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Binding of BAZF and Bc16 to STAT6-Binding DNA Sequences

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BAZF, a family member of Bcl6, can function as a sequence-specific transcriptional repressor. We determined BAZF-binding DNA sequence. The consensus binding sequence (CBS) of BAZF is almost the same as those of Bcl6 previously described. Three nucleotides of T, G and A at position 6, 8, and 9 in the CBS (5′-ATTCCTAGAAAG-3′) are important nucleotides for binding of both BAZF and Bcl6. Since a part (5′-TTC-CTA-GAA-3′) of the CBS resembled the sequence motif (5′-TTC-(N3-4)-GAA-3′) bound by STAT factors, BAZF and Bcl6 can bind to the CD23b-STAT6-binding sequence (5′-TTTC-TTA-GAAAT-3′), the immunoglobulin germline ϵ-STAT6-binding sequence (5′-CTTC-CCAA-GAAC-3′), and the IL4-STAT6-binding sequence (5′-TTTC-CCA-GAAAA-3′) with weak affinity. However, a mutation of C nucleotide to T nucleotide in the IL4-STAT6-binding sequence (5′-TTTC-CTA-GAAAA-3′) strongly increased the binding activity of BAZF and Bcl6. These results suggest that BAZF and Bcl6 can repress some of STAT-induced transcription by binding to DNA sequences recognized by STAT factors.

Key Words: **consensus binding sequence; BAZF; Bcl6; STAT6; GAS motif; transcriptional repressor.**

Chromosomal translocations involving 3q27 were detected in non-Hodgkin's lymphomas, particularly in diffuse large B-cell lymphomas (1, 2). The human protooncogene *BCL6* has been identified from the chromosomal breakpoints (3–5). The *BCL6* gene encodes a 92- to 98-kDa nuclear phosphoprotein that contains the BTB/POZ domain in the NH2-terminal region and *Krüppel*-type zinc finger motifs in the COOH-terminal region. The BTB/POZ domain is important for protein-protein interactions and the zinc finger motifs bind to specific DNA sequence *in vitro*. The *BCL6* gene is well conserved between human and mice, with 100% identity of the zinc finger motifs at the amino acid level (6). Since the NH2-terminal half of the protein contains repressor domains *in vitro* (7–10), BCL6 can function as a sequence-specific transcriptional repressor. Indeed, the BTB/POZ domain of BCL6 can bind to silencing mediator of retinoid and thyroid receptor protein (SMRT) and recruit the SMRT/histone deacetylase (HDAC) complex to specific BCL6-binding regions of target genes to repress expression of those genes (11, 12).

To observe physiological functions of murine Bcl6, this gene was disrupted in the mouse germ line (13–15). Bcl6-deficient ($Bcl6^{-/-}$) mice displayed massive inflammation with eosinophilic infiltration. Since interleukin-5 (IL-5) produced by T helper cell type 2 (Th2) is an important cytokine involved in controlling the growth, differentiation and activation of eosinophils and production of Th2 type cytokines by T cells from $Bcl6^{-/-}$ mice was augmented, mechanisms of this eosinophilic inflammation could be partly explained by a functional dominance of Th2 cells in $Bcl6^{-/-}$ mice. Furthermore, IL-4 stimulation induces differentiation of Th0 cells to Th2 cells (16) by activating signal transducer and activator of transcription (STAT) 6 (17–19), and a part of Bcl6-binding DNA sequences (5′-AT(T/C)C-CTN-GAAAGN(T/A)-3′) (20) resembled the "GAS" motif (5′-TTC-(N3-4)-GAA-3′) bound by the STAT factors. Thus, Dent *et al.* (13) made a hypothesis to explain the mechanisms of Th2 dominance in $Bcl6^{-/-}$ mice as followings; Bcl6 may repress IL-4-induced transcription by competitive binding to DNA sites recognized by the IL-4 activated STAT6. However, the Bcl6-binding DNA sequence is not the same (mismatch, underline) as the STAT6-binding DNA sequence in the *IL-4* gene (5′-TTC-CCA-GAA-3′) (17, 18), the *CD23b* gene (5′-TTC-TTTA-GAA-3′) (13, 21), and the *immunoglobulin germline* ϵ (*I*ϵ) gene (5′-TTC-CCAA-GAA-3′) (19, 21).

We cloned a novel *Bcl6* family gene, BAZF (22). The predicted amino acid sequence of BAZF indicated that the BTB/POZ domain and five repeats of the *Krüppel-*

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type zinc finger motif are located in the NH2-terminal region and the COOH-terminal region, respectively. Since the zinc finger motifs of BAZF are 94% identical to those of Bcl6 at the amino acid level, BAZF can specifically bind to the Bcl6-binding DNA sequence and function as a transcriptional repressor. Therefore, the biochemical character of BAZF may be similar to that of Bcl6. However, BAZF-binding DNA sequence has never been examined and compared with the Bcl6-binding DNA sequence. Here we determined BAZF-binding DNA sequence and critical nucleotides in the sequence for binding of BAZF and Bcl6. We discuss a possibility of repression of gene expression with the STAT6-binding sequences by BAZF and Bcl6.

MATERIALS AND METHODS

Production of glutathione S-transferase (GST)-BAZF and GST-Bcl6 fusion proteins. Construction of GST fusion genes with the zinc finger domain of BAZF or Bcl6 was described previously (20, 22). The constructs were cloned into *Escherichia coli* AD202 cells. Following 3 h incubation of a 10 ml culture with 0.1 mM isopropyl-β-D-thiogalactopyranoside, the bacteria were collected by centrifugation, washed twice in cold PBS, resuspended in 0.5 ml of PBS, and lysed by sonication. The fusion protein was isolated from the supernatant with 500 μl of slurry of 50% glutathione-sepharose beads (Pharmacia) as described in a manufacturer's protocol. The beads were washed with ice cold PBS for 4 times, made aliquot, and stored at 4°C. The protein was obtained from glutathione-sepharose beads by elution buffer (Pharmacia). The protein concentration and size were determined by a protein assay kit (Bio-Rad) and by polyacrylamide gel electrophoresis, respectively.

Selection of GST-BAZF-binding oligonucleotides. Selection of GST-BAZF-binding oligonucleotides from random oligonucleotides was performed according to the method reported by Delwel *et al.* (23). Briefly, random-sequence oligonucleotides, N-26 (5′-GGCTGAGTCTGAACGGATCC (N-26) CCTCGAGACTGAGCGTCG-3′) and N-35 (5′-CTGGATCCTAAGATTCCCTG (N-35) AGGCTCAAAGCTGAATTCCT-3′) with 20 nucleotides flanking sequence, were synthesized and used as a template to make double-stranded DNA. The synthesized single-stranded oligonucleotide (200 pM) was incubated with 600 pM of the reverse primer P26-2 (5′-CGACGCTCAGTCTCGAGG-3′) for N-26 or P35-2 (5′-AGGAATTCAGCTTTGACCT-3′) for N-35, reaction buffer with 2 mM $MgCl_2$, and 5 U of Taq Polymerase in a volume of 50 μl. The mixtures were treated to make double stranded DNA as follows: incubation at 94°C for 1 min, at 50°C for 2 min, and at 72°C for 2 min. The double-stranded DNA (10 μl) was mixed with 50% slurry of GST-BAZF protein and glutathione-sepharose beads in binding buffer (100 mM KCl, 10 mM $MgCl_2$, 0.1 mM $ZnSO_4$, 25 mM Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 0.1% Nonidet P-40, 1 mM ditriothreitol, 5% glycerol), 100 μg of poly(dI-dC) (Boehringer-Manheim) and bovine serum albumin (Sigma Chemical) per ml in a final volume of 125 μl. After 30 min of incubation at room temperature, the sepharose beads were washed four times with binding buffer. The double stranded oligonucleotides (N-26 or N-35) were eluted from the beads in 50 μl of H_2O by boiling for 5 min, and those in the supernatant (1 μl) was amplified in a 50 μl PCR mixture containing 30 pM of P26-1 (5′-GGCTGAGTCTGAACGGATCC-3′) and P26-2 primer for N-26 or 30 pM of P35-1 (5′-CTGGATCCTAAGATTCCCTG-3′) and P35-2 primer for N-35, 1 U of Taq polymerase, 200 μM dNTP, and 2 mM $MgCl_2$. The mixture was denatured at 94°C for 7 min, and then PCR was performed at 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min for 20 cycles. After six rounds of the selection, the double stranded oligonucleotides recovered was cloned into pGEM-T vector (Promega). Each clone was sequenced by T7 primer with DNA sequencing kit (United State Biochemical Corp.).

Electrophoretic mobility shift assay (EMSA). Specific DNA binding activity of BAZF or Bcl6 was determined by EMSA (13). Briefly, synthesized double stranded oligonucleotides were labeled with digoxigenin (DIG) using DIG Gel Shift Kits as a probe (Roche Molecular Biochemical). Binding reactions were performed in the mixture containing appropriate amounts of GST-BAZF or GST-Bcl6, 0.5 μg of poly-(dI-dC), 1 μg of poly-L-lysine and 0.1 pM of DIG-labeled probe in 20 μl of reaction buffer (20 mM Hepes pH 7.6, 30 mM KCl, 1 mM DTT, 1 mM EDTA, 10 mM $(NH_4)_2SO_4$, 1% (w/v) Tween 20). This mixture was then incubated for 15 min at room temperature, separated by electrophoresis on a 6% nondenaturing polyacrylamide gel, transferred to a nylon membrane (Roche Molecular Biochemical), and fixed by UV cross-linking using a Spectrolinker (Schleicher & Schuell). The DIG-labeled probe was detected with sheep anti-DIG antibody conjugated with alkaline phosphatase. The antibody detection reaction was performed using an enhanced chemiluminescent detection system, CSPD (Roche Molecular Biochemical).

Competitive EMSA was done by adding unlabeled double stranded oligonucleotides to the mixture of GST fusion protein and DIG-labeled probe. Sequences of a mutant oligonucleotide (one nucleotide mismatch; underline) of the CBS were as follows: CBS; 5′-cgacATTCCTAGAAAGcata-3′, one nucleotide mismatch at each position (1 to 12); m1 (5′-cgacGTTCCTAGAAAGcata-3′), m2 (5′-cgacACTCCTAGAAAGCATA-3′), m3 (5′-cgacATACCTAGAAAGcata-3′), m4 (5′-cgacATTACTAGAAAGcata-3′), m5 (5′-cgacATTCGTAGAAAGcata-3′), m6 (5′-cgacATTCCGAGAAAGcata-3′), m7 (5′-cgacATTCCTGGAAAGcata-3′), m8 (5′-cgacATTCCTATAAAGcata-3′), m9 (5′-cgacATTCCTAGCAAGcata-3′), m10 (5′-cgacATTCCTAGATAGcata-3′), m11 (5′-cgacATTCCTAGAATGcata-3′), m12 (5′-cgacATTCCTAGAAAAcata-3′). Synthetic oligonucleotides of the STAT6-binding DNA sequence were as follows: IL4-STAT6-binding sequence; 5′-gtaTTTC-CCA-GAAAAggaac-3′ (17, 18), Tm-IL4-STAT6-binding sequence; 5′-gtaTTTC-CTA-GAAAAggaac-3′, CD23b-STAT6-binding sequence; 5′-gtcccTTTC-TTA-GAAATtca-3′ (13, 21) and Iε-STAT6-binding sequence; 5′-agctaaCTTC-CCAA-GAACat-3′ (19, 21).

Preparation of retroviruses and infection. The amphotrophic packaging cell line, PT67 was purchased from CLONTEC Laboratories and maintained in Dulbecco's modified Eagle medium (Sigma Chemical) supplemented with 100 μg/ml streptomycin sulfate, 100 U/ml penicillin G potassium, and 10% (v/v) heat-inactivated fetal calf serum (Sigma Chemical). WIL2-NS cells was purchased from ATCC and maintained in RPMI 1640 medium supplemented with 100 μg/ml streptomycin, 100 U/ml penicillin G potassium, 5×10^{-5} M 2-mercaptoethanol and 10% fetal calf serum.

Murine Bcl6 and BAZF cDNA was cloned into the multicloning site of the bicistronic retrovirus vector (MSCV/IRES-EGFP) (24). PT67 amphotrophic packaging cells were transfected with MSCV/IRES-EGFP, MSCV/IRES-EGFP-Bcl6 or MSCV/IRES-EGFP-BAZF using Fugene6 transfection reagent (Roche Diagnostics) according to the manufacturer's instruction. After 24 h, medium was changed and PT67 cells were cultured another 24 h. Supernatants were collected, and one million WIL2-NS cells were cultured in 4 ml viral supernatants with 10 μg/ml Polybrene (Sigma Chemical) for two days. EGFP fluorescence of the infected WIL2-NS cells was analyzed by a FACS-Vantage (Becton Dickinson), and EGFP positive cells were deposited into 6-well tissue culture plates with CloneCyt (Becton Dickinson). We confirmed expression of Bcl6 and BAZF mRNA in those transfectants by Northern blot analysis. The transfected cells were stimulated with recombinant human IL-4 (10 ng/ml; Genzyme) for 16 h and subsequently stained with mouse anti-human CD23-PE (M-L233, PharMingen) for flow cytometry.

RESULTS

Identification of BAZF-binding DNA sequence. In order to determine an optimal BAZF-binding DNA se-

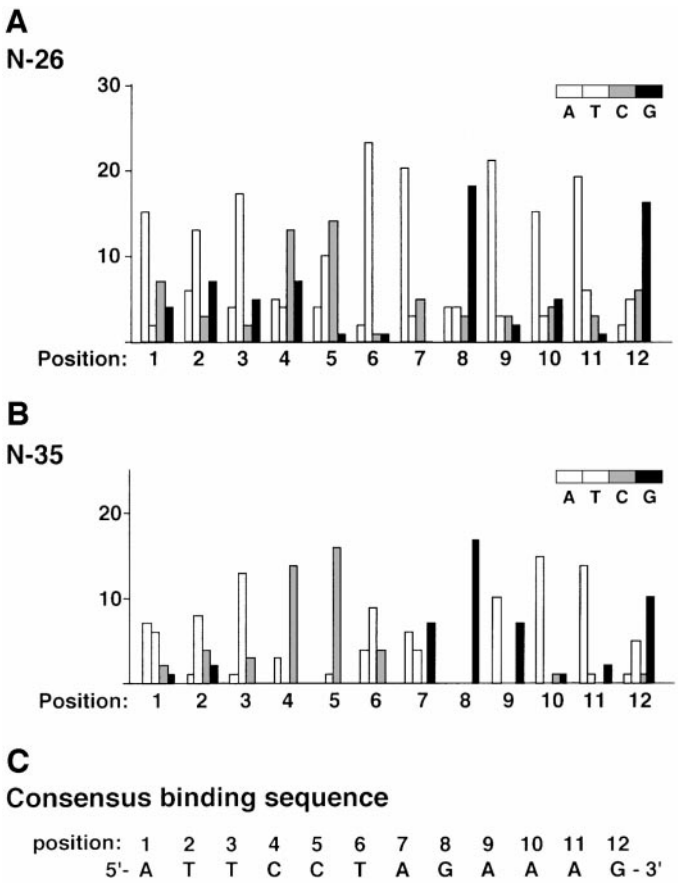

FIG. 1. DNA sequence recognized by BAZF. BAZF-binding DNA sequences were determined using a technique described previously by Delwel *et al.* (24). (A) BAZF-binding DNA sequences from the experiments using 26 random oligonucleotides (N-26). The frequently used nucleotide sequence is 5′-ATTCCTAGAAAG-3′. (B) BAZF-binding DNA sequences from the experiments using 35 random oligonucleotides (N-35). The frequently used nucleotide sequence is 5′-ATTCCTGGAAAG-3′. (C) The most frequent DNA sequence as the CBS (5′-ATTCCTAGAAAG-3′) was decided by the results using N-26 and N-35.

quence, GST-BAZF protein was mixed with double-stranded oligonucleotides containing 26 (N-26) or 35 (N-35) nucleotides of random core sequences. Oligonucleotides bound to GST-BAZF were recovered and amplified by PCR. After 6 sequential rounds of PCR selections, 29 and 17 independent clones were randomly picked up from N-26 and N-35 oligonucleotides, respectively. Random core sequences of those clones were sequenced. Figure 1 shows an alignment of DNA sequences from N-26 (Fig. 1A) and N-35 (Fig. 1B) oligonucleotides. Most frequently used nucleotide sequences from N-26 and N-35 are 5′-ATTCCTAGAAAG-3′ and 5′-ATTCCTGGAAAG-3′, respectively. The core consensus binding sequence (CBS; 5′-ATTCCTAGAAAG-3′) was decided as the most frequently used nucleotide sequences from both N-26 and N-35 (Fig. 1C). These results indicate that the CBS for BAZF is almost the same as those for Bcl6 (5′-AT(T/C)CCTNGAAAGN(T/A)-3′ (20) and 5′-TC(T/C)T(C/A)GAA(A/T)G-3′ (10)).

Binding of GST-BAZF and GST-Bcl6 to the CBS. We confirmed binding of GST-BAZF and GST-Bcl6 to the CBS (5′-cgacATTCCTAGAAAGcata-3′) by EMSA. As shown in Fig. 2, stoichiometric shift bands were observed according to the quantity of recombinant proteins in both GST-BAZF (Fig. 2A) and GST-Bcl6 (Fig. 2B) using the DIG labeled CBS as a probe. Specificity of the binding was further examined by competitive EMSA using cold competitors of the CBS with a single-nucleotide mutation at position 1 to 12 (see Materials and Methods). GST-BAZF bound to the CBS, and 100-fold excess amounts of cold CBS completely inhibited the binding (lane 2). However, 100-fold excess amounts of cold competitors with a single-nucleotide mutation at position 4 (m4; C to A; lane 6), position 6 (m6; T to G; lane 8), position 8 (m8; G to T; lane 10), or position 9 (m9; A to C; lane 11) did not completely inhibit the binding. Since the competitor of m8 was the weakest competitor among them, G nucleotide at the position 8 is the most critical sequence of the CBS. Competitive EMSA for GST-Bcl6 also indicated that four nucleotides of C, T, G, and A at position 5, 6, 8, and 9 of the CBS were also important for binding of GST-Bcl6.

Binding of GST-BAZF and GST-Bcl6 to the IL-4-STAT6-binding DNA sequence. A pair of three nucleotides 5′-TTC-3′ at position 2 to 4 and 5′-GAA-3′ at position 8 to 10 in the CBS (5′-ATTC-CTA-GAAAG-3′) was also shared with STAT6-binding sequence motif (5′-TTC-(N3-4)-GAA-3′) (13). Thus, we analyzed the binding of GST-BAZF and GST-Bcl6 to the IL4-STAT6-binding sequence (5′-gtaTTTC-CCA-GAAAAggaac-3′) by competitive EMSA using the DIG labeled CBS. As shown in Fig. 3, when 100-fold (lanes 2, 4, and 6) excess amounts of cold CBS, m8 or IL4-STAT6-binding sequence was mixed with the DIG labeled CBS, cold CBS but not IL4-STAT6-binding sequence and m8 competed the binding of GST-BAZF (Fig. 3A) and GST-Bcl6 (Fig. 3B) to the probe. Since 200-fold (lane 7) excess amounts of the IL4-STAT6-binding sequence could slightly compete the binding, we reexamined the binding activity of GST-BAZF and GST-Bcl6 to the IL4-STAT6-binding sequence by competitive EMSA. BAZF and Bcl6 can bind effectively to the CBS at least 40-fold stronger than to the IL4-STAT6-binding sequence (data not shown).

Binding of GST-BAZF and GST-Bcl6 to the IL4-STAT6-binding sequence was further confirmed by EMSA using the DIG labeled IL4-STAT6-binding sequence. GST-BAZF (Fig. 4A) and GST-Bcl6 (Fig. 4B) bound to the DIG labeled IL4-STAT6-binding sequence (lane 1), and 50-fold (lane 3) to 100-fold (lane 4) excess amounts of cold IL4-STAT6-binding sequence slightly competed the binding. However, 10-fold excess amounts of cold CBS (lane 5) completely blocked the binding, suggesting weaker binding of GST-BAZF and

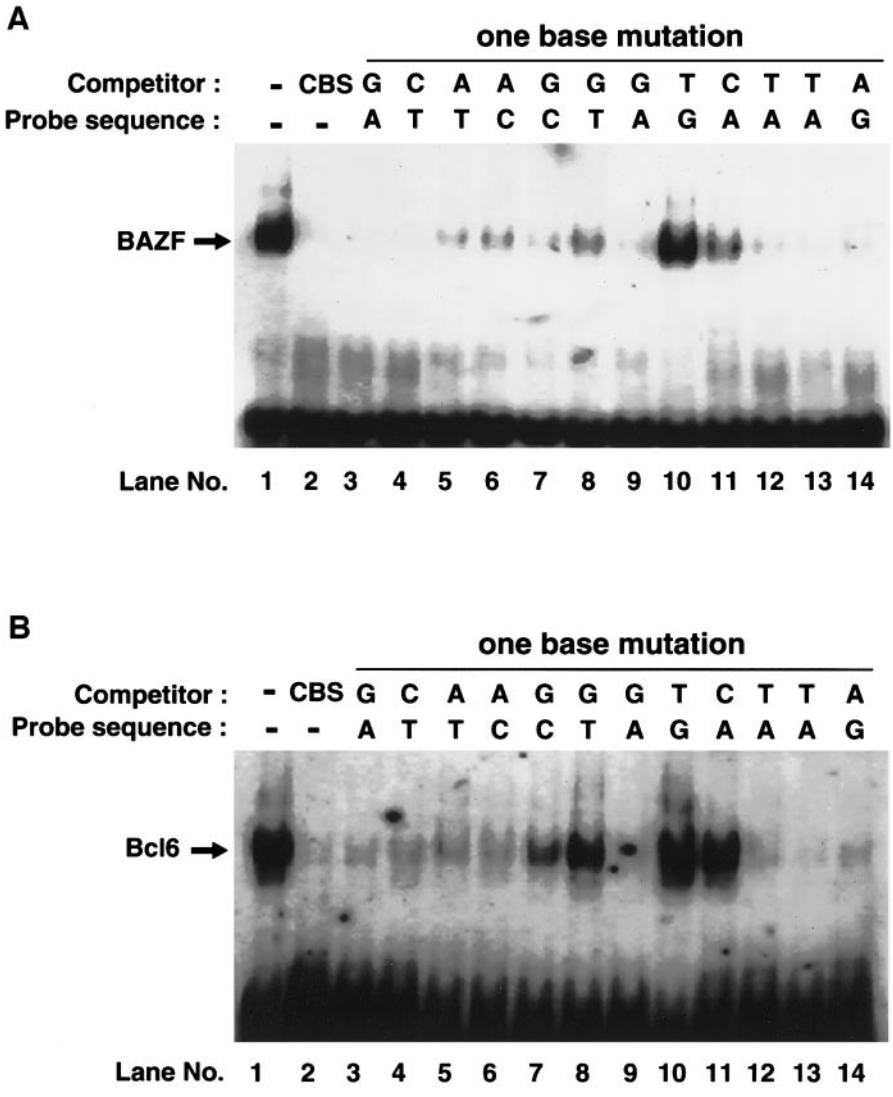

FIG. 2. Binding of BAZF and Bcl6 to the CBS. Binding of BAZF (A) and Bcl6 (B) was examined by EMSA using the DIG-labeled CBS as a probe. Specificity of the binding was analyzed by cold competition assay with 100-fold molar excess amounts of the CBS with a single nucleotide mutation.

GST-Bcl6 to the IL4-STAT6-binding sequence. Furthermore, when we used Tm-IL4-STAT6-binding sequence (5′-gtaTTTC-CTA-GAAAAggaac-3′) with one nucleotide mutation at position 6 (C to T) as a cold competitor (lanes 10–12), the binding was completely inhibited even in 10-fold excess amounts of the competitor, indicating the importance of T nucleotide at position 6 for the binding. These results also suggest that BAZF and Bcl6 may not be able to compete well with STAT6 for binding to the STAT6-binding sequence in the *IL-4* gene.

Binding of GST-BAZF and GST-Bcl6 to other STAT6-binding DNA sequences. Since the Tm-IL4-STAT6-binding sequence strongly blocked the binding of GST-BAZF and GST-Bcl6 to the IL4-STAT6-binding sequence, BAZF and Bcl6 may bind to some other STAT6-binding sequences. Thus, we tried to analyze binding activity of GST-BAZF and GST-Bcl6 to two known DNA elements recognized by STAT6; CD23b-STAT6-binding sequence (5′-gtcccTTTC-TTA-GAAATtca-3′) and Iε-STAT6-binding sequence (5′-agc-taaCTTC-CCAA-GAACat-3′) by competitive EMSA. Figure 5A shows that 200-fold excess amounts of cold CD23b-STAT6-binding sequence (lane 11) or Iε-STAT6-binding sequence (lane 14) inhibited the binding of GST-BAZF and GST-Bcl6 to the DIG labeled CBS. The binding of GST-Bcl6 but not that of GST-BAZF was inhibited by 50-fold excess amounts of cold CD23b-STAT6-binding sequence (lane 13), suggesting that the binding of GST-Bcl6 to CD23b-STAT6-binding sequence is stronger than that of GST-BAZF.

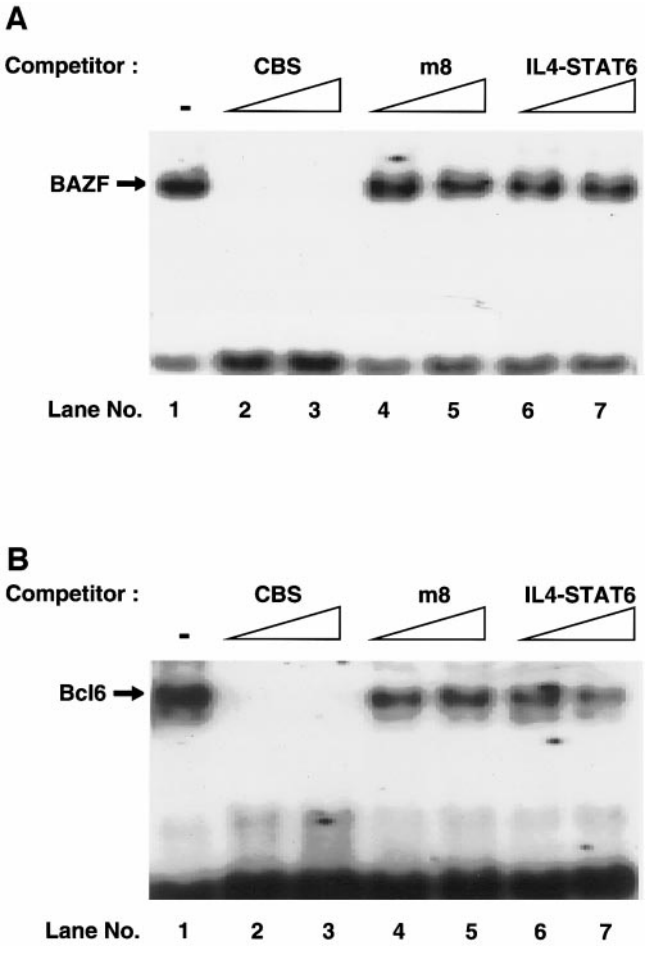

FIG. 3. Binding of BAZF and Bcl6 to the IL4-STAT6-binding sequence. Binding of BAZF (A) and Bcl6 (B) to the IL4-STAT6-binding sequence was analyzed by competitive EMSA using the DIG-labeled CBS as a probe and the IL4-STAT6-binding sequence as a cold competitor. The 100-fold (lanes 2, 4, and 6) and 200-fold (lanes 3, 5, and 7) molar excess amounts of cold CBS, m8 or IL4-STAT6-binding sequence were mixed with the probe.

To confirm the binding, we analyzed binding of GST-BAZF and GST-Bcl6 to DIG labeled CD23b-STAT6-binding sequence, Iε-STAT6-binding sequence, or IL4-STAT6-binding sequence by EMSA (Fig. 5B). GST-BAZF and GST-Bcl6 clearly bound to the CD23b-STAT6-binding sequence (lanes 4–6) and the Iε-STAT6-binding sequence (lanes 7–9), albeit a stronger binding of GST-Bcl6 than that of GST-BAZF. However, the binding to the IL4-STAT6-binding sequence was very weak (lanes 10–12).

It has been reported that overexpression of Bcl6 repressed expression of CD23 on WIL2-NS cells (13). The results shown in Fig. 5 suggested that overexpression of BAZF might suppress expression of CD23 on WIL2-NS cells like as Bcl6 did. Thus, we transfected WIL2-NS cells with BAZF (MSCV/IRES-EGFP-BAZF) or Bcl6 (MSCV/IRES-EGFP-Bcl6). WIL2-NS cells transfected with a control vector (MSCV/IRES-EGFP) did not express the endogenous Bcl6 and BAZF genes, and BAZF or Bcl6 mRNA was strongly detected in those transfectants by Northern blot analysis (data not shown). Expression of CD23 on those transfectants stimulated with or without IL-4 was analyzed by flow cytometry. As shown in Fig. 6, overexpression of BAZF did not repress the expression although overexpression of Bcl6 clearly suppressed the expression even with IL-4 stimulation, suggesting that the binding of BAZF is too weak to repress the expression.

DISCUSSION

In this study, we have determined BAZF-binding DNA sequence using a technique described previously by Delwel *et al.* (23). Most zinc finger proteins contain fewer than 10 repeats of a zinc finger motif that are usually clustered in a single tandem array, and Bcl6

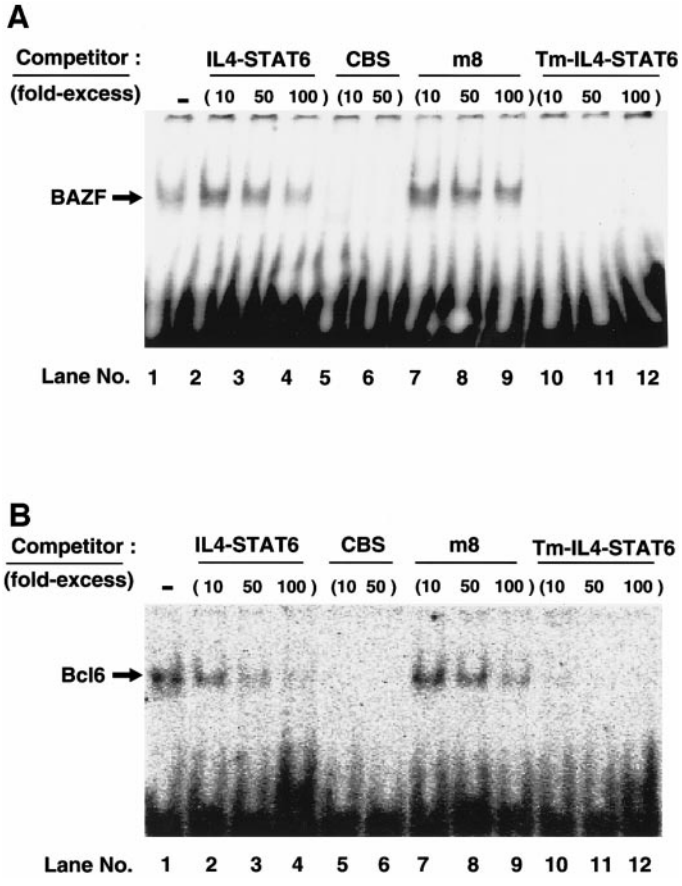

FIG. 4. Binding of BAZF and Bcl6 to the Tm-IL4-STAT6-binding sequence. Binding of BAZF (A) and Bcl6 (B) to the Tm-IL4-STAT6-binding sequence was analyzed by competitive EMSA using the DIG labeled IL4-STAT6-binding sequence as a probe and the Tm-IL4-STAT6-binding sequence a cold competitor. The 10-fold (lanes 2, 5, 7, and 10), 50-fold (lanes 3, 6, 8, and 11) and 100-fold (lanes 4, 9, and 12) molar excess amounts of cold IL4-STAT6-binding sequence, CBS, m8 or Tm-IL4-STAT6-binding sequence were mixed with the probe.

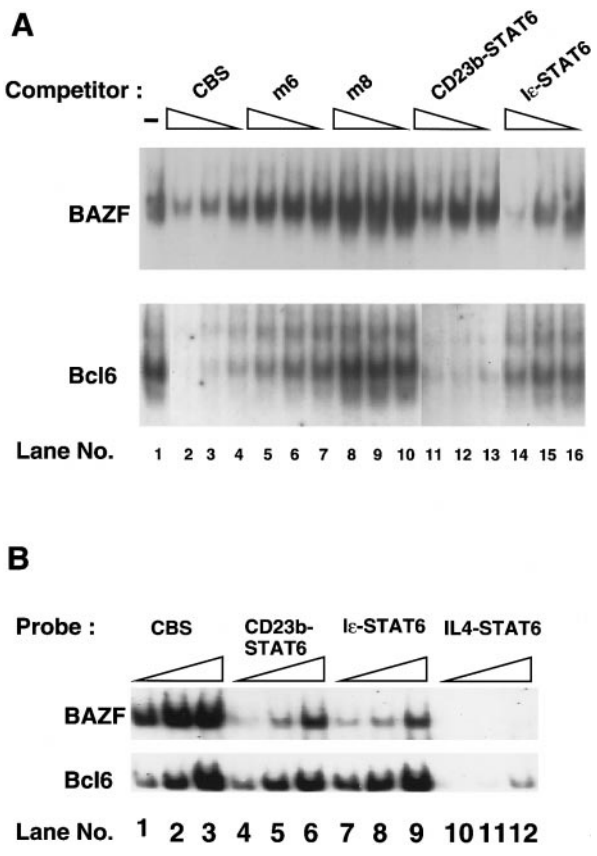

FIG. 5. Binding of BAZF and Bcl6 to several STAT6-binding sequences. (A) Binding of BAZF and Bcl6 to CD23b-STAT6-binding sequence or Iε-STAT6-binding sequence was analyzed by competitive EMSA with the DIG labeled CBS as a probe and 200-fold (lanes 5, 8, 11, and 14), 100-fold (lanes 6, 9, 12, and 15), 50-fold (lanes 2, 7, 10, 13, and 16), 20-fold (lane 3) and 10-fold (lane 2) molar excess amounts of the CD23b-STAT6-binding sequence (lanes 11–13) and the Iε-STAT6-binding sequence (lanes 14–16) as a cold competitor. CBS, m6, and m8 cold competitors (lanes 2–10) were used as controls for specific binding. (B) Binding of BAZF and Bcl6 to the CD23b-STAT6-binding sequence, the Iε-STAT6-binding sequence and the IL4-STAT6-binding sequence was examined by EMSA with the DIG labeled those sequences as a probe. The amounts of probe used were 0.1, 0.2, 0.4 pM probe/reaction from the left.

and BAZF have six and five repeats of a zinc finger motif, respectively. Since five repeats of the zinc finger motif are calculated to interact with 15 to 25 nucleotides (25), N-26 and N-35 random oligonucleotides used are sufficient for identification of recognition sequence for BAZF. BAZF binds to a specific DNA sequence (CBS; 5′-ATTCCTAGAAAG-3′) that is similar to the Bcl6-binding DNA sequences previously described (10, 20). Since the zinc finger motifs of BAZF are 94% identical to those of Bcl6 and BAZF can bind to the Bcl6-binding sequence (22), the similarity of binding DNA sequences between them is reasonable. We further analyzed the important residues in the CBS for binding of BAZF and Bcl6 by competitive EMSA (Fig. 2). Three nucleotides of T, G and A at position 6, 8 and 9 in the CBS are important nucleotides for binding of both BAZF and Bcl6. C nucleotide at position 7 in the CBS is also important for Bcl6-binding but not for BAZF-binding (Fig. 1).

Among transcription factors with zinc finger motifs, each zinc finger will make contact with a nucleotide or a phosphate of DNA or make no contact at all with DNA. For example, GLI (26) has five repeats of a zinc finger motif, and one in the most NH2-terminal side does not make contact with DNA. PLZF (27) has nine repeats of a zinc finger motif and bind to DNA through seven repeats located on the most carboxyl-terminal side. Although the number of repeats of the zinc finger motif is different between BAZF and Bcl6, BAZF and Bcl6 can bind to the similar DNA binding sequences, suggesting that five repeats of the zinc finger motifs of BAZF and Bcl6 on the most carboxyl-terminal side may be important to bind to the DNA sequence. These observations are considered to be important for future study related to transcriptional factors with a zinc finger motif.

BAZF and Bcl6 can bind to the same DNA sequences (Fig. 1). The CBS (5′-ATTC-CTA-GAAAG-3′) of BAZF and Bcl6 resembled the "GAS" motif (5′-TTC-(N3-4)-GAA-3′) bound by STAT factors. Previous reports demonstrated that Bcl6 is capable of recognizing the STAT6-binding DNA sequences (13, 21). However, binding activity of BAZF to the STAT6-binding sequences examined is not strong (Fig. 5). Indeed, overexpression of BAZF could not repress expression of CD23 on WIL2-NS cells (Fig. 6) although overexpres-

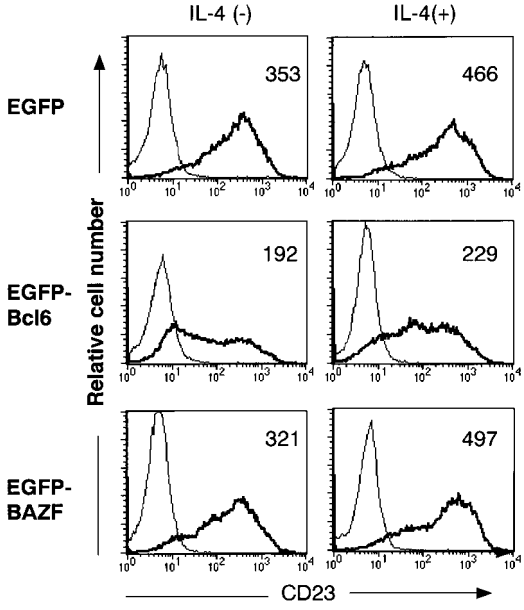

FIG. 6. Repression of CD23 expression on a B cell line by Bcl6 but not by BAZF. WIL2-NS cells transfected with EGFP, EGFP-Bcl6, or EGFP-BAZF were stimulated with IL-4, and cell surface expression of CD23 was analyzed by flow cytometry. Data are representative of three independent experiments. The number in the left side of each quadrant indicates the mean fluorescence intensity.

sion of Bcl6 did as previously reported (13). The binding of BAZF and Bcl6 to the IL4-STAT6-binding sequence strongly increased when the IL4-STAT6-binding sequence with one-nucleotide mutation (T) at two nucleotides upstream of the 5′-GAA-3′ sequence (Tm-IL4-STAT6-binding sequence; Fig. 4), indicating that the binding activity of BAZF and Bcl6 to STAT-binding sequences depends on the nucleotides between the 5′-TTC-3′ sequence and the 5′-GAA-3′ sequence in the "GAS" motif. Therefore, BAZF and Bcl6 may be able to repress some of STAT-induced transcription by binding to the "GAS" motif.

The biochemical character of BAZF is similar to that of Bcl6. Expression of BAZF is induced in mature lymphocytes after stimulation as an immediate early gene (22) such as that of Bcl6 (6). The transcriptional repressor activity of Bcl6 and BAZF is due to recruit the SMRT/HDAC corepressor complex to the CBS in target genes to repress expression of those genes (11, 12, 22). The recent report demonstrated the novel co-repressor protein (BCoR) which binds to the POZ domain of Bcl6 but not to the others POZ family including BAZF (28) although BAZF shares high sequence homology with Bcl6 in its POZ domain (22). Therefore, the transrepressor activity of BAZF and Bcl6 may be distinct in certain cells with BCoR. These findings also suggest that there may be a BAZF-specific corepressor molecule. We have recently reported that Bcl6 may play a role as a stabilizer in protecting spermatocytes from apoptosis induced by stresses using Bcl6-deficient mice (29). Since the tissue expression pattern of BAZF differs from that of Bcl6 and BAZF is strongly expressed in the heart and lung (22), BAZF may play a role in protecting those tissues from apoptosis. This issue is currently studied using BAZF-deficient mice.

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