proteins involved in the formation of AP1 and AP1-like transcription factors.

To evaluate the functional role of AP1 and AP1-like transcription factors in the control of Bcl-3 promoter activity, we mutated both binding sites in Bcl-3 promoter so that it could not bind to nuclear proteins. Binding activity for AP1 and AP1-like factors was detected in nuclear extracts of IL-4-stimulated cells. This binding activity was undetectable using oligonucleotides containing mutated AP1* and AP1*-like binding sites (Fig. 6A). The specificity of the DNA-protein interaction was confirmed by competition with unlabeled oligonucleotides (data not shown). Cells transfected with mu-
tated AP1* or AP1*-like full-length Bcl-3 promoter constructs in the presence of IL-4 showed no transactivation of the luciferase reporter gene compared with cells transfected with the wild-type full-length Bcl-3 promoter construct (Fig. 6B). Similarly, no luciferase activity was detected when cells transfected with wild-type or mutated AP1* or AP1*-like constructs were maintained in the absence of IL-4. The results suggest that AP1 and AP1-like factors play a direct, important role in Bcl-3 promoter transactivation.

Jun proteins induce Bcl-3 expression in the absence of IL-4. Since mutation of AP1* and AP1*-like binding sites in the Bcl-3 promoter abolishes DNA binding and luciferase activity, and since both Jun and Bcl-3 expression are downregulated in the absence of IL-4, we asked whether Jun proteins are involved in the control of Bcl-3 expression. TS1\alphaβ cells were transiently transfected with a mixture of c-Jun, JunB, and JunD expression vectors and analyzed for Bcl-3 expression (Fig. 7A). After IL-4 stimulation, mock transfectants or cells transfected with Jun proteins showed Bcl-3 expression levels comparable to those of control IL-4-stimulated cells. Mock transfectants maintained in the absence of IL-4 showed no Bcl-3 expression. Interestingly, in cells transfected with the Jun protein mixture, Bcl-3 expression was induced without IL-4 addition (Fig. 7A) whereas independent transfection of c-Jun, JunB, or JunD did not restore Bcl-3 expression in the absence of IL-4 (data not shown).
Expression of transiently transfected Jun was confirmed by direct comparison of Jun protein levels in transfected cells and mock controls. Unaltered Ras expression was demonstrated under all transfection conditions as an internal protein loading control. Jun proteins thus appear to induce Bcl-3 expression in the absence of IL-4. We asked whether expression of Jun proteins affects Bcl-3 promoter transactivation. Cotransfection of Bcl-3 promoter and Jun proteins (c-Jun, JunB, and JunD) induced luciferase activity in the absence of IL-4-stimulation (Fig. 7B). Following IL-4-stimulation, cells transfected with the Bcl-3 promoter alone or in combination with the Jun proteins show comparable levels of luciferase activity (Fig. 7B), suggesting that expression of Jun proteins transactivates the Bcl-3 promoter in IL-4-deprived cells.

**Bcl-3 expression prevents apoptosis of IL-4-deprived TS1αβ cells.** IL-4-stimulated cells do not express Bcl-2, while IL-4-stimulated or -deprived cells express Bcl-x (data not shown). Since IL-4 deprivation in TS1αβ cells correlates with down-regulation of Bcl-3 expression and apoptosis, without modification of Bcl-x expression, we hypothesized that Bcl-3 may prevent apoptosis. Mock transfectants or cells transfected with Bcl-3 expression vector were selected from a mixed population of transfected and nontransfected cells. Cells transfected with Bcl-3 and deprived of IL-4 for 24 h showed a strong reduction in the fraction of apoptotic cells compared to IL-4-deprived mock-transfected cells (Fig. 8A). The frequency of apoptotic cells remained similar in all transfected cells in the presence of IL-4. Similar results were obtained for Jun protein expression (data not shown). Taken together, these results suggest that Bcl-3 may act as a survival factor in TS1αβ cells. To analyze the ability of IL-4-deprived Bcl-3-transfected cells to inhibit apoptosis, we performed a proliferation assay (Fig. 8B). Control, mock-transfected, or Bcl-3-transfected cells maintained in the presence of IL-4 after transfection showed thymidine incorporation. Control or mock-transfected IL-4-deprived transfected cells showed strong resumption of thymidine uptake. Interestingly, Bcl-3-transfected cells maintained in the absence of IL-4 showed higher thymidine incorporation than did transfected cells maintained in the absence of IL-4, although they did not reach the level of proliferation detected in IL-4-stimulated cells. This result suggests that IL-4 supplies an additional intracellular signal that complements the Bcl-3 survival signal, allowing cell proliferation.

**DISCUSSION**

For a more complete understanding of Bcl-3 expression regulation by IL-4, we have cloned and characterized the murine Bcl-3 gene promoter region. We have delineated the 5′ regulatory region, which is essential for IL-4-induced promoter activity, and have investigated the role of API and API1-like nuclear proteins in the control of Bcl-3 expression. Stimulation of TS1αβ cells by IL-4, but not IL-2, induces Bcl-3 expression at the RNA and protein levels. The differential control of gene expression by IL-2 and IL-4 has been previously found in TS1αβ cells; IL-2 induces Bcl-2 and NFAT expression, whereas IL-4 does not (18, 19). IL-9, GM-CSF, and Epo also induce Bcl-3 expression in T cells and erythroid cell precursors, as well as stimulating proliferation (35, 46). It has also been found that Bcl-3 is related to genes implicated in cell lineage determination and cell cycle control (31). Another function described for Bcl-3 is the activation of retinoblastoma expression through interaction with E4TF1 (40). Bcl-3 is located preferentially in the cell nucleus (6, 44, 47), although other reports describe its location in both nuclear and cytoplasmic compartments (46), suggesting that nuclear expression may be regulated under physiological conditions. The cytoplasmic retention may be due to physical association with other proteins or to posttranslational modifications. Other members of the NF-kB inhibitor family have been also observed in the nucleus (4, 11, 28, 45).

A remarkable feature of the 1.3-kb promoter is the absence of a TATA box element. The lack of this motif has also been observed in a number of genes whose products have house-
keeping functions (17, 39, 42). Analysis of the Bcl-3 promoter revealed the presence of three transcription start sites; the initiation of gene transcription at multiple sites is consistent with the lack of a canonical TATA box in the promoter. In addition to Bcl-3, the presence of several transcription start sites has been described for other genes (12, 25). The luciferase
activity observed after IL-4 stimulation is consistent with the increased level of Bcl-3 expression. In constructs with endpoints at Xcm1 and BamHI sites, respectively, no activity or nearly undetectable promoter activity was observed. It is interesting that the BamHI deletion retains only one of the AP1-like binding sites and that the Xcm1 deletion has no binding sites for these transcription factors.

Protein binding to AP1 and AP1-like binding sites was induced by IL-4 stimulation. Antibodies against Jun proteins can supershift both AP1 and AP1-like complexes, suggesting that the DNA-protein complexes observed in gel retardation contain Jun proteins. IL-4 deprivation induces downregulation of Jun expression, suggesting that Jun proteins may be the limiting factor in the formation of AP1 and AP1-like transcription factors. IL-4-deprived cells that receive an additional dose of Jun proteins are able to synthesize significant quantities of Bcl-3, suggesting that the presence of AP1 and AP1-like transcription factors may be essential for IL-4-dependent promoter activity and Bcl-3 expression. We do not exclude the possibility that the AP1 and AP1-like factors interact with other proteins to control Bcl-3 expression or, alternatively, that these factors may cooperate in the induction of Bcl-3 expression. In studies on Bcl-3 expression control by IL-9, the effect of IL-9 is controlled by STAT proteins, suggesting differences between IL-4 and IL-9 signaling or, alternatively, synergy between STAT and Jun proteins (35).

Cell death by IL-2 deprivation has been correlated with a decrease in the level of Bcl-x (7), but in our experimental system, Bcl-x protein levels were constant after IL-4 deprivation (data not shown). Although Bcl-x is expressed after IL-4 stimulation, it appears to be insufficient to promote cell survival, since Bcl-x is also expressed in IL-4-deprived cells.

An alternative pathway different of Bcl-2 and Bcl-x may be triggered by IL-4 to prevent cell death and to induce proliferation. IL-4 deprivation induces inhibition of Bcl-3 expression, resulting in apoptotic cell death, which is blocked by Bcl-3 expression, suggesting that Bcl-3 can replace the antiapoptotic role of Bcl-2 and Bcl-x and act as survival factor in IL-4-deprived Th1 cells.

A correlation has been demonstrated between Bcl-3 expression and proliferation of B lymphocytes (5). Similarly, activation of retinoblastoma expression by Bcl-3 protects cells from apoptosis, which may contribute to leukemogenesis following the model suggested for Bcl-2 (20, 40). Bcl-3 downregulation presumably results in a change in the regulation of a gene or genes important in some aspect of cell proliferation, differentiation, or survival. The downregulation of Bcl-3 during IL-4 deprivation-triggered apoptosis, together with the ability of Bcl-3 to act as a cell lineage-specific gene, allowed us to conclude that Bcl-3 may act as a survival gene for Th2 cell differentiation.

Extensive analysis of the response to infections indicated that Bcl-3−/− mice failed to establish a proper antigen-specific Th1 response. In addition, production of IL-12 and gamma interferon, two cytokines necessary for generation of a normal Th1 response, were impaired. The antibody response was also affected in Bcl-3−/− mice. This defect correlates with impaired formation of germinal centers. Such centers are the primary anatomical sites where antigen-specific B cells undergo rapid expansion and finally differentiate into plasma cells or memory cells. These data correlate with our results showing that overexpression of Bcl-3 in T cells, in the absence of complementary signals, allow the survival but not the proliferation of T cells.

Bcl-3 expression is probably controlled by IL-4-regulated transcription factors through binding-site recognition in the promoter region of Bcl-3. Our data demonstrate the significant role of AP1 and AP1-like factors in Bcl-3 promoter transactivation, and their absence provides an explanation for the disruption of Bcl-3 expression in IL-4-deprived cells. Our results also suggest that Bcl-3 can replace the antiapoptotic role of Bcl-2 and Bcl-x in TSIβ cells. We have established the basis of specific molecular Bcl-3 functions and its integration into regulatory pathways. Further studies are needed to determine whether the findings presented here are applicable to other growth factor signaling systems.

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