Human Interferon Consensus Sequence Binding Protein Is a Negative Regulator of Enhancer Elements Common to Interferon-inducible Genes*

Anat Weisz‡, Pierre Marx‡, Rukefet Sharf‡, Ettore Appella§, Paul H. Driggers§, Keiko Ozato¶, and Ben-Zion Levi††

From the ‡Department of Food Engineering and Biotechnology, Technion, Haifa 32000, Israel and the §National Cancer Institute and ¶National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

The promoter regions of many interferon-inducible genes share a short DNA sequence motif, termed the interferon consensus sequence (ICS) to which several regulatory proteins bind. A murine cDNA which encodes an ICS binding protein has been reported (M-ICSBP). The cloning of the human homologue of ICSBP (H-ICSBP) is described. H-ICSBP shares high sequence homology with its murine cognate. The derived sequence of H-ICSBP reveals restricted homology within the first 120 amino acids to three other interferon regulatory factors, IRF-1, IRF-2, and ISGF3γ. Truncated ICSBP lacking the first 33 amino-terminal amino acids fails to bind to the ICS, indicating that at least part of the DNA binding domain is located within the well conserved amino terminus. H-ICSBP is expressed exclusively in cell lines of hematopoietic origin. The results of transient transfection assays carried out either in hematopoietic or nonhematopoietic cells suggest that ICSBP acts as a negative regulatory factor on ICS-containing promoters. Furthermore, either interferon-γ (IFN-γ) or IFN-β can alleviate the repression mediated by ICSBP. Therefore, ICSBP may be involved in maintaining submaximal transcriptional activity of IFN-inducible genes in hematopoietic cells. IFN treatment would then alleviate repression allowing maximal transcriptional activity of these genes.

Interferons (IFNs)§ are a family of species-specific polypeptides that affect a variety of biological responses (1). Two classes of IFNs have been described, IFN-α/β and IFN-γ, which act through distinct cell surface receptors (1–3). IFN-mediated gene regulation is a complex cascade of events that leads to acquisition of the antiviral state. This chain of events is initiated by the binding of ligand (IFN) to its specific receptor, ultimately leading to a signal transduced to the nucleus (4, 5). Such a signal is followed by induction of transcription of specific genes (6). Some IFN-responsive genes are exclusively induced by one type of IFN, whereas others respond to both types.

Promoters that respond to IFN-α have a consensus DNA binding site, called the interferon consensus sequence (ICS), also known as the interferon responsive element, to which several nuclear factors bind (7–14). Treatment of cells with IFN-α/β results in immediate activation of ISGF3γ in the cytoplasm, where it combines with pre-existing ISGF3γ to form an active ISGF3 complex. The ISGF3 complex is rapidly translocated to the nucleus, where it presumably initiates transcription of a family of IFN-inducible genes. While ISGF3γ is expressed in most cell lines tested, it can be induced further by IFN-γ. Two cDNAs encoding interferon regulatory factors, IRF-1 and IRF-2, were recently cloned. These factors bind to the positive regulatory domain I (PRDI) located within the promoter region of IFN-β, as well as to the ICS (18, 19). It was suggested that IRF-1 is a transcriptional activator, while IRF-2 is a repressor (19, 20).

Recently, we reported the cloning of a murine cDNA that encodes another protein which binds to the ICS (ICSBP) (21). ICSBP is a protein of 424 amino acids, of which the amino-terminal 120 amino acids, encompassing the putative DNA binding domain, show significant sequence similarity to the corresponding domain of IRF-1 and IRF-2. Based on this similarity, the ICSBP is likely to be a member of the IRF family of transcription factors.
gene family. ICSBP is expressed predominantly in lymphoid tissues and is preferentially inducible by IFN-γ. However, the induction of ICSBP has not been described.

In this paper we describe the cloning of the human homologue of ICSBP (H-ICSBP). A detailed examination of mRNA expression in cell lines that represent different stages of hematopoietic differentiation shows that H-ICSBP expression is indeed restricted mainly to B cells and monocytic cells. Southwestern analysis with truncated H-ICSBP provides evidence that the presumed DNA binding domain is located within the amino-terminal portion of H-ICSBP. Our transient transfection assays performed in HeLa cells as well as in U937 cells reveal that H-ICSBP is a negative regulatory trans-acting factor. In addition, treatment of cells with either IFN-β or IFN-γ is found to alleviate the negative effect of ICSBP. The possible involvement of ICSBP in mediating cytokine-induced regulation is discussed.

MATERIALS AND METHODS

Cell Culture—Cell lines U937, HeLa, A431, Molt-4, Namalwa, Ramos, and Daudi were received from the ATCC. The cell lines Tenon, RHE, and 2R (kindly provided by Dr. T. Taniguchi, Osaka University, Japan) which is devoid of the putative DNA binding domain of IFN-β; II1 a 1300-bp EcoRI fragment corresponding to the coding region of (2'5')-oligoadenylate synthetase (OAS) (kindly provided by Dr. J. Chebath, Weizmann Institute, Israel).

Southwestern Analysis—Southwestern analysis was carried out as described (23) except that the bacterial supernatants containing 5μg of DNA were mixed with sample buffer, separated on 10% polyacrylamide gels, and electroblotted to nitrocellulose paper (BA85, Schleicher & Schuell, Keene, N.H.). The different constructs used for this analysis are described above.

Transfection and CAT and β-Galactosidase Assays—The different cells were transfected with various CAT constructs using electroporation (32, 33). In general, cells were cotransfected with 4-6μg of CAT vector and 2μg of pMLV3.2 or 5μg of pMLV3.2AS. CAT (2'-5'OAS) (kindly provided by Dr. J. Chebath, Weizmann Institute of Science, Rehovot, Israel) 100 IU/ml recombinant human IFN-γ (Genzyme, Boston), or combined treatment of CHX and IFNs. Following the incubation, the cells were harvested, and RNA was prepared by the manufacturer's instructions with a 1-kb 32P-labeled EcoRI fragment of pMLV3.2 encompassing the whole H-ICSBP coding region into pUC18 digested with the same restriction enzymes. The plasmid pMLV3.2AS is identical to pMLV3.2 except that a 1253-bp BamHI fragment encompassing most of the coding region was flipped into an anti-sense orientation with regard to the lactose promoter. The plasmid pMLV3.2AS is identical to pMLV3.2 except that a 1253-bp BamHI fragment enclosing most of the coding region was flipped into an anti-sense orientation with regard to the lactose promoter.

Northern Blot Analysis—Cells were grown to 75% confluence, and a typical set of experiments included untreated cells and cells treated for 4 h with either 35μg/ml cycloheximide (CHX), 500 IU/ml human IFN-β (fromnu22, kindly provided by Interpharm Laboratories, Ltd., Rehovot, Israel), 100 IU/ml recombinant human IFN-γ (Genzyme, Boston), or combined treatment of CHX and IFNs. Following the incubation, the cells were harvested, and RNA was prepared by the single step method (29). Northern blot analyses were carried out as previously described (31). The following DNA fragments labeled with 32P by random priming, were used: 1) a 262-bp PstI-BglII fragment corresponding to the coding region of the murine H-ICSBP (26); 2) a 620-bp EcoRV-BglII fragment of the plasmid pUCHIRF-1 (kindly provided by Dr. T. Taniguchi, Osaka University, Japan) which is devoid of the putative DNA binding domain of IFN-β; II1 a 1300-bp EcoRI fragment corresponding to the coding region of (2'5')-oligoadenylate synthetase (OAS) (kindly provided by Dr. J. Chebath, Weizmann Institute, Israel).

RESULTS

Cloning of the Human Homologue of ICSBP—Northern blot analysis of different mouse tissues revealed that mRNA corresponding to M-ICSBP is detectable in the lung. Therefore, the resultant plasmid, pICSBP, contained H-ICSBP fused in frame to the lacZα of the plasmid pUC18. The plasmid pICSBP933 was constructed by digesting clone 133, missing the first 33 amino acids of H-ICSBP, with EcoRI-NarI, and subcloning this fragment into pUC18 digested with the same restriction enzymes. The plasmid produced, pICSBP933, contains the open reading frame of clone 133 cloned under the lacZα frame to the lacZα promoter. The clone corresponding to most of the coding region of the murine ICSBP were isolated. In order to isolate clones that contain the missing This library was screened with a 1.2-kb EcoRI-NarI fragment containing the new cDNA insert, was released from the lambda by digestion with EcoRI and subcloned into pUC19. Clone 133, which had the longest insert, was then subjected to DNA sequencing by the dyeoxy chain termination method. Since clone 133 did not contain full-length cDNA, we used a human fresh blood cell line (kindly provided by Dr. K. Robbins, National Institutes of Health) through Dr. D. Ron (Technion, Israel) (27). This library was screened with a 1.2-kb EcoRI fragment containing all of the coding region of clone 133. Thirty positive clones were isolated. In order to isolate clones that contain the missing amino-terminal portion of H-ICSBP, polymerase chain reaction (PCR) analysis was performed. A PCR reaction (28) containing 1p1 of phage DNA, 1.2-kb EcoRI-NarI fragment of pMLV3.2 encompassing the whole H-ICSBP coding region into pUC18, which was cleaved by the same restriction en-
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**A**

Fig. 1. Nucleotide and amino acid sequence of H-ICSBP. A, a 1536-nucleotide sequence containing the coding region of 425 amino acids is illustrated. The underlined amino acids encompass the putative DNA binding domain. B, amino acid comparison of the putative DNA binding domains of H-ICSBP, M-ICSBP, H-IRF-1, and H-IRF-2. Identical amino acid residues among the four proteins are boldface. Underlined amino acids represent identity between the ICSBPs and either IRF-1 or IRF-2.

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**B**

Northern blot analysis of H-ICSBP mRNA in different human cell lines. All the cell lines tested were treated for 4 h with either CHX (100 μg/ml), IFN-β (1000 units/ml), IFN-γ (100 units/ml), or a combination of CHX with either IFN-β or IFN-γ, and total RNA was prepared. Northern blot analysis was performed using 32P-labeled DNA fragments corresponding to either H-ICSBP, (2'--5')OAS, or IRF-1.
The effect of CHX on the expression of H-ICSBP was also examined. This expression was either constitutive or induced following IFN or CHX treatment of the different cell types (Fig. 2, A–D).

Only one message corresponding to ICSBP, with an estimated size of 3 kb, was observed in all Northern blot analyses (Fig. 2). Since H-ICSBP contains the motif that confers instability to mRNA described by Shaw and Kamen (36), the effect of CHX on the expression of H-ICSBP was also examined. In all B-cells and T-cells tested, CHX treatment increased the level of H-ICSBP mRNA expression (Fig. 2, A and C, and Table I). However, in the case of the promonocytic cells U937, CHX treatment reduced both basal and IFN-induced levels of H-ICSBP mRNA (Fig. 2B, Table I). CHX did not induce H-ICSBP mRNA in nonhematopoietic cells (Fig. 2D, Table I).

The effect of IFN-β and IFN-γ treatment on the expression of H-ICSBP mRNA was also tested. A significant increase in mRNA levels was detected in U937 cells treated with IFN-γ for at least 4 h, although some increase following IFN-β treatment was also observed (Fig. 2B). Interestingly, combined treatment with IFN and CHX had an antagonistic effect in U937 cells.

The level of 2′-5′ OAS mRNA following IFN treatment served as evidence for IFN responsiveness in the cells and confirmed the quality of our IFN preparations. Fig. 2 demonstrates that all cell lines tested responded to IFN treatment as is indicated by the elevated expression of 2′-5′ OAS mRNA.

The expression of ICSBP in different cell lines representing different stages of hematopoietic differentiation (22, 23) was further investigated in order to study the possible role of ICSBP in the differentiative pathway. Table I presents the measured levels of ICSBP mRNA in different cell lines. The hematopoietic lineage is composed of two major routes of differentiation: the lymphoid and the myeloid. The data show that ICSBP mRNA is constitutively expressed in a lymphoid precursor cell line (Reh null cells). Among T-cell progeny, ICSBP mRNA is not detected in either the T-blast I or T-blast II stages of differentiation as represented by the cell lines HD-Mar and Molt-4, respectively. ICSBP mRNA was detected following CHX treatment of these cells. However, IFN treatment did not affect the level of ICSBP mRNA. In the B-cell progeny, ICSBP mRNA was constitutively expressed in all of the B-cell lines tested as represented by B-blast I (Daudi and Ramos cells), B blast II (Namalwa cells), and plasma cells (myeloma RPMI 8226 cells). CHX treatment of these cells resulted in augmented expression of ICSBP mRNA, while IFN treatment resulted in a moderate increase. The erythroid lineage precursor cells were represented by the K562 cell line which did not exhibit any detectable ICSBP mRNA under any of the different treatments. As mentioned, U937 cells represent promonocytic cells. This cell line demonstrated a constitutive level of ICSBP mRNA which were significantly elevated following IFN-γ treatment, although some increase with IFN-β was noticed (see also Fig. 2B). On the other hand, no mRNA levels corresponding to ICSBP could be detected in the promyelocytic cell line, HL-60, which is believed to represent a less mature stage of differentiation than U937 cells. Different treatments to drive this cell line to differentiate to either granulocytes or monocytes are being carried out.

### DNA Binding of H-ICSBP to the Interferon Response Element

The putative DNA binding domain of the IRF gene family was assigned to the amino-terminal 120 amino acids. This assignment was partly based on the structural similarity in this region and the fact that all IRF proteins bind to a similar DNA motif (Fig. 1B) (21). Using Southwestern analysis, we previously showed that ICSBP binds to the ICS element of the MHC class I gene and the interferon regulatory element of many IFN-responsive genes (21). In this work, two H-ICSBP fusion proteins were tested by Southwestern DNA binding assay. The proteins were produced from: (i) the full-length coding region of H-ICSBP fused to the lacZα of the plasmid pUC18 (pICSBP); (ii) a truncated H-ICSBP which lacks the first 33 amino acids and fused to the lacZα of PUC18 (pICSBP33). As a control, H-ICSBP cloned in the antisense orientation with regard to the promoter was utilized (see “Materials and Methods”). The ability of the proteins encoded by the different constructs to bind either the intact ICS oligomer or mutated oligomers, in which the binding site was interrupted, was tested (see Fig. 3, bottom part). In accordance with our previous findings (21), only the full length H-ICSBP was capable of binding the ICS oligomer. This binding capability was largely abolished with the M2 and the M3 oligomers. However, the truncated protein in which the amino-terminal 33 amino acids were deleted did not bind any of these oligo-
cis-elements (Fig. 3). As expected, the construct in which ICSBP was in the antisense orientation as well as the plasmid PUC18 alone, did not promote any specific protein binding activity. The presence of either the full-length ICSBP or the truncated ICSBP on the gel. raised in rabbits against ICSBP that was expressed in Escherichia coli under the bacteriophage T7 promoter.3 These results indicate that indeed the amino-terminal portion of ICSBP is essential for DNA binding activity as predicted from the restricted homology to proteins of the IRF family.

H-ICSBP Is a trans-acting Negative Regulator in Transient Transfection Assays—To investigate the role of H-ICSBP as a trans-acting factor, a series of transient cotransfection experiments were performed in both nonhematopoietic cells (HeLa cells, Fig. 4) and hematopoietic cells (U937 cells, Fig. 5) using different CAT constructs containing the ICS. We took advantage of the fact that clone 3.2 which was isolated from the fresh blood monocyte library is already cloned under the Moloney murine leukemia virus long terminal repeats promoter, thus allowing expression in eukaryotic cells (PMLV3.2). Three groups of different CAT constructs were utilized: those that contain the mammalian MHC class I gene promoter (pL237CAT, pL1282CAT, and pL143CAT), those that contain the human MHC class I gene promoter (pV3G, pV3G3.1, pV3G3.6), and those that contain other ICs-like sequences (p-12814r, p-1281RS4r, p-1281114r). Fig. 4 summarizes the results of cotransfection experiments performed in HeLa cells using the three groups of CAT constructs in combination with the ICSBP construct (pMVL3.2). The constructs pL237CAT and pL1282CAT, which contain the murine class I regulatory element and ICS, exhibited higher CAT activity than the construct pL143CAT, which contain only the basal promoter (Fig. 4A). A 30–40-fold decrease in CAT activity was observed when the constructs pL237CAT and pL1282CAT were cotransfected with pMVL3.2. On the other hand, CAT activity produced by pL123CAT was not affected. When the construct pRSV-CAT, in which the CAT gene is driven by the RSV promoter, was cotransfected with pMVL3.2 the CAT activity was either not affected or at most reduced by a factor of four (Fig. 4, A–C).

Similar results were obtained with cotransfection experiments with CAT constructs containing the promoter of the human MHC class I gene, HLA-B7 (Fig. 4B). The CAT activity of pVG3 and pVG3.1, which contain the ICS, was markedly decreased when cotransfected with pMVL3.2. In contrast, the CAT activity of pVG3.6, which does not contain the ICS, was very low but not affected by cotransfection with pMVL3.2.

We next investigated whether other ICs-like sequences might also be affected by H-ICSBP. The data presented in Fig. 4C demonstrate that both IRS- and PRDI-driven CAT activities were decreased in the presence of ICSBP. H-ICSBP had no effect on either PRDII (NFkB-binding element) (31) or RSV promoter. All the above set of cotransfection experiments was also carried out with the construct pMVL3.2AS, in which ICSBP is in the antisense orientation with respect to the MLV promoter. This antisense construct did not promote any significant effect on CAT activity of the different reporter plasmids (data not shown). These results indicate that, in HeLa cells, H-ICSBP is capable of reducing promoter activity of constructs containing the interferon and viral response elements.

Since ICSBP is not expressed in HeLa cells (Fig. 2D) a similar set of experiments was performed in the promonocytic cell line U937 which exhibit constitutive expression of mRNA corresponding to ICSBP (Fig. 2B). Results shown in Fig. 5 demonstrate that cotransfection of ICSBP with IRS (p-12815S3r), PRDII (p-1281Lr), or HLA-promoter (pVG3.1) containing CAT constructs resulted in a significant decrease in the CAT activity when compared to the activity generated by these constructs alone. On the other hand, CAT activities of constructs containing the RSV promoter (pRSV-CAT), PRDII (p-1281Lr), or the basal HLA promoter (pVG3.6) were unaffected when cotransfected with ICSBP into U937 cells. These experiments indicate that the negative effect of ICSBP on ICS-containing promoters also occurs in hematopoietic cells that demonstrate constitutive H-ICSBP expression.

IFNs Can Alleviate the Negative Effect of ICSBP in Transient Transfection Assays—The data presented above indicate that ICSBP might have a role as a negative trans-acting regulator affecting ICS-containing promoters in hematopoietic cells. The impact of IFNs on this effect was studied in a similar experimental protocol using transient cotransfection experiments in HeLa cells (Fig. 6A) and in U937 cells (Fig. 6B). The cotransfection experiments described above were carried out in a molar ratio of 1:1 between the ICSBP construct and the different CAT constructs, respectively. Under these experimental conditions IFN treatment of the transfected cells did not affect the repression mediated by ICSBP on the different CAT constructs. Therefore, the minimal amount of ICSBP needed to exhibit a negative effect on the different CAT constructs was determined. It was found that a molar ratio of 1:1 between the ICSBP expression construct and the different CAT constructs was the minimal sufficient dose to exhibit an inhibitory effect on CAT activity (data not shown). Fig. 6A shows that in HeLa cells, under these experimental conditions, there is a marked decrease in CAT activity of the PRDII containing construct (p-1281Lr) following co-transfection with ICSBP. This repression of CAT activity is almost completely alleviated after treating the cells with either IFN-β (1000 units/ml) or IFN-γ (500 units/ml). The CAT activity of the control constructs, pRSV-CAT and p-1281Lr, was not affected significantly by either ICSBP or by the IFN treatment. The ability of IFNs to reverse the negative

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effect of ICSBP on CAT activity of the PRDI-containing promoter was also true for U937 cells (Fig. 6B). Both IFN-β
and IFN-γ promote enhanced CAT activity in cells cotransfected with ICSBP (5–10-fold).

**DISCUSSION**

Our published data showed that the murine ICSBP specifically binds to the ICS, shares sequence similarity with members of the IRF family of transcription factors, and is expressed in macrophage and lymphoid cell lines (21). Because of the availability of multiple well characterized human cell lines, we sought to obtain the human ICSBP clone in order to analyzed its expression in more detail and to better study its biological significance.

In this report we describe the cloning of the human homologue of ICSBP. The high homology (85%) at the DNA level with the murine cognate is restricted to the coding region and is not significant in the 3′-noncoding region. As demonstrated, the most extensive amino acid similarity is within the putative DNA binding domain (98%). The fact that some of the ICS-binding proteins, i.e. ICSBP, IRF-1, IRF-2, and the recently reported ISGF3γ (37, 38), share significant homology within their DNA binding domains is suggestive that a specific DNA-binding motif exists in this family of proteins. The amino-terminal portions of these proteins exhibit a stretch of highly charged amino acids, 5 tryptophan repeats, and specific conserved amino acids (Fig. 1B). However, the exact mode of interaction of the proteins with DNA is still unknown. Results of the Southwestern analysis with truncated ICSBP are consistent with the assignment of the DNA binding domain to the amino-terminal portion of ICSBP. It provides evidence that at least part of the DNA binding domain is located at the amino terminus.

We show that H-ICSBP expression is restricted to human cell lines of hematopoietic origin. However, unlike mouse cells which exhibit two mRNA species with apparent sizes of 3 and 1.7 kb (21), we found in human cells only a single mRNA species with an apparent size of 3 kb. The restricted expression of ICSBP mRNA in white blood cells suggests that it might have a specific role in mediating IFN response in cells of the immune system. Supporting this notion is the finding that elevated expression of ICSBP mRNA, following exposure to IFN-γ, is detected in monocytic cells (Fig. 2B and Ref. 21).

To study the possible role of ICSBP in hematopoiesis, a detailed analysis of expression patterns of ICSBP mRNA, in a limited panel of cell lines that represent different stages of hematopoietic differentiation, was performed (Table I). The hematopoietic lineage branches into two major routes of differentiation; the lymphoid and the myeloid. The pattern of ICSBP expression during differentiation within the myeloid lineage reveals that constitutive ICSBP mRNA levels can be detected in promonocytic cells, U937 (Fig. 4B and Table I), in fresh blood monocytes, and in macrophage cells (21, 39). On the other hand, no mRNA

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**FIG. 4.** Effect of H-ICSBP on CAT activity driven by ICS-containing promoters in HeLa cells. Cotransfection experiments (striped boxes) in HeLa cells with the vector pMLV3.2 and the different CAT constructs (illustrated at the bottom of each panel) were carried out as described under “Materials and Methods.” Open boxes represent results of transfections with only the CAT constructs. A, murine MHC class I CAT constructs; B, HLA B7 CAT constructs; C, PRDI, PRDII, and IRS CAT constructs.

**FIG. 5.** Effect of H-ICSBP on CAT activity driven by ICS-containing promoters in U937 cells. Cotransfection experiments (striped boxes) in U937 cells were carried out as described under “Materials and Methods.” The CAT constructs p128I4r, p128II4r, and p128IRS4r are illustrated in Fig. 4C. The CAT constructs pVG3.1 and pVG3.6 are illustrated in Fig. 4B. Open boxes represent the results of transfections with the CAT constructs alone.

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expression is detectable in earlier stage(s) of differentiation represented by HL-60 cells (promyelocyte). Therefore, we postulate that H-ICSBP might have a role in the cytokine-induced maturation of monocytes and B-cells.

IRF-1, a positive trans-acting factor, is involved in the regulation of genes induced by both IFN-β and IFN-γ (40). Moreover, it was demonstrated that IRF-1 is regulated by IFN-β, since IRF-1 mRNA levels are also increased by IFN-β treatment (41). Our Northern blot data show that IRF-1 mRNA was induced following exposure to IFN-γ. The increased expression of mRNA was detected in U937, Molt-4, and HeLa cells (Fig. 2, B, C, and D, respectively). These data suggest that both ICSBP and IRF-1 have a role in mediating the response to IFN-γ.

Results of transient CAT assays show for the first time that H-ICSBP acts as a negative trans-acting regulatory factor in both nonhematopoietic cells (HeLa cells) and hematopoietic cells (U937 cells). Both ICS and PRDI-containing promoters were negatively affected by ICSBP. These results are in agreement with our previously published Southwestern data showing that ICSBP is capable of binding different IFN response elements (21). This suggests that, like IRF-2, ICSBP may have a role in reducing the transcriptional activity of IFN-inducible genes in blood cells. The fact that either IFN-β or IFN-γ can reverse the inhibitory effect of ICSBP on CAT activity in a transient assay is indicative of its biological role. It is possible that the constitutive expression of ICSBP in promonocytic cells and B-cells is responsible for the maintenance of the expression of IFN-inducible gene(s) at a submaximal level. This limited repression of IFN-inducible genes can be alleviated following stimulation with IFNs or with IFN-stimulating agents, allowing maximum expression to occur. For example, MHC class I genes can be induced to a higher level of expression following stimulation with both types of IFNs (42). These results might imply that ICSBP plays a role in the regulation of MHC class I expression in monocytes and B-cells which may be involved in potentiating immunocompetence. Our results also suggest that ICSBP may associate with other ICS-binding factors, such as IRF-1 or ISGF3, by either competing for the binding site or by associating directly with them. Cotransfection experiments with expression vectors containing both ICSBP and IRF-1 revealed that IRF-1 can reverse the negative effect of ICSBP on the CAT activity of ICS-containing constructs,5,6 Hoffman and Hauser (43) recently demonstrated that IRF-1, following phosphorylation by casein kinase II, binds to DNA as a homodimer. Bacterially expressed ICSBP can be phosphorylated by casein kinase II.7 It is tempting to postulate that such phosphorylation events can lead to either different biological activities of ICSBP, interaction with other proteins, or a change in cellular localization of ICSBP as described for the ISGF3 complex (for details see Ref. 4). Since H-ICSBP mRNA expression is restricted to cells of hematopoietic origin, several experimental approaches are being pursued in an attempt to better define the role of ICSBP in the complex machinery of IFN-mediated gene regulation.

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