Mechanisms of Signal Transduction:
Role of Nuclear Factor-κB in the Antiviral Action of Interferon and Interferon-regulated Gene Expression

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Role of Nuclear Factor-κB in the Antiviral Action of Interferon and Interferon-regulated Gene Expression*

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Interferons (IFNs) play critical roles in host defense by modulating the expression of various genes via tyrosine phosphorylation of STAT transcription factors. IFN-αβ activates another important transcription factor, nuclear factor-κB (NF-κB), but its role in IFN-mediated activity is poorly understood. Sensitivity to the antiviral and gene-inducing effects of IFN was examined in normal fibroblasts and in NF-κB knockout fibroblasts from p50- and p65-null mice. Antiviral assays demonstrated that NF-κB knockout fibroblasts were sensitized to the antiviral action of IFN. Moreover, analysis of IFN-stimulated gene expression by real-time PCR demonstrated selective effects of NF-κB on gene expression. Our results demonstrate that a subset of IFN-stimulated genes is regulated through an NF-κB-dependent pathway and that NF-κB may regulate the sensitivity of cells to IFN-mediated antiviral activity.

Interferons (IFNs) are a family of multifunctional cytokines that block viral infection, inhibit cell proliferation, and modulate cell differentiation. Whereas type I IFNs (IFN-α, IFN-β, and IFN-ω) bind to a common cell-surface receptor, the receptor for type II IFN (IFN-γ) is a distinct entity (1). IFNs transduce signals from the cell surface, resulting in selective gene induction through the activation of JAK tyrosine kinases and STAT transcription factors (1–3). Upon JAK-mediated tyrosine phosphorylation, the STAT proteins (STAT1, STAT2, and STAT3) dimerize and translocate to the nucleus to activate transcription of IFN-stimulated genes (3). IFN also activates the nuclear factor-κB (NF-κB) transcription factor in a serine/threonine kinase-dependent signaling pathway that protects cells against apoptosis (4, 5).

NF-κB regulates the expression of genes involved in cell signaling, stress responses, growth, survival, and apoptosis by binding to cis-acting κB sites in the promoters and enhancers of these genes. Viruses, cytokines, lipopolysaccharides, and other stimulating agents promote NF-κB translocation to the nucleus and DNA binding. Active DNA-binding forms of NF-κB are dimeric combinations of Rel proteins (p50, p52, c-Rel, RelB, p50/p65, and RelB). In most cell types, the predominant form of NF-κB is the p50–p55 heterodimer. NF-κB dimers are retained in the cytoplasm of unstimulated cells in an inactive state by the binding of a family of inhibitory IκB proteins.

Whereas STAT proteins play crucial roles in the transcriptional response to IFN-αβ and in the induction of antiviral activity, the role of NF-κB in IFN signaling and action has not been studied extensively. To identify the functional role of NF-κB in IFN action, sensitivity to the antiviral effect of IFN was examined in fibroblasts that either had normal NF-κB function or had a functional deletion of the IFN-induced NF-κB pathway by germ line disruption of the Rel p50 and p65 proteins. Antiviral assays demonstrated that NF-κB knockout (KO) fibroblasts were sensitized to the antiviral action of IFN-β. To determine the relationship between gene regulation by IFN-β and antiviral activity, microarray analysis was performed on RNA samples collected from IFN-treated murine fibroblasts. Microarray analysis identified several classical IFN-stimulated genes (ISGs) involved in the antiviral action of IFN. Quantitative real-time PCR demonstrated that, whereas the IFN-induced expression of some ISGs was enhanced in NF-κB KO cells relative to mouse wild-type fibroblasts, the IFN-induced expression of other ISGs was lower in NF-κB KO cells. Our results demonstrate the distinctive role of NF-κB in the regulation of ISGs and in the induction of antiviral activity. Thus, the IFN-activated NF-κB pathway not only counterbalances apoptosis, but also regulates the expression of ISGs and the induction of antiviral activity.

EXPERIMENTAL PROCEDURES

Biological Reagents and Cell Culture—Recombinant Chinese hamster ovary cell-expressed rat IFN-β was obtained from Biogen Idec, Inc. (6). The biological activity of IFN was assayed by protection against the cytopathic effect of vesicular stomatitis virus (VSV) on murine fibroblasts and was expressed in units/ml using the murine National Institutes of Health IFN-β standard for reference. Wild-type mouse embryo fibroblasts (MEFs) and MEFs lacking the Rel p50 and p65 proteins (7), generously provided by Drs. David Baltimore and Alexander Hoffmann (California Institute of Technology, Pasadena, CA), were plated at 3 × 10^5 cells/60-mm dish every 3 days in Dulbecco’s modified Eagle’s medium supplemented with 10% defined calf serum (HyClone Laboratories, Logan, UT).

NF-κB Activity Measurements—Nuclei isolated from control and IFN-β-treated MEFs were extracted with buffer containing 20 mM Tris-HCl (pH 7.85), 250 mM sucrose, 0.4 mM KCl, 1.1 mM MgCl₂, 5 mM β-mercaptoethanol, 1 mM NaF, 1 mM Na₃VO₄, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml soybean trypsin inhibitor, 5 μg/ml leupeptin, and 1.75 μg/ml benzamidine, and extracts were frozen and stored at –80 °C (8). For electrophoretic mobility shift assay (EMSA), the nuclear extracts were incubated with a 32P-labeled κB probe (5’-AGTTGAGGG-3’). This paper is available on line at http://www.jbc.org
assays for the Antiviral Activity of IFN—To determine cellular sensitivity to the ability of IFN to reduce virus titer, cell cultures were preincubated overnight with IFN, followed by infection with VSV for 1.5 h at 0.1 plaque-forming unit/cell. At 24 h post-infection, the virus yield in the medium was assayed by plaque formation on indicator Vero cells (10). To determine the sensitivity to protection against the cytopathic effect of VSV, fibroblasts were cultured in 96-well plates, incubated with serial 2-fold dilutions of IFN for 24 h, infected with VSV (0.1 plaque-forming unit/cell), and scored for viability at 24 h post-infection by uptake of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy- thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium dye (Promega, Madison, WI). Dye uptake was measured by absorbance at 490 nm in an enzyme-linked immunosorbent assay plate reader (Bio-Rad).

RNA Preparation and Microarray Analysis—Total cellular RNA from untreated and IFN-treated (2500 units/ml for 5 h) MEFs was extracted with TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. Approximately 10 μg of RNA was submitted to Genome Explorations Inc. (Memphis, TN) for labeling and hybridization to the murine U74Av2 GeneChip (Affymetrix Inc.) according to the manufacturer’s protocols. Expression values were determined using Affymetrix Microarray Suite Version 5.0 software. The raw expression data for six arrays (per individual untreated and three IFN-treated fibroblast cultures) are given in Supplemental Table I.

Data Analysis—Data analysis was performed using GeneSpring software (Silicon Genetics, Inc.). The Microarray Suite Version 5.0 gene expression values for each gene were normalized across each chip as well as across all experiments. All expression values with an average difference of <20 and those that were flagged absent in all samples were deleted from subsequent analysis. Statistical significance was calculated using Welch’s paired two-tailed t test, which assumes unequal variance. Using a subset of genes that were statistically affected by IFN treatment, hierarchical clustering was performed using standard correlation values. In addition, the expression changes observed in IFN-treated fibroblasts were compared with the expression changes observed in studies using IFN-treated human peripheral blood mononuclear cells (11) and human fibrosarcoma cell lines (12).

Quantitative Real-time PCR—Total RNA was isolated from untreated fibroblasts and fibroblasts treated with IFN for 24 h using TRIzol reagent. RNA (2 μg) was reverse-transcribed using the Omniscript reverse transcriptase kit (QIAGEN Inc.). Quantitative real-time PCR was performed on an iCycler (Bio-Rad) using SYBR Green PCR master mixture (Applied Biosystems) according to the manufacturer’s instructions. The primers were used as follows: mx1, 5′-CTGTGTCGAG-GCCTACGTATGAG-3′ (forward) and 5′-GCCTCTCCACTCTCTCCT-3′ (reverse); Isg15, 5′-TGACGCAGACTGTAGACACGC-3′ (forward) and 5′-TTTGGTCTCCCTCCTGAAGCCT-3′ (reverse); mni, 5′-AGTGGAAAGGC-GTGGATATGAG3′ (forward) and 5′-AATGCGTCTTAAATCGCTGAT-3′ (reverse); Ifi1, 5′-AGGCTGGAGTGTTGCTGAGT-3′ (forward) and 5′-TTTGGTATTACCCGGACAG3′ (reverse); and glyceraldehyde-3-phosphate dehydrogenase, 5′-ATGGATGGATGGATGGATGGAT-3′ (forward) and 5′-GATGCCTGCCCTCACCCCTT-3′ (reverse).

The cycler parameters were as follows: 95 °C for 15 min and amplification at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s for 40 cycles. The product size was initially monitored by agarose gel electrophoresis, and melting curves were analyzed to control for specificity of PCRs. The data on IFN-induced genes were normalized to the expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. (Similar results were obtained by normalization to β-actin.) The relative units were calculated from a standard curve, plotting three different concentrations against the PCR cycle number at which the measured intensity reached a fixed value (with a 10-fold increment equivalent to ~3.1 cycles).

Immunoblotting—At 24 h after IFN-β treatment (1000 units/ml), cells were lysed directly in Laemmli buffer, and equivalent amounts of protein were subjected to SDS-PAGE. Proteins were transferred to polyvinylidene difluoride membranes; immunoblotted with anti-Isg15, anti-nmi, or anti-actin antibody; and visualized by chemiluminescence with ECL reagent (Amersham Biosciences).

RESULTS

NF-κB Activation by Type I IFNs—IFN-α/β promotes NF-κB DNA-binding activity in diverse normal cells as well as in transformed cell lines (4). However, cell type-specific differences were observed in the composition of the IFN-induced Rel-NF-κB complexes. For example, IFN-induced p50-p65 complexes were found in mouse 3T3 fibroblast cells, whereas p50-c-Rel complexes were found in human Daudi cells. To determine whether IFNs promote NF-κB activation, fibroblasts derived from wild-type and p50- or p65 KO mice treated with IFN (1000 units/ml) for varying durations; B, EMSA of nuclear extracts from wild-type fibroblasts treated with 0–10 ng/ml lipopolysaccharide (LPS) for 30 min; C, EMSA of nuclear extracts from wild-type fibroblasts treated with 1000 units/ml IFN for 30 min in the presence or absence of a neutralizing anti-IFN polyclonal antibody. Extracts were incubated with a 5′-labeled promoter probe for the consensus κB site. EMSA results were quantitated on a PhosphorImager using Quantity One software (Bio-Rad). Similar results were obtained in at least two independent experiments.
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**Fig. 2.** IFN-induced antiviral activity in wild-type and NF-κB KO mouse fibroblasts. A, to determine the ability of IFN-β to inhibit virus-induced cytopathicity in wild-type (WT) and NF-κB KO MEFs, fibroblasts were cultured in 96-well plates, incubated with serial 2-fold dilutions of IFN-β for 24 h, infected with VSV, and scored for viability by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium dye uptake. The data represent the average of duplicate determinations from three independent experiments. B, to determine the ability of IFN-β to reduce virus titers in VSV-infected wild-type and NF-κB KO MEFs, fibroblasts were preincubated overnight with IFN-β and infected with VSV, and the virus yield in the medium at 24 h post-infection was assayed by plaque formation. The data represent the average of duplicate determinations from three independent experiments.

**Experimental Procedures**

To further characterize the role of NF-κB in the antiviral action of IFN, we assayed the ability of low IFN concentrations to reduce virus titer. In these assays, MEFs were incubated with 0, 1, or 10 units/ml IFN-β for 24 h prior to infection with VSV at a multiplicity of infection of 0.1 plaque-forming unit/cell. Virus was harvested from culture supernatants at 24 h post-infection and assayed for plaque formation. The titer of virus produced in MEFs not treated with IFN was similar (∼1 × 10^6 plaque-forming units/ml) in wild-type and NF-κB KO cell lines. As shown in Fig. 2B, treatment of wild-type MEFs with 1 unit/ml IFN reduced the virus titer by 3-fold, which is consistent with the theoretical efficacy of IFN, i.e. 1 unit of IFN should reduce the virus titer by 2-fold. In contrast, IFN treatment of NF-κB KO MEFs reduced the virus titer by ∼30-fold. Moreover, whereas treatment of wild-type fibroblasts with 10 units/ml IFN reduced the virus titer by ∼100-fold, it reduced the virus titer by ∼100,000-fold in NF-κB KO fibroblasts. These results indicate that loss of NF-κB activity by KO of p50 and p65 sensitizes fibroblasts to the antiviral action of IFN.

**IFN-regulated Gene Expression**—IFNs produce their biological effects through altering gene expression, most notably through induction of a family of early response genes called ISGs (3). Gene expression profiling using DNA microarrays has revealed a large number of IFN-regulated genes in a variety of cells (11, 12, 14). To investigate the role of specific genes in the IFN-induced antiviral state of murine fibroblasts, we examined the pattern of IFN-regulated gene expression by using high density oligonucleotide arrays. In three separate experiments, fibroblasts from four individual flasks were cultured for 5 h in the absence or presence of IFN-β; RNA was isolated; and cRNA probes were generated for hybridization to the Affymetrix murine U74Av2 GeneChip. The U74Av2 array contains 12,422 non-control probe sets representing ∼6000 annotated genes and ∼6000 expressed sequence tags. After preprocessing of the data (see "Experimental Procedures"), a total of 5400 probe sets were used to compare gene expression between untreated and IFN-treated MEFs. Using Welch’s paired t test, we found 118 probe sets whose expression levels were increased and six probe sets whose expression levels were decreased after IFN treatment (Fig. 3). Many of the 118 probe sets that increased upon IFN treatment represented previously identified ISGs. In some cases, multiple probe sets were represented for a single gene (i.e. Ifi204 (IFN-induced 204 gene), Adar (adenosine deaminase, RNA-specific), and Trim21 (tripartite motif protein 21)). Importantly, the calculated -fold changes by multiple probe sets for the same gene were comparable (for example,
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To further characterize the role of NF-κB in the IFN-β response, we focused on four genes that were identified to be IFN-induced by our microarray analysis: mx1 (myxovirus resistance 1), Ifit1 (IFN-induced protein with tetratricopeptide repeats 1), nmi (N-Myc/STAT-interacting protein), and Isg15 (IFN-stimulated gene 15). These IFN-induced genes were selected for analysis because they were induced by at least 10-fold in wild-type fibroblasts and appeared to be differentially regulated by IFN in NF-κB KO fibroblasts compared with wild-type fibroblasts in preliminary microarray studies. mx1 is a member of the Mx family of GTPases, which block orthomyxovirus replication (15). Ifit1 is a member of the tetratricopeptide (34 amino acids) repeat family of proteins, which interfere with the function of protein kinase R, a serine/threonine protein kinase (16, 17). Protein kinase R is an IFN-inducible protein whose enzymatic activity is activated by double-stranded RNA. Nmi interacts with N-Myc (as well as c-Myc, Max, and Fos). However, more recently, nmi has been shown to interact with STAT proteins and appears to augment transcriptional activity and to inhibit proteasome-mediated degradation of STAT proteins (18). Isg15 is a ubiquitin-like protein that conjugates to numerous proteins in IFN-treated cells (19).

Expression levels of mx1, Ifit1, nmi, and Isg15 were determined by quantitative real-time PCR analysis of RNA from fibroblasts derived from wild-type and p50/p65 KO mice, which had been treated with 0–1000 units/ml IFN-β for 5 h. The mRNA levels for each gene were normalized to glyceraldehyde-3-phosphate dehydrogenase and are expressed as -fold induction relative to the level of expression in the absence of IFN-β treatment. As shown in Fig. 4, MEFs from wild-type and NF-κB KO mice exhibited a dose-dependent induction of all four of the previously identified IFN-induced genes. However, mx1 and nmi were induced by IFN-β at a much higher level in the NF-κB KO versus wild-type fibroblasts. For example, at 1000 units/ml IFN-β, mx1 and nmi levels were both higher (–7- and –5.5-fold, respectively) in NF-κB KO MEFs than in wild-type MEFs. In contrast, Isg15 and Ifit1 were induced at much higher levels in wild-type versus NF-κB KO MEFs. At 1000 units/ml IFN-β, Isg15 and Ifit1 levels were higher (–3- and –5-fold, respectively) in wild-type fibroblasts than in NF-κB KO fibroblasts. These results illustrate that NF-κB plays a selective and distinct role in IFN-induced gene expression.

To further characterize the role of NF-κB, similar experiments were also performed in MEFs solely lacking the p65 protein (p65−). As illustrated in Fig. 4, Isg15 and Ifit1 were induced at relatively similar levels in wild-type and p65− MEFs. nmi levels were somewhat higher in p65− MEFs compared with wild-type MEFs, but lower than induced levels in NF-κB KO MEFs. In contrast, mx1 levels were both significantly higher in NF-κB KO and p65− MEFs compared with wild-type MEFs. These results suggest that the enhancing role that NF-κB plays in some ISGs, such as Isg15 and Ifit1, does not involve p65-containing NF-κB complexes. In con-

three probe sets for Ifi204 yielded 12.2-, 8.2-, and 5.6-fold induction, serving as an internal control for hybridization conditions as well as GeneChip integrity. As an independent confirmation of the expression changes we observed in IFN-treated fibroblasts, we compared our data with those in other studies using IFN-treated human cell lines (11, 12). All of the murine genes whose human homologs were definitively identified showed similar changes, albeit to varying degrees, in response to IFN treatment of mouse fibroblasts, human peripheral blood mononuclear cells, and human fibrosarcoma cell lines (Table I). Interestingly, the expression level of Gbp2 (guanylate-binding protein 2) was increased 400-fold by IFN in fibroblasts and only 2.6-fold in peripheral blood mononuclear cells. These results indicate that the regulation of some genes by IFN is cell type-specific.

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A subset of genes whose expression is modulated by IFN in murine fibroblasts in this study as well as in other studies using human blood or fibrosarcoma cell lines

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DISCUSSION

IFN-α/β has numerous biological activities, including antitumor, anti-proliferative, anti-antibacterial, anti-protozoal, and immunomodulatory functions (20). Moreover, IFN is used clinically in diseases of diverse pathogenesis and manifestation such as hairy cell leukemia, Kaposi’s sarcoma, laryngeal and genital papillomas, chronic viral hepatitis, and multiple sclerosis. The therapeutic potential of IFN and its role as a model for understanding the function of many cytokines make it important to understand the molecular basis for IFN-α/β action. Although the JAK-STAT signaling pathway has been a useful paradigm for the understanding of signal transduction by IFNs, evidence has accumulated that IFN signaling is more complex and involves serine/threonine kinases such as protein kinase C, phosphatidylinositol 3-kinase, Akt/protein kinase B, and ERK (extracellular signal-regulated kinase) (21). Recent studies have established that IFN also signals through a pathway involving the NF-κB transcription factor, which counterbalances pro-apoptotic signals. In this study, we have further characterized the biological significance of NF-κB activation during IFN-mediated responses.

NF-κB functions as a dimer, which consists of different Rel family proteins. We have shown that IFN activation of NF-κB was abolished in fibroblasts derived from mice in which the p50 and p65 members of the Rel family have been knocked out. Moreover, we showed that MEFSs from p50:p65 KO mice were more sensitive to the antiviral action of IFN as demonstrated by higher sensitivity to protection against virus-induced cytopathicity as well as to the inhibition of viral replication as measured by plaque assays. It is of interest that recent studies suggest that NF-κB may down-regulate cellular responsiveness to specific cytokines. For example, enhanced tumor necrosis factor-mediated JNK (c-Jun N-terminal kinase) signaling has been observed in NF-κB KO mice (22, 23).

A number of IFN-induced proteins are believed to be responsible for the antiviral state induced by IFNs. Our microarray analysis of IFN-regulated genes in MEFSs from wild-type mice...
identified 124 probe sets that were differentially regulated upon IFN treatment. This group consisted of many known ISGs such as Ifit1, Gbp2, mx1, Isg15, Stat1, nmi, mx2, If204, Adar, Irf1, and protein kinase R. To further characterize the role of NF-κB in IFN action, we performed a preliminary microarray experiment using MEFs from p50:p65 KO mice. A subset of the ISGs (mx1, Ifit1, nmi, and Isg15) was selected for further analysis based on the following criteria: induced at least 10-fold in wild-type fibroblasts, described previously as IFN-inducible, play a role in IFN action, have κB sites in their promoters, and appeared to be differentially regulated in NF-κB KO cells.

Our results indicate that NF-κB plays a complex role in the regulation of IFN-inducible genes. For instance, NF-κB enhances the IFN-induced expression of Isg15 and Ifit1 (higher levels in wild-type versus KO MEFs) while repressing the IFN-induced expression of mx1 and nmi (higher levels in KO versus wild-type MEFs). NF-κB regulates gene expression by binding to cis-acting κB sites in promoters bearing the consensus sequence 5'-GGGRNYYCC-3' (where R is a purine nucleoside, N is a nucleoside, and Y is a pyrimidine nucleoside). Interestingly, a number of κB sites upstream of the transcription start site are detectable in all four genes using the Genomatix Software Suite. Dimeric complexes of various combinations of the Rel/NF-κB family of polypeptides differ in their preference for certain κB sites on DNA, transactivation potentials, kinetics of nuclear translocation, and levels of tissue expression. The p50:p65 complex is ubiquitously expressed and tends to be

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rapidly translocated to the nucleus in response to activating signals, whereas other complexes tend to accumulate in nuclei more slowly. The p50 homodimer is generally thought of as an inhibitor of NF-κB-dependent transcription (24). Our results suggest that p50, but not p65, contributes to the enhancing role that NF-κB plays in some ISGs such as Isg15 and Ifit1. This is consistent with the recent finding that p50 rather than p65 is involved in the NF-κB-mediated activation of the Gbp1 gene by IFN (25). In contrast, both p50 and p65 apparently contribute to the NF-κB-mediated repression of some ISGs such as msx1 and nmi. Consistent with this notion is the finding that p65, but not p50, inhibits c-maf-dependent interleukin-4 transcription (26). Taken together, our results suggest that NF-κB participates in an intricate gene regulatory network involving a number of other transcription factors that coordinate to regulate the expression of ISGs in the context of other cell signaling pathways. Understanding the exact role of NF-κB subunits in ISG expression will most certainly require further analysis of the interaction of these IFN-activated signaling pathways.

The mouse msx1 protein is induced by IFN and accumulates in the nucleus. msx1 is a member of the Mx family of dynamin-like GTPases, which appear to block viral nucleocapsid transport and inhibit viral RNA synthesis. msx1 alone appears to be sufficient to block viral replication in the absence of any other IFN-inducible genes. msx1 appears to be exclusively regulated by IFN-α/β and is not responsive to other cytokines (27). nmi is an IFN-inducible protein that was originally identified in a two-hybrid screen with N-Myc and hence was named N-Myc-interacting protein. nmi is also induced by double-stranded RNA, an intermediate in viral replication and a potent IFN inducer, but not by the inflammatory cytokines tumor necrosis factor-α and interleukin-1 (28). nmi also interacts with c-Myc, Max, and Fos as well as with all members of the STAT transcription factor family with the exception of STAT2. nmi enhances STAT association with the CBP (cAMP-responsive element-binding protein)-binding protein/p300 coactivator proteins and thereby augments STAT-dependent transcription. We found that both msx1 and nmi were more sensitive to IFN induction in NF-κB KO cells, which is consistent with their presumptive role in mediating IFN action.

Ifit1, also called p58IRF5, is an IFN-inducible member of the tetratricopeptide repeat and J-domain protein families. Ifit1 is also induced by double-stranded RNA, but not by the inflammatory cytokines tumor necrosis factor and interleukin-1 (28). Ifit1 inhibits the double-stranded RNA-activated protein kinase R. Protein kinase R is part of the IFN-induced host response to viral infection and inhibits translation initiation through the phosphorylation of ribosomal eukaryotic initiation factor-2a. Ifit1 may play a broader role in cellular stress response by acting as a co-chaperone for Hsp70 (17). Since Ifit1 is an inhibitor of IFN action and since we observed an increase in antiviral sensitivity in NF-κB KO mice, it is entirely consistent that Ifit1 is induced to a greater extent in MEFs from wild-type mice than in those from NF-κB KO mice. Similar to Ifit1, induction of Isg15 by IFN was also regulated through an NF-κB-dependent pathway. Isg15 encodes a 15-kDa protein that is strongly induced after IFN treatment and that has strong sequence homology to ubiquitin (19). Isg15 can be conjugated to intracellular proteins via an isopeptidase bond in a manner similar to ubiquitin and ubiquitin-like modifiers. The ubiquitin/proteasome pathway functions in the controlled degradation of cellular proteins and regulates cytokine signal transduction through the degradation of specific signaling components. A number of important regulators of signal transduction are modified by Isg15, including JAK1 and STAT1. Although the biochemical function of Isg15 conjugation is unresolved, it is tempting to speculate that Isg15 conjugation may target IFN signaling components for degradation and thereby negatively regulate IFN signaling. If this is the case, the observed increase in antiviral sensitivity to IFN in NF-κB KO mice would be consistent with Isg15 being induced to a greater extent in MEFs from wild-type mice than in those from NF-κB KO mice.

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REFERENCES


