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An interferon γ-regulated protein that binds the interferon-inducible enhancer element of major histocompatibility complex class I genes

(inducible transcription factor/cis DNA element)

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ABSTRACT **Interferons (IFNs) induce transcription of major histocompatibility complex (MHC) class I genes through the conserved IFN consensus sequence (ICS) that contains an IFN response motif shared by many IFN-regulated genes. By screening mouse λZAP expression libraries with the ICS as a probe, we isolated a cDNA clone encoding a protein that binds the ICS, designated ICSBP. Protein blot analysis with labeled oligonucleotide probes showed that ICSBP binds not only the MHC class I ICS but also IFN response motifs of many IFN-regulated genes, as well as a virus-inducible element of the IFN-β gene. The ICSBP cDNA encodes 424 amino acids and a long 3′ untranslated sequence. The N-terminal 115 amino acids correspond to a putative DNA-binding domain and show significant sequence similarity with other cloned IFN response factors (IRF-1 and IRF-2). Because of the structural similarity and shared binding specificity, we conclude that ICSBP is a third member of the IRF gene family, presumably playing a role in IFN- and virus-mediated regulation of many genes. Although IRF-1 and IRF-2 share some similarity in their C-terminal regions, ICSBP shows no similarity to IRF-1 or IRF-2 in this region, suggesting that it is more distantly related. We show that ICSBP mRNA is expressed predominantly in lymphoid tissues and is inducible preferentially by IFN-γ. The induction by IFN-γ appears to be predominant in lymphocytes and macrophages, implying that ICSBP plays a regulatory role in cells of the immune system. The presence of multiple factors that bind common IFN response motifs may partly account for the complexity and diversity of IFN action as well as IFN-regulated gene expression.**

Interferons (IFNs) are potent biomodulators (1, 2). They regulate many cellular genes involved in defense against viral and bacterial infection, enhance immune responses, and control cell growth. We have been interested in IFN-mediated induction of major histocompatibility complex (MHC) class I genes. We and others have observed that a highly conserved upstream cis element, the interferon consensus sequence (ICS), is responsible for IFN-mediated induction of MHC class I genes (3–6). Several nuclear factors that bind to the ICS are either constitutively expressed or induced by IFN treatment (3, 7). A number of groups (8–17) have shown that other IFN-regulated genes have IFN response motifs that are very similar to part of the ICS and to each other. These elements are essential for virus- or IFN-induced transcription of the corresponding genes and bind to the same or very similar factors. These reports put forward the notion that IFN genes and IFN-regulated genes are controlled by shared cis motifs that bind shared transcription factors. We have screened a phage expression library and obtained a murine cDNA clone encoding an ICS-binding protein (ICSBP) that specifically binds the ICS and IFN response motifs. This paper describes the initial characterization of ICSBP.‖

MATERIALS AND METHODS

Library Screening and DNA Sequencing. A λZAP cDNA library was constructed from poly(A)$^+$ RNA prepared from mouse neonatal spleen and liver (Stratagene). This library was screened by the method of Vinson *et al.* (18) with modifications (19) using a concatenated ICS probe (positions −169 to −137 relative to the RNA start site of the mouse *H-2L^d* gene), which identified a single positive clone. The insert of this clone, called 131A, was used as a probe to obtain additional clones with overlapping sequences in the same and another λZAP library. DNA sequences of plasmid inserts (pBluescript SK, Stratagene) were determined for both strands by the dideoxy chain-termination method.

Southwestern Assay. Extracts were prepared from *Escherichia coli* bearing a pBluescript SK plasmid harboring 131A. Three hours after induction with isopropyl β-D-thiogalactopyranoside, bacteria were lysed by lysozyme (1 mg/ml) in 50 mM NaCl/50 mM Tris·HCl, pH 8.0/1 mM EDTA/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride at 4°C for 30 min. An equal volume of 2% (vol/vol) Triton X-100/2 mM EDTA was then added, and after incubation at 4°C for 15 min, the lysates were cleared by centrifugation. Aliquots of the supernatants containing 1.25–5 μg of protein were boiled for 5 min in the presence of 2.5% SDS, 5% (vol/vol) 2-mercaptoethanol, and 0.01% bromophenol blue and electrophoresed, using a Pharmacia Phast system, for 20 min in a 10–15% gradient polyacrylamide gel under denaturing conditions. Proteins were transferred onto nitrocellulose filters (BA85, Schleicher & Schuell). The filters were rinsed with binding buffer [400 mM KCl/200 mM Hepes, pH 7.9/30 mM $MgCl_2$/1 mM dithiothreitol/0.05% Tween 20/5% skim milk (Difco)] and then incubated with ^{32}P-labeled concatenated oligonucleotide probes (3–5 × 10^5 cpm/ml) in binding buffer containing sonicated herring sperm DNA (100 μg/ml) at 4°C

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Abbreviations: IFN, interferon; ICS, IFN consensus sequence; ICSBP, ICS-binding protein; IRF, IFN response factor; MHC, major histocompatibility complex; CRE, class I regulatory element; β_2m, β_2-microglobulin; 2′5′ OAS, (2′-5′)oligoadenylate synthetase; PRD, positive regulatory domain.

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‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M32489).

for 2 hr with gentle rocking. Filters were washed four times in binding buffer at room temperature and exposed to Kodak XAR film overnight at −70°C.

DNA and RNA Blots. Genomic DNA from various species was digested with *Xba* I or *Eco*RI and probed with a 2.8-kilobase (kb) ICSBP insert labeled by the random priming method. Filters were washed twice in 0.3 M NaCl/0.03 M sodium citrate, pH 7/0.1% SDS at room temperature and twice in 0.15 M NaCl/0.015 M sodium citrate, pH 7/0.1% SDS at 65°C. The murine macrophage line PU5-1.8 was stimulated with natural IFN-α/β (Lee Biomolecular Laboratories, San Diego) at 500–1000 units/ml (3, 5), recombinant IFN-γ (a gift from Shionogi Research Laboratories, Osaka) at 100 units/ml, *E. coli* lipopolysaccharide (Sigma) at 20 ng/ml, or phorbol 12-myristate 13-acetate (Sigma) at 100 ng/ml. Total RNA (20 μg per lane) or poly(A)$^+$ RNA (5 μg) was electrophoresed through 1.2% agarose/formaldehyde gels and blots were probed with a ^{32}P-labeled 131A ICSBP insert and washed in the same conditions as above.

RESULTS

Cloning and Sequence of ICSBP. Transcriptional induction of MHC class I genes by IFNs is mediated by the ICS that encompasses nucleotides −167 to −139 relative to the RNA start site (3). The ICS and another adjacent cis element, the MHC class I regulatory element (MHC CRE) (20) are both conserved and involved in regulation of MHC class I gene transcription (21). To isolate a clone encoding a protein that binds to the ICS, we screened a mouse λZAP cDNA library by using a concatenated duplex oligonucleotide containing the ICS as a probe. Screening of ≈10^6 recombinant phage plaques yielded one positive clone containing a 1.0-kb insert, designated 131A. The protein encoded by this clone bound to the ICS but not to the MHC CRE or other unrelated regulatory sequences. Clones with overlapping inserts were obtained and their DNA was sequenced. The cDNA sequence and the deduced amino acid sequence of ICSBP are shown in Fig. 1. A long open reading frame (nucleotides 19–1290) encodes a protein of 424 amino acids. The region immediately upstream of the presumed initiator methionine codon resembles the consensus sequence for translation initiation identified by Kozak (23). A comparison with sequences of other genes indicated significant similarity between ICSBP and IFN response factors 1 and 2 (IRF-1 and IRF-2; refs. 24 and 25) over the first 345 nucleotides of their coding segments. The N-terminal 115-amino acid sequences of the three proteins, representing the region of greatest similarity, are compared in Fig. 2. Amino acid identity between ICSBP and IRF-1 is 45% and that with IRF-2 is 47% in this region. Nucleotide identity of ICSBP with IRF-1 and IRF-2 over the same region is even greater, 54% and 57%, respectively. This

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                                MetCysAspArgAsnGlyGlyArgArgLeuArgGlnTrpLeuIleGluGlnIleAspSerSerMetTyrProGlyLeuIleTrpGluAsnAsp
   1 GTGGGAACCGGCGGCAGGATGTGTGACCGGAACGGCGGGCGGCGGCTGCGGCAGTGGCTGATCGAACAGATCGACAGCAGCATGTACCCGGGGCTGATCTGGGAAAATGAT
                                GluLysThrMetPheArgIleProTrpLysHisAlaGlyLysGlnAspTyrAsnGlnGluValAspAlaSerIlePheLysAlaTrpAlaValPheLysGlyLysPheLys
 112 GAGAAGACCATGTTCCGTATCCCCTGGAAGCATGCCGGCAAGCAGGATTACAATCAGGAGGTGGATGCTTCCATCTTCAAGGCCTGGGCAGTTTTTAAAGGGAAGTTTAAA
                                GluGlyAspLysAlaGluProAlaThrTrpLysThrArgLeuArgCysAlaLeuAsnLysSerProAspPheGluGluValThrAspArgSerGlnLeuAspIleSerGlu
 223 GAGGGAGACAAAGCTGAACCAGCCACGTGGAAGACGAGGTTACGCTGTCGTCTGAACAAGAGCCCAGATTTTGAAGAAGTGACTGACCGGTCCCAGCTGGACATTTCTGAG
                                ProTyrLysValTyrArgIleValProGluGluGluGlnLysCysLysLeuGlyValAlaProAlaGlyCysMetSerGluValProGluMetGluCysGlyArgSerGlu
 334 CCATATAAAGTTTACCGAATTGTCCCCGAGGAAGAACAAAAATGCAAGCTGGGCGTGGCACCTGCAGGCTGCATGAGCGAAGTTCCTGAGATGGAGTGTGGCCGCTCAGAG
                                IleGluGluLeuIleLysGluProSerValAspGluTyrMetGlyMetThrLysArgSerProSerProProGluAlaCysArgSerGlnIleLeuProAspTrpTrpVal
 445 ATTGAGGAGCTGATCAAGGAACCTTCTGTGGATGAGTACATGGGTATGACCAAGAGGAGCCCATCCCCACCAGAGGCCTGCAGGAGCCAGATCCTCCCTGACTGGTGGGTC
                                GlnGlnProSerAlaGlyLeuProLeuValThrGlyTyrAlaAlaTyrAspThrHisHisSerAlaPheSerGlnMetValIleSerPheTyrTyrGlyGlyLysLeuVal
 556 CAGCAGCCCAGTGCAGGCCTGCCACTGGTGACCGGATATGCCGCCTATGACACACACCATTCAGCTTTCTCCCAGATGGTCATCAGCTTCTACTACGGGGGCAAGCTGGTG
                                GlyGlnAlaThrThrThrCysLeuGluGlyCysArgLeuSerLeuSerGlnProGlyLeuProLysLeuTyrGlyProAspGlyLeuGluProValCysPheProThrAla
 667 GGCCAGGCCACCACCACCTCGCTTGAAGGCTGCCGTCTCTCCCTGAGCCAGCCGGGGCTGCCTAAGTTGTATGGGCCGGATGGCCTGGAACCCGTGTGCTTTCCGACGGCC
                                AspThrIleProSerGluArgGlnArgGlnValThrArgLysLeuPheGlyHisLeuGluArgGlyValLeuLeuHisSerAsnArgLysGlyValPheValLysArgLeu
 778 GACACCATCCCCAGTGAGCGGCAGAGGCAGGTGACCCGGAAGCTGTTTGGGCACCTGGAACGTGGCGTGCTACTGCACAGCAACCGCAAGGGCGTGTTCGTGAAGCGGCTG
                                CysGlnGlyArgValPheCysSerGlyAsnAlaValValCysLysGlyArgProAsnLysLeuGluArgAspGluValValGlnValPheAspThrAsnGlnPheIleArg
 889 TGCCAGGGCCGCGTGTTCTGCAGCGGCAACGCGGTGGTGTGCAAGGGCAGGCCCAACAAGCTGGAGCGGGACGAGGTGGTGCAGGTCTTTGACACCAACCAGTTCATCCGA
                                GluLeuGlnGlnPheTyrAlaThrGlnSerArgLeuProAspSerArgValValLeuCysPheGlyGluGluPheProAspThrValProLeuArgSerLysLeuIleLeu
1000 GAGCTGCAGCAATTCTACGCCACCCAGAGCCGCCTACCTGACAGCAGGGTGGTCCTGTGCTTCGGGGAGGAGTTTCCGGACACTGTGCCCTTGCGCTCCAAACTCATTCTG
                                ValGlnValGluGlnLeuTyrAlaArgGlnLeuValGluGluAlaGlyLysSerCysGlyAlaGlySerLeuMetProAlaLeuGluGluProGlnProAspGlnAlaPhe
1111 GTGCAGGTAGAGCAGCTGTATGCCAGGCAGCTGGTGGAGGAAGCGGGCAAGAGCTGCGGTGCTGGCTCCCTGATGCCAGCCCTGGAGGAGCCCCAGCCGGACCAGGCTTTC
                                ArgMetPheProAspIleCysThrSerHisGlnArgProPhePheArgGluAsnGlnGlnIleThrValEnd
1222 CGCATGTTTCCGGATATCTGTACCTCACACCAGAGACCCTTTTTTAGAGAAAATCAACAGATCACCGTCTAAGCCTCAGTCCGGGCACCCCACCTCGCCTGAGCTCAAGCT
1333 TCAAGAGTCTGTGACTAAGAGAATTCCGAAAGGATGTGGAGCCCTCTGACTGGGGTGGGCGGGTGTCCTCCAAGGGGCCTCCGGAAGCCCACAGAGGGATGCGCTCCTGCT
1444 CAGGCAGGTGTCAGAAGCTTGCAGGGGCTGTGGCCGCAACCTGTGATTAAAGCATTCCTTTTCCTTACACTATTTCACCCCTCACCACTAATGGCTGGCCTTTCTGTGTGC
1555 TGAGGTCTTTCGACAGTTCAAATCATCTGGTGGCAGCAGACTCGCCTTTGCCCTTCTGCGGCCGAGGGCGGAGATTTATGACTTTCTCTGCTTGGTTGGAGAAGAAGAATC
1666 TTTACTATTCAGCTTCTTTTCTTTTGGCCAGAACTCTGAAAAAAAAAAAAAAACTCTTTTTTAAGACCAATATTTGTATTCTCACAGGCTCAGCTGTCAATCACTTGAGACC
1777 TTCCCTGTAAAGTGGGGCAGATTTTAAATATGGGTGTAGATACTCGTTGCAGCCTTCGCAGGAATTTTGGTTGTGGTTCATTGATTCACACAGACTCTGTGTCAGCTGACA
1888 GGGCTGTGTGGGGCATCAAAGGAGGACCAGGCACTGTGGAGAAGACCCATTCACTGGCATCTCACCCTTCCTTGTCCAGCTCCATACCCAGTCCTAAGACCCAGTGAAAAG
1999 CCACGTCCAAACTGTGCTCTGGGCTCATCAGTGCCCACCCACGTACCAGGGAAAGGCACACACCCATCCCAGTGGGCACAGAGCGGAATGTCCCCCTACCGCACCATTTGC
2110 GCCCCCAATCTGGCTGTCCAACCTAGTTTGTAAGTAATCTAAATCAGTGACTATAGCCCCGCCTAAGGGACACTTCCCGGAGGAGGGAGCCGCTGAAAAGGAGTTAGTTTG
2221 AGGGTCAGTACACAACAGGGGCAGAAAGCCAAGCAGATGTGGGGGCAGGGAGGTCATCATCTGCTTTTGTCTGAGAGAAGGAGAGCTTCTCCGTTTGTTCAACTTTGTAAC
2332 AAGCTGGGTTACATGCTCCACGCAGCTAGAGAAGCCTAGGTGCTCTGCATTCCCTGGGGAACTGCAGGAAAGCCTTACCTGCTGACTGTTGCTCTGGGGAAAAGCCTGAGG
2443 GTCCAGAGCAGCTACAAGCTACAGGCCATACCTTACAACCTGAAAAGCTAAGGACCACGGTGACCTTCCCGGCTACTGTGTGAAGGTGCTGGGTGGGGCC
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FIG. 1. Nucleotide and deduced amino acid sequences of ICSBP. A 2542-nucleotide sequence containing a translatable region of 424 amino acids is shown. The putative DNA-binding domain is boxed. The ATTTA consensus sequence (22) is underlined.

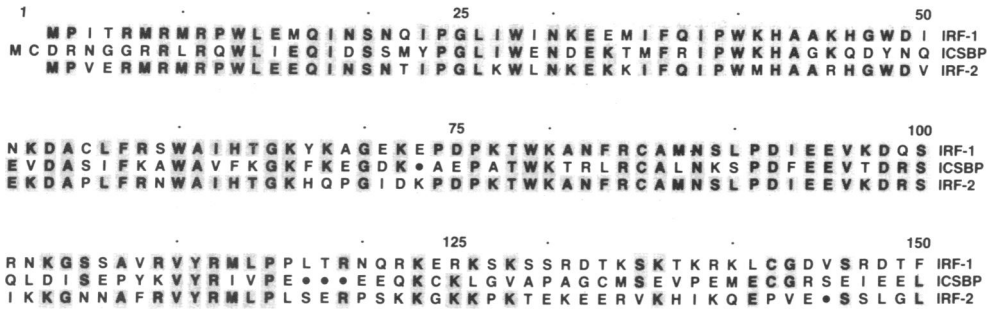

FIG. 2. Amino acid comparison of the putative DNA-binding domain of ICSBP with IRF-1 and IRF-2. The N-terminal 146 amino acids of ICSBP are aligned with the N-terminal sequences of IRF-1 and IRF-2. Amino acid residues identical between proteins are shaded. Filled circles represent gaps inserted for maximal alignment.

sequence similarity and the target DNA specificity prompted us to conclude that ICSBP belongs to the IRF gene family. As presented below (Fig. 4), ICSBP and the IRFs bind a similar (or identical) series of IFN response motifs, which suggests that the DNA-binding domain resides within the N-terminal 115 amino acids. However, the degree of similarity between ICSBP and IRF-1 or IRF-2 is less than that between IRF-1 and IRF-2: IRF-1 and IRF-2 share as much as 75% amino acid identity and 70% nucleotide identity over the same region. The remaining C-terminal 309 amino acids of ICSBP do not show similarity to IRF-1 or IRF-2, despite the fact that IRF-1 and IRF-2 show some similarity even in this region. These findings indicate that ICSBP is a relatively distant member of the IRF gene family. Like IRF-1 and IRF-2, ICSBP does not have sequences characteristic of known DNA-binding domains (e.g., a homeobox, a zinc finger, or a helix–turn–helix motif), nor does it have a leucine zipper as found in some transcription factors. The putative DNA-binding domain is rich in charged residues and presumed to be relatively hydrophilic. ICSBP is substantially larger (48.2 kDa) than IRF-1 (37.3 kDa) or IRF-2 (39.5 kDa), much of the difference being accounted for by the longer C-terminal region of ICSBP. Additionally, the ICSBP cDNA has a long 3′ untranslated region which contains an ATTTA sequence and a number of AT stretches. These sequences are often found in genes whose messages are unstable (22).

Southern analysis of various species (Fig. 3) revealed strong cross-hybridizing bands in human, chicken, and *Drosophila* DNA. Faint bands were detected in *Xenopus* DNA after a long exposure, indicating that ICSBP or similar sequences are conserved widely in vertebrates and invertebrates. Yeast DNA did not have a hybridizable band. The ICSBP gene may exist as a single copy in the mouse, since *Eco*RI digests of mouse DNA yielded a single band of ≈23 kb.

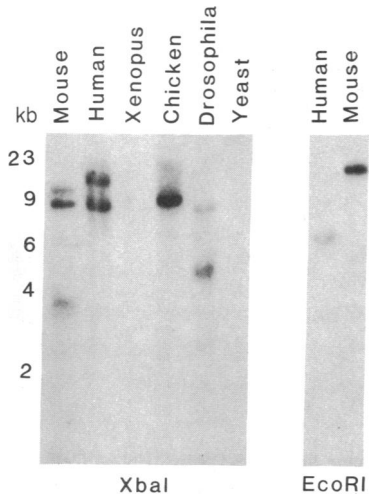

FIG. 3. Southern blot analysis. Genomic DNA was probed with a 2.8-kb ICSBP cDNA.

Southwestern Analysis of Binding Specificity. Various oligonucleotides were tested for their ability to bind an ICSBP fusion protein. These oligonucleotides corresponded to native or mutant forms of the ICS, or to IFN response elements of IFN-regulated genes—i.e., ISG54 (8, 9), (2′-5′)oligoadenylate synthetase (2′5′OAS; refs. 10 and 11), complement factor B (12), 6–16 (13), and β_2-microglobulin (β_2m; ref. 17)—or to a virus- and IFN-inducible element [positive regulatory domain I (PRDI) and the hexamer AAGTGA motif] of the IFN-β gene (16, 24–26) and a similar sequence of the IFN-γ gene (27, 28). Results are shown in Fig. 4 and binding activity is summarized in Table 1. The extracts containing the ICSBP plasmid produced a prominent band of ≈60 kDa with the native ICS probe, while control extracts produced no detectable band with the same probe. The size of the prominent band was that expected for the β-galactosidase–ICSBP fusion protein. In most experiments there was a second, smaller species of ≈30 kDa, probably a product of proteolytic degradation. Two mutant ICS oligonucleotides, m1 and m4, also bound the ICSBP fusion protein. On the other hand, two other mutants, m2 and m3, showed no binding to the fusion protein. This specificity pattern is identical to that observed previously with the naturally occurring nuclear factors expressed in many normal and IFN-treated mouse and human cells (3) and maps the binding site of ICSBP to the AGTTTCACTTCT motif in the ICS. IFN response elements of IFN-inducible genes and IFN-β also bound the ICSBP fusion protein. Binding of the ISG54, 2′5′OAS, IFN-β, and 6-16 oligonucleotides to the ICSBP was strong and comparable to that of the native ICS. Binding of the factor B and β_2m oligonucleotides was substantially weaker. The sequence resembling the IFN response element present in the IFN-γ gene (27, 28) did not show binding. Sequences unrelated to the ICS, such as region I of the MHC CRE (20, 21) and PRDII of the IFN-β gene (29) did not bind ICSBP, as expected. These data suggest a consensus sequence for ICSBP binding, YRGTTTCRYTTYYN. Other proteins expressed from plasmids with sequences overlapping with 131A showed the same binding activity (data not shown).

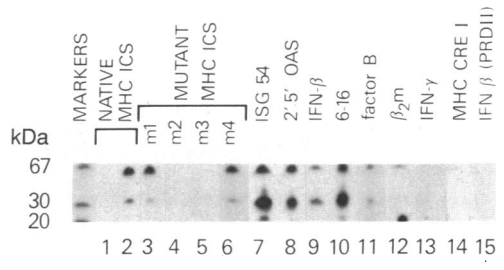

FIG. 4. Binding specificity of ICSBP determined by Southwestern assays. Bacterial extracts containing β-galactosidase–ICSBP fusion protein encoded by clone 131A were tested for binding to ^{32}P-labeled concatenated oligonucleotides corresponding to native and mutant forms of ICS and to various IFN response motifs (see summary in Table 1 also). Lane 1 represents control extracts from *E. coli* harboring plasmids that lacked an insert.

Table 1. Binding of ICSBP to IFN response motifs

Gene*		Sequence†		Binding‡
Mo MHC				
ICS	−157	CCTCAGTTTCACTTCTGCA	−139	++
Mutant ICS				
m1		--CAG----------		++
m2		------TG----------		−
m3		-------GAC---------		−
m4		----------AGC		++
Hu ISG54	−105	CTAGTTTCACTTTCCC	−90	++
Hu 2′5′OAS	−85	GTTGGTTTCGTTTCTTCA	−102	++
Hu IFN-β	−63	CCACTTTCACTTCTC	−77	++
Hu 6-16	−96	TGCAGTTTCATTTTCCCC	−113	++
Hu factor B	−143	TGCAGTTTCTGTTTCCTT	−126	+
Mo β_2m	−131	TTCAGTTTCATGTTCTTA	−148	+
Consensus		YRGTTTCRYTTYYN		
Hu IFN-γ	−239	GGCACTTTTACTTCAC	−254	−
Mo MHC CRE I	−173	TGGGGATTCCCCA	−161	−
Hu IFN-β (PRDII)	−55	GGAATTTCCCAC	−66	−

*Mo, mouse; Hu, human.
†Each oligonucleotide had linker sequences 5′-GCC/GGC-3′. Nucleotide positions relative to the transcription start site of the gene are indicated.
‡Southwestern analysis of binding to the ICSBP fusion protein is presented in Fig. 4.

Induction of ICSBP mRNA Expression by IFN-γ. We examined expression of ICSBP mRNA in various mouse tissues and cell lines. As shown in Fig. 5*C*, Northern blot analysis of spleen and thymus poly(A)$^+$ RNA revealed two message species, one 3 kb and the other 1.7 kb in size. Brain and liver RNA did not exhibit either message. By probing with smaller fragments prepared from the 131A clone, we found that the 3-kb message contained all of the 1-kb ICSBP sequence. The 1.7-kb message was also homologous to the 129 bases at the 5′ end of 131A, suggesting that it was a related species. Among mouse tissue culture cells, low levels of the 3-kb message were detected in RNA of LTK$^-$ fibroblasts, F9 embryonal carcinoma cells, LH8 and EL4 T cells, P3X63 and SP2/0 B cells, as well as a macrophage line, P388D1. Another macrophage line, PU5-1.8, had relatively high levels of the 3-kb message. We tested the effect of IFN-α/β and IFN-γ on expression of ICSBP message in PU5-1.8 (Fig. 5 *A* and *B*). Treatment with IFN-γ increased levels of both 3-kb and 1.7-kb messages within 1–2 hr. Expression of both messages peaked ≈4 hr following IFN-γ treatment, with a subsequent decline in 10 hr. The 1.7-kb message followed different kinetics, as its levels remained constant between 4 and 10 hr. Pretreatment of cells with a protein synthesis inhibitor, cycloheximide, did not inhibit induction of ICSBP mRNA by IFN-γ. Instead, cycloheximide superinduced levels of both mRNA species by 3- to 5-fold. The level of β-actin mRNA, tested as a control, did not change following IFN-γ treatment (data not shown). In contrast, IFN-α/β failed to induce ICSBP mRNA to a measurable degree for over 10 hr. Treatment with cycloheximide did not change the lack of induction by IFN-α/β. These results indicate that (*i*) lymphoid tissues are dominant sites of ICSBP expression, (*ii*) expression of ICSBP is induced preferentially by IFN-γ and not by IFN-α/β, and (*iii*) ICSBP induction by IFN-γ is rapid, does not depend on *de novo* protein synthesis, and therefore is characteristic of an immediate early gene (22). Neither bacterial lipopolysaccharides nor phorbol 12-myristate 13-acetate, both macrophage activators (30, 31), had any effect on ICSBP mRNA expression in PU5-1.8. In addition to PU5-1.8, another macrophage line, P388D1, and a T-cell line, EL4, were induced to express ICSBP message by IFN-γ but not by IFN-α/β (data not shown). ICSBP mRNA levels in F9 embryonal carcinoma cells and L fibroblasts did not increase in response to either IFN-γ or IFN-α/β (data not shown),

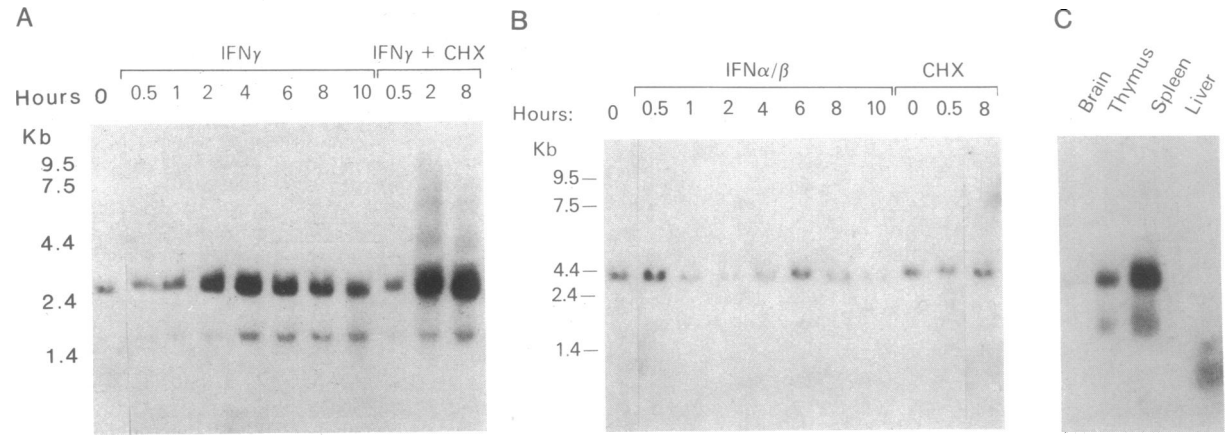

FIG. 5. Expression of ICSBP mRNA. (*A*) Induction of ICSBP mRNA in the macrophage line PU5-1.8 by IFN-γ. Cells were treated with recombinant IFN-γ (100 units/ml) for the indicated periods of time. Cycloheximide (CHX, 35 μg/ml) was added 30 min before IFN-γ. Twenty micrograms of total RNA per lane was tested for hybridization with ^{32}P-labeled 131A probe. (*B*) Effect of IFN-α/β on ICSBP expression in PU5-1.8. Cells were treated with natural murine IFN-α/β (500 units/ml; ref. 5) with or without addition of CHX, and hybridization was carried out as above. (*C*) Tissue distribution. Five micrograms of poly(A)$^+$ RNA was tested for hybridization with the same probe.

suggesting that induction of ICSBP mRNA is restricted to cells of lymphoid/macrophage lineages. The observed selective induction of ICSBP mRNA by IFN-γ may not be absolute, since actions of IFNs vary greatly depending on cell type (1, 2).

DISCUSSION

We have isolated a cDNA clone which encodes a protein, designated ICSBP, that binds a conserved IFN-inducible enhancer, the ICS of MHC class I genes. ICSBP also binds IFN- or virus-response elements of IFN-inducible genes and the IFN-β gene. The ICSBP shares 45–47% sequence identity with IRF-1 and IRF-2 over the N-terminal 115 amino acid residues. This domain, a putative DNA-binding region, is rich in charged residues and likely to be hydrophilic, a feature also shared with IRF-1 and IRF-2. IRF-1 and IRF-2 bind the same or very similar DNA sequences as those bound by ICSBP and are involved in transcriptional regulation of IFN-α and IFN-β genes (24, 25, 32). The striking structural similarity and the common target specificity led us to conclude that ICSBP belongs to the IRF gene family and therefore may play a role in transcriptional control of IFN-regulated genes. A comparison of nucleotide and amino acid sequences of the three genes shows that the level of similarity between ICSBP and IRF-1 and IRF-2 is much lower than that between IRF-1 and IRF-2 (Fig. 2): IRF-1 and IRF-2 share 75% amino acid identity in the first 115 amino acids and are significantly similar between amino acids 116 and 150. Moreover, IRF-1 and IRF-2 share clusters of related amino acid sequences in their C-terminal regions (25). ICSBP shows no similarity to IRF-1 or IRF-2 in its C-terminal region. ICSBP is also substantially larger than IRF-1 and IRF-2. These results suggest that ICSBP is a relatively distant member of this gene family, which may have diverged before the gene duplication event postulated to have resulted in IRF-1 and IRF-2 (25). Sequences homologous to ICSBP are conserved even in *Drosophila*, which does not have defense mechanisms analogous to that of vertebrates (33).

The finding that multiple factors recognize common IFN response motifs would in part explain the well documented complexity and diversity of IFN action (1, 2). Although all three IFNs can act through the IFN response motifs and induce a shared set of genes (2, 34), IFN-γ affects a distinct set of genes and elicits specific biological activities which are unaffected by IFN-α or IFN-β (1, 2). For example, MHC class II (35), Fc receptor (36), P10 (15), and γ.1 genes (37) are induced predominantly by IFN-γ. Further, IFN-γ and IFN-α/β may act synergistically or antagonistically (1, 2, 38, 39). It is noteworthy that ICSBP mRNA is predominantly expressed in lymphoid tissues and is preferentially induced by the immune IFN, IFN-γ. Furthermore, induction of ICSBP mRNA by IFN-γ appears to occur mainly in cells of the immune system. IFN-γ is produced by T cells and, through a distinct receptor, modulates lymphoid functions including macrophage superoxide production (40), macrophage migration (30, 31), T-cell proliferative activity (41), lymphokine production (40), and lymphokine function (39). The prevalence of ICSBP mRNA expression in the immune tissues and its selective inducibility by IFN-γ in cells of lymphocyte/macrophage lineages suggest that ICSBP plays a regulatory role in cells of the immune system.

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