CCAAT/Enhancer-binding Protein β Mediates Interferon-γ-induced p48 (ISGF3-γ) Gene Transcription in Human Monocytic Cells

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Previous studies have identified a novel interferon-stimulated response element-like element, termed γ-interferon-activating transcription element, within the interferon-stimulating gene factor-3 (p48) promoter region that is bound by novel transcription factors in response to stimulation with interferons (IFNs) (Weihua, X., Kolla, V., and Kalvakolanu, D. V. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 103–108). In the present study, we have identified CCAAT/Enhancer-binding protein β (C/EBP-β) as one of the γ-interferon-activating transcription element cognate transcription factors by screening a human monophage-derived cDNA library in a yeast one-hybrid system. Electrophoretic mobility shift assay studies suggest that C/EBP-β dynamically regulates p48 gene expression upon IFN-γ stimulation by undergoing changes in its heterodimerization partners. Transient transfection studies demonstrate that overexpression of C/EBP-β strongly enhanced IFN-γ-induced transcription from the p48 promoter. However, deletion mutants of C/EBP-β that lack the N-terminal transactivation domain were unable to stimulate the p48 promoter. Western blotting revealed that C/EBP-β is induced by IFN-γ stimulation in THP-1-derived macrophages. Collectively, these results suggest that C/EBP-β plays an important role in the human IFN-γ signaling pathway by transcriptional regulation of p48 gene expression, an essential component in the IFN signaling pathway.

Interferon-stimulated gene factor-3 (ISGF3) plays a crucial role in mediating the antiviral, antitumor, and immune responses induced by IFNs. The ISGF3 complex consists of a 48-kDa DNA-binding protein (p48 or ISGF3-γ) and two signal transducer and activators of transcription (STAT) proteins, STAT1 and STAT2. The p48 protein in the ISGF3 complex serves as the adaptor to redirect STAT multimers from their intrinsic palindrome sequence specificity and allows binding to the IFN-stimulated response element (ISRE) that occurs within the promoters of various IFN-stimulated genes (ISGs) (2). In addition to the ISRE element, a distinct cis-element termed IFN-γ-activated site (GAS) is also found within the promoters of many IFN-inducible genes and is bound by the STAT1 homodimer (3). The p48 protein belongs to the interferon regulatory factor (IRF)-myb family of transcription factors. This family of transcription factors can activate (IRF-1 and p48), repress (IRF-2 and interferon consensus sequence-binding protein), both activate and repress (IRF-4), and may activate or repress (IRF-3) transcription of target genes (4). Studies of p48 knockout mice have shown that p48 plays an essential role in both type I and type II IFN responses for activation of IFN-inducible genes and establishment of the antiviral state (5). A recent study showed that an ISRE-like sequence in the hepatitis B viral enhancer-1 could interact with the ISGF3 complex containing p48 and mediate the IFN-α-induced suppression of enhancer activity (6). Other independent studies also demonstrated that overexpression of p48 in NIH-3T3 cells restored IFN responses of adenovirus E1A-expressing cells in which the IFN signaling was blocked by E1A (7, 8). Although IFN and other cytokines induced by the Janus tyrosine kinase-STAT pathway have been well described, the mechanisms regulating expression of the p48 component of ISGF3 remain largely unknown. A number of studies have shown that various cell types, including primary chronic lymphocytic leukemia, human trophoblast, melanoma, and acute promyelocytic leukemia, are resistant to the actions of IFN because the induction of p48 protein is defective (9–12). Additionally, it has been argued that IFN-γ priming up-regulates p48-enhanced antiviral and antitumor effects of class I IFNs on IFN-resistant cells (13, 14). Furthermore, recent studies have shown that p48 protein also binds to virus-inducible elements within the IFN-α/β promoters. This evidence suggests a potentially new role for ISGF3 in that it may participate directly in the activation of IFN-α/β promoters (15, 16). Therefore, understanding the transcriptional regulation of p48 is very important for elucidating the global functions of IFNs and signaling pathway networks among cytokines.

Previously, we demonstrated that a novel IFN-γ-activated transcription element (GATE) within the p48 promoter region and its cognate transacting factors regulate p48 gene expression upon IFN-γ stimulation (1). Although p48 is a member of the IRF family, it does not function like the other IRFs in which expression is regulated via either ISRE or GAS elements within their promoters (4). Instead, induction of p48 gene ex-
pression in response to IFN stimulation occurs through the GATE, which has no significant homologies with GAS and only partial homology to ISRE. Also, it has been demonstrated that the GATE interacts with novel transcription factors instead of the common transcription factors that were found in most ISGs, STATs, or ISGF3 complexes (1). A recent study using a Southwestern screening protocol has identified a member of the CCAAT/enhancer-binding protein (C/EBP) transcription factor family, C/EBP-β, that binds to GATE and enhances the expression of the p48 promoter-luciferase in mouse macrophages (17). However, the mechanism, tissue specificity, and biological contributions to the cells for response to the IFNs of such an event have not been well described. To gain insight into the mechanisms of IFN actions, we have screened a cDNA library derived from human THP-1 macrophages using a yeast one-hybrid system. We demonstrate that C/EBP-β, in association with other undefined transcription factor(s), binds to the GATE and regulates p48 expression in response to IFN-γ stimulation.

The C/EBP belongs to a family of transcription factors with structural as well as functional homologies (18). To date, six members have been identified, each consisting of an activation domain, a basic leucine zipper dimerization domain, and a DNA binding domain. All family members share the highly conserved dimerization domain by which they form homo- and heterodimers with other family members. C/EBP proteins have been linked to a variety of regulatory functions, including normal tissue development, cellular function, cellular proliferation, and differentiation (19). Gene targeting revealed that members of the C/EBP family play critical roles in cellular proliferation and differentiation in various tissues (20). Among the C/EBP protein family members, C/EBP-β is essential for macrophage function and is strongly induced during macrophage differentiation and activation (18). Furthermore, C/EBP-β binding motifs have been found in the functional regulatory regions of genes specifically induced in activated macrophages, such as IL-6, IL-1α, IL-8, granulocyte colony-stimulating factor, and nitric-oxide synthase (19). Mice deficient for the C/EBP-β gene showed that C/EBP-β plays an essential role in the cellular immune defense system, such as acting against foreign pathogens (20, 21). However, the mechanism of such actions of C/EBP-β has not been well described.

MATERIALS AND METHODS

Cell Culture and Reagents—The human THP-1 cell line was maintained at a 1 × 10^6/ml density in RPMI 1640 medium with 10% fetal bovine serum. For IFN-γ stimulation, 20 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) were added into the medium for 8–16 h to induce cell differentiation into macrophages. The differentiated adherent macrophages were then washed twice with phosphate-buffered saline, and additional human recombinant IFN-γ (Genzyme, Cambridge, MA) was added to a final concentration of 200 units/ml for the indicated periods at 37 °C. For Northern blotting, total RNA (10 μg) was separated on 1.0% formaldehyde-agarose gels and transferred to positively charged nylon membranes. After fixation with UV light, the membranes were hybridized with the indicated 32P-labeled cDNA probe in Fast-Hybrid solution (Stratagene, La Jolla, CA) according to the manufacturer’s instructions and visualized by exposing the x-ray film over-night. EMSAs were performed as described previously (22). First, the recombinant vector pLaClGATE was transformed into the yeast strain YM4271, yielding an integrated URA locus yeast strain by selecting the URA marker on SD–Ura selection medium. The His- and lacZ genes under the control of the inserted GATE elements. Therefore, the background level HIS was determined in both engineered yeast strains by culturing the yeast strain on SD–His medium containing various concentrations of 3-AT (from 0 to 60 μM). Both wild type and mutant engineered yeast strains were completely growth-inhibited by at least 45 μM 3-AT. Therefore, 45 μM 3-AT was used in yeast one-hybrid screening. The lacZ background expression level was also checked by β-galactosidase assay, and very low levels of background expression were detected in both strains by the β-galactosidase filter lift assay according to the manufacturer’s instructions (CLONTECH).

The cDNA library was constructed from THP-1-derived macrophages with various periods of IFN-γ stimulations that ranged from 1 to 16 h. After harvesting, the cells were pooled, mRNA were purified from total RNA, and cDNAs were synthesized and cloned into the HybriZAP-2.1 phage vector by using a cDNA library construction kit (Stratagene). Following the manufacturer’s instructions, the cDNA library in phage was converted into phagemid vector pAD-GALA-2.1 using an in vivo excision protocol and was amplified in Escherichia coli. For screening cDNA library, 150 μg of purified cDNA library in yeast expression phagemid (containing about 3 × 10^6 genes) were separately transformed into YM-W-GATE and YM-M-GATE dual selection yeast strains by the LiOAc method (CLONTECH) and plated on SD–His–Ura 45 μM 3-AT agar plates at 30 °C for 5–7 days to select the 3-AT-resistant colonies.

Electrophoretic Mobility Shift Assay (EMSA) and Oligonucleotides—Following the PMA-induced differentiation of THP-1 cells for 16 h, IFN-γ was added at various time points prior to harvesting the cells. THP-1 cells without PMA and IFN-γ treatment were used as the THP-1 control; cells with PMA induction alone were used as the IFN-γ (−) control. After pelleting the cells at 2,000 rpm/min for 2 min at 4 °C, nuclear extracts (NEs) were prepared as described previously (1) and protein assays were performed. EMSAs were performed as described elsewhere (22) except that 2 μg of protein from each sample were mixed with 1× binding buffer containing 1 μg of poly(dI-dC) (Roche Molecular Biochemicals) and 5 × 10^5 cpm [32P]-labeled probe for 15 min at room temperature before loading on an 8% glycerol-polyacrylamide gel.

The following oligonucleotide sequences were used as probe for EMSA (the GATE-w for wild type GATE and GATE-m for mutant GATE were the same as those used for engineered yeast strains); C/EBP-w for wild type C/EBP, 5'-TGC AGA TTG CGC AAT CTG CA-3' and C/EBP-m for mutant C/EBP, 5'-TGC AGA GAC TAG TCT CG-3', in which the mutant nucleotides are italic and underlined. The normal rabbit serum, anti-STAT1 (p91), and anti-STAT2 were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Anti-ISGF3-γ (anti-p48), anti-IRF-1, and anti-C/EBP-β were purchased from Santa Cruz Biotechno-

Plasmids—The plasmids used in transgenic transfection experiments were control vector pMEX, pMEX-C/EBPβ containing the full-length rat C/EBP-β cDNA, pMEX-C/EBPβ-p(132–276) lacking amino acids 1–131, and pMEX-C/EBPβ-p(191–276) lacking amino acids 1–190.
IFN-γ Induces p48 Transcription in THP-1-derived Macrophages—Macrophages are one of the primary targets of IFN-γ. The human monocytic cell line, THP-1, can be differentiated into macrophage-like cells by PMA treatment, and these cells exhibit the characteristics of macrophages, including their immunological functions. To further understand the regulation of p48 by IFN-γ in human macrophages, we performed Northern blots using THP-1 cells that had been differentiated with PMA followed by IFN-γ stimulation. Fig. 1A shows a Northern blot in which the basal level of p48 messenger RNA increased after 5 h of IFN-γ stimulation and continued to increase in a time-dependent manner until maximally induced at 7–12 h. Expression of p48 began to diminish at 16 h post-IFN-γ treatment. The same blot was reprobed for β-actin as a control to ensure equal amounts of RNA were used. These observations are identical to those obtained using murine macrophages (1), i.e., regulation of p48 expression is quite slow (7–10 h) compared with that of ISGF3 formation, which is quite rapid (within minutes (24)) and independent of protein synthesis in the early stage of the response to IFNs.

Previous studies have characterized GATE as the IFN-responsive site within the p48 promoter in murine macrophages (1, 17, 25). To examine the mechanism controlling transcription of p48 by IFN-γ, EMSA was performed using a 32P-labeled GATE probe and NE from IFN-γ-stimulated THP-1 macrophages (Fig. 1B). Three specific binding complexes were observed with different electrophoretic mobilities. Because these binding complexes are not yet defined, we designated them as GATE binding complexes-α, -β, and -γ (GBC-α, -β, and -γ) for the slow-to-fast mobility complexes, respectively. GBC-α was the only complex observed in the control cells (both undifferentiated THP-1 and IFN-γ (−) controls). Its appearance was decreased concomitantly with IFN-γ stimulation. There was no GBC-β observed in untreated cells, but GBC-β was strongly induced by IFN-γ in a time-dependent manner starting at 1 h (Fig. 1B, lane 3), peaking at 7–9 h (lanes 6 and 7), and diminishing at 16 h (lane 8) post-IFN-γ treatment. Another minor binding complex, GBC-γ, was undetectable in control cells but appeared and remained at low levels from 3 to 9 h post-IFN-γ stimulation (Fig. 1B, lanes 4–7). The above observations suggested that GATE binding complexes are induced by IFN-γ stimulation. The close correlation between the time-dependent induction pattern of p48 mRNA expression and GBCs binding to GATE suggests a direct link between GBCs and p48 expression.

Identification of GATE-binding Proteins by the Yeast One-hybrid System—To further identify the transcription factors binding to GATE and activating p48 transcription, we employed the yeast one-hybrid system to screen a cDNA library derived from IFN-γ-stimulated THP-1 macrophages. After transforming the cDNA library into the engineered double selection yeast strains YM-W-GATE and YM-M-GATE (see “Materials and Methods”), SD/-His/-Ura agar plates containing 45 mm 3-AT were used to select transformed yeast colonies that express GATE-binding proteins. After two rounds of selection on the restrictive medium plates, five candidates were selected from the YM-W-GATE strain, and the plasmids were isolated. After retransformation into the YM-W-GATE and YM-M-GATE strains, all five candidates exhibited positive phenotypes in YM-W-GATE compared with the control, YM-M-GATE. Expression of the additional selection marker, β-galactosidase, was tested by filter lift assay. The results confirmed positive phenotypes for all five candidates (data not shown). Sequence analysis of the five clones revealed that none was a full-length cDNA but showed that the five clones belong to three different gene groups. Two of the clones showed very high homologies (98%) to the C/EBP transcription factor family member, C/EBP-β. The other two clones were highly identical to an IFN-inducible gene that has not yet been well characterized. The last clone had no significant matches with any known sequence in the NCBI/GenBank™ database. The two C/EBP homology clones contained similarly sized inserts that encode the DNA binding and leucine zipper domains and the 5′-untranslated region sequence of C/EBP-β. Because C/EBP-β has been well characterized as a nuclear transcription factor and has tissue-specific properties in macrophages (26, 27), our focus...
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Members of the C/EBP protein family can form either homo- or heterodimers with other family members when binding to their target binding site (23, 28). Anti-C/EBP-β was able to supershift both GBC-α and -β, and the cold C/EBP-w probe was able to compete with GBC-α and -β suggesting that GBC-α and -β are both C/EBP-β-containing complexes in which C/EBP-β binds directly or as part of a complex to GATE. Therefore, C/EBP-β is likely to play a critical role in formation of GBC-α and -β complexes for regulation of p48 in THP-1-derived macrophages. However, because GATE is an ISRE-like binding site and IRF-1 also has been shown to be a critical transcription factor in ISGs, the GATE may act as a multiple transcriptional activation site. This is supported by the observation that the weaker IFN-γ-inducible complex, GBC-γ, was removed by anti-IRF-1 and was competed by cold GAS-w element.

**IFN-γ Induces C/EBP-β Expression—**C/EBP-β was originally found to be inducible by IL-6 (29). To determine whether C/EBP-β also responds to IFN-γ stimulation in THP-1-derived macrophages, we performed Western blot analysis of extracts from an IFN-γ time course (Fig. 3). The results show that C/EBP-β protein was induced by IFN-γ as early as 1 h, peaked at 7–9 h, and decreased at 16 h (Fig. 3, lanes 2–7) compared with the basal level in IFN-γ (-) control cells (lane 1). The kinetics of C/EBP-β induction are therefore closely related to the time course of induction of GBCs observed in EMSA and are consistent with those seen in other cells in which C/EBP-β is inducible by IFN-γ (30, 31). In addition, previous studies have reported that C/EBP-β required phosphorylation for activation (35). Our results demonstrated that even the significant constitutive level of C/EBP-β presented in untreated cells may be sufficient, but p48 expression was elevated only when IFN-γ was added. The data suggest that C/EBP-β is possibly activated by an IFN-γ-induced phosphorylation signaling pathway and subsequently interacts with its partner(s) to form GBC-β. Furthermore, inducing C/EBP-β expression by IFN-γ may also enhance the formation of GBC-β.

C/EBP-β Mediates Activation of the p48 Promoter—Luciferase Transcription in THP-1 Macrophages—To further understand the role of C/EBP-β in regulating p48 transcription in response to IFN-γ stimulation, transient transfection assays were performed using a series of luciferase reporter vectors containing various p48 promoter deletions and GATE point mutations. We also analyzed synthetic promoters containing wild type and mutant GATE oligonucleotides, pGL3 promoter-wildGATE and pGL3 promoter-mutantGATE (Fig. 4, A and B). To determine the maximal induction of p48 luciferase activities by IFN-γ, a time course of IFN-γ induction of p48 luciferase reporters was conducted with co-transfection of C/EBP-β full-length cDNA and wild type p48 promoter reporters (data not shown). The results show that the luciferase activities increased corresponding to the time increases as the GBC-β complex appeared in EMSA (Fig. 1B) and the induction of C/EBP-β expression that was observed in Western blot (Fig. 3). Co-transfection of a full-length C/EBP-β expression vector, pMEX C/EBP-β, with various p48 promoter-luciferase reporters into THP-1-derived macrophages resulted in stimulation of p48 promoter-lucif-
**Fig. 4.** C/EBP-β enhances IFN-γ-stimulated p48 transcription in THP-1-derived macrophages. Various p48 promoter-luciferase reporters were used, as illustrated on the left, and the relative luciferase activities are shown in the bar graph. The various C/EBP-β constructs and IFN-γ stimulation status are indicated as "+" for present and "−" for absent. A, serial p48 promoter-luciferase reporters in pGL3-promoter vector (Promega) in which a SV40 promoter was placed upstream of the luciferase gene were co-transfected with various C/EBP-β vectors into THP-1-derived macrophages, with or without IFN-γ stimulation. pGL3 promoter-wildGATE and pGL3 promoter-mutantGATE contain three repeat synthetic sequences of either wild type or mutant GATE, respectively. The lack of stimulation by C/EBP-β was also observed with pGL3-Basic reporter (Fig. 4B, bar 4). However, when the GATE sequences were deleted (pGL3 basic-A8) or mutated (pGL3 basic-GATEpm), the ability of C/EBP-β to induce the luciferase activity was eliminated (Fig. 4B, bars 9–12 and 5–8, respectively). The lack of stimulation by C/EBP-β was also observed using a pGL3 promoter-GATEm reporter instead of pGL3 promoter-GATEw (Fig. 4A, bars 9–16). C/EBP-β deletion mutants that lack the transactive domain (pMEX-C/EBPβ-(132–276) and pMEX-C/EBPβ-(151–276)) did not activate the p48 promoter-luciferase and were not stimulated significantly by IFN-γ (Fig. 4A, bars 5–8, 21–24, and 29–32). Thus, these results demonstrate that C/EBP-β not only contributes to maintaining the basal level of p48 expression but also mediates the induction by IFN-γ. However, because IFN-γ stimulation caused much higher induction of p48 reporters than overexpression of C/EBP-β alone, and taking into account the results from antibody supershift and competition EMSAs (Fig. 2, A and B), the evidence suggests that an additional transcription factor(s) is required to interact with C/EBP-β to fully induce p48 transcription in response to IFN-γ, and such transcription factor(s) seems induced and/or postmodified through phosphorylation mechanisms by IFN-γ to form the GBC-β complex. At present, the identity of such specific transcription factor(s) remains unknown.

**DISCUSSION**

Formation of ISGF3 complexes in the cell in response to IFNs is a rapid process in which preexisting STAT1, STAT2, and p48 (ISGF3-γ) proteins in the cytoplasm assemble into a multimeric complex through mutual interactions. However, the induction of p48 mRNA and other proteins by IFNs appears to be quite slow, taking at least several hours to occur. Therefore, several lines of evidence suggest that regulation of p48 gene expression may involve posttranscriptional mechanisms and require de novo protein synthesis (1, 32). To date, such p48-related posttranscriptional factors have not been well identified. In this report, we demonstrate that a new set of complexes, GBC-β and GBC-γ, appear following IFN-γ stimulation in THP-1-derived macrophages and induce p48 transcription activation, which in the case of GBC-α and GBC-β includes C/EBP-β. Our further studies show that C/EBP-β is mediating the transcriptional activation of the p48 gene, both by main-
are listed on the bottom, and the three mutant nucleotides in the sequence are underlined.

taining the basal level and elevating the expression of p48 in response to IFN-γ stimulation. This occurs by binding to the IFN-γ response element, GATE, within the p48 promoter. The results of EMSA supershifts and transfection assays suggest that C/EBP-β dynamically regulates p48 expression by altering its dimerization partners in response to IFN-γ stimulation. Overexpression of full-length C/EBP-β in THP-1-derived macrophages without IFN-γ stimulation does not result in maximal induction of the p48 promoter. However, stimulation with IFN-γ was demonstrated to produce maximal induction of the p48 promoter as judged by luciferase reporter activity. Furthermore, EMSA experiments show that GBC-β, one of the complexes binding to GATE, was induced by IFN-γ but was absent in untreated cells. Previous studies have suggested that IFN-γ induction of p48 expression requires both de novo protein synthesis and phosphorylation (1). Thus, both our observation and the results of previous studies indicate that the formation of GBC-β is dependent on IFN-γ-induced de novo protein synthesis and/or phosphorylation modifications before interaction with C/EBP-β resulting in the formation of the new GBC-β complex. Also, the data have suggested that gene regulation by IFN-γ has characteristics including the initial transcriptional regulation of additional transcriptional co-factor gene products and that the equally important signal transduction modification of such proteins results in new complex(s) formations, which in turn regulate further specific gene sets. Furthermore, these results also suggest that C/EBP-β regulation of p48 takes place in two distinct states. In the first state, without IFN-γ activation, the C/EBP-β-containing complex, GBC-α, binds to GATE to maintain the basal expression of p48. In the second state, a distinct C/EBP-β-containing complex, GBC-β, further activates p48 expression. However, these two different C/EBP-β partners have not been identified, and further studies are needed to elucidate the exact mechanism.

Sequence alignment analysis using GATE and a number of known C/EBP motifs revealed an imperfect C/EBP binding site within GATE (Fig. 5). Three nucleotide mutations in both the C/EBP-β and IRF-1 recognition core sequence produced a mutant GATE that was unable to recognize the GBCs in competitive EMSAs and severely compromised the ability of GATE to mediate transcriptional induction in luciferase reporter assays. These results further support the findings that C/EBP-β plays an important role in regulating p48 expression by activating the GATE. Also, GATE may act as a multiple transcription activation site in regulating p48 gene induction.

IFNs exert their multifaceted biological functions through the activation of transcription of many IFN-regulated genes. Among them are the IRFs, a family of transcription factors thus far consisting of at least 10 members, such as IRF-1 to IRF-7, vIRF, p48, and Myb. Each of these IRFs serves distinct roles in biological processes (4). The regulation of IRF family proteins by IFNs has been shown to occur mainly through the ISRE or GAS elements found within their promoters by interaction with STAT dimers or ISGF3-like complexes (5). However, in the present study we show that C/EBP-β along with its cognate partners binds to GATE and regulates the expression of p48. These data suggest that the induction of p48 expression by IFN-γ occurs through a posttranscriptional mechanism that is distinct from most IRF family members.

Studies of IL-6 and IFN-γ receptor-deficient mice have demonstrated identical phenotypes in that both types of mice show an increased susceptibility to infection by Listeria monocytogenes and vaccinia virus (33, 34). Such results have suggested that two crucial cytokines in macrophages, IL-6 and IFN-γ, may have overlapping functions. Two types of IL-6 signaling transduction pathways from cell surface to nucleus have been described. C/EBP-β is phosphorylated and activated by a Ras-dependent mitogen-activated protein kinase cascade, whereas STAT3 is tyrosine-phosphorylated directly by Janus tyrosine kinase via the Janus tyrosine kinase-STAT pathway (35). These two distinct pathways have been demonstrated to direct different biological functions according to gene knockout studies. The STAT3 knockout demonstrated that STAT3 is essential for the early development of mouse embryos (36, 37). C/EBP-β knockout mice show that the macrophages from these deficient mice are highly susceptible to infection by L. monocytogenes, even when maximally activated in vitro with IFN-γ and lipopolysaccharide (20). Interestingly, such observations were reported in the p48 knockout mice as well (5). Comparing the observations between mice deficient in STAT3 and IFN-γ receptor with those deficient in C/EBP-β and p48 reveals common biological functions that relate to macrophage-mediated immune defenses against pathogens. Previous studies have demonstrated that IFNs can activate the Ras mitogen-activated protein kinase cascade in various cells (38–40). In the present study, we demonstrate that IFN-γ is capable of inducing C/EBP-β and its transactivation activities in human macrophages. Such actions of IFN-γ presumably go through the Ras mitogen-activated protein kinase pathway. Therefore, a common signal transduction pathway, shared by IL-6 and IFN-γ, could exist according to the present and previously reported studies. It is possible that activation of the Ras-mitogen-activated protein kinase pathway is required to activate C/EBP-β and subsequently mediate the regulation of the expression of p48 to enhance the effectiveness of the antipathogen functions of macrophages.

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