Direct Repression of prdm1 by Bcl-6 Inhibits Plasmacytic Differentiation

Chainarong Tunyaplin, A. L. Shaffer, Cristina D. Angelin-Duclos, Xin Yu, Louis M. Staudt and Kathryn L. Calame

*J Immunol* 2004; 173:1158-1165; doi: 10.4049/jimmunol.173.2.1158

http://www.jimmunol.org/content/173/2/1158

**References**
This article cites 37 articles, 19 of which you can access for free at:
http://www.jimmunol.org/content/173/2/1158.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Direct Repression of prdm1 by Bcl-6 Inhibits Plasmacytic Differentiation


We have identified two intronic regions of mouse prdm1, the gene encoding B lymphocyte-induced maturation protein-1 (Blimp-1), which confer transcriptional repression in response to Bcl-6. The Bcl-6 response element in intron 5, which is conserved between mice and humans, was studied in detail. It binds Bcl-6 in vitro and was shown by chromatin immunoprecipitation to be occupied by Bcl-6 in vivo. Neither Bcl-6 response element functions as a STAT3-response element, showing that STAT3 does not compete with Bcl-6 at these sites. Bcl-6+/− mice confirm the biological importance of Bcl-6-dependent repression of prdm1. These mice have elevated Ab response, increased Ig-secreting cells, and increased Blimp-1+ cells in spleen following immunization and their splenic B cells show accelerated plasmacytic development in vitro. The Journal of Immunology, 2004, 173: 1158–1165.

Although Blimp-1 is found in all plasma cells—those resulting from both T-independent and T-dependent Ags, in both primary and secondary responses—it is not detected in early bone marrow B cells, memory B cells in spleen, and most GC B cells (12). However, a small subset of GC B cells, possibly committed to a plasmacytic fate, express Blimp-1. Little is known about regulation of prdm1 except that developmental stage-specific expression in B cells is determined at the transcriptional level (13). Recent studies have shown that Bcl-6 can repress Blimp-1 expression (5–7). The transcriptional repressor Bcl-6 is abundant in GC B cells and is required for GC formation, affinity maturation, and CSR (14, 15). Ectopic expression of Bcl-6 inhibited induction of Blimp-1 mRNA and plasmacytic differentiation in Bcl-1 lymphoma cells and primary splenocytes (6). A blocking form of Bcl-6 induced Blimp-1 mRNA in the presence of cycloheximide, suggesting direct repression by Bcl-6 (5). Because IL-6 is important for plasma cell differentiation, it was suggested that STAT3, activated by IL-6, might compete with Bcl-6 for binding to sites in prdm1, similar to competition between STAT6 and Bcl-6 on the Ie promoter (16). A recent study by Vasanwala et al. (17) showed that Bcl-6 can repress the human PRDM1 gene by interfering with the AP-1 activator.

In this study, we identify two intronic regions in mouse prdm1 that confer Bcl-6-dependent repression. One of the two Bcl-6 response elements (BREs) was found to be conserved between mice and humans. Bcl-6 binds to this site both in vitro and in vivo. Neither of the two regions that confer Bcl-6-dependent repression functions as a STAT3-response element. These data establish a molecular mechanism for direct repression of prdm1 by Bcl-6. In addition, Bcl-6−/− mice have elevated Blimp-1+ plasma cells in vivo and in vitro, establishing the functional importance of this repression.

Materials and Methods

Plasmids

A reporter containing the Blimp-1 basal promoter was described (13). A series of Blimp-1 reporters was generated by cloning genomic regions (fragments 1–5 in Fig. 1) 5′-TGGAGATGTCAACTGGAATT-3′ and 5′-CCGAGCTCAGCTGACTAATCACAGGAG-3′. The 570-bp fragment containing BRE1 was generated by PCR from fragment 3 using primers 5′-CCGAGCTCAGCTGACTAATCACAGGAG-3′ and 5′-CCGAGCTCAGCTGACTAATCACAGGAG-3′.
PCR products were sequence-verified and cloned upstream of the Blimp-1 basal promoter. The expression vectors for Bcl-6 (pMT2-Bcl-6) and the control vector (pMT2) were described (18). All transfections have been performed with at least two independent preparations of plasmid.

**Site-directed mutagenesis**

Site-directed mutagenesis of the BRED1 was performed as described (19). BRE1 (300 bp) in the context of 330 bp blunted with BglII was generated with mutagens primers 5'-CCGGTCATCTGAACATCTGCATTGCACT-3', 5'-CCCCCTAAAGAACAAGCTGCTAGTGAGCAAACCA-3' and 5'-GCCGACCCTCAGTCAAGATCAGGAG-3'. The mutated BRE1 was then cloned into the Blimp-1 basal promoter reporter. BRED1 deletion in the context of 5-kb Sac1 was generated by using primers 5'-CTGCTTTTCCTGTGCTTGGCGTTCACTGCTTGGATCTGCACTTGGACTGTAATC-3' and 5'-GCCGAGCTCAGATCACCAG-3'.

**Cell lines and transfection**

Plasmacytoma cell line, P3X, was used for Bcl-6 cotransfection experiments. Cells were maintained in RPMI 1640 supplemented with 10% FCS, 50 μM 2-ME, and 10 μg/ml gentamicin. Cells (3 x 10⁶) were pulsed in 300 μl of complete medium at 37°C and 240 V (Bio-Rad, Hercules, CA). The number of different reporters was kept constant at 1.3 pmol. Bcl-6 expression vector (pMT2-Bcl-6) or control expression vector (pMT2) were cotransfected at a constant amount of 0.3 pmol. The total amount of DNA transfected was adjusted to 30 μg with pMT2. After transfection, cells were resuspended in 5 ml of complete media and harvested 16–18 h later. To correct for transfection efficiency, 0.3 μg of RL-tk (Promega, Madison, WI) was included in each transfection. After the incubation period, cells were harvested in 1× passive lysis buffer (Promega). Firefly luciferase activity was measured using standard conditions (25 mM Gly-Gly, 15 mM MgSO₄, 4 mM EGTA, 10.7 mM KPO₄, 1 mM DTT, 1.43 mM ATP, and 0.057 mM luciferin) in a Berthold LB9501 luminometer. The firefly luciferase activity was quenched by the addition of 0.2% SDS. The total luciferase activity was measured in the luminometer. WI-L2-Bcl-6 cells have been described (5). Cells were maintained in Phenol Red-free RPMI 1640 supplemented with 10% charcoal/dextran-treated FCS (HyClone, Logan, UT) and 10 μg/ml gentamicin. Cells are transfected as described (14) with slight modification. Immediately after transfection, cells were divided in halves. One half was fed with complete media while the other half was fed with complete media and 50 nM 4-hydroxytamoxifen. Cells were harvested at 20 h posttransfection and luciferase activity was determined as described above.

**DNase footprinting**

A 332-bp radioactive plus strand probe was generated by PCR from the 5-kb Sac1 using primers 5'-CTGCTTTTCCTGTGCTTGGCGTTCACTGCTTGGATCTGCACTTGGACTGTAATC-3' and 5'-GCCGAGCTCAGATCACCAG-3', of which the former was radioabeled by standard kinase reaction. DNase footprinting: the footprinting was performed as described in SureTrack instruction (Pharmacia, San Diego, CA) with the following modifications. A 2 × 10⁶ cpm probe was used. For each probe, 60, 80, or 100 ng of GST-Bcl-6 or 100 ng of GST were incubated with the probes in a 10-μl reaction (16) at room temperature for 20 min. Then 0.5 μg of XbaI blunted binding buffer was added before being subjected to digestion by 0.006 or 0.012 U of DNase1 (Worthington Biochemical, Lake- wood, NJ). A GA ladder was generated with a 4 × 10⁶ cpm probe as described in the instructions (Pharmacia).

**Chromatin immunoprecipitation (ChIP) analysis**

The WI-L2 B cell lymphoma cell line expressing FLAG-tagged Bcl-6 fused to the estrogen receptor ligand binding domain has been described (5). Twenty million WI-L2 cells were incubated with 1 μM 4-hydroxytamoxifen or left untreated for 2.5 h to induce nuclear localization of Bcl-6. After the induction, ChIP were performed essentially as described (10) except that the formaldehyde cross-linking was performed at 37°C for 30 min. The chromatin was immunoprecipitated in duplicate to control for the variability within the experiment. The conditions for amplifying BRED1 were 94°C, 30 s; 55°C, 30 s; and 72°C, 30 s for 35 cycles. The conditions for amplifying CSF-1 and PRDM1 exon 7 were 94°C, 30 s; 55°C, 30 s; and 72°C, 30 s for 35 cycles. Primers for BRED1 PCR are BRED1-up: 5'-TGGTTTGCATGGTCAATTC-3' and BRED1-down: 5'-TTAAAATGGTCGTAGGCGGAGC-3'. Primers for PRDM1 exon 7 are 5'-GCCAAGTTCAACCTAGTGTGTTG-3' and 5'-GATTCCGGCTGATCATCTC-3'. Primers for CSF-1 PCR have been described (10). The PCR products were separated on 1.5% agarose in 1× Tris-phosphate EDTA blotted, and probed with either BRED1-down, exon 7 internal probe (5'-CAAAGGTTACCTGAGAAAGGGA-3') or CSF-1-up primers.

**Comparison of prdm1 genes**

Genomic sequences for human and mouse prdm1 were retrieved from www.ensembl.org and compared by blast2seq (http://www.ncbi.nlm.nih.gov/blast/bl2seq/bl2html) using the default parameter.

**Bcl-6 knockout (ko) mice and immunization**

Bcl-6 ko mice were described previously (14). Three- to 4-wk-old Bcl-6 heterozygous (control) or ko littermates were injected sterilely i.p. with 100 μg of nitrophenyl-keyhole limpet hemocyanin (NP-KLH) in alum. After the times indicated, mice were sacrificed, their spleens removed, fixed, and stained as described below.

**Immunohistochemistry**

Syndecan-1 and λ single staining: splenic sections were incubated with anti-syndecan-1 (BD Pharmingen, San Diego, CA) or anti-λ (Southern Biotechnology Associates, Birmingham, AL) overnight at room temperature. The primary Abs were detected with alkaline phosphatase (AP)-conjugated secondary Abs and nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate substrate. Bcl-6 and syndecan-1 double-staining: splenic sections were incubated with rabbit anti-Bcl-6 overnight at room temperature. The anti-Bcl-6 Ab was detected with an AP-conjugated secondary Ab and NBT/BCIP as color development, the AP was inactivated by boiling in 10 mM EDTA. Slides were then incubated with antisyndecan-1 (BD Pharmingen) which was detected with AP-conjugated secondary Ab and Fast Red substrate.

**Bcl-6 heterozygous and ko B cell in vitro culture**

Splenic B cells from 3- to 4-wk-old Bcl-6 heterozygous and ko mice (11) were purified using anti-CD19 magnetic beads (Miltenyi Biotec, Auburn, CA) (98% purity, our unpublished data). Cells (5 × 10⁶/ml) were cultured in RPMI 1640 + 10% FCS, 2-ME, t-glutamine, penicillin and streptomycin, and 25 μg/ml LPS (Sigma-Aldrich, St. Louis, MO). After 24 h, aliquots of cells were analyzed by FACS as described (5) using anti-mouse CD19 and anti-mouse syndecan-1 (BD Pharmingen). After 72 h, supernatants were collected and the amount of Igs was quantitated using an isotyping CBA kit (BD Pharmingen).

**Semiquantitative PCR for Bcl-1, cyclophilin A, and β₂-microglobulin**

Bcl-6+/− and Bcl-6−/− splenocytes were stimulated with LPS for 24 h, after which the RNA was purified using the TRIzol method (Invitrogen Life Technologies, Carlsbad CA). The cDNA was generated using the random-primer method and the cDNA was used in the semiquantitative PCR. The primers for Bcl-1 amplification have been described (11). The primers for cyclophilin A are 5'-CAGGATTCGCTGACATCA-3' and 5'-ACCCCTG GCACATGAACTC-3'. The primers for β₂-microglobulin are 5'-AGAC TGATACATACCGCTCGAG-3' and 5'-CGAGGTTCAATAATGA TCTTCGAG-3'.

**Results**

Identification of Bcl-6 response elements in prdm1

Vasanwala et al. (17) reported that Bcl-6 repressed Bcl-1 by associating with AP-1 and interfering with its function in the human PRDM1 promoter. Of the two AP-1 binding sites in the human promoter, only one, located 1748 bp 5' of the transcriptional start site, is conserved in the mouse promoter. However, a luciferase reporter containing the prdm1 transcription initiation sites and the putative AP-1 site (−4086 to +212) did not show
Bcl-6-dependent repression (data not shown). This suggests that in the mouse prdm1 gene, Bcl-6 may use a different mechanism to repress transcription.

Therefore, we sought to map functional BREs(s) in mouse prdm1 by generating a series of luciferase reporters containing the prdm1 promoter (−901 to +212; Ref. 13) and other portions of the gene starting from nucleotide +213 to −6 kb 3′ to the poly(A) site. (Fig. 1a). Reporters were transiently cotransfected into P3X (a Blimp-1−Bcl-6− plasmacytoma) with an expression plasmid encoding Bcl-6 or a control plasmid. Reporters containing either the 6.5-kb SacI fragment (fragment 3) or the 5-kb SacI fragment (fragment 4) were repressed by Bcl-6 (Fig. 1b), suggesting the presence of at least two BREs in mouse prdm1. No other region tested conferred a response to Bcl-6 (Fig. 1b). The location of these BREs was further mapped by additional deletion and cotransfection experiments to a 570-bp fragment in intron 3 and a 330-bp XbaI/PvuII fragment in intron 5 (Fig. 2a). We compared the sequence of these two intronic regions between the mouse and human genes. Only the 330-bp XbaI/PvuII fragment in intron 5 is conserved between mouse and human prdm1 genes (Fig. 2a). No conserved region was found for the 570-bp fragment in intron 3 (Fig. 2a). Therefore, we focused our further studies on the conserved BRE in intron 5, which we call BRE1.

A second system was used to confirm the activity of BRE1. WI-L2-Bcl-6 is a human B cell lymphoma cell line engineered to express, constitutively, a Bcl-6 fusion protein containing the estrogen receptor ligand binding domain (Bcl-6-ERD) (5).

FIGURE 1. Mapping BREs in prdm1. a, Schematic representation of the locus. The arrow denotes transcriptional start site and gray boxes denote the eight exons (13). The scales for exons and introns are indicated. The five genomic fragments tested by transfection are indicated below the diagram. b, Bcl-6 repression by cotransfection. P3X cells were transiently cotransfected with prdm1-based luciferase reporters and a Bcl-6 expression vector (+) or control vector (−). The numbers below the graph denote the genomic fragments in the reporters. The data were corrected for transfection efficiency using Renilla luciferase. The mean ± SD of triplicate data points is shown.

FIGURE 2. Mapping BREs. a, Schematic representation showing genomic fragment 3 (6.5-kb SacI) and fragment 4 (5-kb SacI), various deletion mutants, and Bcl-6-dependent repression as measured by transient cotransfection in P3X. Open boxes are exons. Bars under the gene indicate regions conserved between mouse and human (longer than 200 bp). Fragment 3b is a 570 bp generated by PCR located in intron 3, Fragment 3a is fragment 3 in which a 2.2-kb internal region had been deleted. Fragment 4a is fragment 4 in which the 770-bp internal region had been deleted. Fragment 4b is a 330-bp XbaI/PvuII fragment from intron 5. Fragments Δ4b and Δ4 are fragments 4b and 4 in which a BRE1 footprint had been deleted. The activity of the reporters (after the normalization with Renilla luciferase) in the absence of Bcl-6 cotransfection is adjusted to 100% (not shown in the figure). The activity of the reporters in the presence of Bcl-6 cotransfection is normalized to the percentage of the activity in the absence of Bcl-6. b, Transient transfection of the prdm1-based luciferase reporters containing the Blimp-1 basal promoter and the wild-type or mutated BREs in WI-L2-Bcl-6 cells (see Results). The activity of the reporters (after the normalization with Renilla luciferase) in the absence of Bcl-6 activation with tamoxifen is shown as 100%. The activity of the reporters in the presence of Bcl-6 (+tamoxifen) is normalized to the percentage of the activity in the absence of Bcl-6. The data shown represent the mean ± SD of three to five transfection experiments.
translocation and activation of Bcl-6 can be induced in these cells by the addition of 4-hydroxytamoxifen (4-OHT). Transfection of prdm1-based reporters into WI-L2-Bcl-6 cells and subsequent treatment with 4-OHT removes experimental variability derived from cotransfection of a Bcl-6 expression plasmid. As shown in Fig. 2b, the prdm1 reporter containing the 5-kb SacI fragment with BRE1 is repressed when 4-OHT is added to the culture. 4-OHT addition did not repress a similar reporter containing an internal deletion in the 5-kb SacI fragment that removed the 770-bp XbaI/PvuII fragment containing BRE1 (fragment 4a; Fig. 2). Taken together, cotransfections in P3X and transfections in WI-L2-Bcl-6 are consistent with a BRE located within the 330-bp XbaI/PvuII fragment in intron 5.

DNaseI footprinting, using rBcl-6, was used to identify the exact location of BRE1. Within the 330-bp XbaI/PvuII fragment, one Bcl-6 footprint was observed in a 16-bp sequence located ~2100 bp 3′ of exon 5 (Fig. 3). This binding site has homology to the Bcl-6 binding consensus sequence (Fig. 3b) and a homologous sequence is present in intron 4 of human PRDM1 (the equivalent of mouse intron 5). The binding site was altered using site-directed mutagenesis and tested for its ability to confer Bcl-6-dependent repression. In both P3X and WI-L2-Bcl-6 cells, the 330-bp XbaI/PvuII fragment conferred Bcl-6-dependent repression but a mutated form of the 330-bp XbaI/PvuII fragment containing site-directed deletion in the footprinted region did not confer Bcl-6-dependent repression (Fig. 2b). Further, the 5-kb SacI fragment

![DNase footprinting](http://www.jimmunol.org/Downloaded_from/)
with a deletion of BRE1 was not repressed by Bcl-6 (Fig. 2b). Taken together, these results establish BRE1 as a functional response element mediating direct Bcl-6-dependent repression of prdm1.

**Bcl-6 binds to BRE1 in vivo**

To test whether BRE1 functions in vivo, we performed ChIP assays. When chromatin from WI-L2-Bcl-6 cells was immunoprecipitated using an anti-FLAG (Bcl-6-ERD is FLAG-tagged), BRE1 was specifically coimmunoprecipitated in cells treated with 4-OHT but not from untreated control cells (Fig. 4). A control gene, CSF-1 and exon 7 of PRDM1, was not coimmunoprecipitated with anti-FLAG in induced cells. Thus, we conclude that Bcl-6 binds to BRE1 in vivo, providing strong evidence that BRE1 participates in Bcl-6-dependent transcriptional repression of prdm1.
STAT3 does not compete with Bcl-6 binding at the identified BREs

Interfering with STAT3 activity in Bcl-1 cells inhibits induction of Blimp-1 mRNA and plasmacytic differentiation (6). Because the consensus binding sites for Bcl-6 and STAT3 are similar, it was suggested that STAT3 may compete with Bcl-6 for binding and induce Blimp-1 by relieving Bcl-6-dependent repression. We tested this notion by transfecting reporters containing BRE1 on the 5-kb SacI fragment or in the 330-bp XbaI/PvuII fragment, into HepG2 cells, where STAT3 can be activated by IL-6 (20, 21). Neither the reporter with the 5-kb SacI fragment nor the reporter with the 330-bp XbaI/PvuII fragment (Fig. 5 and data not shown) was activated by IL-6. Furthermore, protein from nuclear extracts of HepG2 cells treated with IL-6 to activate STAT3 did not bind to an oligonucleotide corresponding to BRE1 although there was binding to an IFN-γ activation site control oligonucleotide (data not shown). We also tested the 6.5-kb SacI fragment, which contains the BRE not conserved in the human gene. Although this fragment conferred activation in response to IL-6, the activity was present on mutant reporters lacking BRE activity. Thus, we conclude that the BRE elements identified in this study are unlikely to confer IL-6-dependent activation via competitive binding by STAT3.

Repression of prdm1 by Bcl-6 in vivo

When human tonsils were stained for Bcl-6 and Blimp-1, nearly mutually exclusive expression was observed in GC B cells (data not shown), consistent with previous microarray data (5) and providing evidence that Bcl-6 represses Blimp-1 expression in vivo. To explore Bcl-6-dependent repression of murine prdm1 in vivo, we investigated Bcl-6−− mice. B cells from Bcl-6−− mice do not form GCs and the mice ultimately succumb to a Th2-type inflammatory disease (14, 15). However, their ability to form plasma cells has not been thoroughly examined. We hypothesized that if Bcl-6-dependent repression of prdm1 is important in vivo, activated B cells in Bcl-6−− mice might display exaggerated and/or accelerated plasmacytic differentiation.

Bcl-6−− mice survive poorly, limiting the numbers available for study; however, we were able to immunize a limited number of mice with the T-dependent immunogen NP-KLH. Mice were sacrificed 3 and 9 days postimmunization. Plasma cells in the spleen were analyzed using immunohistochemistry (Fig. 6) for surface syndecan-1 (red), nuclear Blimp-1 (blue), and for cytoplasmic λ (blue). Cells with cytoplasmic λ, used as a measure of Ig-secreting cells formed in response to NP-KLH, increased markedly on day 3 and were still significantly elevated on day 9 (Fig. 6b). At both times, there were 2-fold more Ig-secreting cells in the Bcl-6−−
mice than in Bcl-6+/− littermate controls. Similar increases were observed for syndecan+ cells, which were also Blimp-1+ (Fig. 6, a and c). We measured anti-NP IgM and IgG1 in the sera 3 days postimmunization in two independent experiments. The IgM anti-NP titer in the Bcl-6−/− mice was consistently ~2-fold higher than that of Bcl-6+/− littermate control in both experiments. Thus, a reproducible 2-fold increase in secreting cells and serum Ig was observed in Bcl-6−/− mice following immunization with NP-KLH.

The ability of purified splenic B cells to differentiate ex vivo in response to LPS was also analyzed. After CD19+ splenic B cells were treated with LPS for 24 h, there were ~7-fold more CD19+ syndecan1+ plasma cells in cultures from Bcl-6−/− mice compared with controls (Fig. 6d). This rapid plasmacytic differentiation of Bcl-6−/− B cells correlates with ~4-fold higher levels of Blimp-1 mRNA in LPS-stimulated Bcl-6−/− splenocytes (Fig. 6e). The amount of Ig secreted in cultures from Bcl-6−/− B cells was also significantly elevated, relative to controls, for all isotypes, except IgA and IgE (Fig. 6d). These data are consistent with the in vivo results and show accelerated Blimp-1 mRNA induction and plasmacytic differentiation in the absence of Bcl-6.

**Discussion**

*The mouse prdm1 gene is a direct target of Bcl-6-mediated transcriptional repression*

These studies demonstrate that the prdm1 gene, encoding Blimp-1, is a direct target of the Bcl-6 transcriptional repressor. We have identified two regions, located in introns 3 and 5 of mouse prdm1, that confer Bcl-6-dependent repression. The Bcl-6 response element in intron 5 (BRE1) is conserved in the human gene and was shown to be bound by Bcl-6 both in vitro and in vivo. Our study adds prdm1 to the list of IL-5, IL-18, B7-1, and Bcl-6 itself that are direct targets of Bcl-6 (22–26). Our results are consistent with previous microarray studies which identified PRDM1 as a direct target of Bcl-6 repression (5).

We have provided strong evidence that Bcl-6 directly represses murine prdm1 by binding to BRE1 located in intron 5. Based on the strong conservation of BRE1 in human PRDM1, the data suggest, but do not prove, that Bcl-6 may also bind and directly repress the human gene. Bcl-6 was previously shown to interfere with AP-1 activation in the human gene (17). Although we have no evidence that this also occurs in the murine gene, it is certainly possible that, in addition to the direct repression we have demonstrated, an AP-1-dependent mechanism or another mechanism may also be involved in Bcl-6-dependent repression of prdm1. Given the physiological importance of Bcl-6-dependent repression of Blimp-1 expression, to ensure complete affinity maturation, selection, and CSR in the GC, it is not surprising that multiple mechanisms may be involved.

Recently, an isoform of human and mouse Blimp-1 (Blimp-1β) that is functionally impaired was described (27). This truncated form is transcribed from an alternate promoter located in intron 4 of mouse prdm1 resulting in a truncated protein that lacks the N-terminal acidic domain and part of the PRDI-BFI and RIZ homology domain (PR) domain. We do not know whether this promoter is also regulated by Bcl-6. However, the Blimp-1 antisem we used in the Blimp-1/Bcl-6 double immunofluorescent staining (data not shown) was raised against a portion of Blimp-1 that is common to both full-length Blimp-1 (Blimp-1α) and Blimp-1β (1) and should recognize both isoforms, yet we do not see overlapping expression of Blimp-1 and Bcl-6 (data not shown). Therefore, it seems likely that Bcl-6 represses the expression of both isoforms of Blimp-1.

Dominant-negative STAT3 can functionally reverse IL-2/IL-5-dependent Blimp-1 induction and plasmacytic differentiation in the Bcl-1 cell line, indicating a possible role for STAT3 in the regulation of prdm1 (6). Because the binding sites for all STAT proteins are similar (28) and Bcl-6 was shown to compete with STAT6 for binding and function of CD23b and Ilc genes (14, 16), it was proposed that STAT3 might compete with binding of Bcl-6 to prdm1. This hypothesis is appealing because IL-6 and IL-10, which are important for differentiation of plasma cells, activate STAT3 (29, 30) and STAT3 activation in naive B cells in response to BCR cross-linking is mediated through IL-6 and IL-10 (31). However, we show that neither of the BREs identified in this study are STAT3 response elements in our assay system and STAT3 does not compete for binding to the identified BRE1 on prdm1. Thus, although STAT3 is likely to induce prdm1, induction does not appear to be mediated by the Bcl-6 response elements identified in this study.

**Derepression of Bcl-6 as a trigger for plasmacytic differentiation**

Bcl-6-mediated repression appears to play a critical role in regulating Blimp-1 expression. Shaffer et al. (5) showed that expression of a blocking form of Bcl-6 was sufficient to induce Blimp-1 in Burkitt lymphoma cell lines. Further, treatment with a histone deacetylase inhibitor, which negates Bcl-6-mediated repression (32), induces Blimp-1 and terminal differentiation of the mature B cell line, L10A, and primary splenic B cells (33). By repressing prdm1, Bcl-6 plays a major role in preventing premature plasmacytic differentiation and ensuring the GC reaction is complete.

Ex vivo, purified splenic B cells from Bcl-6−/− mice showed enhanced plasmacytic differentiation and elevated Ig secretion (Fig. 6), suggesting that low levels of Bcl-6 in naive B cells may play a role in regulating plasmacytic differentiation. Bcl-6−/− mice also had more syndecan "Blimp-1" cells (Fig. 5) in spleen after immunization with NP-KLH. We also found increased number of IgM anti-NP secreting cells (data not shown) in the Bcl-6−/− mice. These data provide strong support for the concept that one important function of Bcl-6 in GC B cells is to inhibit plasmacytic differentiation via repression of prdm1 (5–7). Our data extend an earlier report that also showed increased plasmacytoid cells in spleens of Bcl-6−/− mice (15). Our data are also consistent with the finding of Toyama et al. (34) who showed an increase of Ag-specific IgM-secreting plasma cells in the bone marrow of the recipient mice that had received Bcl-6−/− B cells.

The extent of plasmacytic differentiation in vivo could have been underestimated in these studies due to effects of homeostatic mechanisms, including the limited capacity of the spleen to support plasma cells (35), that may limit Ig-secreting cells. Indeed, limited splenic capacity and/or consequences of hyperinflammation probably account for the lack of elevated serum Ig in Bcl-6−/− mice despite observed increases in plasmacytic development in spleen both in vivo and ex vivo.

What signals in normal B cells finally relieve Bcl-6-dependent repression of prdm1 and allow plasma cells to develop? BCR cross-linking activates Erk-1, which phosphorylates Bcl-6 and targets it for proteasome-dependent degradation (36, 37). Perhaps acquisition of a high affinity BCR, via somatic hypermutation and selection, causes sufficient Bcl-6 degradation to derepress prdm1 and allow Blimp-1-dependent plasmacytic differentiation. Another possible mechanism is by acetylation of Bcl-6 because Bcl-6 was shown to be acetylated, resulting in inactivation (32). Regardless of mechanism, once Bcl-6-dependent repression of prdm1 is relieved, plasma cell fate can be maintained by a feedback loop in which Blimp-1 represses Bcl-6 expression (11).
Acknowledgments

We thank Drs. Y. R. Zou and C. Schindler for critically reading the manuscript, Drs. R. Dalla-Favera and C. Schindler for plasmids and cell lines, Dr. G. Siu for assistance on DNasel footprinting, and the members of Calame laboratory for helpful discussion.

References


