A Cell Type–specific Enhancer in the Human B7.1 Gene Regulated by NF-κB

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Summary

The costimulatory molecule B7.1 provides a second signal critical for T cell activation. The distribution of this integral membrane protein is restricted to certain tissues where its level of expression is modulated by multiple exogenous stimuli. To identify the molecular basis for specificity and inducibility, the chromatin configuration of the human B7.1 gene was examined in intact nuclei from various cell types. The identification of a tissue-specific deoxyribonuclease I hypersensitive site ~3kb upstream of the transcription start site led to the characterization of a cell type–specific enhancer region. This 183-bp region was both cell type specific and responsive to two distinct stimuli, lipopolysaccharide and dibutyryl cAMP, known to regulate B7.1 expression. Deletional and site-directed mutagenesis revealed the presence of multiple functionally critical cis elements within this region, one of which was a nuclear factor (NF)-κB consensus sequence. In B7.1-positive B cells, this element bound several members of the NF-κB family, transcription factors already implicated in signal transduction pathways relevant to B7.1 expression. This is the first description, to our knowledge, of regulatory elements that control expression of a gene encoding a B7 costimulatory molecule.

Effective activation of T lymphocytes requires two signals from APCs (for a review see reference 1). The first signal is delivered upon the binding of the clonally distributed TCR to MHC–foreign antigen complexes on APCs. This interaction results in the phosphorylation of cellular tyrosine kinases and the induction of gene expression (2, 3). The second signal is provided by the interaction of costimulatory molecules on T cells with their ligands on APCs (4). The delivery of signal one in the absence of signal two can lead to T cell paralysis or anergy (5–7).

Of the known costimulatory molecules, the B7 family of proteins is arguably the most potent. This family consists of at least two members, B7.1 (CD80) (8) and B7.2 (CD86) (9–11), which interact with similar affinity with their counterreceptors, CD28 and CTLA4, on T cells, although their affinity for CTLA4 is substantially greater than for CD28 (12, 13). Blocking the interaction of the B7 molecules by excess soluble CTLA4 during T cell activation leads to hyporesponsiveness (14). Not surprisingly, B7.1-deficient mice have substantially reduced immune responses (15). Blockade of B7.1 by mAb injection leads to amelioration of autoimmune experimental allergic encephalitis, a murine model of multiple sclerosis (16), and prevents xenograft rejection (17).

The B7.1 and B7.2 molecules have similar but not iden-

tical tissue distribution patterns. Both proteins are expressed on dendritic cells, and B7.1 is expressed on activated but not resting T cells (18, 19). Resting B cells express low levels of B7.2 but not B7.1 whereas upon activation, B cells express substantial levels of both proteins (10, 20). Similarly, macrophages constitutively express B7.2 and can be induced to express higher levels of B7 proteins upon IFN-γ treatment (21). In general, in mice, cell surface B7.2 levels are higher than B7.1 levels, and the kinetics of induction of B7.2 are faster than those of B7.1 (22, 23).

A large number of stimuli can induce B7.1 expression on resting, surface-negative B cells. These include the cytokine IL-4 (24), Abs to the B cell Ig receptor (23), ligation of the B cell activation molecule CD40 (25), the mitogens LPS and dextran sulfate (23), transformation by EBV (26), and agents that activate protein kinase A, such as dibutyryl cyclic AMP (db-cAMP)[1] (27, 28). Since resting B cells do not contain detectable transcripts for B7.1, it is likely that such induction occurs primarily at the transcriptional level. Similarly, B7.1-negative monocytes become B7.1 positive

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[1]Abbreviations used in this paper: db-cAMP, dibutyl cyclic AMP; EMSA, electrophoretic mobility shift assay; HTLV-1, human T cell leukemia virus type-1; NF-κB, nuclear factor κB; NIB, nuclear isolation buffer; tk, thymidine kinase.

after treatment with IFN-γ (29), as do resting T cells upon activation by antigen or transformation by human T cell leukemia virus type-1 (HTLV-1) (26). Many of these stimuli have been shown to activate the nuclear factor (NF) κB transcription factor in other systems.

The cDNAs encoding both human and mouse B7.1 genes have been isolated (8, 30). Using the human cDNA as probe, four transcripts sized 1.7, 2.9, 4.2, and 10 kb were detected in normal and neoplastic B cells. The 2.9 kb transcript was the major species in anti-Ig–activated B cells whereas the 1.7-kb transcript predominated in B tumor cell lines, such as the Raji cell line (30). In the mouse, three transcripts, 2.2, 3.9, and 10 kb were detected in spleen and transformed B cells (8). The molecular basis for the variation in transcript lengths has not been determined. The genomic organization of both the human and mouse genes has also been reported (31, 32). In humans, the B7.1 locus spans ~32 kb. A primer extension analysis of mRNAs from Raji and CH1 B cells has shown that transcription of both human and mouse B7.1 initiates within about 60 nucleotides upstream of the 5' end of the existing cDNA clones (31, 32). However, no obvious promoter elements were observed in the 5' flanking region abutting the putative transcription start sites. Recently, Borriello et al. (33) used RACE (5' reverse amplification cDNA ends) on RNA isolated from CH1 B cells to extend the 5' end of the previously published mB7.1 cDNA by 1.5 kb, suggesting that an additional 5' untranslated sequence may account for the variation in transcript length.

In this report, we describe the analysis of the human B7.1 promoter. We provide evidence for an enhancer region located ~3 kb upstream of the first exon which determines both cell type specificity and inducibility by certain stimuli. Linker-scanning mutagenesis of this region revealed the presence of a NF-κB consensus binding site and several additional sites whose integrity was critical for enhancer function.

Materials and Methods

Isolation, Cloning, and Sequencing of the 5' Flanking Region of the B7.1 Gene. A full-length human B7.1 cDNA was used to screen a leukocyte genomic DNA library in λ EMBL-3 (a gift of Dr. S. Orkin, Children's Hospital, Boston, MA) (34). The screening procedure was as described (35). The DNA from 10 positive clones was prepared, digested with restriction endonucleases, and subjected to agarose gel electrophoresis. The separated DNA fragments were transferred to nitrocellulose filters and probed with ^{32}P-labeled oligonucleotides representing sequences derived from different domains of the B7.1 gene. The procedures of prehybridization, hybridization, and washing of filters were as described (30).

One clone, λUT-1, hybridized to probes from the first and second exons of the B7.1 gene. The DNA of this clone was further mapped by digestion with restriction endonucleases, and various subfragments recovered from agarose gels were subcloned into pBluescript KS (Stratagene Inc., La Jolla, CA). A 3.3-kb SacI subclone containing the first exon was used to generate a series of nested deletions using the Erase-a-Base kit (Promega, Madison, WI). The overlapping deletion clones were sequenced on both

strands using dye-labeled terminator/Taq polymerase chemistry and analyzed on an automated fluorescence DNA sequencer (Applied Biosystems, Inc., Foster City, CA).

In addition, a 2.4-kb BamHI-SacI fragment was sequenced by the dideoxynucleotide chain-termination/extension method (36) and Sequenase (United States Biochemical Corp., Cleveland, OH) using B7.1-specific oligonucleotide primers. The sequence was analyzed with the Genetic Computer Group (GCG) package (37) and compared with those in the GenBank database.

Cell Lines. Human EBV-transformed B cell lines BL30, EBV5, and SKW were obtained from Dr. J. Fingeroth (Dana-Farber Cancer Institute), the Nalm6-transformed pre-B cell line was provided by Dr. J. Ritz (Dana-Farber Cancer Institute), and the BA cell line came from Dr. S. Lederman (Columbia University, New York). Other human cell lines, including the epitheloid carcinoma HeLa, the hepatocellular carcinoma HepG2, the leukemic T cell line Jurkat, the small cell lung carcinoma NCI-H146, the Burkitt lymphoma Raji and EBV-negative Burkitt lymphoma Ramos, and the mouse myeloid cell line M1 were purchased from the American Type Culture Collection (Rockville, MD). The human fibroblast GM00637 was obtained from the Coriell Institute for Medical Research (Camden, NJ). M12.4.1 is a BALB/c B lymphoma cell (38). HeLa, HepG2, and GM00637 were grown in DME, and all other cells were cultured in RPMI 1640 containing 10 mM Hepes, pH 7.3. All culture media were supplemented with 8% heat-inactivated FCS (HyClone Laboratories, Inc., Logan, UT), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-ME. The culture medium for M1 also contained 100 μM nonessential amino acids.

Abs and Flow Cytometry. Anti-hB7.1 mAb 133 (mouse IgM) (39) and anti-mB7.1 mAb 1G10 (rat IgG2a) (27) have been described previously. The mAbs MIgM,κ and RIgG2a,κ were purchased from PharMingen (San Diego, CA) and used as isotype-matched control Abs for human or mouse cells, respectively. Flow cytometry analysis was carried out as described. Briefly, 5 × 10^5 cells were incubated with either control Ab or anti-B7.1 mAb at 4°C in HBSS containing 3% BSA and 0.1% NaN$_3$. After washing, the human and mouse cells were stained with FITC-conjugated goat anti–mouse IgM (Tago, Inc., Burlingame, CA) or FITC goat anti–rat IgG (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD), respectively. The analysis of the cells was performed on a FACS® analyzer (Becton Dickinson & Co., Mountain View, CA).

DNase I Hypersensitive Site Mapping. Isolation and DNase I digestion of nuclei were performed as described (40). Briefly, 10^8 cells in log phase were harvested, washed with PBS, and resuspended in 20 ml of ice-cold nuclear isolation buffer (NIB) (15 mM Tris, pH 7.6, 60 mM KCl, 15 mM NaCl, 300 mM sucrose, 5 mM MgCl$_2$, and 0.5 mM dithiothreitol [DTT]) containing 0.4% NP-40. Cells were permeablized for 5 min on ice and centrifuged at 3,000 g for 5 min. Nuclei collected were resuspended in 0.5 ml of NIB and aliquoted into five tubes containing 100 μl of NIB plus 0–5 μg of DNase I. After 4 min at room temperature, the reactions were stopped by addition of 250 μl of stop solution (63 mM Tris, pH 8, 2.5 mM EDTA and 1.25% SDS) and 50 μl of proteinase K (5 mg/ml). The lysates were incubated overnight at 37°C and proteins were removed by phenol/chloroform extraction. The genomic DNAs isolated were digested by BamHI, SacI, or XhoI and separated by electrophoresis on agarose gels. The gels were blotted and hybridized to a ^{32}P-labeled 295-bp ScaI–BamHI probe, spanning exon 1 of the hB7.1 gene (see Fig. 3).

Expression Plasmid Construction. The 3.3-kb SacI and 5-kb

BamHI fragments containing the first exon of the *B7.1* gene were released from pBluescript clones and purified from agarose gels. These fragments were inserted into pSyncatI, an expression vector containing a promoterless CAT reporter gene (SynapSys, Burlington, MA). Similarly, the 5-kb BamHI fragment and a 2.8-kb EcoRI fragment containing the DH site B (see Fig. 1) were isolated and inserted into pSyncatII, a vector that contains the minimal herpes virus thymidine kinase (tk) promoter upstream of the CAT reporter gene. Subsequently, these clones were digested with a variety of restriction enzymes to systematically remove different parts of the larger fragments. Subclones of the shorter B7.1 DNA fragments were regenerated by ligation of blunted ends.

Transient Transfections, Induction, and CAT Assays. Raji B cells were transfected by electroporation using an electroporator (Bio-Rad Laboratories, Hercules, CA) as described (41). HeLa cells were transfected using the standard calcium phosphate technique (42). Transfections of BA B cells and Jurkat T cells were performed using DEAE-dextran as described (43). Briefly, 10^7 cells were washed in STBS (25 mM Tris, pH 7.4, 137 mM NaCl, 5 mM KCl, 0.6 mM Na$_2$HPO$_4$, 0.7 mM CaCl$_2$, and 0.5 mM MgCl$_2$) and resuspended in 1 ml of DEAE-dextran (200 μg/ml) plus 6 μg of plasmid DNA. Cells were treated with DEAE-dextran/DNA mixture at room temperature for 30 min, washed once with complete medium, and plated. Cells were harvested 48 h after transfection.

M12.4.1 (abbreviated as M12 hereafter) B cells and M1 myeloid cells were transfected using an alternative DEAE-dextran method as previously described (38). Transfected cells were split equally into two sets of plates and incubated for 24 h. db-cAMP and LPS (both from Sigma Chemical Co., St. Louis, MO) were added to appropriate plates of M12 cells at 30 and 5 μg/ml, respectively. M1 cells were treated with LPS at 10 ng/ml. Cells were harvested at 18 h after db-cAMP treatment or at 24 h after LPS stimulation. Protein concentration of cell extracts was measured by a protein assay kit (Bio-Rad Laboratories). Equal amounts of protein were used in CAT assays and CAT activity was measured using thin-layer chromatography and liquid scintillation counting. CAT activities of hB7.1 DNA transfections are expressed as fold increase over those of the vector DNA transfections, which were arbitrarily assigned a value of 1.0. All transfections were repeated three times unless otherwise specified. The NF-κB p50 and p65 expression constructs were the gift of Dr. R. Sen (Brandeis University, Waltham, MA).

Mutagenesis of the B7.1 Enhancer. Deletion and linker-scanning mutants of the 183-bp B7.1 enhancer were made by PCR using a panel of synthesized oligonucleotides (oligos). A 665-bp PvuII fragment was isolated from the 183-bp StuI–ApaI enhancer pSyncatII clone and used as the PCR template. The template contained pSyncatII sequences and restriction sites SacII and BamHI flanking the 5′ and 3′ end of the enhancer, respectively. Oligos used to construct the 5′ and 3′ deletions of the enhancer also contained SacII or BamHI sites, respectively. The 3′ portions of the enhancer were obtained by PCR with SacII-containing oligos and an oligo downstream of the enhancer, whereas the 5′ portions were obtained by PCR with BamHI-containing oligos and an oligo upstream of the enhancer. The central portion of the enhancer was obtained by PCR with a pair of the B7.1 oligos flanking the region. PCR products were digested with SacII and BamHI and different portions of the enhancer were recovered from agarose gels and recloned into pSyncatII.

Linker-scanning mutagenesis was performed by a modified overlap-extension method as described (44). In total, 18 unique oligos were designed to contain a 10-bp mutant sequence flanked by

15-bp template sequences on either side. Each oligo was used in PCR to introduce mutations into a specific 10-bp region of the enhancer without changing the enhancer length. PCR products were digested with SacII and BamHI, purified from agarose gels, and cloned back into pSyncatII. DNA sequencing was used to verify all mutant and flanking sequences.

Electrophoretic Mobility Shift Assay and Ab Inhibition. Small scale nuclear extracts were prepared from 10^7 Raji B cells as described (45). An electrophoretic mobility shift assay (EMSA) was performed as previously described (46) in a 10-μl final volume containing 8 mM Hepes, pH 7.4, 0.3 mM MgCl$_2$, 100 mM NaCl, 10 mM KCl, 0.8 mM EDTA, 0.5 mM DTT, and 9% glycerol. Each binding reaction also included 2.5 μg poly(dI-dC), 2 μg nuclear extract, and ~2 × 10^4 cpm of ^{32}P-labeled probe. For Ab inhibition, antisera against p50, p52, p65, c-Rel and Rel-B (Santa Cruz Biotechnology, Santa Cruz, CA) were added at 1 μg per reaction. Oligo probes and specific or nonspecific competitors used in EMSA included −2983 to −2959 of the hB7.1 enhancer (5′-GGGAAAGGGGTTTTCCCAGCAGTCA-3′), the NF-κB sequence of the Igκ enhancer (5′-CAGAGGGGACTTTC-CGAGA-3′) (47), and mutant 3 of the mIL-4 promoter −79 to −60 region (5′-ATAAAATTTGAACATGTAAA-3′) (45). The reaction mixtures were separated on a 5% nondenaturing polyacrylamide mini-gel at 80 V for 75 min.

Results and Discussion

Isolation and Characterization of a Human B7.1 Genomic Clone. To isolate a genomic clone encoding the human *B7.1* gene, a full-length cDNA was used as a probe to screen a leukocyte genomic DNA library in λ EMBL-3. From ~6 × 10^5 plaques screened, 10 clones were isolated that remained positive on tertiary screening. These clones were then analyzed by Southern blot analysis after restriction enzyme digestion and hybridization with ^{32}P-labeled oligonucleotide probes corresponding to sequences derived from different regions of the cDNA. One clone, λUT-1, hybridized with probes from the first two exons. Further analysis of this genomic clone revealed that it contained ~14 kb of DNA upstream of the first exon with the restriction map shown in Fig. 1.

3.9-kb of DNA flanking the first exon was sequenced on both strands (Fig. 2). A search of this sequence using FASTA of the GCG program in the GenBank database revealed the presence of two alu repetitive sequences (Fig. 1, *I* and *II*) frequently found in flanking regions and introns (48, 49). No other significant similarities betweeen the hB7.1 5′
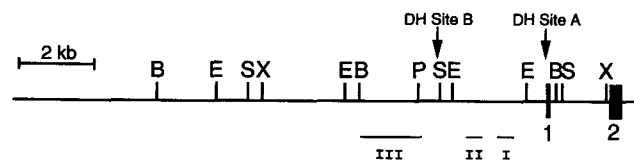
Figure 1. Restriction map of the hB7.1 5′ genomic clone, λUT-1. (*Arrows*) Locations of two DNase I hypersensitive sites, A and B. (*Solid boxes*) Exons 1 and 2. Sequences similar to two alu repeats and one transposon element, Line 1, are marked by horizontal lines and are designated as I, II, and III, respectively. The abbreviations of restriction enzymes used are as follow: BamHI (*B*), EcoRE (*E*), PacI (*P*), SacI (*S*), and XhoI (*X*).

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PacI
TTAATTAAAAAAAAAAGAGTCACAGGCAGG AAGAGTAAGATGCCAAGTATAGGAGGAAGA GTGTGGTGGGTTAGTAAGAAGACGGCCCTC ACTCTGAACGAGGGCTAGCTCTGAAAGTCA CTTGTTTTCATTTTAAGCATTTTGGTGATT  -3287
TCCATCTCAAAGAACCAGAAAGAATGTGAC AGCATTTTAAGAGGAAGAGGTGAACTCTCC CACTTTGATGAGTTTGCTGCCAACTTTAGC AGGCTGTGGCACTCCTGGGAGCACCATAGC CTTAAAGCCTAGATCAGCTGCCCTATGATG  -3137
     StuI     B7.1 Enhancer
GGTGCAGGCCTTATAGACATGCCCAGATGA AGTCATATCTAAAAATGAACTTCTAAACCT CACGGGAGGGTCATCTTAGAACATGAAAAA CTCCCCTGAAAATTTTTGTCAGTGCAACAG GTTTTGTTTACTTGGCAACAGTGAGCCCAC  -2987
        NF-κB                   ApaI                                                                                                                        SacI
TGTGGGAAAGGGGTTTTCCCAGCAGTCAGG CTAGCACCGTGGGCCCTGAGTAGGCAACCT GCAAATGACAGCACTGAGGCCACTGCCAGG AGCCAGCCCAGCCCTCCCTGTGGATGCTGA ACGGAAGGGTAGGAAGGTTGAGCTCAGGCC  -2837
AGCTTTATCAGCATGAGGAGGTGAAGTGGC TCCAAGGTCAAGAGCAGAAAGATGGAACCT TCATTTCCACAGAGATCATCCTGTTCAGGG GTAAAGCAAAGGGCCAGGCTTCCCCTACGG TGTCGCAACAGCCTCTGCACCAACCTCTGA  -2687
                                                                                                                                  EcoRI
GTGACAAAGCTGCATCTCCCTCTCGTCATC TCTTCCTGCTGGGTCCTCTACAGTCTGTGA TTGTGTTTTCATTTCATTTATAATGTCTTT CATTGTACAAATATTTTTAATTTTTATGAA TTCAAATCTGTGTATCTTTTCTTTTTTCGT  -2537
GGCTTCCAAGTTTTGTGTCTTGCTCACCCT AGAATTATAAAAATATACTTCATAAATTTA TCCTTTCAATTTTTCACATAAAAATTTTTA AAAGAATTTTTATGTGAATTATTTTTAAAA GAATTTCTTTTACTTTTATTACAGTAAAGC  -2387
                                                                                                                                                          Alu II
CAAGGATGGATTTACAAAAAAATAAGTGGC ACTAAAGAATTTACACTGAGGAGCAGTAAT TCCTGCTTTATTCTTCTCCAACCCTGGGTC TGCTCCTCAGAACAAACTCATTTAAAGTTT TTTTGTTTTGTTTTGTTTTGTTTTGAAAC  -2237
AGAATCTTGCTCTGTCACCCAGGCTGGAGT GCAATGGCACAATCTTAGCTCACTGCAACC TCCGCCTCTGAAGTTCAAGCAAGTCTCATG TCTCAGCCTTCCCAGTAGCTGGGATTACAG GTATGCACCACTATGCCCAGTTTTTTGGTT  -2087
TTTTTTTTTTTTTGGAGAGCTGGGGTTTCA TCACGTTGGCCAGCTGGCCTCGAACTCCTG GCCTCAAGTGATCCGCCCACCTCAGCCTCT CAAAGTGCTGGGATTACAGGCATGAGCCAC TGTGCCCAGCCTCCTTTTAAATCTTTTACT  -1937
TGGTTTTGTTTTGTTTTGTTTTGTTTTCCA GTAATATAGGTATTCGGGTTCGGCTTCCTT TGCCCGTCTTCATCCATCTCACCTCCAGTG TCCAGAAATTTACCTTAGAAATCTGTGGTC CACAGGTCATGCTCCTAGACACATTGTAGG  -1787
AGGAAGAGGAATTAAACACATCCCATTAGT ACATAATCTTGGAAGAGAAGCCTTCTGAAT TTTACAATCAGGATTGGGGAAACTCTGTAA AGTACCTTCCTGGTGTTCCTTTCTTCCAGC CTCTTTGATTAAGTCACATCAAAGCGCTAA  -1637
                                                                                                                  Alu I
TGGCAAAGTTGTTTAATGTGTGTTTCTCCA AGGCAGGTTAGGATTTCACTGGGATTCTTA AAATTTGAGGGTGTCTGTGATCGTCAAAAT TTGTATTCATGTAGTCCCTCATGAAATTTC TAAAACTCGGCTGGGTGCGGTGGCTCATGC  -1487
CTGTAATCCCAGCACTTTGGGAGGCCGAGG AGGGTGGATCACAAGGTCAGGAGTTTGAGA CCAGCCTGGCCAATATGGTGAAACCCCATC TCTACTAAAAATTTAAAAATTAGCCTGGCA TGGTGGCACACGCCTGTAGTCCCAGCTACT  -1337
CAGGAGGCTGAGGCAGAAGAATTGCTTGAA CCCAGGAGGCGGAGGTTGCAGTGAGCCAAG ATTGCACCACTGCACTCCAGCCTGGGCGGC AGAGAAAGACTTCATCTAAAAAAAAAAAAA GAAAATAAATTTCTAAAACTCTGTATTTCT  -1187
TTGAAAAATCACTTCCAGTGCATTGTAATA ATGTATCAAGTAGAAGTGGCTAAAATCAGT GAAAACATTTCTTTGATCTGAATAAATGTT CAAGAAAATAGAAAACATGAACTTACCTCC AATCTATGTTCCCTTTACTTGAAAATGTAA  -1037
TGTTGATCTACTAGAGGTAAGGCAGGAATT GTCACATCCATATTCCTTGCAACTATTCAA AATATGCATCTGAGACCAGAACAAGACACA GGTGAAGAAGGGTGCTCACTAGGCCCCTTC TGCCAATACAGAAGGAGACAGTGCAGTGGA  -887
GTCCTTTCATAGCAAGTTCTGCCTTCATGA TTTCCAAAGCTTCAGTATTCCAAATCTCAG GTGAATAGGGTGGTATCCACTAAGCTCTGC CACTTGCTCTCTTTCTGGCTGGTGTGGGAA GGATGGCGTCTTTTTTTTTTTTTTTTTTTT  -737
GAGATGGAGTCTTGCTCTGTTACCCAAGCT GGATTGTAGTGTTGTGATCTCGGCTCACTG CAACCCATGCAAGGTGCTGTCAGGTTCCAC CTGTGCAAACAAGTACACTGGTCCAGCATG GGTCAGCTGCTCACTCACAGGGGCTCCAGA  -587
                                                                                                                  EcoRI
ATCCTACAAAGGGACACACAGGGGTGGGTG GCAACCTAGTTGGAAATGGGAGTGATGGGC ATGACTAAAGTCTTCCTCATCCCACCAGAG GCGCACTCAACTGTGGCTAGGGTGACCAGA ATTCACTGTCCAGCGTGGCTGCAGGAGCCC  -437
ACTCTCTGAAGATGGTGGGATTCAGAGGTG GGAGTAGAGTTGGGTGGTCTTGTGAGCTGA GCACTCTCCCAAAAGTGTCCCCTACATATG AGGTTGACAGCTTATTCCTGCAGGGACTCA GGAGTAACTCTGGGTGCTAAAACAAAACTT  -287
                                                                      EcoNI
GAGACCAGCACAGAAACAGCCAGCCCATGA AGCTGGCCTCCCATCACACAGCAAGGCTAG CCAGATGCCCCTCACTCTTCTCGGGCACCA TTCTTCTCCTCCCCTAGGCCGCCTGGTGCT CGTGGTGTTTCATGTTTGTTAGTCCATGCA  -137
                                                                                                                                                           +1
CGGTGGTGAGGGAGGCTGCTGTGTGATTTG CCCCAGCCACAGCCTCTACAAAAGCAAGTA GAAGAAGACGGCAGCAGAGGGCGCTTCTTC AAACACCCTGTCCAACTCCTGGCACTGACA AGTACTGAGTGAACTCAAACCCTCTGTAAA  14
                              5' untranslated region
GTAACAGAAGTTAGAAGGGGAAATGTCGCC TCTCTGAAGATTACCCAAAGAAAAAGTGAT TTGTCATTGCTTTATAGACTGTAAGAAGAG AACATCTCAGAAGTGGAGTCTTACCCTGAA ATCAAAGGATTTAAAGAAAAAGTGGAATTT  164
                                                                                                              BamHI
TTCTTCAGCAAGTAAGTACATTTACTATTA TCTTACTATGGGATAGCTTGGTAGAACTAG TTACCAGCAACCTCACTGCTTTTAAACAA ACAATACCTCTGCTACCACCTCCTGGATCC AGGCTTGCCCTCAAAGGAACCCTTAGGGTC  314
TCATTCCCACAGGAGGCTGTGGCTTCCCGA CATATTTGGAGCATCATAGTGAGAATTGTG GCCCATTATTTTAAAATATCCTAGATCCTA GCAAATATTGTTTTTGTGGGCCGTATTTGA ATTTCTCAAGCCAGTAAAATTCTAGTGAAA  464
   SacI
GTGGGAAAATCGCTTGAGCTC                                                                                                                                          485
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Figure 2. Nucleotide sequence of the hB7.1 5' flanking region. Complete nucleotide sequence of the 3.9-kb PacI-SacI region is listed. The positions of the 5' untranslated region of hB7.1 cDNA, alu repeat I, alu repeat II, and the 183-bp hB7.1 enhancer are underlined. Representative restriction endonuclease sites are shown and the putative transcription start site is underlined and marked as +1. These sequence data are available from EMBL/GenBank/DDBJ under accession number U33208.

flanking region and sequences in the database were found, nor was there any significant similarity to the recently published mB7.1 5' UT region. Further sequencing of a 1.3-kb BamHI-PacI upstream fragment (Fig. 1, *III*) on one strand revealed a region highly similar to the 3' end of a transposon element, line 1, commonly found in intergenic regions of chromosomes (data not shown) (50, 51). The full length of this transposon is ~6 kb, which could specify two transcripts in the same transcriptional orientation as hB7.1 (52). Two consensus TATAA elements were present at positions −2608 and −2501 relative to the putative transcription start site without neighboring 5' consensus CCAAT motifs. No TATAA or initiator sequences were present downstream.

B7.1 Expression Correlates with the Presence of a DNase I Hypersensitive Site in Cultured Cell Lines. As an initial approach to identify tissue-specific regulatory elements in the *hB7.1* gene, the chromatin configuration of this gene was examined in intact nuclei from various cell types by DNase I hypersensitivity assays. Nuclei from a panel of cultured human lines were isolated and digested with various amounts of DNase I. The purified DNA was then digested with the appropriate restriction endonuclease (XhoI, BamHI, or SacI), electrophoresed, blotted, and hybridized to a 295-bp ScaI–BamHI probe spanning the first exon. The cell lines were

examined simultaneously by FACS® analysis for surface B7.1 expression using the anti-hB7.1 mAb 133 (Table 1).

As shown in Fig.3 *A*, XhoI digestion of nuclear DNA gives the expected single 9.5-kb restriction fragment. Treatment of nuclear DNA from all cell lines with increasing amounts of DNase I results in the progressive disappearance of the full-length fragment and the appearance of either one or two nuclease-generated subfragments of ~1.7 (*DH site A*) and 4.6 kb (*DH site B*). Since the 1.7-kb subfragment was present in all cell lines tested, we conclude that this new band represents a non-tissue-specific DNase I hypersensitive site. However, the second 4.6-kb nuclease-generated subfragment appeared only in the cell lines that express surface B7.1. Thus, both fragments are present in the EBV5, SKW, and Raji B7.1-positive B cells, whereas the remaining B7.1-negative cells (Jurkat T cell, fibroblast and carcinoma cell lines, and pre-B cell lines) have only the 1.7-kb subfragment. In only a single case, namely the BL30 B cell, did a B7.1-positive line fail to generate the 4.6-kb DH site B subfragment. It is possible that unique factors interact with other elements in this transformed cell line to control B7.1 expression. Indeed, transfection of the BL30 cell line with a construct containing the enhancer region represented by DH site B (described below) revealed an absence of activity (data not shown). Aside from this one cell line,

Table 1. *Summary of the Correlation between Surface B7.1 Expression and Tissue-specific DNase I Hypersensitive Site B*

|		Surface	
	Cell line	B7.1	DH Site B
Pre-B	Nalm6	−	−
	Ramos	−	−
B	BL30	+	−
	EBV5	+	+
	Raji	+	+
	SKW	+	+
T	Jurkat	−	−
Fibroblast	GM00637	−	−
Epitheloid carcinoma	HeLa	−	−
Hepatocellular carcinoma	HepG2	−	−
Small cell lung carcinoma	NCI-H146	−	−

Surface expression of B7.1 on a panel of human lymphoid and nonlymphoid cell lines was determined by FACS® analysis using anti-hB7.1 mAb 133. Presence or absence of DNase I hypersensitive site B in the hB7.1 5′ flanking region of each cell line was determined by Southern blot analysis and listed as + or −, respectively.

however, DH site B appears to correlate well with the expression of the *B7.1* gene.

To map the location of the two DH sites more precisely, nuclear DNA from the Raji cell line was digested with additional restriction endonucleases and hybridized to the same probe used in the DNase I hypersensitivity assays. Digestion with BamHI revealed that sites A and B are ~0.3 and 3.2 kb, respectively, upstream of the BamHI site located 3′ to exon 1 (Fig. 3 *B*). DH site A, therefore, is located very near the putative transcription start site mapped previously by primer extension. Digestion of DNase I–treated Raji nuclear DNA with SacI revealed a single 0.6-kb fragment representing site A. Since site B is not included in the SacI fragment, it can be mapped between the upstream BamHI and SacI sites to a location ~100 bp 5′ to the SacI site, ~3 kb upstream of the putative start site. The location of the two DH sites is shown schematically in Fig. 3 *C*.

Localization of a Transcriptional Enhancer at DH Site B. The 3.3-kb SacI fragment which includes DH site A did not have transcriptional activity in Raji cells (Fig. 4 *B*). It was possible that negative regulatory elements within this region were responsible for the absence of promoter activity. To test whether DH sites A and B represented B7.1 enhancer elements, we therefore subcloned a variety of fragments encompassing one or both of these sites into pSyncatII, an expression vector containing a minimal herpes virus tk promoter fused to the CAT reporter gene. Fragments tested in preliminary experiments included a large 5-kb BamHI fragment containing both sites A and B and a smaller 2.8-kb EcoRI fragment containing only site B. The former had minimal activity whereas the latter gave a threefold increase in CAT activity over the vector alone (not shown). A 3.7-kb PacI-BamHI fragment which included both DH sites was also inactive (Fig. 4 *A*, lane *12*).
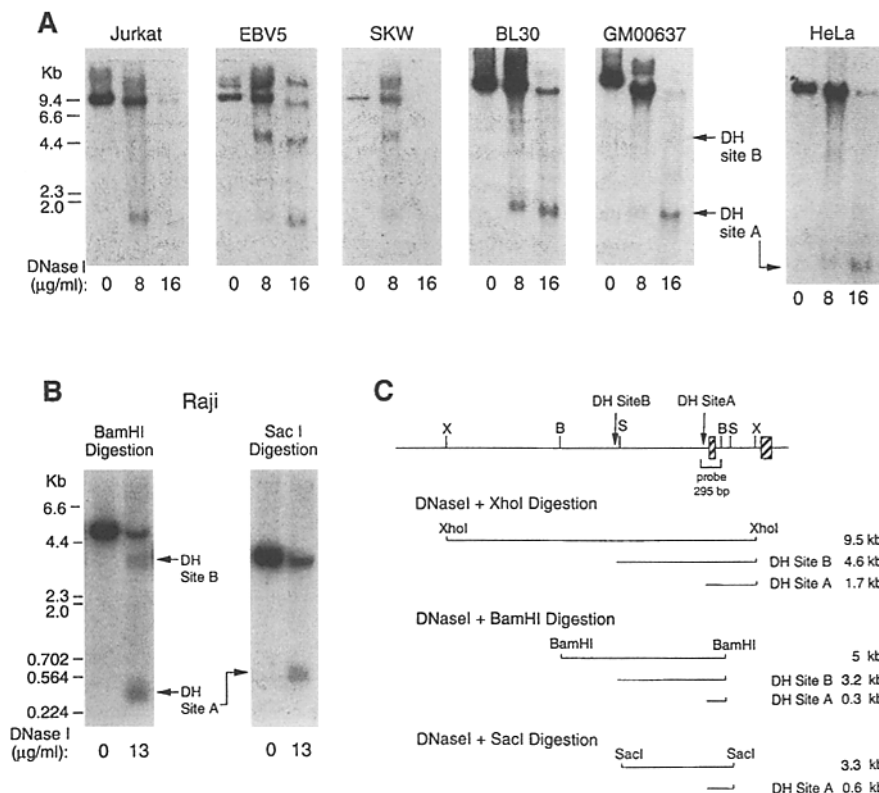
Figure 3. DNase I hypersensitive analysis of the hB7.1 5′ flanking region. (*A*) Nontissue-specific DH site A and tissue-specific site B are shown in Southern blot analysis with XhoI-digested DNAs from both B7.1+ and B7.1− cell lines and (*B*) with BamHI- or SacI-digested Raji DNA. (*C*) The schematic presentation of DH site A and B locations in the 5′ flanking region, derived from three different restriction digestions.
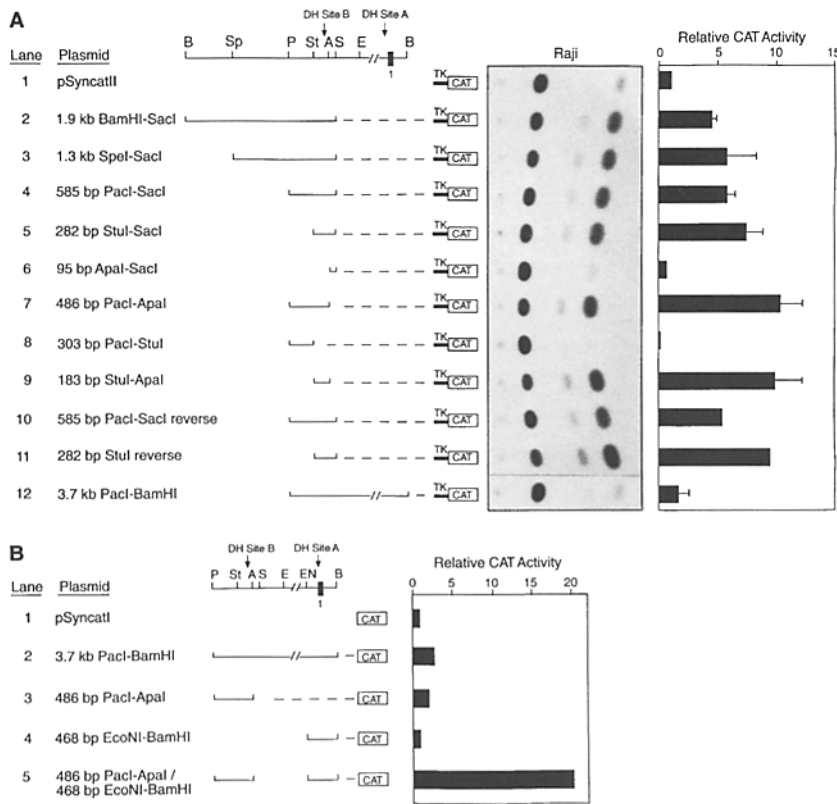
Figure 4. hB7.1 enhancer activity in transfected Raji cells. *(Solid lines)* The 5' flanking region clone and its deletion variants used for transfection. Each clone is indicated by the size of restriction fragment listed and only part of the length between the EcoRI site and exon 1 *(black box)* is shown. The abbreviation of restriction enzymes used are ApaI (*A*), BamHI (*B*), EcoRI (*E*), EcoNI (*EN*), PacI (*P*), SacI (*S*), SpeI (*Sp*), and StuI (*St*). Enhancer activity is shown either with a heterologous tk promoter (*A*) or with the homologous promoter (*B*). Cells were transfected with 20 μg of each plasmid DNA. One representative CAT assay experiment is shown in *A*. The relative CAT activity of lanes *10* and *11* in *A* and lanes *1–5* in *B* are calculated based on two separate experiments.

A series of restriction endonuclease–generated 5' and 3' deletion fragments were therefore constructed and tested for transcriptional activity (Fig. 4 *A*). The 1.9-kb BamHI-SacI fragment displayed fivefold activity above background (Fig. 4 *A*, lane *2*) and further deletions of this fragment from the 5' end resulted in increasing levels of reporter gene activity (lanes *3–5*). A further deletion to the ApaI site deleted DH site B and abolished activity (Fig. 4 *A*, lane *6*), thus establishing the 5' border of the enhancer. Similarly, deletions from the 3' end of the active 486-bp PacI-ApaI fragment (Fig. 4 *A*, lane *7*) to the StuI site 183 bp upstream abolished activity (lane *8*). These experiments localize the enhancer to a 183-bp StuI-ApaI fragment (Fig. 4 *A*, lane *9*), which is active in both forward and reverse orientations (lanes *5, 11* and *4, 10*). Multiple constructs which included the region surrounding DH site A failed to provide evidence for enhancer function in this location (data not shown).

Because the transcription start site of the *hB7.1* gene had been mapped by primer extension to a site 58 nucleotides upstream of the 5' end of the cDNA sequence (31), sequences upstream of the first exon were tested for promoter activity. A 5-kb BamHI fragment and a 3.3-kb SacI fragment containing the first exon were cloned into the promoterless pSyncatI expression vector. Deletion variants of the latter construct generated by restriction enzyme digestion, ranging from ~0.5 to 2 kb of upstream sequence were also produced. This series of constructs was tested for promoter activity by transfection into the B7.1-positive Raji B lymphoma cell line. However, in preliminary experiments, no reporter gene activity was detected. Since

the failure to detect promoter activity might reflect the presence of dominant negative regulatory elements, a construct containing the 486-bp enhancer upstream of a 468-bp EcoNI-BamHI fragment including DH site A in place of the heterologous tk promoter was made. This construct conferred transcription activity, thus demonstrating that sequences surrounding DH site A can act as a minimal promoter. Neither the 468-bp promoter fragment nor the 486-bp enhancer alone were active (Fig. 4 *B*). The absence of transcriptional activity observed with the longer promoter fragments may be due to the presence of negative regulatory elements downstream of the enhancer, possibly including the alu repeat sequences. Indeed, removal of the alu sequences increases promoter activity (Fig. 4 *B*, compare lanes *5* and *2*). The B7.1 promoter has recently been shown to be transcriptionally active and to contain six elements within the first 150 bp of the promoter that are bound by nuclear proteins; one of them is NF-κB (53).

The B7.1 Enhancer is Cell Type Specific and Inducible by cAMP and LPS. B7.1 expression is strictly cell type specific. To test the tissue specificity of the enhancer region defined above, transient transfections of several constructs which include this region were performed (Fig. 5 *A*). Cell lines tested included the B7.1-negative Jurkat T lymphoma and HeLa epithelial carcinoma and the B7.1-positive human B Lymphoma BA (54). The 585-, 282-, and 183-bp constructs shown to be active in the B7.1-positve Raji B cell line (Fig. 4 *A*) are also active in the BA cells. No enhancer activity from any of the constructs was observed in the Jurkat or HeLa cell lines, demonstrating that, in non-B
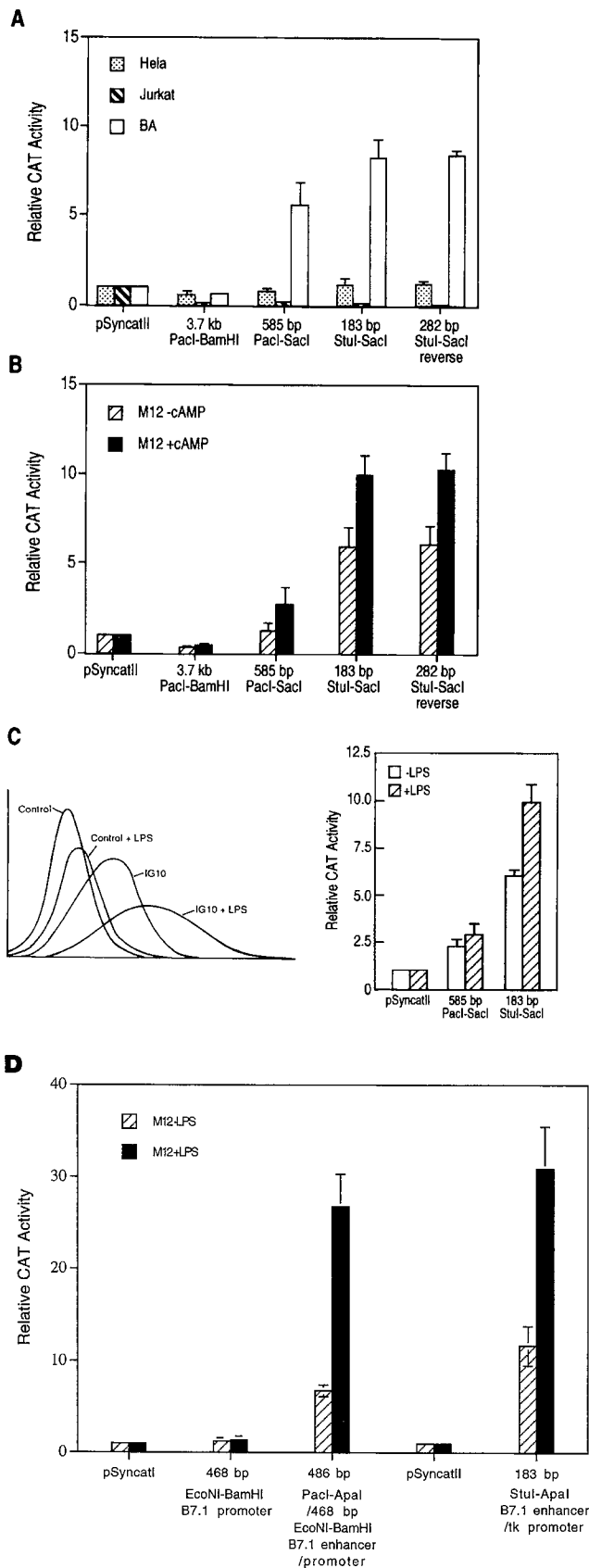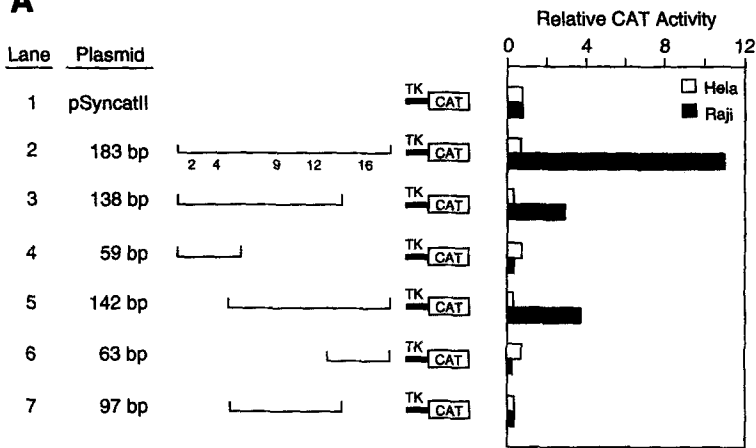
cells, this 183-bp region is cell type specific. Additional B7.1-negative cell lines (Hut78 T cell and G82 glioblastoma) also showed no activity (data not shown). Some modest (about twofold) enhancer activity was observed in a B7.1-negative pre-B cell line Nalm 6 with these constructs, but no activity was present when the longer 1.3-, 1.9-, and 3.7-kb constructs were used (data not shown). This suggests that in B7.1-negative B cells, there may also be a role for negative elements, outside of the 183-bp enhancer, in mediating tissue specificity. We conclude that the enhancer located at DH site B within the 183-bp StuI-ApaI fragment is cell type specific.

Multiple stimuli increase B7.1 expression in B cells and macrophages. We have previously shown that the M12 cell line expresses low levels of cell surface B7.1 that can be markedly increased by treatment with db-cAMP (27). Treatment of M12 with 30 μg/ml db-cAMP after transfection of the 585-, 282-, and 183-bp constructs results in a modest but reproducible increase in CAT activity (Fig. 5 B). We were also able to test the induction of transcription activity of the enhancer by LPS in both M12 and in the mouse B7.1-positive myeloblast/monocyte-like cell line M1. Treatment of these cell lines with LPS for 48 h results in a modest but reproducible increase in both surface B7.1 expression for M1 (not tested for M12) (Fig. 5 C, left) and CAT activity in both cell lines (Fig 5 C, right and Fig.5 D). We also tested the native B7.1 promoter fused to the 486-bp enhancer in the M12 cell line. Induction of transcription by LPS was also observed in this instance (Fig. 5 D). We conclude that elements within the 183-bp region confer responsiveness to stimuli known to increase B7.1 expression. Further, enhancer activity is not limited to cells of the B lineage, but can be observed in B7.1-positive, non-B cells, including the HTLV-1–transformed B7.1-positive T cell MT2 (data not shown). We could not examine other stimuli such as IL-4 or Abs to CD40, known to induce B7.1 expression in B cells, because of the absence of appropriately inducible, transfectable cell lines. To establish whether this enhancer region also controls responsiveness to other stimuli such as IL-4 and anti-CD40 may require the production of mice transgenic for the enhancer to allow the examination of B7.1 regulation in normal cells.

Deletional and Mutational Analysis of the 183-bp Enhancer Reveals Several Functional cis Elements. Initial experiments (Fig. 6 A) employing a series of enhancer deletion constructs with subsequent transfection into Raji cells revealed that nucle-

Figure 5. Tissue-specific hB7.1 enhancer activity in transfected B and non-B cells and induction by db-cAMP and LPS. (A) Selected clones of the hB7.1 5' flanking region and its deletion variants were transfected into B7.1-positive or -negative cell lines. (B) Transfected M12 cells were either induced or uninduced with 30 μg/ml db-cAMP. (C) Enhancer activity in murine myeloid M1 cells and inducibility by LPS. FACS® analysis of B7.1 surface expression on M1 cells either stimulated or unstimulated by 10 ng/ml LPS for 48 h and stained with Ab to mB7.1 (1G10) or control Ab are shown at left. (D) Comparison of LPS induction of the native B7.1 promoter and the heterologous tk promoter fused to the enhancer in M12 cells. The activity is expressed as fold increase over that of the respective cloning vectors.

A

Relative CAT Activity

Lane	Plasmid
1	pSyncatII
2	183 bp
3	138 bp
4	59 bp
5	142 bp
6	63 bp
7	97 bp

□ Hela
■ Raji

B

```
        -3128           -3108            -3088            -3068            -3048            -3028           -3009
        |               |                |                |                |                |               |
B7.1    CCTTATAGACATGCCCAGATGAAGTCATATCTAAAAATGAACTTCTAAACCTCACGGGAGGGTCATCTTAGAACATGAAAAACTCCCCTGAAAATTTTTGTCAGTGCAACAGGTTTTGTT
Mut 1   .TA.GACC.G................................................................................................................
Mut 2   ..........C.ATGACACG......................................................................................................
Mut 3   ...................T.CTGACC.G.............................................................................................
Mut 4   ....................................TG.CCAG..............................................................................
Mut 5   ..........................................TAC.GACC.G.....................................................................
Mut 6   ....................................................ATGACC..............................................................
Mut 7   ...............................................................CTATGA.CAG................................................
Mut 8   ...........................................................................CT.TGACC.G....................................
Mut 9   .........................................................................................CTA.GA..A......................
Mut 10  ..............................................................................................CT.TGACA..................
Mut 11  .......................................................................................................CT.TGA.C.G.........
Mut 12  ...................................................................................................................CTA.GACCAG
Mut 13  .........................................................................................................................
Mut 14  ...............................................................O.........................................................
Mut 15  .........................................................................................................................
Mut 16  .........................................................................................................................
Mut 17  .........................................................................................................................
Mut 18  .........................................................................................................................
```
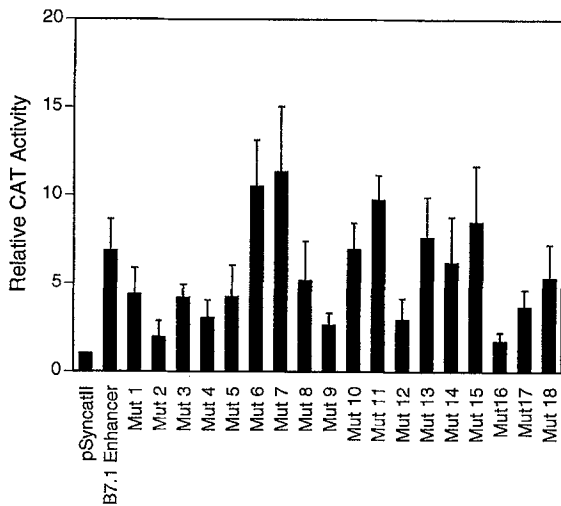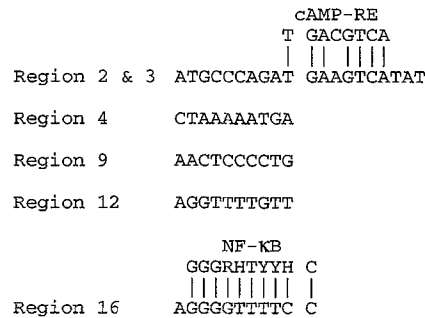
```
        -3008           -2988            -2968            -2946
        |               |                |                |
B7.1    TACTTGGCAACAGTGAGCCCACTGTGGGAAAGGGGTTTTCCCAGCAGTCAGGCTAGCACCGTG
Mut 1   ..............................................................
Mut 2   ..............................................................
Mut 3   ..............................................................
Mut 4   ..............................................................
Mut 5   ..............................................................
Mut 6   ..............................................................
Mut 7   ..............................................................
Mut 8   ..............................................................
Mut 9   ..............................................................
Mut 10  ..............................................................
Mut 11  ..............................................................
Mut 12  ..............................................................
Mut 13  ....GAC..G....................................................
Mut 14  .........TA...C.AG............................................
Mut 15  ...................CTATGACC.G.................................
Mut 16  .............................CTAT.ACCAG.......................
Mut 17  .......................................TACTG.CCAG............
Mut 18  .................................................CTA.GA.CAGTG.
```

otides at both the 5' (lanes 5 and 7) and 3' (lanes 3 and 7) ends of the enhancer were important. Furthermore, sequences internal to these were also involved in enhancer activity (compare lanes 5 with 6 and lanes 3 with 4). Thus, multiple cis elements appeared to be present within the 183-bp fragment. To precisely delineate the sequences within the 183-bp region responsible for enhancer function, linker-scanning mutagenesis was performed. Each linker-scanning mutation was 10 bp in length with the exception of mut 18

which was 13 bp in length. Several bases in the 10-bp stretch were mutated to one of the three remaining nucleotides (see Fig. 6 B) with each resulting sequence checked to ascertain that no known promoter motif had been inadvertently created. The wild-type and mutation constructs were transfected into the Raji cell line and the results of three to five independent experiments are shown in Fig. 6 C. Two of the 18 mutations (mut 2 and 16) led to particularly striking decreases in transcriptional activity of the en-
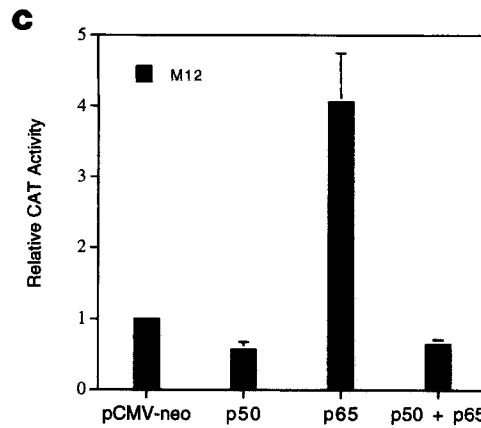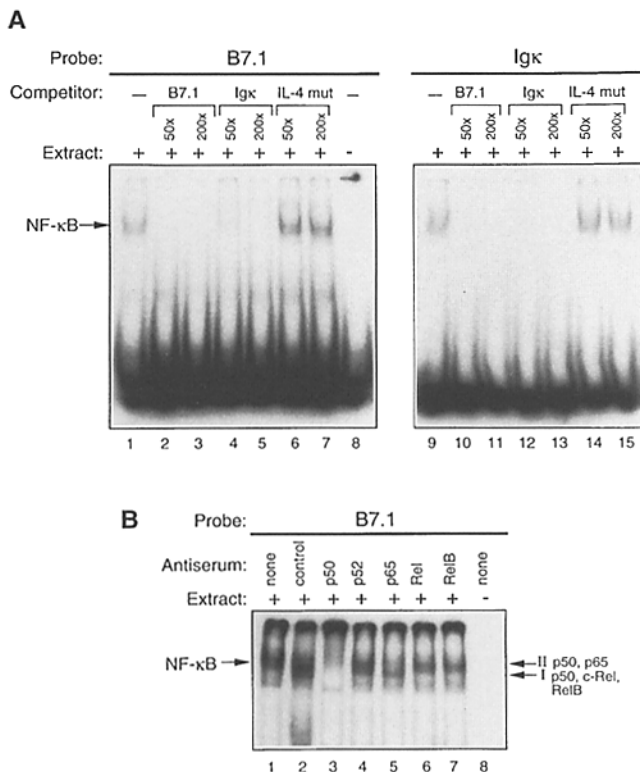
C

D

```
                                        cAMP-RE
                                      T  GACGTCA
                                      |  ||  ||||
Region 2 & 3    ATGCCCAGAT GAAGTCATAT

Region 4        CTAAAAATGA

Region 9        AACTCCCCTG

Region 12       AGGTTTTGTT

                                NF-κB
                             GGGRHTYYH C
                             ||||||||| |
Region 16       AGGGGTTTTC C
```

Figure 6. Mutagenic analysis of the 183-bp B7.1 enhancer. (A) Regions of the enhancer were analyzed by deletion mutations as shown in one initial experiment. The regions corresponding to linker-scanning mutations 2, 4, 9, 12, and 16 are marked by numbers. (B) The sequence of the hB7.1 enhancer and mutations indicated by nucleotide changes. The NF-κB binding site is underlined. (C) The enhancer activities of linker-scanning mutants in transfected Raji cells. 15 μg of plasmid DNA was used for each transfection. The relative CAT activity of mut 2, 4, 9, 12, and 16 is calculated based on five separate experiments. (D) Nucleotide sequences of these regions as well as that of region 3.

hancer, whereas three additional mutations (mut 4, 9, and 12) also reproducibly decreased enhancer activity in five independent experiments. The locations of these mutations (Fig. 6 B) are consistent with the deletion data in Fig. 6 A. Several additional mutations also modestly decreased activity (mut 3, 5, and 17). Inspection of the mut 2, 4, 9, 12, and 16 sequences (Fig. 6 D) reveals that site 16 is a consensus NF-κB element that is identical both in its core nucleotides and in the flanking nucleotides to the NF-κB site in the Igκ enhancer. Mutation of this element resulted in >70% abolition of enhancer activity. The remaining four sequences do not bear homology to known promoter motifs as checked against sequences in the database. A sequence with a 7/8 nucleotide match to a cAMP response element is also present in the enhancer, but mutation of this element (mut 3) has minimal effect on enhancer activity in Raji cells.

Members of the NF-κB Family Bind to the B7.1 NF-κB Site. Using an oligonucleotide corresponding to the consensus NF-κB element mutated in site 16 as a probe, EMSAs were performed with nuclear extracts from the B7.1-positive Raji cell line (Fig. 7 A, left). A DNA–protein complex could be observed with extracts from Raji (Fig. 7 A), but not with nuclear extracts from Jurkat (not shown) that migrates similarly to a complex observed with the Igκ NF-κB site (Fig.7, right). That this complex was specific was shown by the ability of a 10–100-fold molar excess of unlabeled wild-type NF-κB oligonucleotides, but not an oligonucleotide from the unrelated IL-4 promoter, to prevent complex formation (Fig. 7 A). The multimember NF-κB family includes homologous proteins termed p50, p52, p65, c-Rel, and RelB. Various combinations of homo- and heterodimers of these family members may activate or repress

NF-κB–dependent gene transcription (55, 56). To determine which family members present in Raji cells bound to this site, supershift EMSA experiments were performed using antisera to the p50, p52, p65, c-Rel, and RelB proteins. Fig. 7 B demonstrates that two complexes can be observed (arrows). The faster migrating lower complex (Fig. 7 B, I) is supershifted by Abs to p50, c-Rel, and RelB, suggesting it is a homo- or heterodimer of these proteins whereas the slower migrating upper complex (II) is supershifted by the Abs to p50 and p65, suggesting it is a heterodimer composed of p50 and p65 proteins. Such homo- and heterodimers of various NF-κB family members have been previously observed to bind NF-κB target sequences in many promoters (47, 57, 58). The p52 protein is not involved in binding to the B7.1 NF-κB site as judged by the failure of the anti-p52 Ab to affect complex formation. Cotransfection of M12 B cells with the 183-bp enhancer and NF-κB subunits p50 or p65 expression constructs demonstrated that p65 transactivates the enhancer, whereas p50 does not (Fig. 7 C). Further, p50 decreases transactivation by p65, consistent with the known role of p65 as a transcriptional activator and p50 as a repressor.

This is the first description to our knowledge of regulatory elements that control expression of the gene encoding the B7.1 costimulatory molecule. The presence of a tissue-specific DNase I hypersensitive site ~3 kb upstream of the transcription start site of the human *B7.1* gene led to the identification of a cell type–specific enhancer region. This 183-bp region was both cell type specific and responsive to two distinct stimuli (LPS and db-cAMP) known to regulate B7.1 expression. Site-directed mutagenesis revealed the presence of multiple *cis* elements within this region, one of which was a NF-κB consensus sequence. This element binds

A

Probe: B7.1   Igκ

Competitor: — B7.1 Igκ IL-4 mut —   — B7.1 Igκ IL-4 mut

Extract: + + + + + + + -   + + + + + + +

NF-κB→

1 2 3 4 5 6 7 8   9 10 11 12 13 14 15

B

Probe: B7.1

Antiserum: none | control | p50 | p52 | p65 | Rel | RelB | none

Extract: + + + + + + + -

NF-κB →

←II p50, p65
←I p50, c-Rel, RelB

1 2 3 4 5 6 7 8

C

Relative CAT Activity

■ M12

pCMV-neo | p50 | p65 | p50 + p65

Figure 7. EMSA of the NF-κB site in the hB7.1 enhancer. (A) EMSA complexes are shown using Raji nuclear extract and ^{32}P-labeled probes of the hB7.1 and Igκ enhancer NF-κB sequences. The complexes are competed by excess amounts of unlabeled competitors as indicated. (B) Inhibition of NF-κB protein binding to the hB7.1 enhancer by antisera to NF-κB family member p50, p52, p65, c-Rel (Rel), and Rel-B. The fast-migrating NF-κB complex I was blocked by mAb to p50, c-Rel, and Rel-B, and the slow-migrating complex II was blocked by mAb to p50 and p65. (C) Cotransfection of M12 cells with the 183-bp StuI-ApaI enhancer construct (1 μg) and NF-κB p50 and p65 (5 μg) constructs demonstrates transactivation by p65 and repression by p50.

several members of the NF-κB family in B7.1-positive cells and can be transactivated by the p65 subunit.

The NF-κB family of transcription factors has been shown to be critical for cell development, differentiation, and the response to multiple stimuli. The five members of this family—p50, p52, p65, c-Rel, and RelB—all contain a Rel-homology domain required for binding to DNA. NF-κB members are present ubiquitously in the cytosol of many different cell types where they form a stable complex with the IκBα and IκBβ inhibitor proteins (59, 60). Activation by mitogenic factors leads to phosphorylation and degradation of IκB and subsequent translocation of NF-κB proteins into the nucleus (61–64). Various homo- and heterodimers of nuclear NF-κB members are potent transactivators or repressors for many developmentally regulated genes. B7.1 expression is also developmentally regulated and responsive to stimuli known to increase nuclear NF-κB activity. These stimuli include LPS (65), cross-linking of CD40 on B cells by soluble CD40 ligand (66, 67), or ligation of B cell

surface Ig (68). The induction of NF-κB activity in T cells is regulated by HTLV-1 Tax (69). Mature activated B cells which contain constitutive nuclear NF-κB activity are the major site of expression of B7.1 (56, 70, 71). Thymic dendritic cells, the other cell type that constitutively expresses B7.1, are critically dependent of Rel-B activity since Rel B–deficient mice have recently been shown to lack mature dendritic cells (72). Thus, B7.1 expression appears to parallel NF-κB activity, consistent with a convergence of signal transduction for B7.1-inducing stimuli on this family of transcription factors. However, our data clearly indicate that other cis elements also control B7.1 expression. Several additional cis elements with no obvious homology to known promoter motifs were critical for functional enhancer activity. Now that a B7.1-specific enhancer that functions in concert with a mimimal promoter has been delineated, identification of the factors that bind to these additional elements will be key in further understanding the regulation of this important costimulatory molecule.

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