cis and *trans* Elements Differ among Mouse Strains with High and Low Extrahepatic Complement Factor B Gene Expression

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Summary

Factor B (Bf), an enzyme of the alternative pathway of complement activation, is one of four major histocompatibility complex (MHC) class III genes. To ascertain the genetic mechanism for tissue-specific constitutive and regulated expression of Bf, we sequenced the regulatory regions 5′ of the gene from mice of different H-2 MHC haplotypes and assessed *trans*-acting factors, specific DNA binding nucleoproteins, in liver and kidney. Striking tissue-specific differences in constitutive expression of Bf were demonstrated in mice of H-2^f or H-2^z haplotypes when compared with H-2^d or H-2^u (kidney and intestinal Bf in H-2^d or H-2^u >> H-2^f or H-2^z). These differences correlated with a point nucleotide substitution 3 bp downstream of the upstream Bf initiation site that affects interaction with a DNA binding protein. This and additional *cis* differences localize the sequence substitutions responsible for previously identified restriction fragment length polymorphisms among inbred mouse strains and also reveal two previously unrecognized polymorphisms generated by SmaI and HinfI digestion. Evidence for differences in *trans* was found in a comparison of DNA binding nucleoproteins from kidney, but not liver, of B10.PL when compared with B10.M. These data, together with the high degree of sequence homology between human and mouse Bf 5′ flanking regions, should prompt a search for polymorphic restriction sites and *cis* binding elements in the Bf promoter that could serve as markers of human MHC–associated renal pathology and variants in local MHC class III gene expression.

Factor B (Bf),[1] a 93-kD single chain glycoprotein serine proteinase, is a constituent of the antibody-independent alternative pathway of complement activation, an important mechanism for host defenses against microbial infection (reviewed in reference 1). In addition, Bf activation cleavage products affect B lymphocyte proliferation and differentiation (2, 3), and participate in monocyte-mediated cellular cytotoxicity (4–6) and macrophage spreading (7). The Bf gene is located within the MHC, immediately 3′ to the homologous complement gene C2 on chromosome 6 in humans (8) and 17 in mice (9, 10). Bf is synthesized in liver (11), hepatoma cells (12), and at many extrahepatic sites (13), in mononuclear phagocytes (14, 15), fibroblasts (16), endothelial (17), and epithelial (18) cells. The expression of Bf is upregulated by bacterial products (endotoxin) (19) and by several cytokines, including IL-1 (20, 21), IL-6 (22), TNF-α (23), and IFN-γ (24). A map of the sequence 5′ to the Bf gene and extending into the 3′ untranslated region of C2 gene has revealed domains necessary for constitutive and IL-1- and IFN-γ-regulated expression (25–27).

[1] *Abbreviations used in this paper:* Bf, factor B; SAA, serum amyloid A; UIS, upstream transcriptional initiation site.

Among inbred mouse strains, differences in Bf serum concentrations are paralleled by differences in hepatic Bf mRNA expression (28). Constitutive expression of Bf is tissue specific within a given strain. That is, hepatic Bf mRNA in mice of H-2^u strains is greater than in H-2^d or H-2^f. On the other hand, in H-2^d peritoneal macrophages, Bf mRNA is greater than in either H-2^u or H-2^f strains. Moreover, in kidney and small intestine, two Bf transcripts resulting from utilization of alternative sites of transcriptional initiation (302 bp from one another) (29) are expressed in approximately equal amounts, but in liver, the Bf long mRNA represents <5% of mature Bf transcripts (30). The purpose of the present study was to identify and elucidate the relative importance of *cis*- and *trans*-regulatory elements on this strain- and tissue-specific expression of Bf.

Materials and Methods

Mice. 6–8-wk-old male mice of BALB/cJ, B10.D2/nSnJ, B10.PL (73NS)/Sn, B10.M/Sn, NZW/LacJ, and A.CA/Sn strains were purchased from The Jackson Laboratory (Bar Harbor, ME).

RNA Isolation and Analysis. Mice were injected intraperitoneally with 200 μl of saline alone, saline containing 10 μg of sonicated

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endotoxin (LPS from *Escherichia coli* 0111-B4, phenol extract; Sigma Chemical Co., St. Louis, MO), or were uninjected. The animals were killed by cervical dislocation 24 h. later; liver, kidney, and small intestine were immediately removed, cleaned, snap frozen in liquid nitrogen, and stored at −70°C. The frozen tissues were pulverized in liquid nitrogen, and total RNA was extracted by lysis with guanidinium thiocyanate and cesium chloride density gradient ultracentrifugation (31). RNA was quantitated by absorbance at 260 nm.

RNA samples (10 μg) were denatured and subjected to electrophoresis in formaldehyde-containing 1% agarose gels as previously described (32). The gels were rinsed overnight and RNA was transferred to nitrocellulose sheets (Bio-Rad Laboratories, Richmond, CA). The membrane were baked, prehybridized, and hybridized overnight at 65°C with the appropriate cDNA probe in 5× SSC, 0.2% Ficoll 400, 0.2% BSA, 0.2% polyvinyl pyrrolidone, 0.2% SDS, 0.05% Na pyrophosphate, 0.1 mg/ml boiled sonicated salmon sperm DNA, pH 6.5. The blots were washed twice at 65°C in 0.2× SSC, 0.2% SDS, and exposed to x-ray film at −70°C using an intensifying screen.

The probes were radiolabeled (33) with 100 μCi of α-[^{32}P]dCTP (New England Nuclear, Boston, MA), using the Random Primed DNA Labeling Kit (Boehringer-Mannheim Biochemicals, Indianapolis, IN) according to the manufacturer's protocol. For Bf and serum amyloid A (SAA) cDNA probes, the 2-kb EcoRI fragment from pmBf5/70 clone (30) and the 195-bp PstI fragment from pA1 clone (34), respectively, were used as template. To detect Bf long mRNA, the RNA probe (−269 to −48 of Bf short) previously described (29) was generated using α-[^{32}P] CTP (New England Nuclear) and the SP6-T7 transcription kit (Boehringer-Mannheim Biochemicals). With this probe, 50% deionized formamide was added to the hybridization solution.

PCR Amplification, Cloning, and Sequencing of the Murine C2-Bf Intergenic Region. High molecular weight DNA was prepared from mouse liver as described (35). The genomic segment −724 to +118 (nucleotide positions relative to the downstream Bf cap site) from each strain was amplified by a 30-cycle Taq PCR (36) from 1 μg of DNA in a TempCycler (Coy Laboratory Products, Ann Arbor, MI), using the GeneAmp DNA Amplification Reagent Kit (Perkin Elmer Cetus, Norwalk, CT), as described in the manufacturer's protocol. The primers, designed from the previously published sequences (25, 30), included a 26-base genomic sequence (−724 to −699 of the coding strand and +118 to +93 of the noncoding strand) flanked with XhoI (coding strand primer) and BglII (noncoding strand primer) cloning sites. The conditions for denaturation, annealing, and polymerization were 2 min at 95°C, 2 min at 65°C, and 5 min at 72°C, respectively. The first denaturation step was extended to 5 min, and after the last cycle, the synthesis was completed by an additional 7-min incubation step at 72°C. The PCR product was digested with XhoI and BglII, gel purified, and cloned into pSP72 (Promega Biotec, Madison, WI). The plasmid was then purified (37) and the sequence determined on both strands after alkaline denaturation (38) by the dideoxy-termination method (39), according to the manufacturer's protocol (Sequenase Version 2.0 Kit; United States Biochemical Corp., Cleveland, OH).

The sequence differences among strains were confirmed by direct sequencing and restriction mapping (SmaI, HinfI, HindIII, KpnI) in several PCR reactions. For each strain, three to five PCR-generated clones were mapped and interstrain mutations were confirmed in duplicate clones. Direct sequencing of the PCR products were performed with slight modification: the annealing mixture, containing 1 pmol of the gel-purified DNA and 1 pmol of primer, was heat denatured in boiling water for 7 min, cooled to room temperature for 1 min, transferred to ice, and labeled for 3 min, using half the amount of unlabeled nucleotides as for cloned DNA. Annealing and labeling reactions were performed in the presence of 10% (vol/vol) DMSO.

Gel Retardation Assay. Nuclei were freshly prepared from mouse tissues as described (40), lysed with NaCl and spermidine (41), and the resulting extracts dialyzed overnight (42). The soluble fraction was measured for protein content (43) and stored at −70°C. The binding reaction (25 μl) was performed with 8 μl (4 μg) of nuclear extract, 1 ng/200 bp of end-labeled probe, and 1 μg of poly[dI-dC]-poly[dI-dC] (Sigma Chemical Co.), in a buffer containing 10 mM Tris-HCl, 50 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, pH 7.5. After 20 min at room temperature, a bromphenol blue/xylene cyanol solution was added and electrophoresis carried out in 4.5% polyacrylamide gel in 1× TAE (40 mM Tris acetate, 1 mM EDTA, pH 8.0) at 4°C. The gel was fixed, dried, and exposed at −70°C to x-ray film, using an intensifying screen. Probes were end labeled using Klenow enzyme (Boehringer Mannheim Biochemicals) and α-[^{32}P]dCTP (New England Nuclear). Competition experiments were performed with 50 times molar excess of unlabeled DNA fragments. The probes, generated from restriction fragments, and competitors consisting of restriction fragments or PCR-amplified segments or annealed complementary oligonucleotides, are described in Results. PCR segments were synthesized as above, but using 18-mer primers and the XhoI-BglII cloned fragment (see above) as template.

Results

Strain- and Tissue-specific Variation in Bf mRNA Content. Bf-specific mRNA content of liver, kidney, and small intestine from mice of BALB/c (H-2^d haplotype), B10.D2 (H-2^d), B10.PL (H-2^u), B10.M (H-2^f), and NZW (H-2^z) strains was estimated by Northern blot analysis. The results of one of the replicate (up to four mice of each strain and multiple RNA determinations from each) experiments are shown in Figs. 1 and 2. Constitutively, approximately equal amounts of two distinct Bf mRNA species (2.4 and 2.7 kb) were present in kidney of strains of the H-2^d and H-2^u haplotypes, whereas no Bf message was detected in strains of the H-2^f or H-2^z haplotype (Fig. 1 *A*). After LPS injection, in the strains expressing detectable kidney Bf mRNA, the content of the shorter message (Bf short) increased, while the longer transcript (Bf long) slightly decreased. The rank order of Bf mRNA level among these strains was: BALB/c < B10.D2 < B10.PL. In B10.M and NZW strains, even the Bf short mRNA was barely detectable after LPS injection.

Small intestine contained both Bf mRNA species in B10.D2 (Fig. 1 *B*), BALB/c, and B10.PL (not shown). Bf long mRNA was more abundant than Bf short; as in kidney, the latter increased after LPS administration. NZW and B10.M intestine failed to express Bf mRNA constitutively, and minimal amounts of Bf short were detected after LPS stimulation.

The difference in kidney Bf mRNA between B10.M or NZW and the other strains was not paralleled by differences in liver Bf mRNA among the corresponding strains (Fig. 2). Indeed, hepatic Bf mRNA was constitutively expressed and upregulated by LPS in all strains examined (Fig. 2 *A*). While the constitutive level of liver Bf mRNA showed minor strain-specific variation (the rank order was: B10.M ~ B10.D2

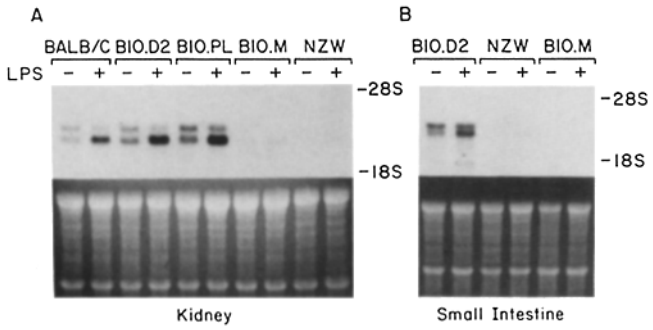

Figure 1. Strain-specific expression of murine Bf gene in (*A*) kidney and (*B*) small intestine. Total RNA was isolated from tissues of various strains 24 h after intraperitoneal injection of saline (−) or 10 μg of LPS (+), subjected to electrophoresis in agarose gel (10 μg of RNA per lane), transferred to a nitrocellulose membrane, and probed with a 2-kb cDNA sequence spanning up to the 3′ end of the transcripts (*top*). (*Bottom*) Ethidium bromide stain of the gels is shown.

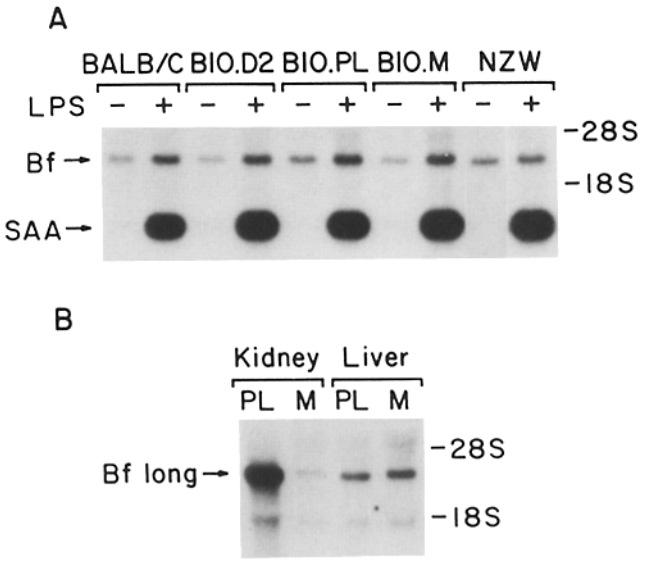

Figure 2. Hepatic Bf gene expression among strains. (*A*) Constitutive (−) and LPS-regulated (+) expression of Bf mRNA; same protocol as in Fig. 1. Liver Bf mRNA was probed along with SAA mRNA as a control for the hepatic response to LPS. (*B*) Constitutive expression of Bf long mRNA in B10.PL (PL) and B10.M (M) liver (and kidney as control). In this experiment, Bf long was specifically detected by an RNA probe from a genomic sequence 5′ to the transcription start site for Bf short.

~BALB/c <NZW <B10.PL), no significant difference was found among the LPS-treated animals, except for NZW, for which the upregulated level was lower than in the other strains. The induction of hepatic SAA mRNA, used as an index for the LPS response, was similar in all strains (Fig. 2 *A*). Analysis of Bf long mRNA with a 5′ probe that does not overlap Bf short mRNA sequence shows that, in contrast to kidney, the constitutive hepatic expression of the long message was similar in B10.PL and in B10.M (Fig. 2 *B*). Constitutive expression of Bf mRNA in all tissues examined was similar in A.CA and B10.M strains that share the H-2^f haplotype but differ in background (A/Wy instead of C57BL/10). A comparison between BALB/c and A.CA is shown in Fig. 3. Within each strain the constitutive level of Bf mRNA in saline-injected mice was indistinguishable from that of untreated animals (not shown).

Strain-specific Sequences of the 5′ Flanking Region of Factor B Gene. For nucleotide sequencing of the upstream region of Bf (−698 to +92) in the different mouse strains, the −724 to +118 genomic segments (nucleotide numbering refers to the downstream initiation site of transcription), encompassing the 3′-untranslated region of C2 gene and the 5′-untranslated region of C2 Bf gene, were amplified by PCR. The differences among the six strains are shown in Fig. 4. In the strains with H-2^d (BALB/c, B10.D2) or H-2^u (B10.PL) haplotype (group I), the sequences were identical to previous reports (25, 30). The strains B10.M and A.CA shared the same sequence (group II); a sequence that differed from the first group in four regions (Fig. 4, *A*, *B*, *C*, and *D*). At position −553 (*A*), a point substitution (G→A) abrogates an SmaI restriction site. Next to the upstream transcriptional initiation site (−302; *B*), three point substitutions were found at positions −327 (T→C, which abrogates a HinfI restriction site), −307 (G→A), and −299 (T→C, which generates a HinfI restriction site). In strains of group II, the segment −203 to −198 (*C*), corresponding to the sequence AGGGAG in the group I, is deleted. This deletion generates a HindIII restriction site. Two point substitutions (*D*) were found at positions +22 (G→A) and +52 (C→T, which generates a KpnI restriction site in group II). NZW (H-2^z) was included in group II because the sequence is identical to that for the H-2^f haplotype, except that the nucleotide at position +52 (*D*) is not substituted.

Interactions between Sequences Upstream to Bf Gene and Mouse Liver and Kidney Nuclear Factors. Nuclear extracts as a source of DNA-binding factors were prepared from liver and kidney of untreated B10.PL and B10.M strains. Bf mRNA was monitored by Northern blot analysis in the same animals for each tissue studied to assure that the extracts were obtained from tissues expressing Bf constitutively. Four overlapping genomic fragments (designated fragments A, B, C, and D) encompassing the intergenic region between murine C2 and Bf (from the last exon of C2 to the first exon of Bf) were generated (Fig. 5) from group I (H-2^d, H-2^u) and group II (H-2^f,

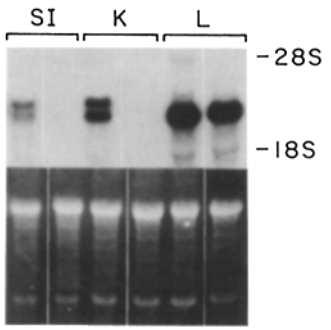

Figure 3. Constitutive Bf gene expression in A.CA strain. A comparison of Bf mRNA from small intestine (SI), kidney (K), and liver (L) of untreated mice of BALB/c and A.CA strains (first and second lane of each pair, respectively) was assessed by Northern blot analysis (*top*) using the same probe as in Fig. 1. (*Bottom*) Ethidium bromide stain of the gel is shown.

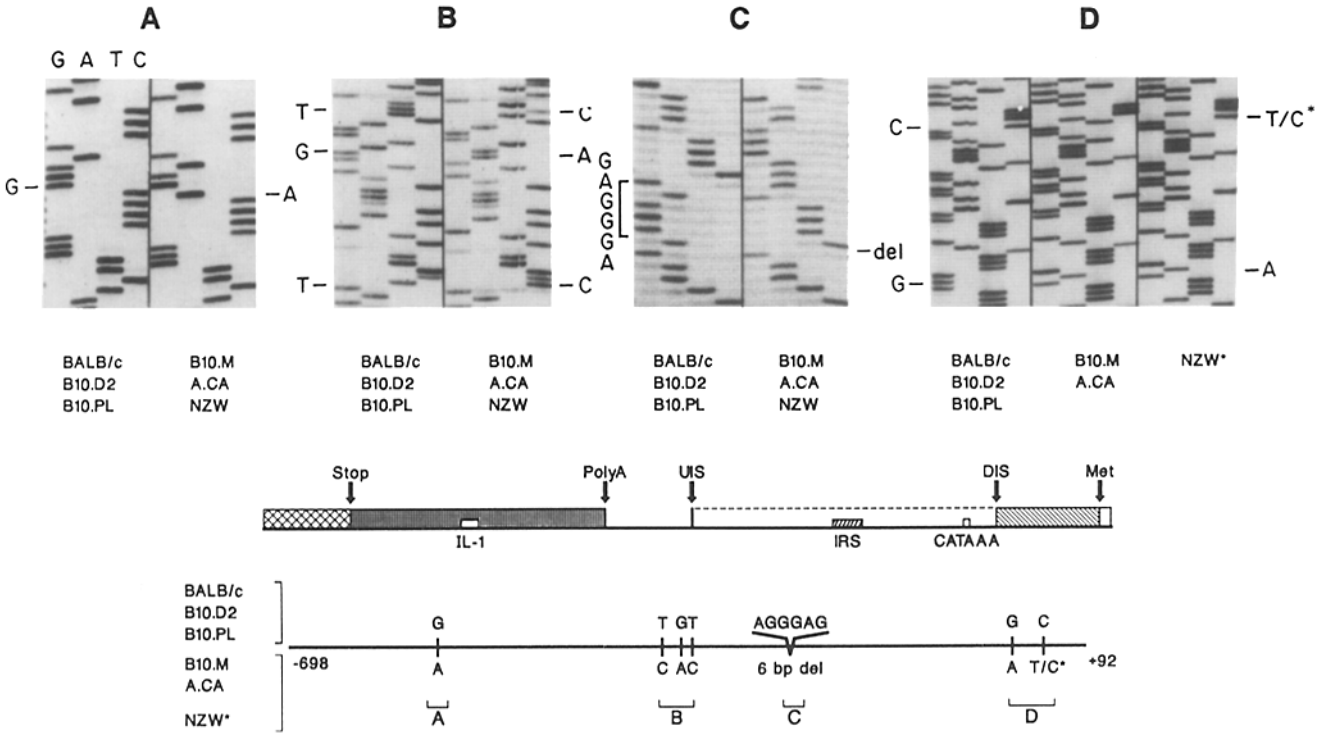

Figure 4. Sequence polymorphism in the 5′-flanking region of murine Bf gene. For each of the six strains studied, the −698 to +92 (+1 is the transcription start site for Bf short) genomic segment was PCR amplified, cloned, and the nucleotide sequence determined. (*A*, *B*, *C*, and *D*) The four areas of representative sequencing gels (coding strand) presenting differences among strains. Their relative positions are indicated in the bottom and aligned with the main features of the C2-Bf genomic region. The strains were split into two groups according to the sequence. Group I (BALB/c, B10.D2, and B10.PL) and group II (B10.M, A.CA, and NZW) -specific nucleotides are shown above and under the line, respectively. *The difference between NZW and the other group II strains at +52 (*D*) with a cytosine (like group I) instead of a thymidine. The positions of the upstream (UIS, at −302), and downstream (DIS, at +1) transcriptional initiation sites for Bf long and short mRNAs, respectively, are indicated. The C2 stop codon (Stop) and polyadenylation site (PolyA), the interleukin 1 (IL-1), and IFN-γ (IRS) response elements for Bf gene, and the putative Bf TATA box (CATAAA) and first methionine (Met) are also represented.

H-2^z) clones (see paragraph above) and labeled for use in gel retardation assays.

Two regions of major sequence-specific interaction were found in group I sequence for B10.PL liver and kidney factors, as demonstrated by retardation of probes B and D and competition with the respective unlabeled fragments (Fig. 5). With either probe B or D, the major complexes formed in the presence of kidney extracts had the same mobility as with liver. Fragments A and C showed much lesser binding activity with either tissue and no difference was found using the corresponding sequences of group II nor with B10.M extracts (not shown).

To assess whether strain-specific nuclear elements (DNA or proteins) participate in *cis* or in *trans* to the interactions with fragments B and D, liver and kidney extracts from B10.PL and B10.M strains were probed with fragments B and D of group I and group II sequences (Fig. 6).

As shown in Fig. 6 *A*, when fragment B (which includes the upstream transcription start site) of group I sequence was used as a probe, one or two major complexes were formed with liver and kidney nuclear factor(s) from each strain. Two distinct retarded bands were clearly visible when kidney extracts from either strain were used. In all cases, the sequence specificity of the interaction was demonstrated by competition with excess of unlabeled probe. Using fragment B of group II sequence as a probe, no major retarded band could be detected in the presence of liver or kidney extracts from either strain. The interaction at fragment B of group I was only partially competed by an excess of unlabeled fragment B of group II (Fig. 7 *A*, lane *4*). A 35-mer double-stranded synthetic oligonucleotide defined by group I sequence, namely Bs1 (−323 to −289, with G at −307 and T at −299), encompassing Bf upstream transcriptional initiation site (UIS; at −302), was able to compete for this interaction (Fig. 7 *C*, lane *4*) and to form a major specific complex with liver and kidney extracts when used as a probe (not shown). A series of oligonucleotides with combinations of substitutions at −307 and −299, namely Bs2 (A and C = group II sequence), Bs3 (A and T), and Bs4 (G and C), were tested as competitors against group I fragment B as a probe for binding liver extracts (Fig. 7 *C*). Partial competition was observed with Bs2 and Bs4, whereas Bs3, like Bs1, completely competed for the interaction. The same observations were made with kidney extracts (not shown). Therefore, the T → C substitution at −299 observed in group II strains and located 3 bp downstream from the UIS prevents the interac-

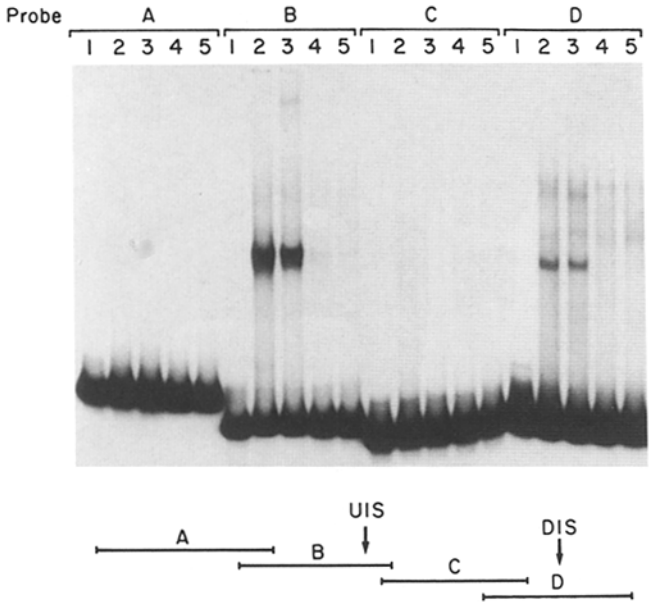

Figure 5. Interactions of liver and kidney constitutive nuclear factors along the Bf gene promoter region. Nuclear extracts from liver (lanes *2* and *4*) and kidney (lanes *3* and *5*) of untreated B10.PL mice were probed for gel retardation with (*A*) XhoI-AspHI (−727 to −449), (*B*) AvaII-DraI (−502 to −262), (*C*) NcoI-PstI (−281 to −49), and (*D*) PvuII-BglII (−118 to +116) genomic restriction fragments of the group I sequence (BALB/c, B10.D2, and B10.PL). (Lane *1*) Probe alone; (lanes *2* and *3*) binding assay; (lanes *4* and *5*) binding assay in the presence of a 50-fold molar excess of cold probe. The four fragments, designated A, B, C, and D, are depicted relative to Bf transcription start sites (UIS and DIS).

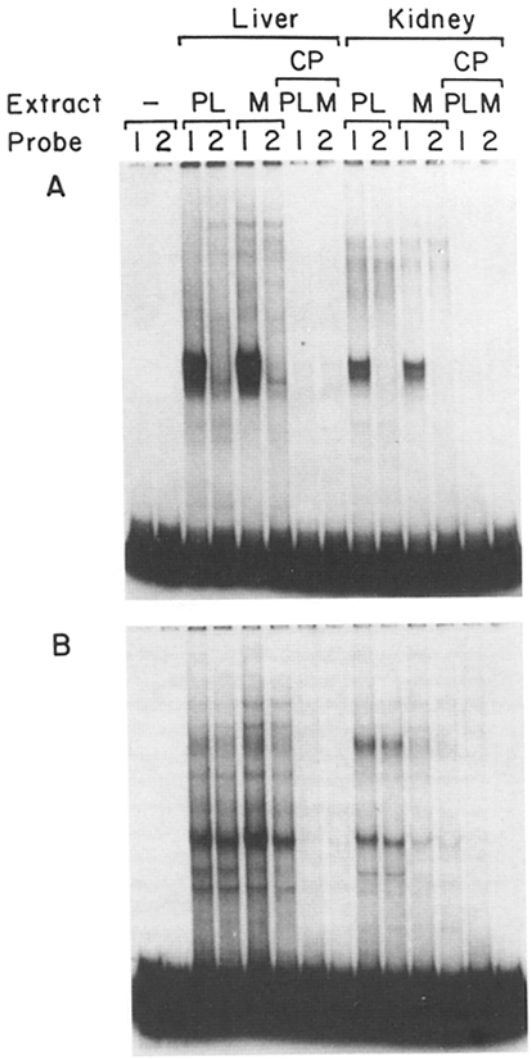

Figure 6. *Cis* and *trans* differences in protein-DNA interactions among strains in fragments B and D. Liver and kidney nuclear extracts from B10.PL (PL) and B10.M (M) strains were probed for gel retardation with fragments B (*A*) and D (*B*) of group I (probes 1) and group II (probes 2) sequences. (*B*) Probe 2 is of the H-2^f sequence (two substitutions vs. group I). CP denotes competition with cold probe. The nucleotides specific for group I and group II and other features of fragments B and D are shown in Figs. 7 and 8.

tion of a *cis* element, located within the Bs segment (−323 to −283), with one or more sequence-specific nuclear factors constitutively expressed in liver and kidney of B10.PL and B10.M strains.

Using fragments D of group I (H-2^d, H-2^u) and group II (H-2^f) sequences as probes and the same nuclear extracts from B10.PL and B10.M strains as above, the gel retardation patterns were similar for each probe and each tissue (Fig. 6 *B*). However, the intensity of the three major retarded bands was significantly lower with B10.M than with B10.PL kidney extracts. The major retarded bands using liver extracts from either strain and each kidney extract were quantitatively similar irrespective of the probe. Competition with the unlabeled probes indicated that the corresponding interactions were sequence specific. Four subsegments of fragment D of group I sequence (namely D1, D2, D3, and D4) were generated by PCR and tested as competitors to localize the interaction on fragment D (Fig. 8 *B*). The main retarded band was completely competed by segment D3 (−4 to +75) and only partially by segments D2 (−63 to +14) and D4 (+56 to +116). A 50-mer oligonucleotide, Ds (+33 to +82), overlapping D3 was also able to compete for the interaction (Fig. 8 *C*) and to form a major specific complex when used as a probe (not shown).

Using probes and competitors of group I sequence and liver extracts, partial cross-competition with probe B (Fig. 7, *A* and *B*) was observed with fragment D and segment D3. Fragments A and C and segments D1, D2, and D4 failed to compete with this probe. Reciprocally, the interaction at probe D (Fig. 8 *A*) was partially competed with fragments A and B, in contrast with fragment C. The same cross-competition phenomenon was observed using kidney extract (not shown).

Discussion

In the present report we demonstrate that among inbred mouse strains, striking tissue-specific differences in the level of Bf gene expression correlate with *cis* differences in the Bf

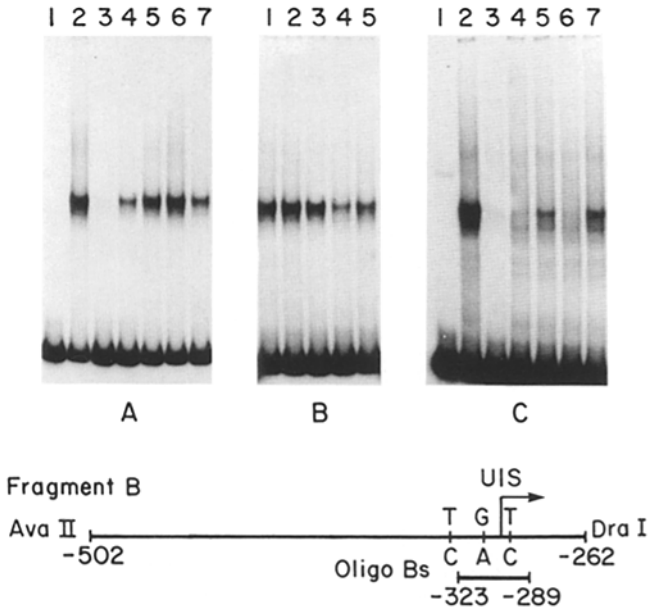

Figure 7. Competition assays for binding on fragment B by various DNA sequences. Gel retardations were performed using fragment B of group I sequence as a probe and B10.PL liver nuclear extract. (*A*) Competition with fragment B of group I (lane *3*) and group II (lane *4*) sequences and fragments A, C, and D (lanes *5*, *6*, and 7, respectively) (see Fig. 5) of group I. (Lane *1*) Probe alone; (lane *2*) no competitor. (*B*) Competition assays with subfragments of fragment D (group I sequence). (Lane *1*) no competitor; (lanes *2*, *3*, *4*, and *5*) competition with D1, D2, D3, and D4, respectively (see Fig. 8). (*C*) Localization of the binding site of fragment B and identification of the base substitution affecting it in group II. (Lane *1*) Probe alone; (lane *2*) no competitor; (lane *3*) competition with cold probe; (lane *4*, *5*, *6*, and 7) competition with double-stranded oligonucleotides Bs1 (G at −307, T at −299), Bs2 (A,C), Bs3 (A,T), and Bs4 (G,C), respectively. The representation of fragment B shows group I– and group II–specific nucleotides (T,G,T and C,A,C, respectively) at −327, −307, and −299, and the position of the UIS (−302) and of the oligonucleotides (oligo Bs) used in *C*.

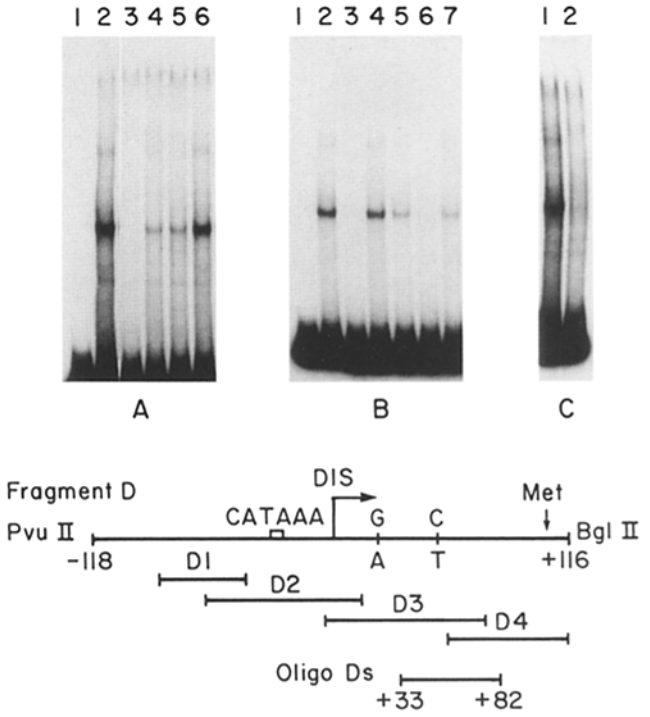

Figure 8. Competition assays for binding on fragment D. Gel retardation assays were performed with probe D and B10.PL extract. Probe and all competitors were of group I sequence. (*A*) Competition with fragments A, B, and C (lanes *4*, *5*, and *6*, respectively) (see Fig. 5). (Lane *1*) Probe alone; (lane *2*) no competitor; (lane *3*) competition with cold probe. (*B* and *C*) Localization of the binding site of fragment D. (*B*) (Lanes *1–3*) Same as in *A*; (lanes *4*, *5*, *6*, and 7) competition with D1 (−86 to −43), D2 (−63 to +14), D3 (−4 to +75), and D4 (+56 to +116), respectively. (*C*) (Lane *1*) No competitor; (lane *2*) competition with oligonucleotide Ds (+33 to +82). Fragment D is depicted along with competitors. Group I– and H-2^f–specific nucleotides (G,C and A,T, respectively) and DIS (at +1) are indicated.

gene 5′ regulatory region. These include a point substitution, 3 bp from the transcription start site for Bf long mRNA, affecting the binding activity of a *cis* element for a constitutive, sequence-specific nuclear factor(s).

In the past few years, the importance of extrahepatic complement synthesis in the local inflammatory process has been increasingly exemplified by the ubiquity of cytokine-induced complement production (16, 17, 21, 23, 24, 28), and particularly emphasized in murine models of SLE by the observation that several complement genes are upregulated at sites of inflammation concomitant with the development of the disease (44, 45). Increasing evidence was also accumulated that the control of constitutive and regulated expression of complement genes in extrahepatic tissues is independent from that in liver. This concept was illustrated in a study reporting quantitative tissue-specific differences for Bf, C3, and C2 mRNAs among several inbred murine strains (28). By analyzing Bf mRNAs in tissues and strains displaying quantitatively more profound differences, we provide evidence that pretranslational, tissue-specific mechanisms controlling constitutive and LPS-regulated expression of Bf gene differ in inbred strains carrying H-2^f or H-2^z haplotype, when compared with H-2^d and H-2^u. That is, in kidney and small intestine, both Bf short and long mRNAs are constitutively expressed at relatively high levels in BALB/C, B10.D2 (both H-2^d), and B10.PL (H-2^u), but are nearly undetectable in B10.M, A.CA (both H-2^f), and NZW (H-2^z); this difference was maintained even after LPS stimulation. In contrast, the difference in hepatic expression of Bf is quantitatively less.

Several lines of evidence support the hypothesis that the strain- and tissue-specific Bf expression originates in part from a *cis* regulatory element at the transcriptional initiation site for Bf long mRNA. The parallel between Bf gene expression in kidney and small intestine indicates a similar tissue-specific mechanism generating high steady-state levels of Bf long mRNA in H-2^d and H-2^u strains, and a similar strain-specific mechanism resulting in the lower level of both Bf mRNAs in H-2^f and H-2^z strains. A relationship between the two mechanisms is strongly suggested by the observation that the strain-specific expression (*a*) is also tissue-specific

and (*b*) correlates with sequence differences at the Bf long mRNA transcriptional initiation site. The point substitution located 3 bp downstream of this transcription start site is the only *cis* difference within a 790-bp genomic segment encompassing the end of C2 gene and Bf 5′ untranslated region that affects a DNA-protein interaction. This genomic region, previously sequenced in H-2^d strains (25, 30), includes a promoter activity, functional IL-1 and IFN-γ response elements (25, 26), and both transcription start sites (29), and therefore represents an essential *cis* component in the control of Bf gene expression. Moreover, the corresponding human genomic region was found to contain an enhancer activity that could contribute to the cell-type specificity of Bf gene expression (27). Because the Bf gene is located within the S region of the H-2 complex (9, 10), the correlation between strain-specific Bf expression and H-2 haplotype reinforces the possibility that the strain-specific expression originates from a *cis* regulatory element of the Bf gene itself. Similarities in Bf expression among strains that differ genetically including in the H-2 complex (NZW, compared with B10.M or A.CA; BALB/C, compared with B10.PL), but share the same *cis* specificity of binding, supports this assertion. However, the possibility that strain-specific Bf expression could be determined in *trans* by H-2-dependent, tissue-specific regulatory factors, or by more distal *cis* elements, is not excluded. In any case, the H-2 haplotype is most likely determinant since, among three H-2 congenic strains sharing the same B.10 background, only the H-2^f strain (B10.M) displays low extrahepatic expression, like the A.CA strain, another congenic line with H-2^f haplotype but a different genetic background.

The comparisons among strains for Bf expression and *cis/trans* interactions show that the *cis* element at the UIS, although not involved in the hepatic expression of either Bf mRNA, might be essential in kidney and small intestine. However, the gel retardation assays could not clearly distinguish liver from kidney nuclear factors, since the interaction with extracts from both tissues yields a major DNA-protein complex of same mobility. The hypothesis that Bf tissue-specific expression is mediated through this interaction requires the involvement of additional *trans*-acting factors and a more complex set of protein-DNA or protein-protein interactions. In several reported cases (46–48), the promoter activation by a transcription regulatory factor does not reflect a change in the primary DNA protein interaction but correlates with phosphorylation of the factor already bound to its recognition DNA sequence. Thus, the ability of a regulatory factor to bind to the *cis* element is not always sufficient to affect transcription. Such secondary events after DNA-protein interaction may not always be detectable by gel retardation analysis. Moreover, we cannot exclude that the interaction at the UIS could involve distinct factors, or combinations of factors, of very similar structure in liver and in kidney, or whether differential concentration of the same factor(s) could determine the tissue specificity of Bf expression.

The other major *cis* binding element, localized downstream to the transcription start site for Bf short mRNA, revealed in kidney (but not in liver) a lower concentration or affinity of the sequence-specific nuclear factor(s) in B10.M as compared with B10.PL. The difference was specific for this factor since the same kidney extracts showed identical patterns at the UIS *cis* element. The role of the factors binding 3′ to the downstream promoter and their possible association with H-2 remain unexplained. Nevertheless, the difference in *trans* is tissue specific and parallels the difference in Bf gene expression among strains. However, for reasons similar to those cited above, the tissue-specific expression of Bf long mRNA in B10.PL cannot be assigned to this primary DNA-protein interaction alone. Partial, but reciprocal, competition for binding between the regions of the upstream and downstream promoters in vitro could reflect protein-mediated interactions between the two regions in vivo. In such a molecular interaction, the binding site of the UIS could possibly be used in kidney to control the transcription at either initiation site, depending on other cell physiological variables. It is more difficult to envision that the assembly of a preinitiation complex or any stable interactions at the downstream promoter region could allow the elongation of transcription initiated at the upstream promoter and be necessary to this initiation. Analysis of Bf mRNAs in H-2 recombinant congenic strains and functional transcription studies in cell-free or transient transfection systems could elucidate the relative contribution of each binding site to the strain and tissue specificity of Bf gene expression, and define the promoter element(s) controlling the transcription of Bf long mRNA.

The differences observed among strains and the isolation of both binding sites on short synthetic oligonucleotides provide tools to isolate and identify these specific nuclear proteins and investigate the spectrum of their target genes. Such studies will provide new insights in understanding the mechanisms generating tissue specificity in complement gene expression and its physiological significance in host defenses and immune dysfunction.

In a murine model of SLE, the H-2^z haplotype of NZW is determinant in the exacerbation of the autoimmune traits and earlier mortality of the (NZB × NZW)F_1 hybrids vs. NZB (49–51). Interestingly, during the evolution of the disease, C2 and Bf mRNA levels in kidney remain several fold lower in NZB × NZW than in NZB and, in contrast to other complement transcripts, show little or no increase (45). The role of local complement expression in murine SLE is still unclear but a low expression at the Bf locus of H-2^z may contribute to such differences.

Previous RFLP studies of H-2 complement genes identified HindIII and KpnI polymorphisms detectable with both C2 and Bf cDNA probes (52, 53). Our sequence data localize both restriction sites and reveal in addition SmaI and HinfI polymorphic sites. The localization of these markers will be useful for further studies of the C2-Bf intergenic region and fine mapping of H-2 recombinant strains. Although among human kidney samples examined to date, a single Bf mRNA species has been observed (29), the high sequence homology with mouse in the Bf 5′ region, at critical sites for promoter activity and regulation (25, 27), raises the possibility of similar

tissue-specific control mechanisms in humans and occurrence of individuals with markedly decreased Bf expression in kidney. A search for polymorphic restriction sites and *cis* binding elements in the human C2-Bf intergenic region could generate important markers of Bf local expression for human renal diseases associated with the MHC.

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