JOURNAL OF INTERFERON AND CYTOKINE RESEARCH 22:145–152 (2002)
Mary Ann Liebert, Inc.

REVIEW

ICSBP/IRF-8: Its Regulatory Roles in the Development of Myeloid Cells

TOMOHIKO TAMURA and KEIKO OZATO

ABSTRACT

Interferon (IFN) consensus sequence binding protein (ICSBP)/IFN regulatory factor (IRF)-8 is an IFNγ-inducible transcription factor of the IRF family and regulates transcription through multiple target DNA elements, such as IFN-stimulated response element (ISRE), Ets/IRF composite element, and IFN-γ activation site (GAS). ICSBP$^{-/-}$ mice are immunodeficient and susceptible to various pathogens. They have defects in the macrophage function, including the ability to induce interleukin-12 (IL-12) p40 and some IFN-γ-responsible genes. In addition, ICSBP$^{-/-}$ mice develop a chronic myelogenous leukemia (CML)-like syndrome, where a systemic expansion of granulocytes is followed by a fatal blast crisis. ICSBP$^{-/-}$ mice harbor an increased number of myeloid progenitor cells, and the $-/-$ progenitors preferentially give rise to granulocytes, although they cannot efficiently generate another descendant of the myeloid lineage, macrophages. Studies with myeloid progenitor cells have shown that ICSBP drives their differentiation toward macrophage, whereas it inhibits granulocyte differentiation. Furthermore, myeloid cells from ICSBP$^{-/-}$ mice are resistant to apoptosis. These results illustrate the mechanism by which the loss of ICSBP leads to immunodeficiency and CML-like syndrome and suggest ICSBP's critical role in the development of myeloid cells.

CLONING, STRUCTURE, AND EXPRESSION OF ICSBP

Interferon (IFN) consensus sequence binding (ICSBP), also designated IFN regulatory factor-8 (IRF-8), was originally cloned as a nuclear protein that binds to IFN consensus sequence (ICS) in the major histocompatibility complex (MHC) class I genes.[1] It belongs to the family of IRF transcription factors, which includes nine mammalian members and four virally encoded homologs.[2,3] ICSBP has the DNA-binding domain (DBD) conserved in the IRF family at the N-terminal region and the IRF association domain (IAD) in the C-terminal region (Fig. 1).[2–5] The IAD is also conserved in several IRF members (IRF-3, IRF-4, IRF-5, IRF-8, and IRF-9). To date, human and chicken homologs of ICSBP/IRF-8 have been identified.[6–8] ICSBP is expressed in the hematopoietic cells, including cells of the monocyte/macrophage lineage, B lymphocytes, and activated T lymphocytes, and is inducible by IFN-γ in these cells, suggesting its role in immune cells.[6,9–13] Lipopolysaccharide (LPS) also induces ICSBP, albeit to a lesser degree, and synergizes with IFN-γ in macrophages.[13–15] Recently, we found that ICSBP is expressed in lineage marker-negative (Lin$^-$) hematopoietic progenitor cells in bone marrow. The expression is relatively high in macrophages and very low in granulocytes (H. Tsujimura, T. Nagamura-Inoue, T.T., and K.O., unpublished observations). The expression of ICSBP and its induction by IFN-γ in lens cells and retinal pigment epithelial cells also have been reported.[16–19]

TARGET DNA ELEMENTS, INTERACTING PROTEINS, AND REGULATION OF TRANSCRIPTION

Subsequent studies have shown that ICSBP is a transcriptional repressor acting through the IFN-stimulated response element (ISRE).[6,20,21] ISRE and ICS contain the IRF recognition sequence (IRS), AANNGAAA, to which the DBD of the IRF family binds.[22,23] This sequence is present in many IFN-α/β-inducible genes, as well as other genes.

Several studies indicate that ICSBP acts by associating with other transcription factors. ICSBP interacts with IRF-1, IRF-2, or IRF-4 on the ISRE and negatively regulates some IFN-

Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

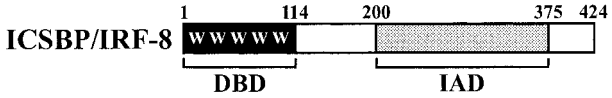

FIG. 1. Structure of ICSBP/IRF-8. A diagram of mouse ICSBP/IRF-8 is shown. The DBD contains a repeat of five conserved tryptophans (W).

inducible genes, such as *ISG15*, which carries a functional ISRE.[4,24,25] It also associates with PU.1 and stimulates activity of the Ets/IRF composite element (EICE), which also contains IRS and is present in the immunoglobulin (Ig) κ and λ, gp91^{phox}, scavenger receptor, toll-like receptor-4 (TLR-4), and interleukin (IL)-1β genes.[5,23,26–30] Interactions with IRF-1/2 and PU.1 significantly enhance DNA-binding activity of ICSBP and are mediated through the IAD.[5] PU.1 also interacts with another IRF member, IRF-4, to activate transcription through the EICE.[31] Among IRF family members, IRF-4 has the highest similarity in amino acid sequence with ICSBP. ICSBP was also shown to interact with E47 (encoded by the *E2A* gene) on the element composed of IRS-like sequence and the *E2A* binding site found in the Igκ 3′ enhancer to activate transcription, again like IRF-4.[5,32] As many of these studies relied on activities of transfected reporters containing ISRE or EICE, it will be important to determine if ICSBP regulates endogenous genes through these elements. Among many ISRE-carrying genes, only *ISG15* has been reported to be upregulated by the loss of ICSBP. Stable overexpression of ICSBP in U937 cells did not inhibit the IFN-α induction of endogenous 2′,5′-oligoadenylate synthetase (2′,5′-OAS), double-stranded RNA (dsRNA)-dependent kinase, IRF-2, and promyelocytic leukemia genes, all of which carry functional ISRE.[33] Among genes carrying EICE, it was shown that coexpression of PU.1 and either ICSBP or IRF-4 significantly induces endogenous IL-1β gene expression, whereas PU.1 alone is able to induce the expression only modestly.[30]

Recent studies indicate that ICSBP can act on a wide range of target elements even beyond the IRS, such as the Ets site in IL-12 p40 promoter and the IFN-γ activation site (GAS).[15,34] In these cases, ICSBP binds to the elements indirectly through protein-protein interactions and activates promoter activity. Clearly, ICSBP can act either as a repressor or as an activator, depending on the target DNA sequence, presumably by interacting with different proteins.

PHOSPHORYLATION OF ICSBP

It has been reported that ICSBP is constitutively phosphorylated on tyrosine (Tyr) residues *in vivo*. Although this Tyr phosphorylation negatively regulates ICSBP's own DNA binding activity, it is essential for the formation of heterodimer with IRF-1/2, and Tyr-phosphorylated ICSBP can bind target DNA only through association with IRF-1/2.[35]

ICSBP was shown to bind Trip15 (CSN2), which is a component of the COP9/signalosome (CSN) complex. The CSN complex was able to phosphorylate a serine residue within the IAD of ICSBP, the residue of which phosphorylation is essential for efficient association with IRF-1.[36]

IMMUNODEFICIENCY OF ICSBP$^{-/-}$ MICE

The phenotype of ICSBP$^{-/-}$ mice suggested a previously unpredicted role of ICSBP in myeloid cell development.[37] ICSBP$^{-/-}$ mice not only are immunodeficient because of defects in macrophage function but also develop a chronic myelogenous leukemia (CML)-like syndrome.

ICSBP$^{-/-}$ mice were found to display increased susceptibility to infection with various pathogens, control of which requires type II IFN (IFN-γ)-mediated immunity. These pathogens include vaccinia and lymphocytic choriomeningitis viruses,[37] such bacteria as *Listeria monocytogenes* and *Yersinia enterocolitica*,[38,39] and such parasites as *Leishmania major* and *Toxoplasma gondii*.[40,41] However, ICSBP$^{-/-}$ mice were able to survive infection with vesicular stomatitis virus (VSV) or influenza A,[37,40] which are controlled primarily by type I IFN (IFN-α/β)-mediated immunity and humoral immunity, respectively.

Regulation of IL-12 p40 gene by ICSBP

ICSBP$^{-/-}$ mice were shown to be deficient in producing IL-12 and IFN-γ and were unable to mount a Th1-mediated response.[37,40,41] IL-12 is composed of the p35 and p40 subunits, forming a heterodimer p70.[42] IL-12 p40 is expressed specifically in macrophages/dendritic cells, B cells, and neutrophils and induced on stimulation by pathogens or their components. IFN-γ priming of cells greatly enhances the inducibility of the IL-12 p40 gene. IL-12 governs production of IFN-γ in natural killer (NK) cells and CD4$^+$ T cells. IFN-γ, in turn, induces expression of effector molecules that elicit potent antiviral, antibacterial, and antiparasitic activities.[43] Strikingly, ICSBP$^{-/-}$ macrophages or cells from draining lymph nodes were severely impaired in both basal and induced expression of the IL-12 p40 gene after infection.[40,41] A similar impairment was noted for peritoneal macrophages in response to various *in vitro* stimuli, such as LPS, *Staphylococcus aureus* Cowan strain 1 (SAC), or soluble tachyzoite antigen (STAg) from *T. gondii* with and without IFN-γ pretreatment.[40,41]

On the other hand, splenocytes or purified CD4$^+$ T cells from ICSBP$^{-/-}$ mice could produce IFN-γ *in vitro* in response to concanavalin A (ConA), IL-12, or anti-CD3 plus anti-CD28 antibodies.[40,41,44] Administration of IL-12 induced IFN-γ production in ICSBP$^{-/-}$ mice *in vivo* in *Yersinia* infection.[39] ICSBP$^{-/-}$ splenocytes were able to eliminate *Listeria* in the Rag2$^{-/-}$ background, where ICSBP was absent in T and B cells but was normally expressed in macrophages and NK cells.[38] Therefore, expression of ICSBP in T cells appears not to be required for responding to these stimuli to secrete IFN-γ. It is apparent that ICSBP regulates IL-12 p40 gene activation and, as a consequence, promotes IFN-γ-dependent host resistance.

It was shown that ICSBP alone is able to activate mouse and human IL-12 p40 promoters. In the RAW 264.7 macrophages line, transfection of ISCBP alone led to induction of endogenous IL-12 p40 mRNA in the absence of IFN-γ and LPS. Analysis of human IL-12 p40 promoter indicated that ICSBP acts through the Ets site, known to be important for activation by IFN-γ/LPS.[15] Although exogenous IRF-1, also shown to be necessary for IL-12 p40 gene expression,[45,46] had no effect on its own, it synergized with ICSBP. A DNA affinity binding assay revealed that endogenous ICSBP is re-

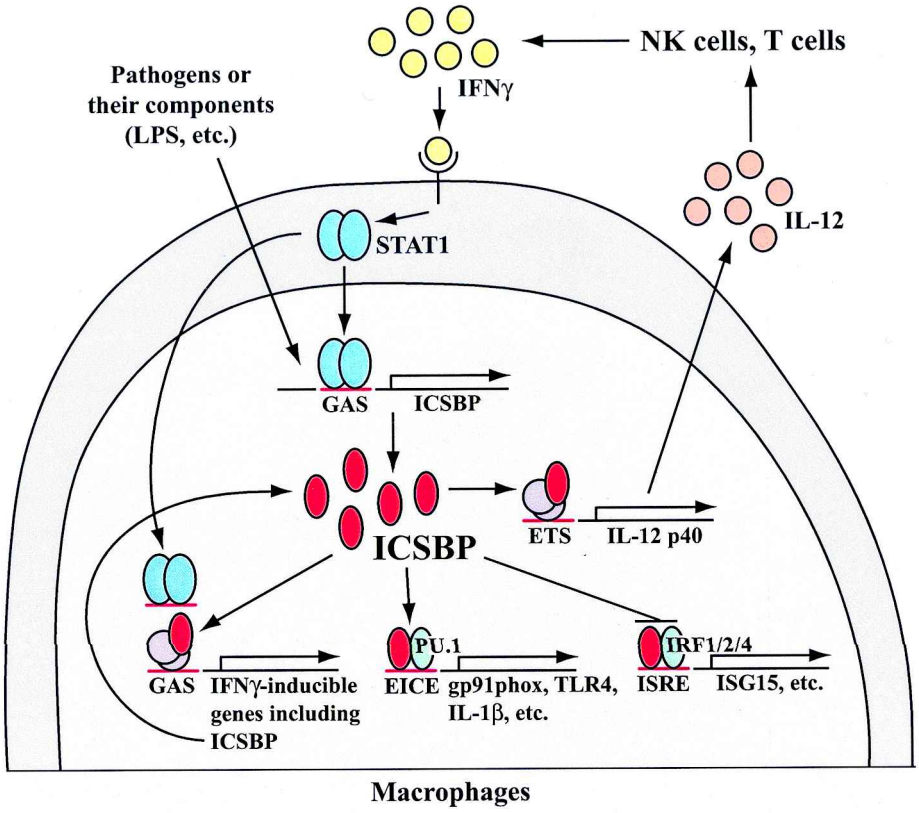

FIG. 2. A model for the role of ICSBP in immune responses against pathogens. ICSBP regulates multiple stages of immune responses mainly through macrophage function. ICSBP induces IL-12 p40 gene expression after various stimuli, such as pathogens, their components (LPS, SAC, and STAg), and IFN-γ. IL-12 promotes differentiation of NK and Th1 cells that secrete IFN-γ. ICSBP also potentiates induction of some IFN-γ-inducible genes including the *ICSBP* gene itself. This, in turn, likely makes a positive feedback circle to achieve efficient immunity.

cruited to the Ets site through protein-protein interaction. Interestingly, IRF-1 was also detected in this multiprotein complex on the Ets site. The complex on the Ets site also contained PU.1 and c-Rel.[47] Studies with the mouse IL-12 p40 promoter, however, suggest that an additional element may participate in activating IL-12 p40 transcription (unpublished observations). Thus, ICSBP, induced by IFN-γ/LPS, acts as a principal activator of IL-12 p40 transcription in macrophages.

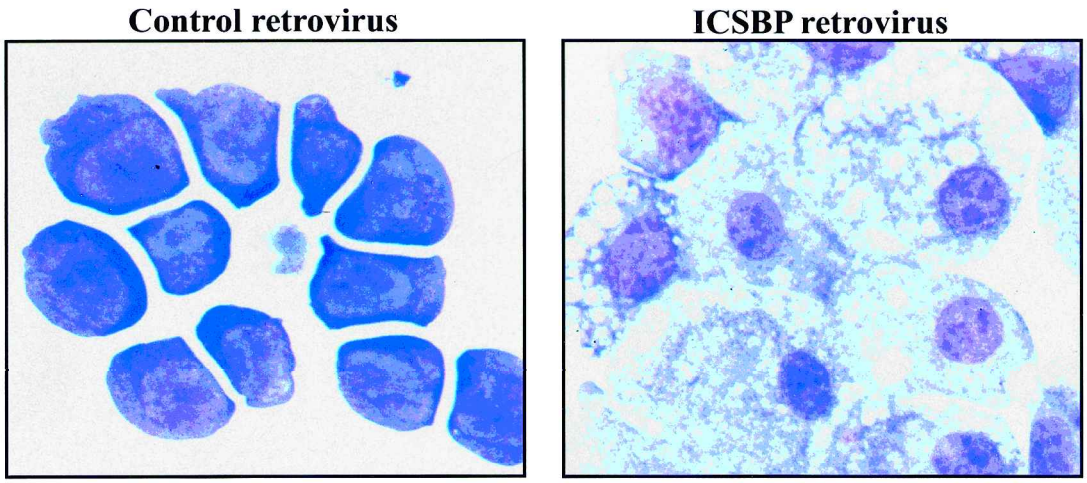

FIG. 3. ICSBP induces macrophage differentiation in the Tot2 ICSBP$^{-/-}$ myeloid progenitor cell line. Tot2 cells, the representative line of established ICSBP$^{-/-}$ myeloid progenitor cells, were transduced with the control retrovirus MSCV-puro **(left)** or MSCV-ICSBP-puro **(right)**. Transduced cells were stained with Wright-Giemsa 6 days after the transduction. ×600.

ICSBP-dependent activation of IFN-γ-inducible genes

IFN-γ, once called macrophage-activating factor, stimulates many genes in macrophages, leading to elicitation of macrophage activities that are fundamental to host defense against infections.[43] These activities include phagocytosis, antimicrobial activities, cytokine production, and antigen presentation. The functions of IFN-γ depend on activation of signal transducer and activator of transcription (Stat)1, which stimulates transcription of IFN-γ-inducible genes, including ICSBP, through the GAS element. A recent study suggests that ICSBP potentiates activation of GAS in macrophages.[34] Macrophages from ICSBP$^{-/-}$ mice were found to be defective in inducing some IFN-γ-responsive genes, such as Fcγ receptor I (FcγRI) and nitric oxide synthase (iNOS) genes. However, they were capable of activating Stat1 in response to IFN-γ. Accordingly, IFN-γ activation of luciferase reporters fused to the GAS element was severely impaired in ICSBP$^{-/-}$ macrophages, but transfection of ICSBP resulted in marked stimulation of these reporters, even in the absence of IFN-γ treatment and in Stat1-negative cells. DNA affinity binding assay revealed that endogenous ICSBP was recruited to a multiprotein complex that bound to GAS. Therefore, it is suggested that ICSBP, when induced by IFN-γ through Stat1, in turn generates a second wave of transcription from GAS-containing promoters, thereby contributing to the elicitation of the unique activities of IFN-γ in immune cells. Supporting this idea, in ICSBP$^{-/-}$ mice infected with *Toxoplasma*, administration of IL-12 prolonged their survival, but only partially.[41] Similarly, although exogenous IL-12 or IFN-γ could improve the course of *Yersinia* infection in ICSBP$^{-/-}$ mice, it failed to completely restore the impaired immunity,[39] suggesting that the *in vivo* response to IFN-γ may be impaired in ICSBP$^{-/-}$ mice.

Another piece of evidence pointing to a defect in responding to IFN-γ in ICSBP$^{-/-}$ macrophages is that they produce a reduced amount of reactive oxygen intermediates on IFN-γ stimulation.[38] Consistent with this, EICE-like elements in the promoters of the *CYBB* and *NCF2* genes were shown to be bound and activated by ICSBP together with PU.1 and IRF-1 in U937 cells.[28,48] The *CYBB* and *NCF2* genes encode p97^{phox} and p67^{phox}, respectively, which are IFN-γ-inducible subunits of the phagocyte respiratory burst oxidase catalytic unit.

Together, ICSBP regulates multiple stages of immune responses in macrophages. By virtue of regulating IL-12 p40 gene induction, ICSBP clearly affects differentiation of NK and Th1 cells that secrete IFN-γ. ICSBP is also involved in the subsequent phase of infection by potentiating activation of IFN-γ-inducible genes (Fig. 2).

ROLE OF ICSBP IN MYELOID CELL DIFFERENTIATION

A CML-like syndrome in ICSBP$^{-/-}$ mice

Unexpectedly, ICSBP$^{-/-}$ mice were found to develop a syndrome resembling CML in humans.[37] The most significant alteration was a systemic expansion and infiltration of granulocytes (predominantly mature neutrophils), causing lymphadenopathy and hepatosplenomegaly, which was observed in essentially 100% of ICSBP$^{-/-}$ mice. The appearance of pseudo-Gaucher cells and hyperplasia of plasma cells also were observed. The bone marrow exhibited a marked hypercellularity due to an increase in mature granulocytes and their precursors. The disease was noticeable by day 17 of embryogenesis, progressed with age, and became neutrophilia in peripheral blood. Other organs infiltrated by these cells included liver, myocardium, lungs, and kidneys. It was also shown that ICSBP$^{-/-}$ hematopoietic progenitor cells display significantly reduced adhesion to fibronectin.[49] These alterations simulate the chronic phase of CML in humans. Similar hematopathologic alterations, although in a quantitatively reduced form, were observed also in ICSBP$^{+/-}$ mice.

Transition of the chronic phase to a fatal blast crisis is a characteristic feature of human CML. Approximately 33% of ICSBP$^{-/-}$ and 9% of ICSBP$^{+/-}$ mice died by 50 weeks of age, with features indicating a transition to a blast crisis.[37] Systemic expansion of immature leukemic cells accompanied striking lymphadenopathy, hepatosplenomegaly, a marked enlargement of all abdominal organs, and severe leukocytosis ($>100{,}000/\mu l$). Cytochemistry results indicated that many of the immature cells belonged to the granulocyte lineage. In addition, immunochemistry and Southern blot analyses of Ig heavy chain gene J_H organization indicated the involvement of B lymphoid cells, clonality of the leukemic cells, and a biphenotypic or triphenotypic nature of blast transformation. Normal mice injected with cells from mice in blast crisis developed acute leukemia within 6 weeks of transfer. Therefore, it was suggested that ICSBP regulates the proliferation and differentiation of hematopoietic cells and may act as a tumor suppressor.

It has been reported that ICSBP expression is reduced in CML and acute myeloid leukemia (AML) patients.[50] IFN-α, the effective cytokine for therapy of CML, induced ICSBP expression *in vivo*, and the ICSBP expression correlated with pretreatment risk features and cytogenetic response to IFN-α in CML.[51] In the mouse model with a CML-like disease induced by Bcr-Abl, ICSBP expression was also downregulated. Interestingly, the mouse disease was ameliorated by coexpression of ICSBP.[52]

Furthermore, it was shown that forced expression of ICSBP induces potent immunity against Bcr-Abl-induced leukemia in mice.[53] Although ectopic expression of ICSBP did not alter *in vitro* growth of Bcr-Abl-transformed BaF3 cells, *in vivo* leukemia caused by the transformed BaF3 was completely inhibited by coexpression of ICSBP. Mice that survived a challenge with ICSBP-coexpressing transformed cells gained long-lasting immunity against parental leukemia cells. This immune rejection was associated with T cell-mediated reactivity against cell surface antigens specific to Bcr-Abl-transformed cells. The immune-mediated rejection of leukemic cells induced by ICSBP was rapid, potent, and effective against even preestablished disease, suggesting that ectopic ICSBP expression may provide a novel immunotherapeutic strategy for Bcr-Abl-positive leukemia patients. ICSBP may play an important role in the pathogenesis of leukemia not only as an intrinsic regulator in the leukemic cells but also as an immune regulator.

Regulation of myeloid cell growth and differentiation

It was shown that adult bone marrow and fetal liver of ICSBP$^{-/-}$ mice harbor increased numbers of progenitor cells,

which are hyperresponsive to both granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) *in vitro*, as assessed by colony-forming assay.[49] Cell transfer studies showed an intrinsic leukemogenic potential and long-term reconstitution capability of ICSBP$^{-/-}$ progenitors. In contrast, their response to macrophage colony-stimulating factor (M-CSF) was strongly reduced, and, surprisingly, ICSBP$^{-/-}$ colonies formed in the presence of M-CSF were mostly granulocytes. Consistent with this, cells of macrophage lineage (M-CSFR^{+} F4/80^{+} cells) in ICSBP$^{-/-}$ bone marrow were significantly fewer than wild-type counterparts.[49]

Myeloid progenitor cells give rise to granulocytes and macrophages. Several ICSBP$^{-/-}$ myeloid progenitor cell lines were established from mice undergoing blast crisis.[54] These progenitor cells grew in a GM-CSF-dependent manner and were capable of differentiating into mature granulocytes in response to G-CSF. Transduction of a retrovirus carrying the ICSBP gene caused marked differentiation in the progenitor cells, leading to generation of mature macrophages with phagocytic activity, which coincided with the induction of DNA-binding activity specific for ISRE and EICE (Fig. 3).[54] Domain analysis of ICSBP showed that the ability of ICSBP to induce macrophage differentiation precisely correlated with its ability to bind to the ISRE and EICE through interaction with IRF-2 or PU.1. Similar to macrophages *in vivo*, ICSBP-transduced cells were growth arrested at G_1 phase. They expressed many macrophage-specific genes such as c-*fms*, *Egr*-1, and scavenger receptor, and were positive for F4/80 on the cell surface. They responded to the classic macrophage-activating stimulus with IFN-γ/LPS and induced IL-12 p40, iNOS, and FcγR genes. In contrast, ICSBP transduction led to repression of granulocyte-specific genes, such as C/EBPα and ϵ and G-CSF receptor genes. Furthermore, ICSBP inhibited G-CSF-mediated granulocyte growth and differentiation in the ICSBP$^{-/-}$ progenitor cells and in the 32Dcl.3 myeloid progenitor cell line.[54] Recently, we observed very similar results using freshly isolated Lin^{-} bone marrow progenitor cells (H. Tsujimura, T. Nagamura-Inoue, T. T., and K. O., unpublished observations). These results suggest that whereas ICSBP stimulates expression of genes important for macrophage differentiation, it represses a series of genes required for granulocyte differentiation and that the two events are internally coupled through the action of ICSBP (Fig. 4). The dual mode of ICSBP action indicates that it plays a critical role in the lineage selection of myeloid progenitor. This fits well with *in vivo* expression profiles of ICSBP. ICSBP expression begins early in hematopoiesis and is expressed at higher levels in macrophages than in granulocytes (unpublished observations).[55]

ICSBP regulation of apoptosis in myeloid cells

Myeloid cells (Gr1^{+} as well as Mac1^{+} cells) from ICSBP$^{-/-}$ were shown to exhibit reduced spontaneous apoptosis. Further, in ICSBP$^{-/-}$ cells, there was a significant decrease in the sensitivity to apoptosis induced by DNA damage.[56] In contrast, apoptosis in ICSBP$^{-/-}$ thymocytes was unaffected. Overexpression of ICSBP in the human U937 myelomonoblastic leukemia cell line enhanced the rate of spontaneous apoptosis and increased the sensitivity to apoptosis induced by etoposide, LPS plus ATP, or rapamycin. Programmed cell death induced by etoposide was specifically blocked by peptides inhibitory for the caspase-1 or caspase-3 subfamilies of caspases. Cells overexpressing ICSBP had enhanced expression of caspase-3 precursor protein and decreased expression of Bcl-X(L). These results indicate that ICSBP modulates survival of myeloid cells by regulating the expression of apoptosis-related genes. The reduced apoptosis may contribute to quantitative and qualitative changes in the ICSBP$^{-/-}$ myeloid progenitor cells, as well as to their progeny, further promoting the overgeneration of granulocytes (Fig. 4).

Transcriptional pathways regulated by ICSBP

At present, immediate target genes activated or repressed by ICSBP that can trigger differentiation in the progenitor cells have not been identified. It is interesting to note that ICSBP induces a zinc finger transcription factor Egr-1 that has been implicated in macrophage differentiation,[57] although studies of Egr-1$^{-/-}$ mice indicated the presence of redundant mechanisms capable of compensating Egr-1 activity.[58] ICSBP may act upstream of Egr-1, and once Egr-1 is induced by ICSBP, it may generate a secondary cascade of gene expression emanating from Egr-1.

One of the proteins that directly interact with ICSBP is the Ets transcription factor PU.1, which regulates the expression of numerous myeloid-specific genes.[59] PU.1$^{-/-}$ mice do not produce mature macrophages and have very few granulocytes.[60] In view of the fact that ICSBP and PU.1 cooperate to activate a number of myeloid-specific gene promoters through the EICE, PU.1 is an obvious candidate partner with which ICSBP regulates myeloid cell development.

ICSBP also interacts with IRF-1 and IRF-2 and antagonizes IRF-1 activation of promoters through the standard ISRE. However, ICSBP and IRF-1 cooperate to activate transcription through some target elements.[15,28,30,48] Although neither IRF-1$^{-/-}$, IRF-2$^{-/-}$, nor IRF-1$^{-/-}$/IRF-2$^{-/-}$ mice were reported to have defects in myeloid differentiation, in light of well-known anti-oncogenic and proapoptotic functions of IRF-1 and the oncogenic potential of IRF-2,[61] ICSBP may regulate growth/apoptosis-related genes in myeloid cells by interacting with IRF-1 or IRF-2 or both.

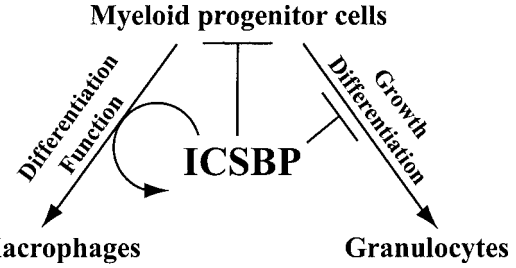

FIG. 4. Model for the role of ICSBP in myeloid cell development. ICSBP starts to affect the proliferative potential of myeloid cells at the progenitor cell level. Further, ICSBP regulates lineage selection of myeloid progenitor cells. It directs them to differentiate into macrophages and facilitates their functional maturation while it inhibits growth and differentiation of granulocytes. ICSBP positively regulates apoptosis in myeloid cells.

Several studies have investigated the expression and potential role of IRF-4 in macrophages.[25,27,30,62] IRF-4 is similar to ICSBP in structure, interacts with some common partners (PU.1 and E47), and regulates an overlapping set of target promoters.[31,32,63,64] IRF-$4^{-/-}$ mice develop progressive generalized lymphadenopathy and have severe defects in B and T lymphocyte function.[65] However, information on the development of myeloid cells in these mice is not available. It is possible that ICSBP and IRF-4 have an overlapping role and can compensate the loss of each other to a limited degree. It would be of interest to generate and examine ICSB$P^{-/-}$/IRF-$4^{-/-}$ double mutant mice.

In conclusion, ICSBP is an IRF member dedicated to the growth and differentiation of myeloid cells and functional activation of macrophages. Its expression profoundly affects various disease processes ranging from infections to leukemia. Further investigation, including a genome-wide gene expression study, may clarify the precise molecular mechanism by which ICSBP regulates myeloid cell development.

NOTE ADDED IN PROOF

Recently, we found that another Ets transcription factor, TEL, interacts with ICSBP to be recruited onto the ISRE (C. Gongora, T. Kuwata, T.T., and K.O., submitted for publication). Our recent data also show that ICSBP plays a critical role in the maturation of dendritic cells (H. Tsujimura, T.T., and K.O., submitted).

ACKNOWLEDGMENTS

We are indebted to Dr. Ivan Horak (Berlin, Germany), in whose laboratory ICSB$P^{-/-}$ mice were originally created. We also thank Drs. Ben-Zion Levi, Xiaojing Ma, Herbert C. Morse, III, John O'Shea, Robert A. Seder, Alan Sher, Giorgio Trinchieri, and Stefanie N. Vogel for valuable collaborations and successive members of the Ozato laboratory who contributed to research on ICSBP.

REFERENCES

1. DRIGGERS, P.H., ENNIST, D.L., GLEASON, S.L., MAK, W.H., MARKS, M.S., LEVI, B.Z., FLANAGAN, J.R., APPELLA, E., and OZATO, K. (1990). An interferon gamma-regulated protein that binds the interferon-inducible enhancer element of major histocompatibility complex class I genes. Proc. Natl. Acad. Sci. USA **87,** 3743–3747.
2. NGUYEN, H., HISCOTT, J., and PITHA, P.M. (1997). The growing family of interferon regulatory factors. Cytokine Growth Factor Rev. **8,** 293–312.
3. TANIGUCHI, T., OGASAWARA, K., TAKAOKA, A., and TANAKA, N. (2001). IRF family of transcription factors as regulators of host defense. Annu. Rev. Immunol. **19,** 623–655.
4. SHARF, R., AZRIEL, A., LEJBKOWICZ, F., WINOGRAD, S.S., EHRLICH, R., and LEVI, B.-Z. (1995). Functional domain analysis of interferon consensus sequence binding protein (ICSBP) and its association with interferon regulatory factors. J. Biol. Chem. **270,** 13063–13069.
5. MERARO, D., HASHMUELI, S., KOREN, B., AZRIEL, A., OUMARD, A., KIRCHHOFF, S., HAUSER, H., NAGULAPALLI, S., ATCHISON, M.L., and LEVI, B.-Z. (1999). Protein-protein and DNA-protein interactions affect the activity of lymphoid-specific IFN regulatory factors. J. Immunol. **163,** 6468–6478.
6. WEISZ, A., MARX, P., SHARF, R., APPELLA, E., DRIGGERS, P.H., OZATO, K., and LEVI, B.-Z. (1992). Human interferon consensus sequence binding protein is a negative regulator of enhancer elements common to interferon-inducible genes. J. Biol. Chem. **267,** 25589–25596.
7. JUNGWIRTH, C., REBBERT, M., OZATO, K., DEGEN, H.J., SCHULTZ, U., and DAWID, I.B. (1995). Chicken interferon consensus sequence-binding protein (ICSBP) and interferon regulatory factor (IRF) 1 genes reveal evolutionary conservation in the IRF gene family. Proc. Natl. Acad. Sci. USA **92,** 3105–3109.
8. DOSCH, E., ZOLLER, B., REDMANN-MULLER, I., NANDA, I., SCHMID, M., VICIANO-GOFFERGE, A., and JUNGWIRTH, C. (1998). The genomic structure of the chicken ICSBP gene and its transcriptional regulation by chicken interferon. Gene **210,** 265–275.
9. POLITIS, A.D., SIVO, J., DRIGGERS, P.H., OZATO, K., and VOGEL, S.N. (1992). Modulation of interferon consensus sequence binding protein mRNA in murine peritoneal macrophages. Induction by IFN-gamma and down-regulation by IFN-alpha, dexamethasone, and protein kinase inhibitors. J. Immunol. **148,** 801–807.
10. KANNO, Y., KOZAK, C.A., SCHINDLER, C., DRIGGERS, P.H., ENNIST, D.L., GLEASON, S.L., DARNELL, J.E., Jr., and OZATO, K. (1993). The genomic structure of the murine ICSBP gene reveals the presence of the gamma interferon-responsive element, to which an ISGF3 alpha subunit (or similar) molecule binds. Mol. Cell. Biol. **13,** 3951–3963.
11. POLITIS, A.D., OZATO, K., COLIGAN, J.E., and VOGEL, S.N. (1994). Regulation of IFN-gamma-induced nuclear expression of IFN consensus sequence binding protein in murine peritoneal macrophages. J. Immunol. **152,** 2270–2278.
12. NELSON, N., KANNO, Y., HONG, C., CONTURSI, C., FUJITA, T., FOWLKES, B.J., O'CONNELL, E., HU-LI, J., PAUL, W.E., JANKOVIC, D., SHER, A.F., COLIGAN, J.E., THORNTON, A., APPELLA, E., YANG, Y., and OZATO, K. (1996). Expression of IFN regulatory factor family proteins in lymphocytes. Induction of Stat-1 and IFN consensus sequence binding protein expression by T cell activation. J. Immunol. **156,** 3711–3720.
13. KANTAKAMALAKUL, W., POLITIS, A.D., MARECKI, S., SULLIVAN, T., OZATO, K., FENTON, M.J., and VOGEL, S.N. (1999). Regulation of IFN consensus sequence binding protein expression in murine macrophages. J. Immunol. **162,** 7417–7425.
14. BARBER, S.A., FULTZ, M.J., SALKOWSKI, C.A., and VOGEL, S.N. (1995). Differential expression of interferon regulatory factor 1 (IRF-1), IRF-2, and interferon consensus sequence binding protein genes in lipopolysaccharide (LPS)-responsive and LPS-hyporesponsive macrophages. Infect. Immun. **63,** 601–608.
15. WANG, I.M., CONTURSI, C., MASUMI, A., MA, X., TRINCHIERI, G., and OZATO, K. (2000). An IFN-gamma-inducible transcription factor, IFN consensus sequence binding protein (ICSBP), stimulates IL-12 p40 expression in macrophages. J. Immunol. **165,** 271–279.
16. EGWUAGU, C.E., SZTEIN, J., CHAN, C.C., MAHDI, R., NUSSENBLATT, R.B., and CHEPELINSKY, A.B. (1994). Gamma interferon expression disrupts lens and retinal differentiation in transgenic mice. Dev. Biol. **166,** 557–568.
17. LI, W., NAGINENI, C.N., GE, H., EFIOK, B., CHEPELINSKY, A.B., and EGWUAGU, C.E. (1999). Interferon consensus sequence-binding protein is constitutively expressed and differentially regulated in the ocular lens. J. Biol. Chem. **274,** 9686–9691.

18. LI, W., NAGINENI, C.N., EFIOK, B., CHEPELINSKY, A.B., and EGWUAGU, C.E. (1999). Interferon regulatory transcription factors are constitutively expressed and spatially regulated in the mouse lens. Dev. Biol. **210,** 44–55.
19. LI, W., NAGINENI, C.N., HOOKS, J.J., CHEPELINSKY, A.B., and EGWUAGU, C.E. (1999). Interferon-gamma signaling in human retinal pigment epithelial cells mediated by Stat1, ICSBP, and IRF-1 transcription factors. Invest. Ophthalmol. Vis. Sci. **40,** 976–982.
20. NELSON, N., MARKS, M.S., DRIGGERS, P.H., and OZATO, K. (1993). Interferon consensus sequence-binding protein, a member of the interferon regulatory factor family, suppresses interferon-induced gene transcription. Mol. Cell. Biol. **13,** 588–599.
21. WEISZ, A., KIRCHHOFF, S., and LEVI, B.-Z. (1994). IFN consensus sequence binding protein (ICSBP) is a conditional repressor of IFN inducible promoters. Int. Immunol. **6,** 1125–1131.
22. DARNELL, J.J., KERR, I.M., and STARK, G.R. (1994). Jak-Stat pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science **264,** 1415–1421.
23. FUJII, Y., SHIMIZU, T., KUSUMOTO, M., KYOGOKU, Y., TANIGUCHI, T., and HAKOSHIMA, T. (1999). Crystal structure of an IRF-DNA complex reveals novel DNA recognition and cooperative binding to a tandem repeat of core sequences. EMBO J. **18,** 5028–5041.
24. BOVOLENTA, C., DRIGGERS, P.H., MARKS, M.S., MEDIN, J.A., POLITIS, A.D., VOGEL, S.N., LEVY, D.E., SAKAGUCHI, K., APPELLA, E., COLIGAN, J.E., et al. (1994). Molecular interactions between interferon consensus sequence binding protein and members of the interferon regulatory factor family. Proc. Natl. Acad. Sci. USA **91,** 5046–5050.
25. ROSENBAUER, F., WARING, J.F., FOERSTER, J., WIETSTRUK, M., PHILIPP, D., and HORAK, I. (1999). Interferon consensus sequence binding protein and interferon regulatory factor-4/Pip form a complex that represses the expression of the interferon-stimulated gene-15 in macrophages. Blood **94,** 4274–4281.
26. BRASS, A.L., ZHU, A.Q., and SINGH, H. (1999). Assembly requirements of PU.1-Pip (IRF-4) activator complexes: inhibiting function *in vivo* using fused dimers. EMBO J. **18,** 977–991.
27. MARECKI, S., ATCHISON, M.L., and FENTON, M.J. (1999). Differential expression and distinct functions of IFN regulatory factor 4 and IFN consensus sequence binding protein in macrophages. J. Immunol. **163,** 2713–2722.
28. EKLUND, E.A, JALAVA, A., and KAKAR, R. (1998). PU.1, interferon regulatory factor 1, and interferon consensus sequence-binding protein cooperate to increase gp91 (phox) expression. J. Biol. Chem. **273,** 13957–13965.
29. REHLI, M., POLTORAK, A., SCHWARZFISCHER, L., KRAUSE, S.W., ANDREESEN, R., and BEUTLER, B. (2000). PU.1 and interferon consensus sequence-binding protein regulate the myeloid expression of the human toll-like receptor 4 gene. J. Biol. Chem. **275,** 9773–9781.
30. MARECKI, S., RIENDEAU, C.J., LIANG, M.D., and FENTON, M.J. (2001). PU.1 and multiple IFN regulatory factor proteins synergize to mediate transcriptional activation of the human IL-1beta gene. J. Immunol. **166,** 6829–6838.
31. EISENBEIS, C.F., SINGH, H., and STORB, U. (1995). Pip, a novel IRF family member, is a lymphoid-specific, PU.1-dependent transcriptional activator. Genes Dev. **9,** 1377–1387.
32. NAGULAPALLI, S., and ATCHISON, M.L. (1998). Transcription factor Pip can enhance DNA binding by E47, leading to transcriptional synergy involving multiple protein domains. Mol. Cell. Biol. **18,** 4639–4650.
33. THORNTON, A.M., BULLER, R.M., DEVICO, A.L., WANG, I.M., and OZATO, K. (1996). Inhibition of human immunodeficiency virus type 1 and vaccinia virus infection by a dominant negative factor of the interferon regulatory factor family expressed in monocytic cells. Proc. Natl. Acad. Sci. USA **93,** 383–387.
34. CONTURSI, C., WANG, I.M., GABRIELE, L., GADINA, M., O'SHEA, J., MORSE, H.C., 3rd, and OZATO, K. (2000). IFN consensus sequence binding protein potentiates Stat1-dependent activation of IFNgamma-responsive promoters in macrophages. Proc. Natl. Acad. Sci. USA **97,** 91–96.
35. SHARF, R., MERARO, D., AZRIEL, A., THORNTON, A.M., OZATO, K., PETRICOIN, E.F., LARNER, A.C., SCHAPER, F., HAUSER, H., and LEVI, B.-Z. (1997). Phosphorylation events modulate the ability of interferon consensus sequence binding protein to interact with interferon regulatory factors and to bind DNA. J. Biol. Chem. **272,** 9785–9792.
36. COHEN, H., AZRIEL, A., COHEN, T., MERARO, D., HASHMUELI, S., BECH-OTSCHIR, D., KRAFT, R., DUBIEL, W., and LEVI, B.-Z. (2000). Interaction between interferon consensus sequence-binding protein and COP9/signalosome subunit CSN2 (Trip15). A possible link between interferon regulatory factor signaling and the COP9/signalosome. J. Biol. Chem. **275,** 39081–39089.
37. HOLTSCHKE, T., LOHLER, J., KANNO, Y., FEHR, T., GIESE, N., ROSENBAUER, F., LOU, J., KNOBELOCH, K.P., GABRIELE, L., WARING, J.F., BACHMANN, M.F., ZINKERNAGEL, R.M., MORSE, H.C., 3rd, OZATO, K., and HORAK, I. (1996). Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell **87,** 307–317.
38. FEHR, T., SCHOEDON, G., ODERMATT, B., HOLTSCHKE, T., SCHNEEMANN, M., BACHMANN, M.F., MAK, T.W., HORAK, I., and ZINKERNAGEL, R.M. (1997). Crucial role of interferon consensus sequence binding protein, but neither of interferon regulatory factor 1 nor of nitric oxide synthesis for protection against murine listeriosis. J. Exp. Med. **185,** 921–931.
39. HEIN, J., KEMPF, V.A., DIEBOLD, J., BUCHELER, N., PREGER, S., HORAK, I., SING, A., KRAMER, U., and AUTENRIETH, I.B. (2000). Interferon consensus sequence binding protein confers resistance against *Yersinia enterocolitica*. Infect. Immun. **68,** 1408–1417.
40. GIESE, N.A., GABRIELE, L., DOHERTY, T.M., KLINMAN, D.M., TADESSE-HEATH, L., CONTURSI, C., EPSTEIN, S.L., and MORSE, H.C., 3rd (1997). Interferon (IFN) consensus sequence-binding protein, a transcription factor of the IFN regulatory factor family, regulates immune responses *in vivo* through control of interleukin 12 expression. J. Exp. Med. **186,** 1535–1546.
41. SCHARTON-KERSTEN, T., CONTURSI, C., MASUMI, A., SHER, A., and OZATO, K. (1997). Interferon consensus sequence binding protein-deficient mice display impaired resistance to intracellular infection due to a primary defect in interleukin 12 p40 induction. J. Exp. Med. **186,** 1523–1534.
42. TRINCHIERI, G. (1998). Interleukin-12: a cytokine at the interface of inflammation and immunity. Adv. Immunol. **70,** 83–243.
43. STARK, G.R., KERR, I.M., WILLIAMS, B.R., SILVERMAN, R.H., and SCHREIBER, R.D. (1998). How cells respond to interferons. Annu. Rev. Biochem. **67,** 227–264.
44. WU, C.Y., MAEDA, H., CONTURSI, C., OZATO, K., and SEDER, R.A. (1999). Differential requirement of IFN consensus sequence binding protein for the production of IL-12 and induction of Th1-type cells in response to IFN-gamma. J. Immunol. **162,** 807–812.
45. TAKI, S., SATO, T., OGASAWARA, K., FUKUDA, T., SATO, M., HIDA, S., SUZUKI, G., MITSUYAMA, M., SHIN, E.H., KOJIMA, S., TANIGUCHI, T., and ASANO, Y. (1997). Multistage regulation of Th1-type immune responses by the transcription factor IRF-1. Immunity **6,** 673–679.
46. SALKOWSKI, C.A., KOPYDLOWSKI, K., BLANCO, J., CODY, M.J., McNALLY, R., and VOGEL, S.N. (1999). IL-12 is dysreg-

ulated in macrophages from IRF-1 and IRF-2 knockout mice. J. Immunol. **163,** 1529–1536.
47. GRAZIA, CAPPIELLO, M., SUTTERWALA, F.S., TRINCHIERI, G., MOSSER, D.M., and MA, X. (2001). Suppression of IL-12 transcription in macrophages following Fc gamma receptor ligation. J. Immunol. **166,** 4498–4506.
48. EKLUND, E.A., and KAKAR, R. (1999). Recruitment of CREB-binding protein by PU.1, IFN-regulatory factor-1, and the IFN consensus sequence-binding protein is necessary for IFN-gamma-induced $p67^{phox}$ and $gp91^{phox}$ expression. J. Immunol. **163,** 6095–6105.
49. SCHELLER, M., FOERSTER, J., HEYWORTH, C.M., WARING, J.F., LOHLER, J., GILMORE, G.L., SHADDUCK, R.K., DEXTER, T.M., and HORAK, I. (1999). Altered development and cytokine responses of myeloid progenitors in the absence of transcription factor, interferon consensus sequence binding protein. Blood **94,** 3764–3771.
50. SCHMIDT, M., NAGEL, S., PROBA, J., THIEDE, C., RITTER, M., WARING, J.F., ROSENBAUER, F., HUHN, D., WITTIG, B., HORAK, I., and NEUBAUER, A. (1998). Lack of interferon consensus sequence binding protein (ICSBP) transcripts in human myeloid leukemias. Blood **91,** 22–29.
51. SCHMIDT, M., HOCHHAUS, A., NITSCHE, A., HEHLMANN, R., and NEUBAUER, A. (2001). Expression of nuclear transcription factor interferon consensus sequence binding protein in chronic myeloid leukemia correlates with pretreatment risk features and cytogenetic response to interferon-alpha. Blood **97,** 3648–3650.
52. HAO, S.X., and REN, R. (2000). Expression of interferon consensus sequence binding protein (ICSBP) is downregulated in Bcr-Abl-induced murine chronic myelogenous leukemia-like disease, and forced coexpression of ICSBP inhibits Bcr-Abl-induced myeloproliferative disorder. Mol. Cell. Biol. **20,** 1149–1161.
53. DENG, M., and DALEY, G.Q. (2001). Expression of interferon consensus sequence binding protein induces potent immunity against Bcr/Abl-induced leukemia. Blood **97,** 3491–3497.
54. TAMURA, T., NAGAMURA-INOUE, T., SHMELTZER, Z., KUWATA, T., and OZATO, K. (2000). ICSBP directs bipotential myeloid progenitor cells to differentiate into mature macrophages. Immunity **13,** 155–165.
55. PHILLIPS, R.L., ERNST, R.E., BRUNK, B., IVANOVA, N., MAHAN, M.A., DEANEHAN, J.K., MOORE, K.A., OVERTON, G.C., and LEMISCHKA, I.R. (2000). The genetic program of hematopoietic stem cells. Science **288,** 1635–1640.
56. GABRIELE, L., PHUNG, J., FUKUMOTO, J., SEGAL, D., WANG, I.M., GIANNAKAKOU, P., GIESE, N.A., OZATO, K., and MORSE, H.C., 3rd (1999). Regulation of apoptosis in myeloid cells by interferon consensus sequence-binding protein. J. Exp. Med. **190,** 411–421.
57. NGUYEN, H.Q., HOFFMAN, L.B., and LIEBERMANN, D.A. (1993). The zinc finger transcription factor Egr-1 is essential for and restricts differentiation along the macrophage lineage. Cell **72,** 197–209.
58. LEE, S.L., WANG, Y., and MILBRANDT, J. (1996). Unimpaired macrophage differentiation and activation in mice lacking the zinc finger transplantation factor NGFI-A (EGR1). Mol. Cell. Biol. **16,** 4566–4572.
59. TENEN, D.G., HROMAS, R., LIGHT, J.D., and ZHANG, D.E. (1997). Transcription factors, normal myeloid development, and leukemia. Blood **90,** 489–519.
60. SIMON, M.C. (1998). PU.1 and hematopoiesis: lessons learned from gene targeting experiments. Semin. Immunol. **10,** 111–118.
61. TANAKA, N., and TANIGUCHI, T. (2000). The interferon regulatory factors and oncogenesis. Semin. Cancer Biol. **10,** 73–81.
62. MARECKI, S., and FENTON, M.J. (2000). PU.1/interferon regulatory factor interactions: mechanisms of transcriptional regulation. Cell Biochem. Biophys. **33,** 127–148.
63. YAMAGATA, T., NISHIDA, J., TANAKA, S., SAKAI, R., MITANI, K., YOSHIDA, M., TANIGUCHI, T., YAZAKI, Y., and HIRAI, H. (1996). A novel interferon regulatory factor family transcription factor, ICSAT/Pip/LSIRF, that negatively regulates the activity of interferon-regulated genes. Mol. Cell. Biol. **16,** 1283–1294.
64. BRASS, A.L., KEHRLI, E., EISENBEIS, C.F., STORB, U., and SINGH, H. (1996). Pip, a lymphoid-restricted IRF, contains a regulatory domain that is important for autoinhibition and ternary complex formation with the Etx factor PU.1. Genes Dev. **10,** 2335–2347.
65. MITTRUCKER, H.W., MATSUYAMA, T., GROSSMAN, A., KUNDIG, T.M., POTTER, J., SHAHINIAN, A., WAKEHAM, A., PATTERSON, B., OHASHI, P.S., and MAK, T.W. (1997). Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. Science **275,** 540–543.

Address reprint requests to:
Dr. Tomohiko Tamura
Laboratory of Molecular Growth Regulation
National Institute of Child Health and Human Development
National Institutes of Health
Building 6, Room 416
9000 Rockville Pike
Bethesda, MD 20892-2753

Tel: (301) 496-2518
Fax: (301) 480-9354
E-mail: tamurat@mail.nih.gov

Accepted 1 August 2001