Synergistic Activation of Interleukin-12 p35 Gene Transcription by Interferon Regulatory Factor-1 and Interferon Consensus Sequence-binding Protein

Interferon regulatory factor-1 (IRF-1) and interferon consensus sequence-binding protein (ICSBP or IRF-8) are two members of the IRF family of transcription factors that play critical roles in interferon signaling in a wide range of host responses to infection and malignancy. Interleukin-12 (IL-12) is a key factor in the induction of innate resistance and generation of T helper type 1 cells and cytotoxic T lymphocytes. In this work, we find that ICSBP-deficient macrophages are highly defective in the production of IL-12. The defect is also observed at the level of IL-12 p40 and p35 mRNA expression. Transcriptional analyses revealed that ICSBP is a potent activator of the IL-12 p35 gene. It acts through a site localized to −226 to −219, named ICSBP-response element (ICSBP-RE), in the human IL-12 p35 promoter through physical association with IRF-1 both in vitro and in vivo. Co-expression of ICSBP and IRF-1 synergistically stimulates the IL-12 p35 promoter activity. Mutations at the ICSBP-RE results in the loss of protein binding as well as transcriptional activation by ICSBP alone or together with IRF-1. This study provides novel mechanistic information on how signals initiated during innate and adaptive immune responses synergize to yield greater IL-12 production and sustained cellular immunity.

Interferon regulatory factors (IRFs) constitute a family of nine mammalian transcription factors (IRF-1–9) that commonly possess a novel helix-turn-helix DNA binding motif. Members of IRF family are typically induced following microbial infections. The first discovered member of this family, IRF-1, has a remarkable functional diversity in the regulation of cellular response in host defense. IRF-1 selectively targets different sets of genes in various cell types in response to diverse cellular stimuli and evokes appropriate innate and adaptive immune responses (1). It has been firmly established as a critical effector molecule in IFN-γ-mediated signaling and in the development and function of NK, NK T cells, and cytotoxic T lymphocytes (2–7). IRF-1 also has direct anti-proliferative effects, thus acting as a tumor suppressor and tumor susceptibility gene. Interferon consensus sequence-binding protein (ICSBP)/IRF-8 is restricted in its expression to myeloid and lymphoid cell lineages. It can function both as a transcriptional repressor and an activator depending on the partners with which it interacts, and it plays crucial roles in myeloid differentiation, generation of plasmacytoid dendritic cells (8), macrophage activation, and tumor suppression (1, 9).

IL-12 is a heterodimeric cytokine produced primarily by macrophages and dendritic cells in both innate and adaptive immune responses. It is a key factor in the induction of T cell-dependent and independent activation of macrophages, NK cells, generation of T helper type 1 cells and CTL, induction of opsonic complement-fixing antibodies, and resistance to intracellular infections (10). The genes encoding the two heterologous chains of IL-12, p40 and p35, are located on different human and mouse chromosomes. Together, p40 and p35 form the biologically active IL-12 (also called p70). The highly coordinated expression of p40 and p35 genes to form IL-12 p70 in the same cell type at the same time is essential for the initiation of an effective immune response. The production of IL-12 p40 in activated professional antigen-presenting cells is generally in great excess over that of the p35 chain, making the latter molecule a limiting step in the formation of bioactive IL-12 (11).

Previous studies by us and others have revealed an intimate relationship between IRF-1, ICSBP, and IL-12 in that IRF-1 acts as a critical component of IFN-γ signaling in the selective activation of the IL-12 p35 transcription (12) in synergy with LPS-mediated events (13, 14) and that IRF-1 and ICSBP cooperatively regulate the transcription of IL-12 p40 gene in IFN-γ-amplified IL-12 production (15, 16). In this work, we show that ICSBP and IRF-1, activated primarily by IFN-γ, also cooperate in their transcriptional activation of the IL-12 p35 gene by directly binding to a specific site in the human IL-12 p35 promoter. This study provides additional mechanistic information regarding how innate and adaptive immune activation jointly lead to greater IL-12 production and persistent inflammatory responses.

MATERIALS AND METHODS

Mice—ICSBP−/− mice breeding pair were originally obtained from Dr. Keiko Ozato (National Institutes of Health). All of the mice used in the experiment were female and 6–8 weeks old. Mice were housed in cages with filter tops in a laminar flow hood and fed food and acid water ad libitum.

Cells—The murine macrophage cell line RAW 264.7 (RAW cells hereafter) was obtained from American Type Culture Collection and main-
tained in RPMI 1640 medium supplemented with 2 mm glutamine, 100 units/ml penicillin and streptomycin, and 10% FBS (endotoxin <1 ng/ml; Hyclone). Mouse inflammatory peritoneal exudate macrophages were obtained by lavage 4 days after injection of sterile 3% thioglycollate broth (1 ml of intraperitoneal). Cells were washed and resuspended in RPMI 1640 medium containing 10% fetal calf serum and standard supplements. Macrophages were plated in 24-well tissue culture dishes (0.5 x 10^5 cells/well). After 2 h of incubation to allow for adherence of macrophages, monolayers were washed three times to remove nonadherent cells and incubated with RPMI 1640 medium containing 10% fetal calf serum and standard supplements. The next day, some of the wells were treated with 1 μg/ml LPS and 10 ng/ml IFN-γ in a final volume of 1 ml or 10 ng/ml IFN-γ first for 16 h (priming) followed by LPS.

Human blood-derived monocytes were isolated from fresh blood by Ficoll/Hypaque gradient centrifugation. Mononuclear cells were incubated for 1 h in polyethylene tissue culture flasks (Falcon, BD Biosciences). After 1 h of incubation to allow for adherence of monocytes, monolayers were washed three times to remove nonadherent cells and incubated with RPMI 1640 medium containing 10% fetal calf serum and standard supplements. The treatment of human monocytes is the same as above unless otherwise stated.

Reagents—All of the antibodies used in this study were purchased from Santa Cruz Biotechnology. Inc. Expression vector ICSBP and IRF-1 were provided by Keiko Ozato. All of the plasmid DNA used in this study were prepared using Qiagen Endo-free Maxi-Prep kits. Recombinant human and murine IFN-γ was purchased from Genzyme (Boston, MA). LPS from Escherichia coli 0217:B8 and thioglycollate medium were purchased from Sigma.

ELISA—Supernatants from macrophage cultures were harvested at 6, 12, and 24 h after LPS stimulation and stored at -80 °C. IL-12 p40 and p70 were detected using the OPT-EIA ELISA kit (BD Biosciences) according to the manufacturer’s instructions. Concentrations were calculated by regression analysis of a standard curve.

RNAse Protection Assay (RPA)—Mouse peritoneal macrophages were pretreated with IFN-γ for 16 h followed by treatment with LPS for an additional 4 h. 10 μg of total RNA for each determination was subjected to reverse transcription. cDNA was amplified for 34 cycles by PCR and analyzed by electrophoresis on 1.2% agarose gel. The samples were amplified for 34 cycles by PCR and analyzed by electrophoresis on a 1.2% agarose gel or analyzed by quantitative real-time PCR as described above.

Statistical Analysis—Student’s t test was performed wherever possible. Mean ± S.D. is shown unless otherwise indicated.

RESULTS

IL-12 p40 and p70 Production Is Defective in ICSBP+/− mice—To determine the role of ICSBP in the regulation of IL-12 production, we obtained inflammatory peritoneal macrophages from ICSBP+/− mice (on C57BL/6 background) and control wild type animals and stimulated them in vitro with LPS or primed with IFN-γ followed by LPS stimulation. IL-12 p40 and p70 production at various times over a period of 24 h was measured by specific ELISA. Because of the lack of a p35-specific ELISA due to the fact that it is secreted only as a precursor, the measurement of p70 production is a generally accepted indicator of IL-12 p35 production due to the one to one ratio of the p40-p35 interaction. As shown in Fig. 1, IL-12 p40 production stimulated by LPS alone (A) or IFN-γ plus LPS (B) was strongly impaired in ICSBP+/− macrophages compared with wild type or ICSBP+/+ controls. Similarly, IL-12 p70 production induced by LPS (C) or IFN-γ plus LPS (D) was virtually absent in ICSBP+/− cells. These results indicate that ICSBP is an essential regulator of IL-12 p40 and p70 production.

IL-12 p40 and p35 mRNA Expression Is Impaired in ICSBP+/− Macrophages—To determine the effects of ICSBP deficiency on IL-12 p40 and p35 gene expression, we examined their steady-state mRNA expression by RPA and by qRT-PCR. The RPA (Fig. 2A) shows that the level of IL-12 p40 mRNA induced by LPS alone or IFN-γ plus LPS was comparable between wild type and ICSBP+/− peritoneal macrophages (lanes 3 versus lanes 7 and lane 4 versus lane 8), whereas its level was significantly reduced in ICSBP−/− cells activated by IFN-γ and LPS (lanes 4 and 8 versus lane 12), a result consistent with our
FIG. 1. Impact of ICSBP deficiency on IL-12 p40 and p70 production. IL-12 p40 (A and B) and p70 (C and D) were measured by ELISA from cell-free supernatants of thioglycolate-elicited inflammatory mouse peritoneal macrophage cultures (0.5 × 10⁶ cells in 1 ml) stimulated with LPS (A and C) or primed with IFN-γ for 16 h followed by LPS (B and D) for indicated times in hours. Filled square, wild type macrophages (WT); asterisk, ICSBP+/− cells; open circle, ICSBP−/− macrophages. Results shown are the mean ± S.D. of four independent experiments.

FIG. 2. Impact of ICSBP deficiency on IL-12 p40 and p35 mRNA expression. Total RNA were isolated from peritoneal macrophages of wild type (WT) (+/+), ICSBP heterozygous (+/−), and ICSBP homozygous (−/−) mice and subjected to analysis by RPA (A) or by qRTPCR (B). 10 μg of RNA were used in the RPA for the expression of the indicated cytokines using MCK2b (BD Biosciences). The RPA data are representative of two separate experiments with very similar results. The qRT-PCR data are the mean ± S.D. from three experiments. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. MIF, macrophage migration inhibitory factor.
ICSBP was drastically reduced in ICSBP induced by IFN-γ. ICSBP protein expression revealed that ICSBP was primarily as we have shown previously (12). An analysis of endogenous (10–20-fold) without exogenous ICSBP, as is the case here and but by far the greatest stimulation was seen in IFN-γ in a dose-dependent manner in RAW cells under all conditions, although not LPS activated cells (lane 12). The qRT-PCR analysis (Fig. 2B) shows more definitively that both LPS- and IFN-γ-LPS-induced IL-12 p35 mRNA levels were drastically reduced in ICSBP−/− macrophages. These data strongly indicate that ICSBP regulates both IL-12 p40 and p35 mRNA expression.

ICSBP Activates the IL-12 p35 Gene Transcription through a Proximal Promoter Region.—We had previously established the molecular mechanism whereby ICSBP regulates IL-12 p40 gene transcription (16). In this work, we sought to elucidate how ICSBP regulates IL-12 p35 gene transcription by first examining the response of the human IL-12 p35 promoter to the effects of ICSBP in a well-established transient transfection system in RAW cells (12). Fig. 3A shows that ICSBP expression stimulated the IL-12 p35 promoter-driven luciferase activity in a dose-dependent manner in RAW cells under all conditions, but by far the greatest stimulation was seen in IFN-γ-LPS-activated cells (filled diamonds). Note that, although difficult to discern in this graph because of the relatively low scale, the IL-12 p35 promoter was highly inducible by IFN-γ and LPS (10–20-fold) without exogenous ICSBP, as is the case here and as we have shown previously (12). An analysis of endogenous ICSBP protein expression revealed that ICSBP was primarily induced by IFN-γ in the nucleus, not in the cytoplasm, and that LPS treatment alone had little effect (Fig. 3B). These data clearly demonstrate the ability of ICSBP, primarily as an IFN-γ-inducible transcription factor, to activate IL-12 p35 gene transcription.

To further explore the molecular mechanism whereby IFN-γ regulates IL-12 p35 transcription through ICSBP, we dissected the human IL-12 p35 promoter by sequentially deleting the full-length promoter, a 1143-bp genomic fragment (12), from the 5′ end. The transcriptional start site of the human IL-12 p35 gene in macrophages has been mapped previously (21). Because ICSBP is primarily induced by IFN-γ, not LPS treatment, in macrophages (Fig. 3B), we stimulated RAW 264.7 cells with LPS in all of our transfection studies to provide the other essential set of transcription factors for maximally induced IL-12 p35 gene expression (12). As shown in Fig. 3C, the full-length IL-12 p35 promoter (−1082 to +61) was highly responsive to stimulation by ICSBP in resting cells (−8-fold compared with control vector-transfected cells) and in LPS-activated cells (−27-fold). Sequential deletion of the full-length promoter down to −231 had no adverse impact on the inducibility of the p35 promoter by ICSBP. Note that the −231/+61 construct has lost a previously identified element, IRF-E (−245 to −236), that is critical for response to IRF-1 (12). The loss of the IRF-E apparently had no effect on the response to ICSBP. A further deletion of 17 bp (to −214) resulted in a moderate loss of the ICSBP response in resting cells and a dramatic loss of response in LPS-stimulated cells, indicating the presence of a critical
cis-element(s) in this region involved in response to ICSBP. By this approach, we identified the 17-bp region between −231 and −214 as a site containing a ICSBP-RE. Within this region, the GeneTool™ program (Double Twist, now defunct) predicted an IFN-γ-regulated element consensus sequence (γ-IRE_CS), 5′-CTGGAGGC-3′, from −225 to −219.

Localization of ICSBP-RE—To assess whether the predicted sequence 5′-CTGGAGGC-3′ located at −226 to −219 was critical for ICSBP response, we introduced 2-bp substitutions into this site in the context of the minimal promoter (−231/+61). The wild type, mutant, and a downstream (−214/+61) construct were transfected into RAW cells, respectively, together with the ICSBP expression vector or its control. Cells were stimulated with LPS (7 h). Luciferase activity was measured from cell lysates and expressed as fold of stimulation over that of the same reporter co-transfected with the empty vector. Student’s t test was performed for the comparisons indicated by the inclusive brackets. Results are the mean ± S.E. from 9–11 independent experiments.

Fig. 4. Localization of an ICSBP-RE. A, sequence of wild type (WT) and mutant (Mut) of the putative ICSBP-RE in the IL-12 p35 promoter located at −226 to −219. The 2-bp mutation in the mutant ICSBP-RE is underlined. B, site-specific mutations (2 bp) were introduced into the ICSBP-RE at −226 to −225 in the context of the minimal promoter (−231/+61). The wild type, mutant, and a downstream (−214/+61) construct were transfected into RAW cells, respectively, together with the ICSBP expression vector or its control. Cells were stimulated with LPS (7 h). Luciferase activity was measured from cell lysates and expressed as fold of stimulation over that of the same reporter co-transfected with the empty vector. Student’s t test was performed for the comparisons indicated by the inclusive brackets. Results are the mean ± S.E. from 9–11 independent experiments.

cis-element(s) in this region involved in response to ICSBP. By this approach, we identified the 17-bp region between −231 and −214 as a site containing a ICSBP-RE. Within this region, the GeneTool™ program (Double Twist, now defunct) predicted an IFN-γ-regulated element consensus sequence (γ-IRE_CS), 5′-CTGGAGGC-3′, from −225 to −219. Localization of ICSBP-RE—To assess whether the predicted sequence 5′-CTGGAGGC-3′ located at −226 to −219 was critical for ICSBP response, we introduced 2-bp substitutions into this site in the context of the minimal promoter (−231/+61). The wild type, mutant, and a downstream (−214/+61) construct were transfected into RAW cells, respectively, together with the ICSBP expression vector or its control. Cells were stimulated with LPS (7 h). Luciferase activity was measured from cell lysates and expressed as fold of stimulation over that of the same reporter co-transfected with the empty vector. Student’s t test was performed for the comparisons indicated by the inclusive brackets. Results are the mean ± S.E. from 9–11 independent experiments.

cis-element(s) in this region involved in response to ICSBP. By this approach, we identified the 17-bp region between −231 and −214 as a site containing a ICSBP-RE. Within this region, the GeneTool™ program (Double Twist, now defunct) predicted an IFN-γ-regulated element consensus sequence (γ-IRE_CS), 5′-CTGGAGGC-3′, from −225 to −219. Localization of ICSBP-RE—To assess whether the predicted sequence 5′-CTGGAGGC-3′ located at −226 to −219 was critical for ICSBP response, we introduced 2-bp substitutions into this site in the context of the minimal promoter (−231/+61). The wild type, mutant, and a downstream (−214/+61) construct were transfected into RAW cells, respectively, together with the ICSBP expression vector or its control. Cells were stimulated with LPS (7 h). Luciferase activity was measured from cell lysates and expressed as fold of stimulation over that of the same reporter co-transfected with the empty vector. Student’s t test was performed for the comparisons indicated by the inclusive brackets. Results are the mean ± S.E. from 9–11 independent experiments.

DISCUSSION

IFN-γ-mediated enhancement of IL-12 production represents the convergence of signals derived from innate and adaptive immune responses. IFN-γ is produced mainly by NK cells during innate responses and by activated T lymphocytes during adaptive immunity. The exposure of professional antigen-presenting cells to IFN-γ activates IRFs including IRF-1 and ICSBP that are critical for maximal IL-12 synthesis when coupled with microbial stimuli such as LPS. This work builds on our previous studies and further elucidates the mechanism whereby the two signals initiated by innate and adaptive stimuli, respectively, converge to induce high levels of IL-12 production and ensure highly active and persistent NK and T helper type 1 responses.

The critical role of ICSBP in IL-12 production is illustrated in the observation that IL-12 p35 and p40 mRNA expression is impaired in ICSBP-deficient macrophages (Fig. 2) and that IL-12 p40 synthesis is severely reduced (Fig. 1, A and B), whereas IL-12 p70 production is virtually absent (Fig. 1, C and D). There is a quantitative discrepancy between reduced IL-12 p35 and p40 mRNA expression in ICSBP−/− macrophages on the one hand and the total lack of IL-12 p70 synthesis by these
cells on the other. This may be explained by the possibility that the reduced levels of IL-12 p40 and p35 proteins in ICSBP−/− cells fall below a certain “threshold” for the formation of IL-12 heterodimers. Alternatively, although less likely, the ICSBP deficiency causes an indirect effect on the assembly or secretion of the IL-12 p70 heterodimer. Post-translational regulation of IL-12 production has been reported previously (22–24). That in our transfection system, LPS coupled with forced ICSBP ex-
pression failed to induce significant activation of the IL-12 p35 promoter compared with unstimulated cells (Fig. 3A) is consistent with the fact that IL-12 p35 transcriptional activation also requires IRF-1, which is induced by IFN-γ but not by LPS (12). It should be stressed that the transcriptional activation of IL-12 p35 requires two signals, one typically provided by LPS

FIG. 6. ICSBP and IRF-1 bind to IL-12 p35 promoter in vivo. A, sequence of human IL-12 p35 promoter containing ICSBP-RE. The sequences of the PCR primers used to perform ChIP and ICSBP-RE are indicated. B, ChIP analysis was performed in human blood derived monocytes as described under “Materials and Methods.” The amplified human genomic fragment derived from the endogenous IL-12 p35 promoter encompassing the ICSBP-RE is indicated. Gel size markers are labeled in bp. The control antibody was an isotype-matched IgG. Input DNAs were used as controls, which should not vary significantly across all of the samples. C, qRT-PCR was performed with the newly extracted DNA samples, the same as that used in B, to quantify the binding activity of IRF-1 (top) and ICSBP (bottom) to the ICSBP-RE. Data are expressed as relative expression, i.e., fold increase of all of the conditions versus medium alone with anti-IRF-1 or anti-ICSBP antibody (Ab). M, medium; L, LPS; γ, IFN-γ. D, ICSBP expression in the nucleus. Nuclear extracts were isolated from primary human monocytes following stimulation with IFN-γ (20 h) or LPS (4 h) or both and then subjected to Western blot analysis using an anti-ICSBP Ab. Afterward, the membrane was stripped and reprobed with anti-PU.1 Ab to confirm equivalent protein loading.
and the other by IFN-γ. The LPS-initiated signal induces the activation of such transcription factors as NFκB and Sp-1 that are essential for IL-12 p35 transcription (12–14). The IFN-γ-initiated signal induces IRF-1 and ICSBP that are critical for IL-12 p35 transcription and p70 production (12, 14). Both signals are necessary (either signal alone is not sufficient) to activate IL-12 p35 transcription. The fact that the ICSBP response of the IL-12 p35 ICSBP-RE mutant promoter was reduced by ~70% regardless of the absence or presence of LPS (Fig. 4B) suggests that the LPS response of the promoter is not dependent on the integrity of the ICSBP-RE. In other words, the ICSBP and LPS responses of the IL-12 p35 promoter are separate.

We were unable to detect a direct interaction of ICSBP with the ICSBP-RE in vitro but able to do so in vivo by ChIP (Fig. 6B). This could be due to a low abundance of ICSBP in the complex or due to the shielding of ICSBP from antibody contact by IRF-1 in vitro. This “hindrance” by IRF-1 may not occur in vivo on the chromatin-wrapped IL-12 p35 promoter because, presumably, the conformation in which IRF-1 and ICSBP bind to the ICSBP-RE in the presence of the surrounding chromatin structures does not obstruct access by their respective antibodies during the ChIP procedure (Fig. 6B). Furthermore, other lines of evidence suggest that ICSBP functionally interact with IRF-1. They can synergize to stimulate IL-12 p35 transcription through the ICSBP-RE (Fig. 5C) by binding together to the ICSBP-RE in vivo (Fig. 6B). It should be pointed out that the ICSBP-RE we identified in the human IL-12 p35 promoter does not look like a conventional IRF-E (GAAA(G/C)(T/C)GAAA(G/C)(T/C)) (25) or ISRE ((A/G)NGAANN(G/A)ACT) (26). Thus, it is possible that ICSBP may interact with this element through an unidentified factor in addition to IRF-1. A third possibility is that ICSBP could enhance IRF-1 binding to the ICSBP-RE through an intermediary but itself does not come into direct contact with DNA.

Based on several studies by us as well as other groups, a model is emerging that schematizes the molecular events that lead to IL-12 production by antigen-presenting cells (Fig. 7 for the IL-12 p35 subunit gene). During the innate phase of an immune response, microbial antigens activate the NFκB pathway via Toll-like receptors (Toll-like receptor 4 for LPS) in a MyD88-dependent manner (27, 28). Activated NFκB alone stimulates moderate transcription of IL-12 p35 and p40 genes primarily through p50 and c-Rel (13, 29–31). The initial small amount of IL-12 is able to stimulate NK cells to produce IFN-γ. IFN-γ can also be produced by activated T cells. IFN-γ then activates IRF-1 and ICSBP, which can bind two sites in the IL-12 p35 promoter, either alone to the IRF-E by IRF-1 (12) or together (ICSBP-RE, Figs. 5 and 6), resulting in much greater levels of transcription of IL-12 p40 (16) and p35 genes (12) and the production of IL-12. The combined activity of IFN-γ and LPS also leads to the activation of Sp1 and its binding to a site in the p35 promoter as part of selective remodeling of a single nucleosome within the −310 to −160 region (14). The combination of signals derived from innate and adaptive immune events stimulate synergistically the production of IL-12 and sustain the inflammatory response and cell-mediated immunity against pathogens.

REFERENCES
1. Taniguchi, T., Ogawara, K., Takaoka, A., and Tanaka, N. (2001) Annu. Rev. Immunol. 19, 623–655
2. Matsuyama, T., Kimura, T., Kitagawa, M., Pfeffer, K., Kawakami, T., Watanabe, N., Kundig, T. M., Amakawa, R., Kishihara, K., Wakeham, A., et al. (1995) Cell 75, 83–97
3. Duncan, G. S., Mittrucker, H. W., Kagi, D., Matsuyama, T., and Mak, T. W. (1996) J. Exp. Med. 184, 2043–2048
4. Taki, S., Sato, T., Ogawara, K., Fukuda, T., Sato, M., Hida, S., Suzuki, G., Mitsuoka, M., Shin, E. H., Kojima, S., Taniguchi, T., and Asano, Y. (1997) Immunity 6, 673–679
5. Ogawara, K., Hida, S., Azimi, N., Tagaya, Y., Sato, T., Yokochi-Fukuda, T., Waldmann, T. A., Taniguchi, T., and Taki, S. (1998) Nature 391, 700–703
6. Ohzeki, T., Yoshida, H., Matsuyama, T., Duncan, G. S., Mak, T. W., and Ohashi, P. S. (1998) J. Exp. Med. 187, 967–972
7. Lehoff, M., Duncan, G. S., Perrick, D., Mittrucker, H. W., Bischof, S., Prezchtl, S., Rollinghoff, M., Schmitt, E., Pahl, A., and Mak, T. W. (2000) J. Exp. Med. 192, 325–336
8. Tsujimura, H., Tamura, T., and Ozato, K. (2003) J. Immunol. 170, 1131–1135
9. Tamura, T., Kong, H. J., Tanyapin, C., Taniguchi, H., Calame, K., and Ozato, K. (2003) Blood 102, 4547–4554
10. Trinchieri, G. (1995) Annu. Rev. Immunol. 13, 251–276
11. Snijders, A., Hilkens, C. M., van der Pouw Kraan, T. C., Engel, M., Aarden, L. A., and Kapseenberg, M. L. (1996) J. Immunol. 156, 1207–1212
12. Liu, J., Cao, S., Herman, L. M., and Ma, X. (2003) J. Exp. Med. 198, 1265–1276
13. Grumont, R., Hochstein, H., O’Keefe, M., Gagasyan, R., White, C., Caminschi, I., Cook, W., and Gerondakis, S. (2001) J. Exp. Med. 194, 1021–1032
14. Goriely, S., Demonte, D., Nizet, S., De Wit, D., Willems, F., Goldman, M., and Van Lint, C. (2003) Blood 101, 4984–4902
15. Masumi, A., Tamaoki, S., Wang, I. M., Ozato, K., and Komuro, K. (2000) FEBS Lett. 531, 348–353
16. Wang, I. M., Contursi, C., Masumi, A., Ma, X., Trinchieri, G., and Ozato, K. (2000) J. Immunol. 165, 271–279
17. Rajeevan, M. S., Ranamukhaarachchi, D. G., Vernon, S. D., and Unger, E. R. (2001) Methods 25, 443–451
18. Schreiber, E., Matthäus, P., Muller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419
19. Laemmli, U. K. (1970) Nature 227, 680–685
20. Ma, X., Chow, J. M., Gri, G., Carru, G., Gerosa, F., Wolf, S. F., Dziale, R., and Trinchieri, G. (1996) J. Exp. Med. 183, 147–157
Synergistic Activation of Interleukin-12 p35 Gene Transcription

21. Hayes, M. P., Murphy, F. J., and Burd, P. R. (1998) Blood 91, 4645–4651
22. Babik, J. M., Adams, E., Tone, Y., Fairchild, P. J., Tone, M., and Waldmann, H. (1999) J. Immunol. 162, 4069–4078
23. Murphy, F. J., Hayes, M. P., and Burd, P. R. (2000) J. Immunol. 164, 839–847
24. Carra, G., Gerosa, F., and Trinchieri, G. (2000) J. Immunol. 164, 4752–4761
25. Tanaka, N., Kawakami, T., and Taniguchi, T. (1993) Mol. Cell. Biol. 13, 4531–4538
26. Darnell, J. E., Jr., Kerr, I. M., and Stark, G. R. (1994) Science 264, 1415–1421
27. Seki, E., Tsutui, H., Tsuji, N. M., Hayashi, N., Adachi, K., Nakano, H., Futatsu-gi-Yumikura, S., Takeuchi, O., Hoshino, K., Akira, S., Fujimoto, J., and Nakanishi, K. (2002) J. Immunol. 169, 3863–3868
28. Edwards, A. D., Manickasingham, S. P., Sporri, R., Diebold, S. S., Schulz, O., Sher, A., Kaisho, T., Akira, S., and Reise Sousa, C. (2002) J. Immunol. 169, 3652–3660
29. Murphy, T. L., Cleveland, M. G., Kulesza, P., Magram, J., and Murphy, K. M. (1995) Mol. Cell. Biol. 15, 5258–5267
30. Gri, G., Savio, D., Trinchieri, G., and Ma, X. (1998) J. Biol. Chem. 273, 6431–6438
31. Sanjabi, S., Hoffmann, A., Liu, H. C., Baltimore, D., and Smale, S. T. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12705–12710