Interferon (IFN) β Acts Downstream of IFN-γ–induced Class II Transactivator Messenger RNA Accumulation to Block Major Histocompatibility Complex Class II Gene Expression and Requires the 48-kD DNA-binding Protein, ISGF3-γ

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Summary

Interferon (IFN) γ, a cardinal proinflammatory cytokine, induces expression of the gene products of the class II locus of the major histocompatibility complex (MHC), whereas IFN-α or -β suppresses MHC class II expression. The mechanism of IFN-β–mediated MHC class II inhibition has been unclear. Recently, a novel factor termed class II transactivator (CIITA) has been identified as essential for IFN-γ–induced MHC class II transcription. We studied the status of IFN-γ–induced CIITA messenger RNA (mRNA) accumulation and CIITA-driven transactivation in IFN-β–treated cells and used cell lines that had defined defects in the type I IFN response pathway to address the roles of IFN signaling components in the inhibition of MHC class II induction. IFN-β treatment did not suppress IFN-γ–induced accumulation of CIITA mRNA. After cells were stably transfected with CIITA, endogenous MHC class II genes were constitutively expressed, and MHC class II promoters, delivered by transfection, were actively transcribed in CIITA-expressing cells. Expression of these promoters was significantly impaired by pretreatment with IFN-β. These results suggest that IFN-β acts downstream of CIITA mRNA accumulation, and acts in part by reducing the functional competence of CIITA for transactivating MHC class II promoters. IFN stimulated gene factor 3 (ISGF3) γ was essential for IFN-β to mediate inhibition of MHC class II induction, regardless of whether MHC class II transcription was stimulated by IFN-γ or directly by CIITA expression. Results of these experiments suggest that inhibition of MHC class II in IFN-β–treated cells requires expression of gene(s) directed by the ISGF3-IFN-stimulated response element pathway, and that these gene product(s) may act by blocking CIITA-driven transcription of MHC class II promoters.

Biochemical and genetic studies of signaling by the IFNs recently culminated in a convincing and lucid description of the process by which type I IFNs induce transcription of a set of immediate response genes, termed the IFN-responsive genes (ISGs)[1] (1). This pathway has been proposed as a paradigm for a "direct effector" model of transcriptional regulation by cytokines, involving protein–protein interactions to form a transcriptional activator, IFN-stimulated gene factor 3 (ISGF3), which can activate transcription via an inducible enhancer, the IFN-stimulated response element (ISRE). Upon receptor binding by IFN-α or IFN-β, latent cytoplasmic transcription factors, collectively termed ISGF3-α, were shown to be phosphorylated on tyrosine residues and to accumulate in the nucleus, where they formed a complex with a 48-kD DNA-binding protein, designated ISGF3-γ. These components were designated ISGF3, which was shown to be essential to induce

[1] *Abbreviations used in this paper:* CAT, chloramphenicol acetyltransferase; CIITA, class II transactivator; EMSA, electrophoresis mobility shift assay; GAS, γ-activated sequence; ISG, IFN-responsive gene; ISGF3, IFN-stimulated gene factor 3; ISRE, IFN-stimulated response element; mRNA, messenger RNA; MS, multiple sclerosis; PTK, protein tyrosine kinase; STAT, signal transducer and activator of transcription.

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transcription from ISG promoter/enhancer elements containing ISREs.

Unexpectedly, this research revealed that type I (IFN-α or IFN-β) and type II (IFN-γ) IFNs, despite signaling through unique receptors, share certain transcriptional regulatory components (2). In particular, a 91-kD ISGF3-α component, designated signal transducer and activator of transcription (STAT) 1α, and a nonreceptor protein tyrosine kinase (PTK) termed JAK1, proved to be absolutely required for response to both types I and II IFNs (2–4). Other components, such as the transcription factors ISGF3-γ and p113/STAT-2, as well as the PTK tyk2, were implicated particularly in signaling by the type I IFNs. The PTK JAK2 was used specifically by IFN-γ (1, 5, 6). These results explained previously described signaling interactions between the partially overlapping pathways used by the two IFN types (7).

The ISGs encode products that mediate the biological consequences of IFN treatment, including antiviral, growth-regulatory, and immune-modulatory effects (8). Prominent among these effects, IFN treatment regulates expression of MHC antigens (8). Either of the two IFN subtypes, type I or type II IFN, up-regulate expression of MHC class I antigens (9). It has been shown that MHC class II antigens were responsive to IFN-γ, whereas IFN-α and -β were typically unable to induce MHC class II expression (9). In fact, for many cell types, type I IFN was shown to block the induction of MHC class II expression by IFN-γ (10–12). These contrasting consequences of IFN-γ and IFN-β treatment for MHC class II expression might be of pathogenetic significance, as IFN-γ and IFN-β recently exhibited highly divergent effects in regulating the human inflammatory demyelinating disorder multiple sclerosis (MS). MS patients treated with IFN-γ experienced elevated disease activity, accompanied by immune stimulation (13, 14). Patients treated with IFN-β experienced fewer and milder attacks, with diminished disease progression as monitored by magnetic-resonance brain scanning (15, 16). IFN-β may ameliorate MS by down-regulating pathogenic MHC class II expression in the affected central nervous system tissues (17).

In this regard, we and others have shown that IFN-β–mediated blockade of IFN-γ–induced MHC class II expression occurred at the transcriptional level (18, 19). Transcriptional suppression of MHC class II by IFN-β was relatively gene specific and did not require sequence content, beyond the conserved MHC class II *cis*-regulatory elements that were also required for response to IFN-γ (20).

MHC class II expression has been intensely investigated in recent years (9, 21–24). Expression of MHC class II was shown to be regulated developmentally and environmentally, in a remarkably specific and stringent fashion. Transcriptional activity of the MHC class II genes has been shown to determine their expression, and several lines of evidence have indicated selective transcriptional control of the MHC class II genes. The strongest support for this notion came from human patients with bare lymphocyte syndrome, an autosomal-recessive deficiency of MHC class II gene expression (25). Further, two groups generated cell lines with selective defects in the IFN-γ pathway for MHC class II expression (26, 27). MHC class II gene induction by IFN-γ required intermediary protein synthesis in most cells (28, 29). Additionally, the regulatory *cis* element for MHC class II transcription was quite different from the γ-activated sequence (GAS) element that governed the direct (protein synthesis–independent) response to IFN-γ (1). Taken together, studies of IFN-γ induction of MHC class II transcription indicated an indirect mechanism requiring synthesis of a protein factor, in addition to the direct JAK–STAT pathway. Class II transactivator (CIITA), a novel transcription factor deficient in one bare lymphocyte syndrome complementation group, has recently been identified by a variety of genetic and biochemical strategies as the IFN-γ–inducible factor that was necessary for MHC class II transcription (30–33).

Based on recent reports, CIITA is proposed to activate MHC class II transcription by interacting (directly or indirectly) with factors bound to regulatory DNA elements upstream of the structural genes (34, 35). Furthermore, CIITA contains a potent NH_2-terminal transactivation domain that functions in heterologous context (34, 35). Taken in aggregate, these observations prompted the attractive hypothesis that physical interaction between CIITA and promoter-bound components positions the transactivator domain to provide a stimulatory interface between the MHC class II gene–specific factors and the basal transcription apparatus (34, 35).

Experiments described in this report addressed the role of CIITA in the pathway by which IFN-β inhibited MHC class II expression. Additionally, cell lines with defined defects in type I IFN signaling were used to address the functions of individual ISRE–ISGF3 signaling components. Initial experiments were performed in the parental fibrosarcoma cell line 2fTGH, which responds to IFN-γ with expression of MHC class II antigens, and this induction can be readily suppressed by IFN-β. As anticipated, CIITA messenger RNA (mRNA) accumulated in 2fTGH cells after induction with IFN-γ. We found that IFN-β treatment did not suppress IFN-γ–induced CIITA mRNA accumulation. 2fTGH cells that were stably transfected with a CIITA expression construct displayed MHC class II antigens in the absence of IFN-γ, and MHC class II promoter/reporters were strongly activated in CIITA-transfected cells. IFN-β pretreatment impaired the expression of MHC class II promoter/reporter constructs in CIITA-transfected cells, and this inhibition was observed at modest concentrations of IFN-β. Mutant cell lines were used to show that functional ISGF3-γ was required for IFN-β to mediate inhibition of MHC class II expression, either induced by IFN-γ treatment or directly by CIITA expression.

Taken together, these results indicate that IFN-β treatment blocks MHC class II expression at a point subsequent to the accumulation of CIITA mRNA. Our observations in cell lines with defined defects in IFN signaling suggest the hypothesis that an IFN-β–induced gene product, expressed via the ISRE–ISGF3–dependent pathway, is responsible for the ability of IFN-β to down-regulate MHC

class II expression. Data reported here also demonstrate that IFN-β impairs the expression of MHC class II promoters in CIITA-transfected cells. We propose that this inhibitory effect for CIITA-driven MHC class II transcription accounts at least in part for the ability of IFN-β to abrogate IFN-γ–induced MHC class II expression.

Materials and Methods

Cytokines. Purified human recombinant IFN-γ (1.9×10^7 U/mg protein) was generously provided by Genentech, Inc. (South San Francisco, CA). Recombinant human IFN-β (10^8 U/mg protein) was generously provided by Biogen (Cambridge, MA). Wellferon, a purified mixture of IFN-α subtypes (10^8 U/mg protein), was kindly provided by Wellcome Research Laboratories (Kent, UK).

Plasmids. Construction of pCIITA.2.11 and its use to direct expression of functional CIITA have been described (31, 35). Construction of pDRA(267)CAT was previously described (20). This promoter–reporter construct contains 267 bp of 5′-flanking sequence of the human HLA-DRA gene and 27 bp of transcribed sequence, fused to a splice cassette, and the bacterial chloramphenicol acetyltransferase (CAT) gene. pGL2, an expression plasmid containing a luciferase reporter gene under control of an SV40 promoter, was obtained from Promega Corp. (Madison, WI).

Cell Culture. 2fTGH and all derivative cell lines were grown in complete medium: DME with 10% heat-inactivated FCS (GIBCO/BRL, Gaithersburg, MD) and 250 μg/ml of hygromycin B (Sigma Chemical Co., St. Louis, MO) (36).

Mutant Cell Lines Defective in Response to IFN-α/β. The 2fTGH fibrosarcoma cell line and derivative mutants exhibiting defects in their response to IFN-α were generated by lethal selection (37). Recessive mutant cell lines were organized into complementation groups, designated U (unresponsive)-1 through U-6 (1, 37). Each complementation group contains one or more cell lines that are genetically defective for individual components of the IFN signaling pathway (1, 2, 36–38). The parental cell line 2fTGH exhibits wild-type response to types I and II IFN (36). U1A (initially designated 11.1) lacks tyk2 and is unresponsive to IFN-α, but retains partial response to IFN-β and full response to IFN-γ (6, 36). U2A lacks functional ISGF3-γ and is unresponsive to IFN-α and IFN-β for gene expression directed by the ISRE, but retains full response to IFN-α for an ISRE/ISGF3-independent pathway (1, 6, 36, 38, 39). U2A cells activate all IFN-γ–responsive genes tested (including the MHC class I and II antigens) except 1-8 and 9-27 (1, 38–40).

Cell Lines Expressing CIITA 2.11. For these studies, 2fTGH and derivative cell lines were cultured in 10-cm^2 dishes to ~70–80% confluence and incubated with prewarmed complete medium containing pCIITA.2.11 and pSV2neo (41) plasmid DNAs (in 10:1 molar ratio) and 10 μg/ml hexadimethrine bromide (Polybrene; Sigma Chemical Co.) for 6 h. Cells were washed once with PBS and shocked for 2 min with 30% DMSO (Sigma Chemical Co.). The cells were incubated in 10 ml complete medium for 2 d and subjected to selection in complete medium supplemented with 500 μg/ml G418 (Sigma Chemical Co.) for 2–3 wk.

Restoration of ISGF3-γ Activity in U2A Cells. U2A cells were transfected with pDRISGF3γ (kindly provided by Dr. Ganes Sen, Research Institute, Cleveland Clinic Foundation) and pSV2neo DNA in 10:1 molar ratio, subjected to selection for G418 (500 μg/ml) resistance for 2 wk, and then selected for restoration of type I IFN response in DME containing IFN-α (Wellferon, 500 U/ml) and 1× hypoxanthine–aminopterin–thymidine medium (GIBCO BRL) for 1 wk, incubated without aminopterin in DME containing 1× hypoxanthine–thymidine medium (GIBCO BRL) for 4 d, and passaged routinely in complete DME (36). IFN-α responsiveness was confirmed by demonstrating MHC class I induction.

Northern Blot Hybridizations. Northern blots were performed as described previously (19). A CIITA cDNA fragment was generated by PCR from a Daudi cell cDNA library kindly provided by Dr. Taolin Yi (Cleveland Clinic Foundation) using primers that amplified residues 2909–3534 of the published sequence (42). The identity of the amplified segment was confirmed by sequence analysis, after which a gel-isolated fragment was labeled by random priming for hybridization.

Normalized Transient Transfections. Cells were cultured in 10-cm^2 dishes to ~70–80% confluence in the presence or absence of IFN-β. Medium was replaced with 3 ml of prewarmed complete medium containing 10–20 μg of pDRA(267)CAT plasmid DNA, 2–4 μg of pGL2 luciferase expression plasmid DNA (Promega Corp.), and 10 μg/ml Polybrene for 6 h, and the cells were washed once with PBS and shocked with 30% DMSO in 3 ml of serum-free media for 2 min at room temperature. Cells were washed twice with incomplete medium and incubated for 48 h in complete medium before harvesting and normalized CAT assay.

Cells were scraped in 1.4 ml PBS, pelleted for 2 min in a microcentrifuge, and resuspended in 70 μl of 1× luciferase lysis buffer (Promega Corp.). Cell extracts were prepared by lysis with three cycles of freezing and thawing and clarified for 10 min in a microcentrifuge. 10 μl of extract was subjected to luciferase assay according to the manufacturer's instructions. Extract input to CAT assays was normalized to luciferase activity to correct for transfection efficiency.

Flow Cytometry. Cells were immunostained for HLA-DR and an isotype-matched control and analyzed on a FACscan® (Becton Dickinson & Co., Mountain View, CA) as described (12). For each data point, the percentage of cells staining with isotype-matched control was subtracted from the experimental value, and results were expressed as the percentage of HLA-DR–positive cells.

Because of variability in HLA-DR induction, data for these experiments were not normally distributed; however, the percentages of inhibition by IFN-α and IFN-β were highly reproducible. Representative experiments are presented in the figures. Mean inhibitions of HLA-DR induction in preliminary experiments were as follows: by IFN-α in 2fTGH cells, 71.3% ($n = 2$); by IFN-β in 2fTGH cells, 94.2% ($n = 6$); by IFN-α in U1A cells, 20% ($n = 2$); by IFN-β in U1A cells, 48.8% ($n = 2$); by IFN-α in U2A cells, 0% ($n = 1$); by IFN-β in U2A cells, 5% ($n = 3$).

Results

IFN-γ–induced CIITA mRNA Accumulation Is Not Affected by IFN-β. It has recently been shown that CIITA was necessary for IFN-γ–induced MHC class II transcription (30, 31, 33). This surprising result provided a unitary explanation for distinctive characteristics of IFN-γ regulation of MHC class II expression, including the requirement for ongoing protein synthesis.

We considered the possibility that IFN-β might inhibit synthesis of CIITA as a plausible mechanism for suppressing MHC class II expression. Northern blot hybridizations showed that CIITA mRNA was induced in 2fTGH fibro-

sarcoma cells by IFN-γ. CIITA mRNA accumulation was unaffected by IFN-β treatment at saturating concentrations that, in preliminary experiments, completely abrogated MHC class II expression (Fig. 1). This result indicated that the inhibition of MHC class II expression by IFN-β was not mediated by blocking CIITA mRNA accumulation.

IFN-β Blocks Expression of MHC Class II Promoter/Reporters in CIITA-expressing Cells. Expression of CIITA through DNA-mediated gene transfection can induce MHC class II transcription in the absence of IFN-γ (30, 31, 33). CIITA.2.11, an expression construct for CIITA that directs the synthesis of functional protein, was stably transfected into 2fTGH cells (31). Endogenous MHC class II genes were induced in CIITA-expressing 2fTGH/CIITA.2.11 cells, as monitored by flow cytometry (data not shown). This MHC class II expression was dependent on expression of the transfected CIITA gene, as transfection with CIITA.10, which encodes a nonfunctional protein, did not produce MHC class II expression (data not shown) (35).

In cells stably transfected with CIITA.2.11, endogenous MHC class II expression was constitutive. To determine the effect of IFN-β treatment on constitutive CIITA-driven MHC class II expresson, 2fTGH/CIITA.2.11 cells were left untreated or exposed to IFN-β (1,000 U/ml) for 48–72 h and were assayed by flow cytometry for MHC class II antigen. Under these conditions, IFN-β did not inhibit CIITA-driven MHC class II expression (data not shown).

It remained possible that IFN-β treatment blocked an early stage of MHC class II gene expression in CIITA-transfected cells, and that FACscan® assay of HLA-DR protein was insensitive to this effect. To address this point, CIITA-driven expression of MHC class II promoter/reporters was assayed after normalized transient transfection. For these experiments, a promoter–reporter construct derived from the 5′-flanking region of the human MHC class II HLA-DRA gene, pDRA(267)CAT was used (20). This construct contains all sequence content required for the induction and inhibition of MHC class II transcription. (20). Expression of this MHC class II reporter, induced by CIITA expression, was equivalent to that produced by optimal concentrations of IFN-γ. Activity of a GAS-containing promoter–reporter construct, pGBP.CAT, was unaffected by CIITA expression, documenting specificity for MHC class II (data not shown).

2fTGH/CIITA.2.11 cells were preincubated with IFN-β or left untreated before transient transfection with pDRA-(267)CAT. Pretreatment with IFN-β (125 U/ml) caused significant inhibition of MHC class II transcription in 2fTGH/CIITA.2.11 cells (Fig. 2; $P = 0.007$ for medium vs. 125 U/ml IFN-β by paired means comparison). Further, we observed a significant inhibition in cells exposed to 25 U/ml IFN-β (Fig. 2; $P = 0.025$ for medium vs. 25 U/ml). The magnitude and dose dependency of this inhibition were closely related to the IFN-β–mediated inhibition of IFN-γ–induced expression of the endogenous HLA-DR MHC class II antigen.

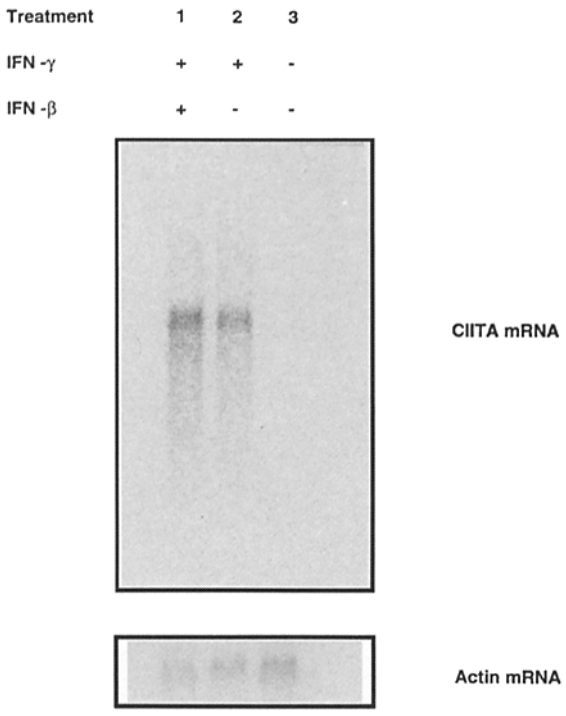

Figure 1. Northern blot analysis of CIITA mRNA in 2fTGH cells: regulation by IFN-γ and IFN-β. Cells were exposed for 6 h to IFN-γ (500 U/ml) alone or in combination with IFN-β (1,000 U/ml) as indicated, before preparation of polyadenylated RNA and blot hybridization analysis. The filter was rehybridized with β-actin probe (*inset*) to normalize for loading variation. Exposure to PhosphorImager screen for CIITA: 48 h; exposure for β-actin: 30 min.

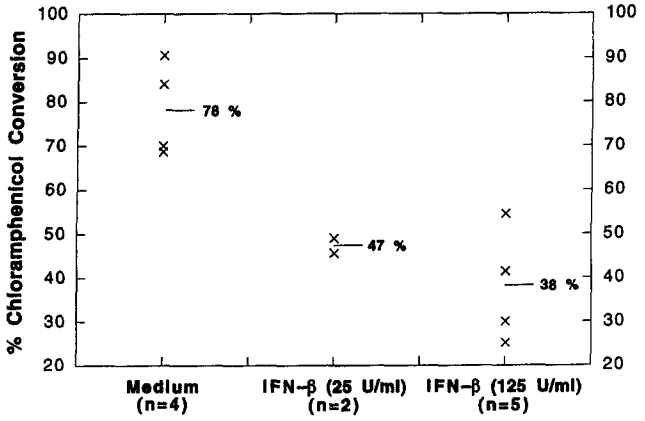

Figure 2. IFN-β pretreatment impairs expression of MHC class II promoters in CIITA-expressing 2fTGH cells. 2fTGH cells were stably transfected with CIITA.2.11 by cotransfection with a selectable marker (20). These 2fTGH/CIITA.2.11 cells were exposed to IFN-β at indicated concentrations overnight or left as untreated controls before washing and transient cotransfection with pDRA(267)CAT (10 μg) and pGL2 (5 μg). 2fTGH/CIITA.2.11 cells were then incubated with or without IFNs as indicated for 48 h before harvest. An aliquot of the cell lysate was subjected to luciferase assay, and luciferase activity was used to correct extract inputs to CAT assays, to normalize for transfection efficiency. Each data point represents one assay; two overlapping data points are present in the column showing IFN-β treatment at 125 U/ml. Mean percentage of chloramphenicol acetylation is indicated. IFN-β pretreatment significantly inhibited expression of pDRA(267)CAT.

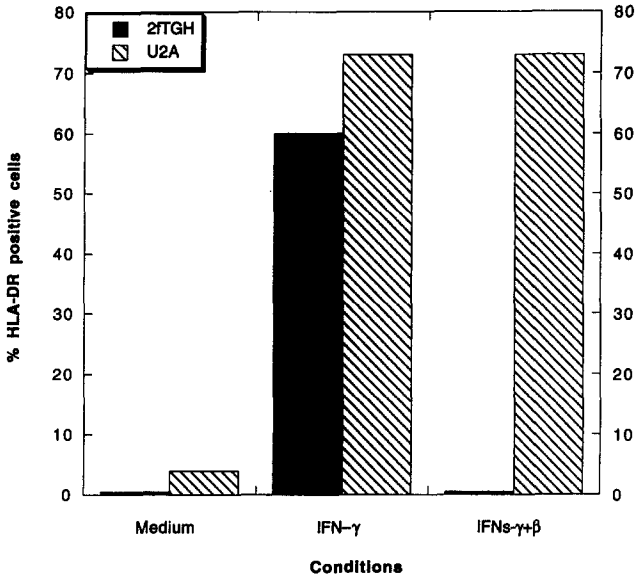

Figure 3. ISGF3-γ is required for IFN-β suppression of IFN-γ–induced MHC class II expression. Cells were treated for 48 h with IFN-γ (100 U/ml) alone or in the presence of IFN-β (1,000 U/ml) before immunostaining with mAb to HLA-DR or isotype-matched control and analysis by FACScan®.

ISGF3-γ Is Required for IFN-β–mediated Inhibition of IFN-γ–induced MHC Class II Expression. IFN-β treatment activates a signaling cascade that culminates in expression of gene products, which in turn mediate many of the biological effects attributed to IFN-β. One possible mechanism by which IFN-β could inhibit MHC class II transcriptional induction was by activation of a preexisting signaling component (e.g., protein kinase) in the IFN-β pathway. Alternatively, IFN-β could require de novo gene expression to impair transcriptional induction of MHC class II genes.

It was possible to test the role of IFN-β–activated signaling components in the inhibition of MHC class II through the use of cell line U2A, which lacked functional ISGF3-γ (1, 38). Despite the absence of functional ISGF3-γ in U2A cells, IFN-α/β signaling leading to the generation of activated STAT-1α/STAT-2 heterodimers (ISGF3-α) has been documented (1, 38). Furthermore, studies in U2A cells showed that IFN-α induces expression of the IRF-1 gene through an alternative pathway that generates DNA-binding complexes containing STAT-1α (39). These results documented that U2A cells have intact IFN-α/β–activated signaling components, which include the IFN-α/β receptor complex, JAK1, tyk2, STAT-1α, and STAT-2 (39). U2A was shown in these studies to be deficient only in ISRE-regulated gene expression.

IFN-β failed to inhibit IFN-γ-induced MHC class II antigen expression in mutant U2A cell line (Fig. 3). This result suggests that ISGF3-γ is required for down-regulation of IFN-γ–induced MHC class II expression; however, one possible trivial explanation for this observation would be a second site mutation in U2A cells. To address this issue, we restored functional ISGF3-γ by transfection and back-selection, producing cell line U2A/p48, which was fully responsive to IFN-β. In U2A/p48 cells, IFN-β suppressed MHC class II induction equally as observed in the wild-type 2fTGH line, demonstrating formally that ISGF3-γ was required for IFN-β to inhibit MHC class II expression (Fig. 4). These observations suggest that IFN-β acts through the ISGF3/ISRE-dependent pathway to induce the expression of gene(s) whose product(s) mediate the inhibition of MHC class II transcriptional induction.

To address further the role of gene expression in IFN-β inhibition of MHC class II, we performed studies in mutant cell line U1A, which was deleted for PTK tyk2, defective in IFN-α binding to the receptor, and completely un-

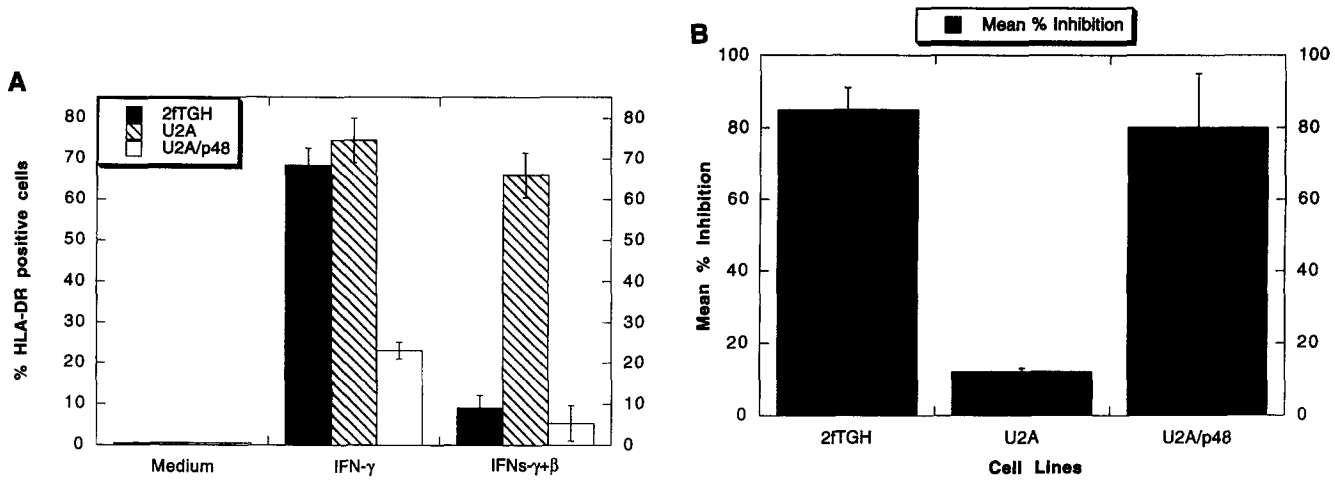

Figure 4. Restoration of ISGF3-γ to U2A cells repairs the defect for MHC class II inhibition by IFN-β. (*A*) 2fTGH, U2A, and U2A/p48 cells were exposed to cytokines as indicated for 48 h before harvest, immunostaining with mAbs to HLA-DR or isotype-matched control, and FACS® analysis. Means and SE of two independent experiments are shown. (*B*) Mean percentage of inhibition of HLA-DR induction in each experiment was calculated as:

$$\left\langle 1 - \left(\frac{[\%\text{HLA-DR-positive cells in IFN-}\gamma \oplus \text{-}\beta\text{-treated sample}]}{[\%\text{HLA-DR-positive cells in IFN-}\gamma\text{-treated sample}]} \div \right) \right\rangle \times 100.$$

responsive to IFN-α for induction of ISGs (6, 36). However, U1A cells retained a weak (~50%) gene regulatory response to IFN-β (36). IFN-β inhibited MHC class II induction in U1A cells by 49% (n = 2) vs. 94% inhibition in 2fTGH cells (n = 6; data not shown). IFN-β caused a 45% reduction of mean peak channel relative fluorescence intensity of HLA-DR–positive U1A cells, in addition to reducing the number of positive cells. This observation excluded the possibility that one subpopulation of U1A cells responded with wild-type competence to IFN-β, whereas a separate population of cells remained entirely refractory. We concluded that essentially all U1A cells responded weakly to IFN-β for inhibition of MHC class II induction. As expected, induction of HLA-DR by IFN-γ was resistant to blockade in U1A cells by IFN-α (20% inhibition vs. 71% inhibition in 2fTGH cells, n = 2; data not shown). These results indicate that PTK tyk2 is dispensable for IFN-β–mediated inhibition of MHC class II induction and further support a correlation between ISGF3/ISRE-dependent gene expression and IFN-β inhibition of MHC class II.

ISGF3-γ Is Required for IFN-β–mediated Inhibition of CIITA-induced MHC Class II Transcription. Our results suggest that IFN-β inhibits IFN-γ–induced MHC class II expression by impairing transcriptional induction from MHC class II promoters, even in the presence of CIITA (Fig. 2). U2A cells were used to study the role of IFN-β–activated signaling components in inhibiting MHC class II induction (Figs. 3 and 4). To address the role of these signaling components in suppressing CIITA-driven MHC class II expression, U2A cells that stably expressed CIITA.2.11 were constructed. Strikingly, IFN-β failed to inhibit expression of pDRA(267)CAT in U2A/CIITA.2.11 cells in normalized transient transfections (Fig. 5). This observation further supports the conclusion that IFN-β induces expression of gene(s), whose product(s) impairs IFN-γ–induced MHC class II expression, acting downstream of CIITA mRNA accumulation.

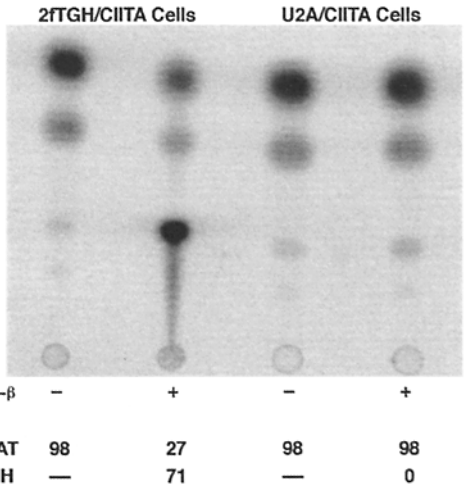

Figure 5. ISGF3-γ is required for IFN-β suppression of CIITA-driven MHC class II promoter/reporter expression. 2fTGH and U2A cells were stably transfected with CIITA.2.11 by cotransfection with a selectable marker (20). 2fTGH/CIITA.2.11 cells or U2A/CIITA.2.11 cells were exposed to IFN-β (500 U/ml) overnight or left as untreated controls before washing and transient cotransfection with pDRA(267)CAT (10 μg) and pGL2 (5 μg). Cells were then incubated in complete medium with or without IFNs as indicated for 48 h before harvest. An aliquot of the cell lysate was subjected to luciferase assay, and luciferase activity was used to correct extract inputs to CAT assays to normalize for transfection efficiency. An autoradiogram is shown; the percentage of conversion of CAT to acetylated product (*% CAT*) was determined by autoradiography on a PhosphorImager. The percentage of inhibition of promoter activity by IFN-β (*% INH*) was determined as described in the legend to Fig. 4 and is indicated below.

Discussion

CIITA has been identified as a novel IFN-γ–inducible factor required for MHC class II transcription, providing new insight into the intricate regulation of these important gene products. The selective function of CIITA for regulating MHC class II expression corresponded to the specific inhibition of MHC class II (among IFN-γ–inducible genes) by IFN-β. This relationship prompted the hypothesis that IFN-β might suppress CIITA mRNA accumulation or act downstream of CIITA expression to block its function in mediating MHC class II induction. In this report, we describe studies of the effect of IFN-β on IFN-γ induction of CIITA mRNA; we further characterize IFN-β treatment effects on the function of CIITA, after expression by DNA-mediated gene transfer. It was found that IFN-β did not block the accumulation of the CIITA mRNA. This result was consistent with the status of CIITA as an immediate response gene for IFN-γ (33). Among IFN-γ–induced immediate response genes, only one (ICSBP) has been shown to be inhibited by type I IFNs, specifically in macrophage cells (43, 44). Our data localize the inhibitory effect of IFN-β for MHC class II expression subsequent to IFN-γ–induced CIITA mRNA accumulation.

We observed that IFN-β treatment significantly impaired expression of MHC class II promoters in CIITA-expressing cells in transient transfection experiments (Fig. 2). This result indicates that CIITA protein is not fully functional in IFN-β–treated cells. The most straightforward explanation for this effect of IFN-β treatment would be that accumulation or competence of CIITA protein is modulated. Potential mechanisms include destabilization or posttranslational modification of the CIITA protein, abrogation of CIITA interaction with MHC class II–specific factors associated with promoter elements, or blockade of nuclear accumulation of CIITA. A plausible alternative is that IFN-β treatment could result in modification or displacement of any of the components required for MHC class II transcription, including the factors (such as RF-X, X2BP, NF-Y) that contact DNA directly. The interference with these factors would then secondarily produce an apparent abrogation of CIITA function.

In other systems, IFN-mediated transcriptional inhibition has shown varied characteristics. Vilcek and colleagues showed that the IFN-mediated inhibition of TNF-α–induced IL-8 did not require protein synthesis, and the ef-

fect was shortlived (45). We used mutant cell lines for IFN signaling components to address whether gene expression was required for the inhibition of IFN-γ–induced and CIITA-driven MHC class II transcription. The most informative mutant was U2A, a cell line lacking ISGF3-γ but otherwise competent for IFN signaling. IFN-β failed to inhibit MHC class II transcription in U2A cells, whether stimulated by CIITA or by IFN-γ. Restoration of ISGF3-γ by transfection and back-selection for IFN response also repaired the ability of IFN-β to block MHC class II expression. This experiment confirmed that a fortuitous second mutation did not determine the U2A phenotype of resistance to MHC class II inhibition. IFN-β mediated a partial inhibition of HLA-DR induction in U1A cells. In experiments reported previously, ISG expression was stimulated by IFN-β in U1A cells with ~50% of wild-type efficiency (36).

The observations described in this report establish a close relationship between IFN-β–inducible gene expression via the ISGF3–ISRE pathway and inhibition of MHC class II expression. IFN-β was inert for inhibition of MHC class II in U2A cells, where all components of IFN type I signaling were intact except for gene expression through the ISGF3–ISRE pathway. Conversely, IFN-β mediated weak inhibition of MHC class II expression in U1A cells, where there remained a partial gene-regulatory response directed through the ISRE. The inducible component that mediates inhibition of MHC class II transcription remains to be identified. Several DNA-binding factors (YB-1, NF-X1) and a novel cytokine, IK, were recently described as inhibitors of MHC class II expression or transcription (46–48). We did not find IFN-β–inducible soluble factors that blocked MHC class II expression in prior studies (20). Induction of YB-1 or NF-X1 by IFN-β has not been addressed.

IFN-β–mediated inhibition was not observed for the endogenous MHC class II genes that were constitutively expressed in CIITA-transfected cells. Furthermore, when IFN-β treatment was introduced 12 h after transfection with MHC class II promoter–driven constructs, no inhibition was observed (data not shown). These characteristics were similar to those we reported previously for IFN-β effects on expression of the MHC class II genes. In particular, IFN-β failed to inhibit constitutive MHC class II expression by human monocytes (19, 49). Further, we previously observed that IFN-β did not block MHC class II induction in human astrocytoma cells that were highly sensitive to such inhibition, when added ≥6 h after IFN-γ (Ransohoff, R., and G.T. Babcock, unpublished observations). These observations may indicate that CIITA is not susceptible to the inhibitory effects of IFN-β after its production and activation. In this context, it is not certain whether CIITA expression by DNA-mediated gene transfer is identical in all respects to its induction by IFN-γ, and in particular whether posttranslational modifications occur in IFN-γ–induced cells. The stoichiometric relationship between CIITA and IFN-β–induced inhibitor(s) may also be critical for interpretation of these experiments. It is possible that CIITA levels in stably transfected cells are sufficient to titrate IFN-β–induced components, so that inhibition of MHC class II promoter expression is relatively modest (Fig. 2).

Recent information about functional domains of CIITA has come from detailed structure–function analysis (34, 35). A surprisingly strong NH_2-terminal acidic transactivation domain functions efficiently for heterologous promoters and in yeast cells, indicating general competence as a eukaryotic transactivator. CIITA requires the conserved MHC class II DNA elements to drive transcription, a property that is conferred by the COOH terminus of the protein, possibly through physical interaction with factors that contact DNA directly. Our finding that CIITA function is sensitive to IFN-β action suggests the prospect that CIITA-mediated transactivation or interaction with DNA-binding factors may be targeted by an IFN-inducible component. These possibilities can be experimentally addressed.

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