Interferon consensus sequence binding protein: Not essential for interferon α-mediated antiviral response to vesicular stomatitis virus infection in HL-60 cells

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= Abstract =

Background: The role of the interferon consensus sequence binding protein (ICSBP), a member of interferon regulatory factor family, in protecting against a vesicular stomatitis virus (VSV) infection has not been firmly elucidated. Thus, it was investigated utilizing the human promyelocytic leukemia HL-60 cells which do not express ICSBP. **Methods:** HL-60 cells were stably transfected with plasmid containing cDNA for either ICSBP or DNA binding domain (DBD) and tested for their VSV-susceptibilities. The susceptibility of each transfectant group to a VSV infection was determined by a plaque assay at 1 h, 24 h, and 48 h post-infection in the presence (500 IU/ml) or absence of interferon α (IFNα). **Results:** In the absence of IFNα, the three groups showed similar sensitivities to a VSV infection. However, when pre-treated with IFN, the viral titers in both the ICSBP and control clones steadily decreased over 48 h of incubation, indicating the existence of IFNα-mediated protection against VSV infection. The IFNα-treated ICSBP clones appeared to be more resistant to infection compared with the control clones, although the difference was not great. On the contrary, the viral titers in the IFNα-treated DBD clones increased at 24 h then decreased by 48 h. **Conclusion:** The expression of truncated ICSBP (DBD) does not appear to underlie the impaired protection against a VSV infection in the DBD clones, since even the control clones lacking ICSBP were protected from a VSV infection. This suggests that ICSBP does not play a critical role in the IFNα- mediated anti-VSV response of HL-60 cells, although it appears to confer some resistance to a VSV infection.

Key Words: interferon consensus sequence binding protein, DNA binding domain, interferon α, vesicular stomatitis virus infection

INTRODUCTION

Interferon (IFN) plays a key role in mediating the antiviral and antiproliferative responses as well as in modulating the immune response (1,2). These responses are elicited largely through the transcriptional activation of the IFN-regulatory genes. These genes possess specific consensus sequences within their promoters and are regulated in part by binding of the interferon regulatory factors (IRFs), a growing family of transcription factors (3,4), to the consensus sequences.

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The interferon consensus sequence binding protein (ICSBP), a member of the IRF family, was originally isolated as the protein that recognized the interferon-stimulated response element (ISRE) motif present in the promoter region of the MHC class I, H-2L^{D} gene (5,6). Unlike most of other IRF proteins, ICSBP exhibits a tissue-restricted pattern of expression and is expressed exclusively in immune cells, particularly in the macrophage and lymphoid lineages. ICSBP is induced by IFNα but not by IFNα/β (7), and represses the IFNα/β and IFNα/β-inducible promoters through the ISRE (8). Conversely, ICSBP is capable of stimulating the transcription of certain IFNγ-inducible promoters in a gamma- activated sequence (GAS)-dependent manner (9).

Studies on the role of ICSBP in the antiviral response have been made through several approaches. A recent work on ICSBP knockout mice demonstrated that these mice are sensitive to particular viral infections and ICSBP thus plays a critical role in the establishment of an antiviral state (10). However, sensitivity to viral infections is not a generalized phenomenon and a vesicular stomatitis virus (VSV) infection was well controlled in these mice. In contrast, another study showed that U937 monocytic cells transfected with truncated ICSBP cDNA, which retains the DNA-binding domain (DBD) but lacks the regulatory domain, did not have antiviral activity against VSV upon IFN treatment (11). This suggests that ICSBP plays an important role in controlling VSV infections.

As seen in these two reports, the protective role of ICSBP against VSV infection has not been firmly established. Thus the function of ICSBP in VSV infection was studied in a similar way as reported previously (11) utilizing the ICSBP and DBD stable clones of HL-60 human promyelocytic leukemia cells. However, in the current study, HL-60 cells, another model of the monocyte-macrophage lineage, were used since they were reported to express little or no ICSBP (6,12), while U937 cells obviously did. This character obviously renders HL-60 cells superior to U937 cells for evaluating the effect of exogenously introduced ICSBP.

The present study reports that both the ICSBP and control clones of HL-60 cells are protected from VSV infections in the presence of IFNα, although the former clones are more resistant. This indicates that ICSBP is not essential for the IFNα-mediated anti-VSV response, though it does play a certain role, since even the control clones lacking ICSBP were protected from a VSV infection. In addition, the results indicate that the DBD clones of HL-60 cells show an impaired anti-VSV response to IFNα, which is probably due to the inhibitory effects of DBD on the action of other IRF proteins.

MATERIALS AND METHODS

1. Cell culture

HL-60 cells were obtained from the American Type Culture Collection (Rockville, MD). They were cultured in a RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine sera, 2 mM glutamine, 50 μg/ml gentamicin sulfate, and 50 μM β-mercaptoethanol. The cells were maintained at 37℃ in a humidified incubator with 5% CO_2. The transfected HL-60 cells were cultured in the G418 (Geneticin, Life Technologies) containing medium at 400 μg/ml.

2. Construction of ICSBP expression vectors

An expression plasmid for the full-length ICSBP was constructed by subcloning the *Bam*HI fragment of a full-length mICSBP into the *Xho*I site of pCXN2 (13). An expression plasmid for DBD was constructed by subcloning the *Bam*HI/*Hind*III fragment of DBD (8) into pCXN2.

3. Transfection and cloning of stable transfectants

The HL-60 cells (1×10^{7}) were transfected with 50 μg of control pCXN2 (without insert), pCXN2 containing ICSBP or DBD cDNA by electroporation with a Cell-Porator (Life Technologies) as previously described (11). The cells were selected with G418 at 400 μg/ml for 14 days then cloned by limited dilution at 0.5 cells/96 microtiter well.

4. Immunoblot analysis

The cells (1×10^7) were incubated with a lysis buffer (10 mM Tris, pH 7.5, 10 mM NaCl, 15 mM $MgCl_2$, 1 mM AEBSF, and 0.05% Nonidet P-40), and nuclei were then pelleted (14). The nuclei were washed and the nuclear extracts were prepared as previously described (15), then resuspended in a buffer solution containing 20 mM HEPES, pH 7.9, 0.4 M NaCl, 1 mM EDTA, pH 8.0, 1 mM EGTA, 1 mM DTT, and 1 mM AEBSF. Thirty micrograms of nuclear proteins were resolved on 10% (for ICSBP analysis) or 15% (for DBD analysis) SDS-PAGE, electroblotted to Immobilon P nitrocellulose (Millipore, Bedford, MA), and reacted with the appropriate antibodies according to the ECL protocol provided by the manufacturer (Amersham, Arlington Heights, IL). The primary antisera were either rabbit anti-peptide antibodies that react with the C-terminal domain of ICSBP (7) or polyclonal rabbit anti-rICSBP antibodies that react with the DBD epitopes. Each antibody was diluted either 1:500 (for ICSBP) or 1:300 (for DBD), respectively, in a blocking solution containing PBS, pH 7.1, 5% nonfat dry milk, and 0.05% Tween-20. The target proteins were detected with peroxidase-coupled goat anti-rabbit IgG diluted 1:30,000.

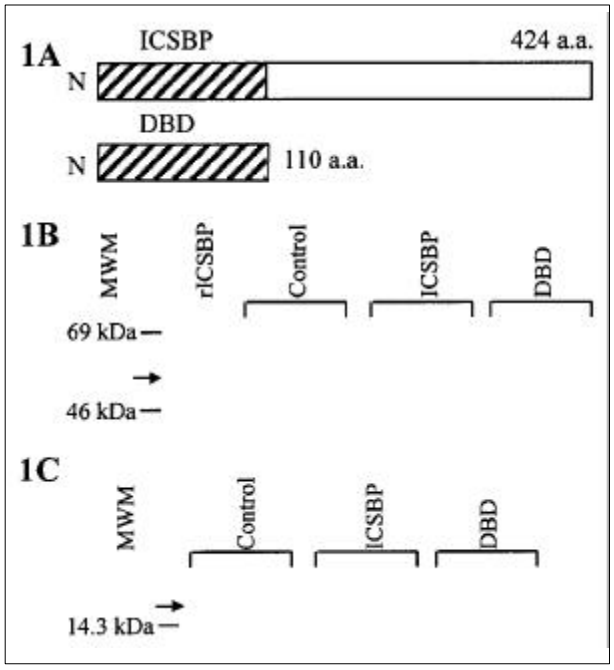

Fig. 1. Immunoblot detection of ICSBP and DBD peptides in HL-60 stable clones. **(A)** Schematic illustration of ICSBP and DBD. The N-terminal 110-aa sequence (hatched) represents the DBD. **(B)** Nuclear extracts (30 μg) from each transfectant clone were resolved on 10% SDS-PAGE and reacted with the appropriate antibodies. Rabbit antibodies to the C-terminal peptide of ICSBP were diluted 1:500 in a blocking solution and the ICSBP was detected with peroxidase-coupled goat anti-rabbit IgG diluted 1:30,000. ICSBP (indicated by an arrow) is strongly expressed only in the ICSBP clones. MWM, molecular weight marker; rICSBP, recombinant ICSBP protein generated in a baculovirus vector[22]. **(C)** DBD was similarly detected as in (B), except that proteins were resolved on 15% SDS-PAGE and the primary antibodies were diluted 1:300. DBD (indicated by an arrow) is distinctly expressed in the DBD clones.

5. Viral infection

The cells (3×10^6) were incubated with the Indiana strain of VSV at 5 plaque-forming units (PFU) per cell for 1 h at 37℃. They were subsequently washed and cultured in a complete medium for the indicated times, and the viral yields from the supernatants were determined by a plaque assay (16). Where indicated, the cells were pretreated with recombinant human IFNα2A (Lee Biomolecular Laboratories, San Diego) at 500 units/ml overnight and cultured with IFN after infection.

RESULTS

1. Expression of ICSBP or DBD in HL-60 transfectants

Clones expressing either ICSBP or DBD (Fig. 1A) were screened by immunoblot analysis. The twenty clones that were propagated from each of three transfection groups were screened.

Using immunoblot analysis, the ICSBP (Fig. 1B) or DBD peptide (Fig. 1C) was confirmed in the respective clones, while none of these peptides were expressed in the control clones. Three representative clones from each

transfection group, shown in Fig. 1, were used for further investigation.

2. Susceptibilities of IFNα-treated stable clones to VSV infection

To compare the susceptibilities of ICSBP, DBD, and control clones to a VSV infection, three clones from each group were infected with VSV and the viral titers were determined by a plaque assay at various times following infection.

In the absence of IFNα, the ICSBP, DBD, and control clones showed similar sensitivities to a VSV infection over 48 h of incubation (Fig. 2A). When these clones were pre-treated with IFNα prior to the VSV challenge and cultured with IFN after infection, the viral titers steadily decreased for up to 48 h of incubation in both the ICSBP and control clones. This suggests that IFN can protect the cells from a VSV infection (Fig. 2B). When the viral titers in the control and ICSBP clones were compared, the titers from the control clones were higher than those from the ICSBP clones by 1.5-fold (24 h) and 2.2-fold (48 h). This suggests that there is an additional resistance to a VSV infection conferred by ICSBP. Unlike the two other clones, the viral titers in the DBD clones increased at 24 h then decreased by 48 h post-infection, indicating an impaired IFN-mediated resistance to a VSV infection. The amount of viral shedding in the DBD clones was 7.9-fold (24 h) and 9.0-fold (48 h) greater than that from the control clones.

DISCUSSION

It has been reported that ICSBP is necessary for establishing a resistance to various pathogens (10,17-19), despite it being a transcriptional repressor of IFN-regulatory genes. ICSBP-deficient mice succumbed upon challenge with certain viruses such as the vaccinia virus and the lymphocytic choriomeningitis virus as well as other intracellular pathogens. A defective T helper cell type 1 (Th1) response, a decrease in cytotoxic T

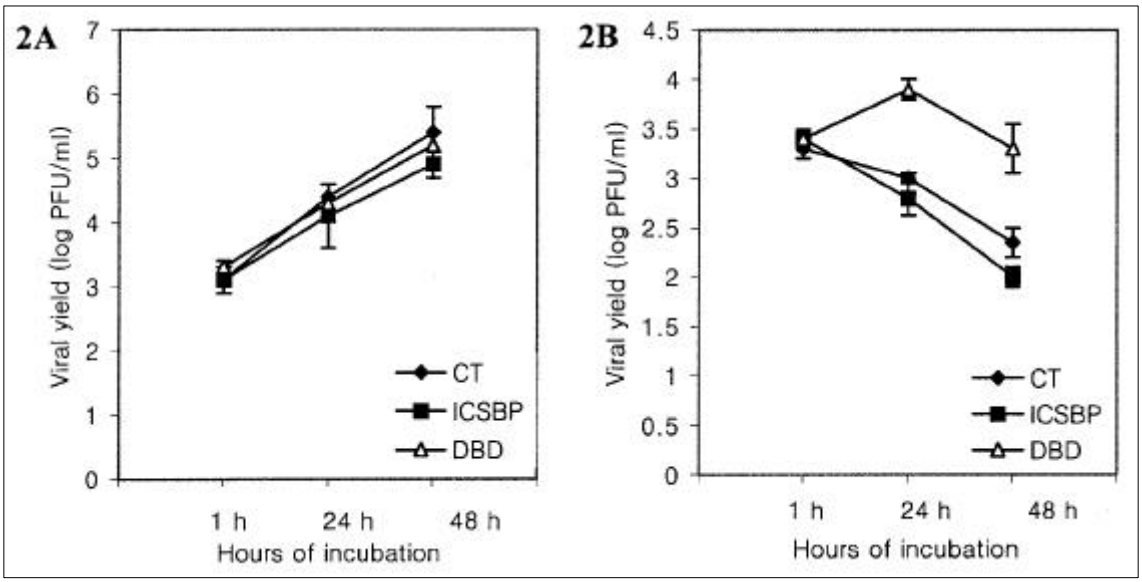

Fig. 2. IFNα-induced protection against VSV infection in the HL-60 control and ICSBP clones. **(A)** Cells (3×10^6) of the clones from each group were infected with VSV at 5 PFU per cell and the viral titers in the resulting supernatants were determined at the indicated times. Viral titers from each of three transfectant groups increased steadily over 48 h of incubation. Values are the mean of viral titers from three clones for each group. Bars represent the range of the three separate measurements. **(B)** When incubated in the presence of IFNα(500 IU/ml), the viral titers in the ICSBP and control clones decreased steadily over 48 h of incubation while those in the DBD clones increased at 24 h then decreased by 48 h. Values and bars are same as above.

lymphocyte activity, as well as the deficient production of IFN γ after stimulation of T cells or macrophages appear to underlie the inability to control these pathogens (10,18). Defects in these IFN γ- mediated immune responses that are observed in ICSBP null mice could be explained by a very recent observation that ICSBP acts as a transcriptional activator for IFN γ-inducible promoters (9). In contrast, these mice survived the VSV infection and mounted a normal neutralizing antibody response, which implies that the B and T helper cell compartments as well as the IFN type I system was normal in these mice. In the present study, IFN γ treatment effectively reversed the sensitivities of HL-60 control and ICSBP clones to a VSV infection (Fig. 2). In a certain viral infection, one of the two IFN systems (type I and II) usually dominates (20). Although attempts to examine the effect of IFN γ on the anti-VSV response were not made, it can be said that at least the IFN type I system was indispensable for an anti-VSV response.

The HL-60 control clones that did not express ICSBP on the immunoblot analysis (Fig. 1B) could control the VSV infection in the presence of IFN γ, even though the ICSBP clones appeared to do it better (Fig. 2B). This indicates that ICSBP is not essential for protecting against a VSV infection in response to IFN, though it is helpful to a certain extent. In a previous study (11), both the control and ICSBP clones of U937 cells exhibited a similar resistance to a VSV infection. The presumable reason for this difference is that U937 cells obviously express ICSBP while HL-60 cells do not. Accordingly, exogenously introduced ICSBP might not have produced as much effect on the U937 cells as it did on HL-60 cells. Unlike two other clones, the DBD clones failed to elicit effective antiviral activity against VSV in response to IFN (Fig. 2B). As the control clones mounted an anti-VSV response effectively, the impaired viral resistance in the DBD clones is not likely to be caused by the expression of truncated ICSBP in these clones. Similarly, defective ICSBP in U937 DBD clones does not appear to be responsible for the impaired anti-VSV response. In this regard, the results of two previous studies on the role of ICSBP in VSV infections (10,11) might not be discordant.

The IRF family proteins are composed of a conserved DBD in the N-terminal region and a divergent C-terminal region that serves as the regulatory domain (3,4) (Fig. 1A). Since the proteins bind the target genes through the DBD, truncated ICSBP lacking the regulatory domain (DBD) blocks the function of other IRF family proteins by occupying their DNA binding sites, producing a dominant negative effect (21). Considering this, the failure of the DBD clones to mount an antiviral response is probably due to the repression of IFN α-inducible antiviral genes by DBD. As discussed above, the lack of intact ICSBP might well be excluded as a reason for the impaired antiviral response in the DBD clones. It is of note that although the DBD clones did not elicit an effective antiviral response, their viral titers were lower in the presence of IFN α than in its absence (Fig. 2). One speculation is that the dominant negative effect by DBD was not sufficient to completely block the binding of other IRF proteins induced by IFN α. Subsequently they might have generated some antiviral activity.

In summary, the present data suggest that ICSBP does not play a crucial role in controlling VSV infections and at least the IFN type I system is important for protecting against a VSV infection.

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