The human programmed cell death-2 (*PDCD2*) gene is a target of BCL6 repression: Implications for a role of BCL6 in the down-regulation of apoptosis

Beverly W. Baron*†, John Anastasi*, Michael J. Thirman‡, Yoichi Furukawa§, Scott Fears‡, David C. Kim*, Federico Simone‡, Mark Birkenbach*¶, Anthony Montag*, Annamma Sadhu*, Nancy Zeleznik-Le‡‖, and Timothy W. McKeithan*,

Departments of *Pathology and ‡Medicine (Section of Hematology–Oncology), University of Chicago, Chicago, IL 60637; and §Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan

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***BCL6*, a gene on chromosome 3 band q27, encodes a *Kruppel*-type zinc finger transcriptional repressor. Rearrangements of this gene are frequent in various kinds of lymphomas, particularly of the large-cell B-cell type. The BCL6 nuclear phosphoprotein is expressed in a variety of tissues and is up-regulated particularly in lymph node germinal centers. The zinc fingers of BCL6 bind DNA in a sequence-specific manner. To identify targets of the BCL6 repressive effects, we used a VP16-BCL6 fusion protein containing the zinc fingers but devoid of the repressor domains to compete with the binding of endogenous BCL6 in a transiently transfected B-cell line and then performed subtractive hybridization by using a method to selectively amplify sequences that are differentially expressed. We found that the programmed cell death-2 (*PDCD2*) gene is a target of BCL6 repression. This gene is the human homolog of *Rp8*, a rat gene associated with programmed cell death in thymocytes. Immunohistochemistry reveals the anticipated inverse relationship between BCL6 and PDCD2 expression in human tonsil. PDCD2 is detectable in cells of the germinal center in areas where there is less BCL6 expression as well as in the mantle zone, where there is little or no BCL6 expression. These results raise the possibility that BCL6 may regulate apoptosis by means of its repressive effects on PDCD2. BCL6 deregulation may lead to persistent down-regulation of PDCD2, reduced apoptosis, and, as a consequence, accumulation of BCL6-containing lymphoma cells.**

BCL6 is a gene on chromosome 3 band q27 that we and others identified (1–4) in association with chromosomal rearrangements that accompany a variety of lymphoid malignancies, particularly diffuse large-cell B-cell lymphomas (5). Although the most common translocations involving *BCL6* are the t(3;14)(q27;q32) and the t(3;22)(q27;q11), many other chromosomal partners have been identified in association with *BCL6* rearrangements (6, 7). The breakpoints cluster around the noncoding first exon of *BCL6* (2–4), and it is believed likely that BCL6 expression becomes deregulated by translocations in which heterologous promoters/enhancers are substituted for normal BCL6 regulatory sequences (8).

BCL6 encodes a transcription factor containing six C-terminal Cys_2-His_2 zinc fingers (3, 4). Two domains upstream of the zinc finger domain (ZF) independently convey transcriptional repressor activity (9–12)—an N-terminal evolutionarily conserved POZ domain and a more central domain. BCL6 is a nuclear phosphoprotein whose expression is up-regulated 3–34-fold in lymph node germinal center B cells (13–15). The BCL6 ZF binds DNA readily in a sequence-specific manner (9–11, 16, 17).

We sought to identify genes that are the targets of the BCL6 repressive effects. We used a construct expressing the BCL6 ZF fused to the VP16 activating domain of herpes simplex virus (VP16-BCL6ZF) to compete with the binding of endogenous BCL6 in BJAB cells, an Epstein–Barr virus-negative Burkitt lymphoma cell line expressing high levels of BCL6 (15). Electrophoretic mobility-shift assay (EMSA) showed that the VP16-BCL6ZF fusion protein could bind specifically to the BCL6 consensus binding site. Luciferase assays revealed an 18-fold elevation of luciferase expression when VP16-BCL6ZF was transiently transfected in BJAB cells along with a luciferase reporter construct containing the BCL6 consensus binding site sequences, as compared with controls transfected with the vector containing VP16 (vVP16) but not BCL6ZF. Because BCL6 is a repressor, competition for endogenous binding of BCL6 by the exogenously transfected ZF should result in up-regulation of BCL6 targets. We used subtractive hybridization methodology permitting selective amplification of differentially expressed sequences to compare RNA from BJAB cells transiently transfected with the VP16-BCL6ZF expression construct and selected on beads at harvest with a selected control cell population transiently transfected with vVP16. These studies led to the identification of the programmed cell death-2 (*PDCD2*) gene (18) as a target of BCL6. Northern blot analysis was supportive of this finding. Further, immunohistochemical studies of human tonsil showed, as would be predicted, that the localization of PDCD2 expression is inversely related to that of BCL6 in germinal center and follicular mantle cells.

Materials and Methods

Preparation of Constructs, Transient Transfections, and Luciferase Assays. To identify genes repressed by BCL6, we developed a cell system in which the BCL6 repressive effects are inhibited, resulting in up-regulation of genes that are ordinarily its targets of repression. We used the BCL6 ZF, which readily binds DNA but lacks repressive effects, to compete with the binding of the endogenous BCL6 wild-type protein in a cell line that expresses BCL6 at high levels. To convert the BCL6 ZF into a transcriptional activator, we fused nucleotides 1418–2181 of the *BCL6* cDNA, which encompass the ZF domain devoid of the upstream repressor domains, to the VP16 activating domain of herpes simplex virus that had been hemagglutinin (HA)-tagged and subcloned in pcDNA3 (gift of J. Leiden, Abbott Laboratories, North Chicago, IL). To test whether the VP16 fusion had

Abbreviations: ZF, zinc finger domain; EMSA, electrophoretic mobility-shift assay; HA, hemagglutinin; RT, reverse transcription.

†To whom reprint requests should be addressed at: Blood Bank, MC0007, University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637. E-mail: bbaron@uchospitals.edu.

¶Present address: Department of Pathology and Anatomy, Eastern Virginia Medical School, Norfolk, VA 23507.

‖Present address: Cardinal Bernardin Cancer Center, Loyola University Chicago, Maywood, IL 60153.

**Present address: Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198-6495.

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converted the ZF into a transcriptional activator, BJAB cells were grown under 5.5–7% CO_2 in RPMI medium 1640 supplemented with 10% FCS (Mediatech, Herndon, VA) and transiently cotransfected by electroporation with this construct and a luciferase reporter construct (the BCL6 consensus binding site sequences subcloned in TK-LUC, a gift of R. Dalla-Favera, Columbia University, New York).

Expression of VP16-BCL6ZF and EMSA. To determine whether we could detect RNA from VP16-BCL6ZF, transient cotransfections of BJAB cells (electroporation) were performed along with pHook-1 (Invitrogen), a vector that directs synthesis of a single-chain antibody, sFv, which is expressed on the surface of transfected cells. The cells were harvested 22–47 h after transfection. We separated transfected cells from the total cell population with magnetic beads (Invitrogen) coated with the hapten (phOx) toward which the sFv is specifically directed. Total RNA was prepared, DNase-I-digested, reverse transcribed, then subjected to reverse transcription (RT)-PCR using β-actin primers that amplify 285 bp of cDNA (vs. 396 bp for genomic DNA) and primers that amplify a 370 bp cDNA fragment across the VP16-BCL6ZF junction.

For EMSA, nuclear extracts (NE) were prepared from COS-7 cells grown under 5.5–7% CO_2 in DMEM supplemented with 10% FCS and transiently transfected by electroporation/diethylaminoethyl-dextran with the VP16-BCL6ZF HA-tagged expression construct. The NE were incubated with ^{32}P-labeled double-stranded oligomers containing the BCL6 consensus binding site and electrophoresed on a polyacrylamide gel as described (17).

cDNA Subtraction. We performed subtractive hybridization by using cells in which BCL6 repressive effects are inhibited by the BCL6 ZF as the "tester" population and cells containing vVP16 as "driver." The tester population should contain genes that are up-regulated because BCL6 repression is inhibited in these cells. BJAB cells were transiently cotransfected with VP16-BCL6ZF and pHook-1 as described above ("study" cell population); a "control" BJAB cell population was transiently cotransfected with pHook-1 and vVP16. The study and control cells were harvested at the same time points (22–48 h) and selected on magnetic beads as described above. Poly(A)$^+$ mRNA was prepared from study- and control-transfected cells with an Oligotex Direct mRNA kit (Qiagen). CLONTECH's PCR-Select cDNA Subtraction Kit was used to synthesize tester cDNA from mRNA of the study cells and driver cDNA from mRNA of the control cells, and subtractive hybridization was performed according to the manufacturer's protocol (CLONTECH).

Amplification of Differentially Expressed Sequences and Nucleotide Sequencing. Two rounds of PCR were performed to amplify differentially expressed cDNAs, which were subcloned in the pT-Adv vector (AdvanTAge PCR cloning kit, CLONTECH), transformed using TOP10F′ *Escherichia coli* competent cells, and plated on LB/X-Gal/IPTG kanamycin plates; 25 clones were screened. Clones containing inserts of varying sizes were selected for nucleotide sequencing.

Confirmation of Differential Expression by Northern Blotting. BJAB cells were transiently cotransfected with the study or control construct and pHook-1 and selected on magnetic beads. Total RNA was purified and used to prepare Northern blots. The inserts of the cDNA clones obtained from subtractive hybridization/PCR were ^{32}P-labeled and hybridized to the blots which were washed under stringent conditions and exposed to film. These probes then were stripped, and the blots were rehybridized with a ^{32}P-labeled human β-actin cDNA control probe (CLONTECH), washed under stringent conditions, and reautoradiographed. Quantitation of relative band intensity was normalized to β-actin by the IMAGEQUANT computer program (Molecular Dynamics).

Production of Polyclonal Antibodies to PDCD2 and Confirmation of Specificity by Western Blotting. A peptide corresponding to amino acids 332–343, at the C terminus of PDCD2, was synthesized, conjugated to keyhole limpet hemocyanin through a cysteine residue added at the N terminus, and injected into New Zealand White rabbits to produce polyclonal antibodies (Sigma–Genosys). RT-PCR performed on mRNA from normal human colon (Invitrogen) was used to clone nucleotides 21–1061 (GenBank accession no. 002598) of the *PDCD2* gene, encompassing its full-length coding region, in the pFLAG-CMV5 vector (Eastman Kodak). Nucleotide sequencing confirmed intactness of the cDNA and proper insertion in the vector. Whole-cell extracts of human 293 cells transfected with the Flag-tagged PDCD2 construct or a ≈43 kDa unrelated Flag-tagged protein, EAF1 (19), were electrophoresed on an SDS/7.5% PAGE and transferred to a nitrocellulose membrane (Bio-Rad). Membranes were blocked in 5% nonfat dry milk in Tris-buffered saline with 0.05% Tween 20 and incubated with the rabbit polyclonal antiserum to PDCD2 or with a Flag monoclonal antibody (Sigma). The membranes were washed and incubated with biotin-conjugated goat anti-rabbit antibody (Santa Cruz Biotechnology), washed again, then incubated with horseradish peroxidase-conjugated streptavidin (Jackson ImmunoResearch). Protein bands were detected with an enhanced chemiluminescence protocol (Amersham Pharmacia).

Immunohistochemistry. Frozen sections (4 μm) of human tonsil were fixed in acetone:methanol (1:1) and stained with a mouse monoclonal antibody to BCL6 (#M7211, Dako), rabbit polyclonal antibodies to PDCD2, or preimmune rabbit serum (control), rinsed, and incubated with anti-mouse or anti-rabbit IgG, respectively. Antigen–antibody binding was detected by the DAB substrate (Ventana Basic DAB Detection Kit with Amplification Kit). Slides were counterstained with hematoxylin.

Promoter Analysis. Sequences flanking the first exon of PDCD2 were identified by a BLAST search (20) of the human genome database. Using the MACVECTOR program (Oxford Molecular Group), we determined the closest matches to two identified high-affinity sites (TTCCTAGAA and TTCCTCGAA; ref. 21), which together comprise 9 nucleotides of an 11-nucleotide BCL6 binding site consensus—TTCCT (A/C) GAA (A/T) G (22).

Results

Luciferase Assays. When BJAB cells were transiently cotransfected with VP16-BCL6ZF and the BCL6-TK-LUC reporter, an 18-fold elevation of luciferase levels was noted as compared with vVP16-transfected controls ($P < 0.0025$). Because the endogenous BCL6 protein in BJAB cells would be expected to bind to the luciferase reporter and repress luciferase levels, these results implied that the VP16-BCL6ZF protein competed effectively for binding with full-length BCL6 at the BCL6 consensus site and further activated luciferase expression.

VP16-BCL6ZF Is Expressed and Binds Specifically to the BCL6 Consensus Binding Site. RT-PCR on total RNA from BJAB cells transiently transfected with VP16-BCL6ZF and selected on beads confirmed that RNA from this construct was present (Fig. 1). EMSA revealed that the VP16-BCL6ZF protein binds specifically to the BCL6 consensus binding site (Fig. 2). This binding is specific because an excess of the cold double-stranded oligomer containing the BCL6 consensus site competed for binding with the radioactive double-stranded oligomer, whereas an excess of a double-stranded oligomer containing point mutations that block

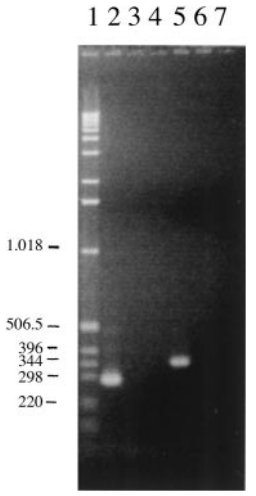

Fig. 1. RT-PCR of RNA from VP16-BCL6ZF-Transfected BJAB Cells. Lane 1, 1 Kb-DNA Ladder; lane 2, RT-PCR with β-actin primers that amplify 285 bp; lane 3, negative control, same primers, no RT; lane 4, negative control, same primers (water template); lane 5, RT-PCR with primers across the VP16-BCL6ZF junction that amplify 370 bp of cDNA; lane 6, negative control, VP16-BCL6ZF primers, no RT; lane 7, negative control, same primers (water template).

BCL6 binding did not displace the heavy shifted band. Antibodies to the BCL6 carboxy terminus and the HA epitope tag reduced binding and caused a supershift.

Subtraction/Amplification of Differentially Expressed cDNAs and Nucleotide Sequencing. Differentially expressed bands were noted on an ethidium bromide-stained 2% agarose gel when primary and secondary PCR products of the experimental subtracted sample were compared with the unsubtracted tester control. Nucleotide sequences of two of the clones analyzed were identical to overlapping regions of the *BCL6 ZF*, implying that subtractive hybridization had been successful, because it could identify the BCL6 ZF mRNA that had been overexpressed in the study cells. Sequences of another clone matched a human gene of potential interest: *PDCD2* (18).

Confirmation of Differential Expression by Northern Blotting. Hybridization of the cDNA fragment of *PDCD2* obtained from cDNA subtraction/amplification with a Northern blot prepared from total RNA of selected BJAB cells transiently transfected with VP16-BCL6ZF (study cells, S lane) or vVP16 (controls, C lane) revealed the expected ≈1.5-kb transcript (ref. 18; Fig. 3 *Upper*). IMAGEQUANT Analysis of relative band intensity normalized to β-actin (Fig. 3 *Lower*) showed that differential expression of PDCD2 (S lane) as compared with control (C) is 5-fold.

Specificity of PDCD2 Antibodies. The polyclonal rabbit antiserum is directed specifically against the PDCD2 protein, because on Western blots it detects a band at ≈45 kDa in whole-cell extracts from cells transfected with Flag-tagged PDCD2, which comigrates with the band recognized by the Flag antibody; however, this band is not visualized by the rabbit antiserum in whole cell extracts from cells transfected with a Flag-tagged unrelated protein, EAF1 (ref. 19; Fig. 4).

Immunohistochemistry. Frozen sections of human tonsil stained with BCL6 or PDCD2 antibodies reveal that, whereas BCL6 stains the nuclei of lymphocytes in germinal centers most heavily in centroblasts, PDCD2 localizes in the cytoplasm of cells in the opposite pole of the germinal center where BCL6 stains least

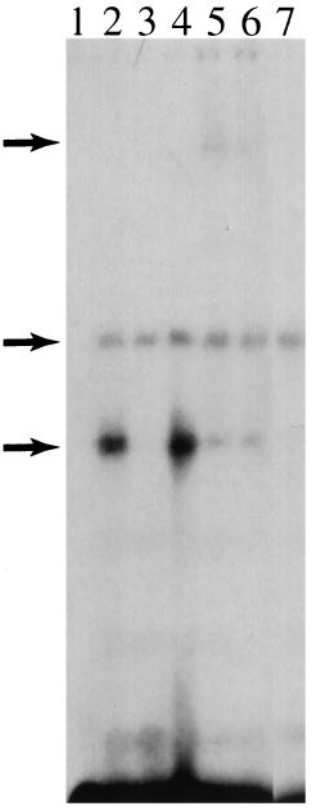

Fig. 2. EMSA. Lane 1, nonprotein control (no shifted bands). The VP16-BCL6ZF protein binds specifically to the BCL6 consensus site, because the heavy shifted band (lane 2, bottom arrow) is displaced by mixing the radiolabeled double-stranded oligomer containing the BCL6 binding site with a 338-fold molar excess of the same nonradiolabeled double-stranded oligo (lane 3), is not displaced by mixing the hot probe with a 715-fold molar excess of cold mutant double-stranded oligomer containing point mutations that block BCL6 binding (lane 4), and its binding is reduced (bottom arrow) and supershifted (top arrow) by addition of polyclonal rabbit antibodies to the BCL6 carboxy terminus (lane 5) and polyclonal antibodies recognizing the HA epitope tag (lane 6). Nuclear extracts of untransfected COS cells do not bind specifically to the probe (lane 7). A nonspecific cold DNA competitor was added to each reaction. The central arrow depicts a nonspecifically binding band.

(Fig. 5). PDCD2 also stains cells in the mantle zone, where BCL6 does not stain (23). Negative controls (isotype control and omission of primary antibody) showed no staining. Staining with the preimmune (control) serum did not reveal a differential (polar) staining pattern similar to that noted with the PDCD2 antibodies. Findings were best observed on follicles that showed the most polarization. Because BCL6 is a transcriptional repressor, one would anticipate that PDCD2 would be repressed in cells containing high levels of BCL6. Thus, our data are consistent with the notion that PDCD2 is a valid target of BCL6.

Promoter Analysis. Analysis of the sequences flanking the first exon of *PDCD2* revealed an exact match to a previously identified BCL6 binding site. This sequence, TTCCTAGAA, lies ≈3.4 kb upstream of exon 1. An exact match to one of the two high-affinity sequences (21) would be expected to occur once per 65.5 kb, assuming equal abundance among the four nucleotides, or once per 52.5 kb, taking into account the 58.1% A+T average abundance of the 5-kb 5′ of the *PDCD2* first exon.

Discussion

BCL6 encodes an evolutionarily conserved *Kruppel*-type zinc finger protein that functions as a strong transcriptional repressor

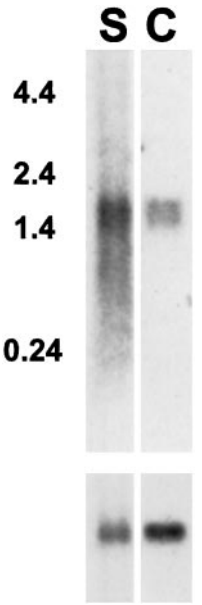

Fig. 3. The upper panel is an autoradiograph of a Northern blot prepared from total RNA of BJAB cells transiently transfected with the study (S) expression construct (contains VP16-BCL6ZF-HA) or the control (C, contains vector with VP16-HA); RNA sizes (kb) are indicated on the left. (*Upper*) Hybridization with a cDNA fragment of *PDCD2* obtained from subtractive hybridization; its ≈1.5-kb transcript is noted. The blot was stripped and rehybridized with a human β-actin probe (*Lower*). Differential expression of *PDCD2* normalized to β-actin is 5-fold.

and is required for germinal center development (24–26). Rearrangements of the *BCL6* gene are frequent in human B-cell malignancies, particularly in diffuse large cell B-cell lymphomas, and are associated with many different partner chromosomes. Such translocations involve promoter substitutions that leave the BCL6 coding region intact (8). The BCL6 protein has been shown to interact with a number of corepressors—e.g., N-CoR, SMRT, mSIN3A, and BCoR (27–30). It is believed likely that the repressive effects of BCL6 are mediated through multiprotein repression complexes with histone deacetylase activity.

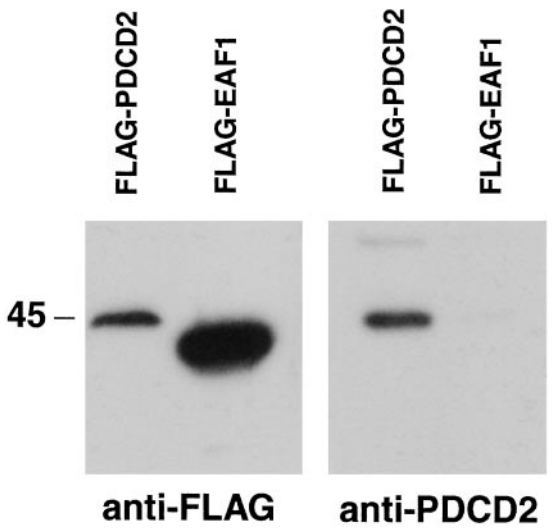

Fig. 4. Western blots prepared from whole-cell extracts of human 293 cells transfected with a Flag-tagged PDCD2 expression construct or an unrelated Flag-tagged expression construct, EAF1 (control). (*Left*) Western blot incubated with anti-Flag; the expected ≈43-kDa band is present in the EAF1 extract. A band at ≈45 kDa is recognized by anti-Flag in the extract from the PDCD2-transfected cells. (*Right*) The same ≈45-kDa band is recognized by anti-PDCD2, but, as expected, no band is recognized by this antibody in the EAF1-transfected cells.

To gain further insight into the biological function of BCL6 and its role in the pathogenesis of lymphoma, we sought to identify BCL6-regulated genes. We developed a dominant-negative cell system in which the BCL6 repressive effects are inhibited, resulting in up-regulation of genes that are ordinarily repressed. We used the BCL6 DNA-binding domain—its ZF, devoid of the upstream repressor domains, to compete with the binding of the full-length wild-type endogenous BCL6 protein expressed at high levels in a B-cell lymphoma line. To enhance the competition, we converted the BCL6 ZF into a transcriptional activator by fusing its cDNA to the VP16 activating domain of herpes simplex virus. A test of this construct showed that the VP16-BCL6ZF protein competed very effectively for full-length BCL6 binding at the BCL6 consensus site, resulting in significantly elevated luciferase levels. The RNA from this construct could be detected readily in transiently transfected cells. EMSA revealed that the VP16-BCL6ZF protein binds specifically to the BCL6 consensus binding site.

With the use of subtractive hybridization methodology that permits selective amplification of differentially expressed sequences to detect up-regulated messages of BCL6-targeted genes in our study cell population, we identified a BCL6-regulated gene—*PDCD2*. Northern blots showed differential expression of PDCD2 (5-fold) in an experimentally transfected cell population as compared with vVP16-transfected controls. Because selection on beads using pHook-1 methodology is not perfect, it is likely that the repressive effects of BCL6 on PDCD2 *in vivo* may be of even greater magnitude than what we report here. Immunohistochemical staining with polyclonal antibodies specifically directed against the PDCD2 protein revealed the anticipated inverse relationship between the expression of BCL6, which is a nuclear protein predominantly expressed in centroblasts located largely at one pole of the germinal center, and PDCD2, which has not been studied previously by immunohistochemical analysis. We showed that PDCD2 is localized predominantly in the cytosol of cells situated at the opposite pole of the germinal center from the centroblasts as well as in cells in the mantle zone—two regions in which lymphocytes show less or no expression of BCL6 (23).

Further, the promoter region of the *PDCD2* gene contains an exact match to a 9-nucleotide sequence (TTCCTAGAA) to which BCL6 has been shown to bind with high affinity (21). BCL6 can compete for binding by suppression of signal transducer and activator of transcription 3 (STAT3) (31), which is expected also to bind this site with high affinity. It is striking that the sequence TGAGTTA, a 6/7 match to the AP1 consensus, lies 10 bp following the putative BCL6/STAT3 site. Jun family members bound to AP1 sites can functionally interact with STAT3 (32, 33). Unless they are functional, the proximity of the two sites would be a remarkable coincidence.

The human gene *PDCD2* was isolated from fetal lung by Kawamaki *et al.* (18) and contains an ORF encoding 344 aa, predictive of a 38-kDa protein, with a single zinc finger (MYST) domain. It has close homologs in *Schizosaccharomyces pombe*, *Drosophila*, and *Caenorhabditis elegans* (34) and is highly homologous to *Rp8*, a rat gene that was identified in association with programmed cell death in immature thymocytes (35). The PDCD2 protein is expressed in a variety of tissues and was localized to chromosome 6q27 (18). Interestingly, this is a region where translocations have been reported in acute nonlymphocytic leukemias and where deletions may occur in cutaneous T-cell lymphomas, acute lymphoblastic leukemia (18), and non-Hodgkin lymphomas, including follicular B-cell lymphoma (36).

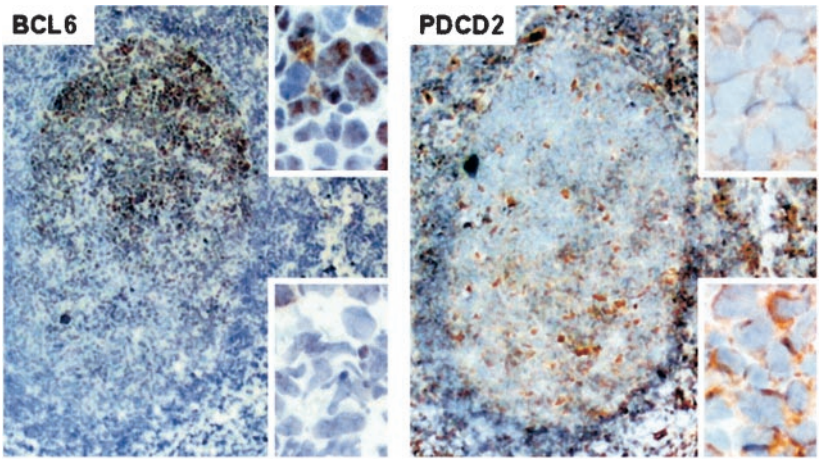

Fig. 5. Immunohistochemistry. Frozen sections (4 μm) of human tonsil were stained with a mouse monoclonal antibody to BCL6 (*Left*) or rabbit polyclonal antibodies to PDCD2 (*Right*). The anticipated inverse relationship between BCL6 and PDCD2 localization is noted. The antibody to BCL6 stains nuclei of lymphocytes in the germinal center most heavily in larger cells (centroblasts, toward top of *Left* and detail in top *Inset*—dark brown nuclei), whereas PDCD2 localizes in the cytoplasm of germinal center cells (toward bottom of *Right* and detail in bottom *Inset*) in areas where there is less BCL6 staining (*Left*, bottom of germinal center and detail in bottom *Inset*). There is little PDCD2 staining (detail, *Right*, top *Inset*) in areas where BCL6 stains heavily (*Left*, top *Inset*). PDCD2 is also noted in a number of follicular mantle cells which do not express BCL6.

Recent reports have described other potentially down-regulated target genes of BCL6, including *CD69*, *CD44*, *Leu-13*, *CXCR4*, *EB12*, *ld2*, *STAT1*, *blimp-1*, *MIP-1*α, *IP-10*, *p27kip1*, and *cyclin D2* (37). These genes, which were identified with the use of DNA microarrays, have roles in B-cell activation and differentiation, inflammation, and cell cycle control (37). BCL6 also has been found to regulate chemokine gene transcription in macrophages (21). Finally, with a cDNA hybridization technique, *CXCR4* was again noted to be a down-regulated BCL6 target gene, and, in addition, *cyclin A2* as well as insulin-like growth factor binding protein-4 were newly identified down-regulated targets of BCL6 (38). *PDCD2* has not been described previously as a target of BCL6 repression. With our subtractive hybridization technique, we did not identify any of the previously detected BCL6-regulated genes. In the course of our studies, we noticed, as have others (37), that there were consistently fewer surviving cells in cultures transfected with the BCL6 ZF than in the controls. As the rat homolog of PDCD2 has been shown to be associated with programmed cell death in thymocytes (35), we propose that inhibition of BCL6 repressive effects resulted in up-regulation of PDCD2, which may have been responsible, at least in part, for the decreased numbers of the experimental cells in culture. Further work will be required to confirm this hypothesis.

Several prior reports have addressed the relationship of BCL6 to apoptosis. Two of these studies noted that high expression or overexpression of BCL6 induces apoptosis. Albagli *et al.* (39) used a tetracycline-regulated human osteosarcoma cell line stably transfected with BCL6 that could be induced several hundred-fold by removal of tetracycline. These authors thought it unlikely that the effects of BCL6 (dose-dependent growth suppression correlated with delayed S phase progression and triggering of apoptosis) might be nonspecific because of vast overexpression, because a truncated BCL6 derivative used as a control failed to induce apoptosis, although it did slow cell growth and delay S phase progression, albeit much less efficiently. In another report, Yamochi *et al.* (40) used a recombinant adenovirus to express BCL6 in CV-1 and HeLa cells. They found that viability of these cells was markedly reduced secondary to apoptosis. In their studies BCL6-overexpressing cells accumulated at the subG_1 and G_2/M phase. BCL6-induced apoptosis was preceded by down-regulation of BCL2 and BCLX, and they suggested that BCL6 might regulate the expression of these apoptosis repressors.

However, two other reports find that BCL6 protects cells from apoptosis. Kumagai *et al.* (41) cultured differentiating mouse myocytes (C2C12 cell line) in serum-starved medium and showed that BCL6 induction is related to terminal differentiation of these cells. Adenovirus-mediated overexpression of BCL6 prevented apoptosis of the differentiating cells. Whereas high levels of BCL6 antisense mRNA expression induced apoptosis during differentiation, apoptosis was prevented by infection with adenovirus expressing BCL6 sense mRNA. These authors suggest that BCL6 may protect myocytes from specific stresses—e.g., serum starvation—and that deregulation of the expression of BCL6 may contribute to lymphoma development through its influence on apoptosis. In agreement with this notion, Kojima *et al.* report that BCL6 may protect spermatocytes from stress-induced apoptosis (42).

Our findings support the concept that BCL6 can repress a gene whose rat homolog is associated with programmed cell death and thus are consistent with the latter reports (41, 42). Whether apoptosis is induced or inhibited by BCL6 may depend on its level of expression and the cellular context. It seems plausible that one of the physiological roles of BCL6 in germinal center cells may be inhibition of apoptosis, permitting these cells to generate and amplify an immune response. As Kumagai *et al.* imply, deregulation of BCL6 during lymphomagenesis may, in turn, result in perturbation of the proteins that control apoptosis, leading to an abnormally enhanced growth and expansion of cells that are normally limited to germinal centers. We hypothesize that repression of PDCD2 by BCL6 plays a role in lymphomagenesis associated with BCL6 deregulation. Analysis of the normal function of the PDCD2 protein and study of its potential role in lymphomagenesis may be fruitful areas for future investigation.

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