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Interferon regulatory factor-2 physically interacts with NF-κB in vitro and inhibits NF-κB induction of major histocompatibility class I and β2-microglobulin gene expression in transfected human neuroblastoma cells

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Abstract

Most neural cells constitutively lack major histocompatibility complex (MHC) class I and β2-microglobulin gene expression. Cytokines and viruses may, however, induce expression of these genes in some neural cells, and this correlates with factor binding to the NF-κB and interferon stimulated response elements of these genes. Here, we demonstrate that NF-κB is capable of inducing MHC class I and β2-microglobulin gene expression when transiently co-transfected into CHP-126 neuroblastomas, and that IRF-2 represses this induction. Interferon regulatory factor-2 (IRF-2) repression of MHC class I and β2-microglobulin gene expression in CHP-126 neuroblastomas may demonstrate a mechanism by which virus persists in neural cells. We show here that IRF-2 physically interacts in vitro with NF-κB. This interaction may contribute to the repression of the expression of these genes. Our demonstration that IRF family members, in addition to IRF-2, physically interact in vitro with NF-κB (p50 and p65), provides a general mechanism by which these transcription factors may, in concert, regulate the expression of a variety of genes involved in immune responses in the brain.

Keywords: Major histocompatibility complex; Neuroblastoma; NF-κB; Interferon regulatory factor-2; Protein–protein interaction

1. Introduction

Major histocompatibility complex (MHC) class I genes encode a series of polymorphic glycoproteins which, together with β2-microglobulin, present self and foreign peptides to $CD8^+$ class I-restricted T cells. MHC class I restricted CTL are essential for immune surveillance of virally infected as well as transformed tumor cells (Townsend and Bodmer, 1994). MHC class I genes are expressed broadly with the highest levels of expression in lymphoid organs (David-Watine et al., 1990). Cells of the central nervous system express low levels of MHC class I molecules (Lampson and Hickey, 1986) and this has been associated with persistence of virus in cells of neural origin (Oldstone, 1991). However, some neural cells express MHC class I in response to cytokines and upon viral infection (Lavi et al., 1988; Massa et al., 1989; Gogate et al., 1991), and these cells may be recognized by class I-restricted CTL.

MHC class I gene expression is principally regulated by an upstream enhancer region termed the Class I regulatory complex and the adjacent interferon consensus sequence (reviewed in Ting and Baldwin, 1993). Region I, located within the CRC, is an NF-κB-like site shown previously to enhance MHC class I gene expression (Burke et al., 1989). Region I may be bound by a variety of transcription

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factors. For example, Region I is bound by NF-κB (p50 and p65 heterodimer) as well as KBF-1 (NF-κB p50 homodimer) (reviewed in Siebenlist et al., 1994). Members of the C2H2-type zinc finger family of proteins including PRDII BF-1 are also capable of binding Region I (Singh et al., 1988; Fan and Maniatis, 1990; Nakamura et al., 1990). The MHC class I ICS contains the consensus interferon stimulated response element (ISRE) present in a variety of interferon-stimulated genes (reviewed in Darnell et al., 1994). Members of the IRF family of proteins have been shown to be capable of binding ISREs. The IRF family consists of IRF-1, IRF-2, interferon consensus sequence binding protein (ICSBP), and the DNA-binding subunit of ISGF3 termed ISGF3γ (Miyamoto et al., 1988; Harada et al., 1989; Driggers et al., 1990; Veals et al., 1992).

MHC class I and β2-microglobulin gene expression are commonly coordinately regulated. Interestingly, the β2-microglobulin promoter contains an NF-κB-like site and an ICS which act as enhancers of β2-microglobulin gene expression (Drew et al., 1993). NF-κB and ICS elements have been shown to mediate the cooperative induction of MHC class I and β2-microglobulin gene expression (Israel et al., 1986; Drew et al., 1993; Johnson and Pober, 1994). TNFα induces binding of NF-κB (p50 and p65) to Region I and IFNγ induces binding of IRF-1 to the ICS of the MHC class I promoter (Drew et al., 1993; Johnson and Pober, 1994; Drew et al., submitted). NF-κB and IRF-1 are capable of synergistically inducing MHC class I gene expression in transient transfection assays (Drew et al., submitted). Other IRF family members such as ISGF3γ activate, while IRF-2 and ICSBP repress expression of interferon-responsive genes. However, with the exception of IRF-1, the function of IRF family members in mediating the induction of MHC class I and β2-microglobulin gene expression has not been thoroughly investigated.

This report demonstrates that IRF-2 is capable of repressing the NF-κB induction of MHC class I and β2-microglobulin gene expression in transient co-transfection studies. It has been demonstrated that some virus-infected neural cells are capable of avoiding lysis by MHC class I restricted T cells by suppressing MHC class I surface expression (Joli et al., 1991). Thus, IRF-2 suppression of MHC class I gene expression may play a role in viral persistence in neural cells. We demonstrate that IRF-2 inhibits NF-κB induction of MHC class I and β2-microglobulin genes when transfected into CHP-126 neuroblastomas. We further show that IRF-2 physically interacts with NF-κB (p50 and p65) in vitro. This interaction may be important in IRF-2 suppression of MHC class I and β2-microglobulin gene expression. In addition, we demonstrate that a variety of IRF family members, in addition to IRF-2, interact in vitro with NF-κB. Interaction of these molecules may be important in regulation of the expression of a large number of cytokine-responsive genes, and may function in regulating inflammatory responses in the central nervous system.

2. Materials and methods

2.1. Cell culture

CHP-126 cells were maintained as described previously (Drew et al., 1993) and, where indicated, treated with 100 U/ml of human IFNγ (Amgen Biologicals, Thousand Oaks, CA). CHP-126 was derived from human neuroblastoma, a tumor of the sympathetic neuroblast (Lampson and Fisher, 1984).

2.2. RNA-blot analysis

RNA was separated on 1.2% agarose gels containing formaldehyde, blotted on nylon membranes (MSI, Westboro, MA), and hybridized to cDNA probes ^{32}P-labeled by the random priming method (Stratagene, La Jolla, CA) as described previously (Drew et al., 1993). The IRF-2 and B-actin probes were described previously (Harada et al., 1989; Drew et al., 1993). Following hybridization, blots were washed twice in 2 × SSC, 0.1% SDS for 30 min at room temperature and then twice in 1 × SSC, 0.1% SDS for 30 min at 65°C, and autoradiography was performed.

2.3. Transient transfection and CAT assays

CHP-126 cells were seeded at a density of 7.5×10^5 cells per 100-mm dish the day prior to transfection. Cells were transfected with 5 μg of MHC class I or β2-microglobulin-CAT reporter constructs (Drew et al., 1993) (see Fig. 1), and the indicated amount of NF-κB p50, NF-κB p65, and IRF-2 expression vectors (Harada et al., 1990; Bours et al., 1992). Carrier DNA (pUC-19) was added to normalize the amount of DNA per culture dish (approx. 20 μg). Transfections were performed by the calcium phosphate co-precipitation method. Cells were washed with PBS approx. 12 h following transfection and fresh medium was added. Following a 48 h incubation, cells were harvested and CAT activities determined (Drew et al., 1993). CAT activities were normalized for protein concentrations of the samples.

2.4. Construction of GST fusion protein and binding assays

The pGEX-p50 and pGEX-p65 constructs were prepared in the bacterial expression vector pGEX (Glutagene) as described previously (Franzoso et al., 1992, 1993). The GST-p50 and p65 fusion proteins and the GST protein were purified with glutathione-coated beads from bacterial sonicates as reported previously (Franzoso et al., 1992, 1993; Bours et al., 1993). The ICSBP, ISGF3γ, and IRF-2 plasmids used to program in vitro transcription/translation reaction (TNT-Promega) are described elsewhere (Bovolenta et al., 1994). The ^{35}S-methionine labelled translation products were assayed for binding to the GST-p50 and

GST-p65 fusion proteins attached to glutathione-coated beads as detailed (Franzoso et al., 1992, 1993; Bours et al., 1993). Briefly, in vitro translated ^{35}S-labelled proteins were mixed for 1 h at room temperature with GST, GST-p50, or GST-p65 fusion proteins attached to glutathione-Sepharose beads in buffer containing 20 mM Tris · HCl, pH 7.4, 150 mM NaCl, and 0.2% Triton X-100. The beads were precipitated, washed four times in the same buffer, and the attached material was separated on 10% polyacrylamide gels and detected by autoradiography.

3. Results

3.1. IRF-2 production in human neuronal cells

We previously demonstrated that CHP-126 neuroblastomas exhibit low transcription factor binding activity to NF-κB and ISRE sites present in the promoters of MHC class I and β2-microglobulin genes (Fig. 1). This explains, at least in part, the low surface expression of the encoded antigens on these cells (Drew et al., 1993). Factor binding to these elements correlates with the synergistic induction of MHC class I and β2-microglobulin gene expression in transient co-transfection assays of cytokine (TNFα and IFNγ) treated neuroblastomas. Furthermore, transiently co-transfected NF-κB and IRF-1 are capable of synergistically inducing MHC class I promoter activity in the neuroblastomas (Drew et al., submitted).

IRF-2 has been demonstrated to function as a repressor of interferon-stimulated genes (Harada et al., 1989, 1990; Nelson et al., 1993). Here, we show that IFNγ induces the expression of IRF-2 RNA levels in CHP-126 neuroblastomas. IRF-2 RNA levels are only slightly elevated at the earliest time (4 h) following IFNγ stimulation of the neuroblastomas, but remained elevated throughout the remainder of the assay (48 h) (Fig. 2). In contrast, IRF-1

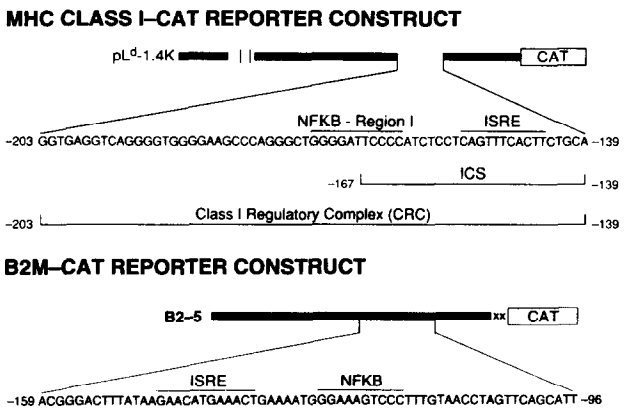

Fig. 1. Schematic diagram of MHC class I and β2-microglobulin reporter constructs. MHC class I pL^d 1.4K CAT contained 1.4 kb of MHC class L^d promoter and B25 contained approx. 500 bp of mouse β2-microglobulin promoters. Both the MHC class I and β2-microglobulin CAT constructs contained ISRE and NF-κB elements.

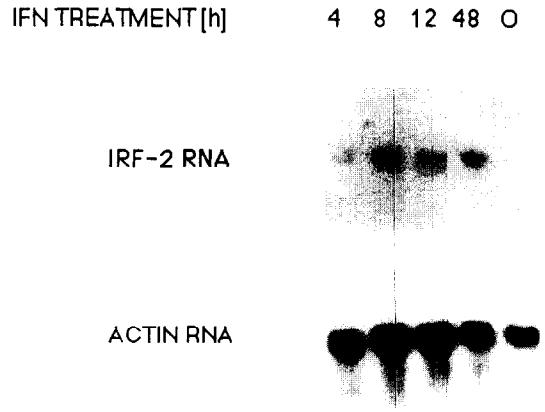

Fig. 2. INFγ induction of IRF-2 RNA in human neuroblastomas. Northern analysis using PolyA$^+$ RNA (5 μg/lane) from untreated CHP-126 neuroblastomas and from cells treated for the indicated time (h) with IFNγ.

RNA levels were markedly induced by 4 h and returned to constitutive levels by 48 h (Drew et al., submitted). These data are consistent with a model in which IRF-1 rapidly activates the expression of interferon-stimulated genes including MHC class I and IRF-2 later represses further transcription of these genes. In addition, our preliminary data suggest that IFNγ induces the production of IRF-2 protein in CHP-126 neuroblastomas as determined by electrophoretic mobility antibody super-shift assays with IRF-2 specific antibodies. Collectively, these data indicate that IRF-2 is produced in IFNγ stimulated CHP-126 neuroblastomas, and suggest that IRF-2 may regulate the expression of IFN-stimulated genes.

3.2. IRF-2 represses NF-κB induction of MHC class I and β2-microglobulin promoter activity

IRF-2 has previously been shown to repress the expression of interferon-stimulated genes including MHC class I (Harada et al., 1989, 1990; Nelson et al., 1993). Here, we investigated the effect upon MHC class I and β2-microglobulin promoter activity of NF-κB and IRF-2 expressed following transient transfection into the neuroblastomas. We demonstrate that NF-κB (p50 and p65) is capable of inducing MHC class I and β2-microglobulin promoter activity (Fig. 3). Furthermore, IRF-2 represses the NF-κB induction of these genes (Fig. 3). Neurons infected with virus have been demonstrated to lack MHC class I expression (Joli et al., 1991), which protects these cells from lysis by cytotoxic T cells (Griffin et al., 1992). Our studies suggest that IRF-2 may play a role in suppressing MHC class I and β2-microglobulin expression in neuroblastoma cells.

3.3. Physical interaction between NF-κB and IRF proteins

NF-κB and IRF proteins bind distinct regulatory elements of the MHC class I gene promoter. Yet, IRF-2

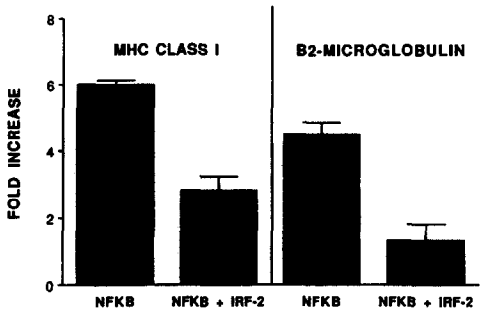

Fig. 3. IRF-2 inhibition of NFκB induction of MHC class I and β2-microglobulin promoter activity. Left panel: CHP-126 neuroblastomas were transiently transfected with MHC class I L^d 1.4K CAT (see schematic in Fig. 1). Where indicated, cells were also transfected with NF-κB p50 (5 μg), NF-κB p65 (1 μg), and IRF-2 (10 μg). Right panel: CHP-126 neuroblastomas were transfected with β2-microglobulin CAT (see schematic in Fig. 1). Where indicated, cells were transfected with NF-κB p50 (5 μg), NF-κB p65 (5 μg), and IRF-2 (10 μg). In each case, pUC19 was added to equilibrate the amount of DNA transfected into cells. Values are expressed relative to cells transfected with reporter CAT constructs (MHC class I L^d 1.4K CAT or β2-microglobulin CAT) alone. Experiments were performed three times independently and values represent the average of triplicate determinations ± one standard deviation.

represses NF-κB induction of MHC class I promoter activity. One mechanism by which NF-κB and IRF proteins could coordinately regulate the expression of MHC class I and other genes, is through physical interaction between these transcription factors. To test this, we performed GST binding analyses. Briefly, in vitro translated, radiolabelled IRF proteins were mixed with glutathione S-transferase (GST) or NF-κB-GST fusion proteins and precipitated with glutathione coated beads. The beads were washed and bound material was analyzed by SDS polyacrylamide gel electrophoresis followed by autoradiography. Our studies indicate that in vitro translated IRF-2 physically interacts with NF-κB fused to GST but not to control GST beads alone (Fig. 4). This provides a potential mechanism by which IRF-2 may regulate NF-κB activation of MHC class I gene expression. Our studies further indicate that in addition to IRF-2, the IRF family members, ICSBP and ISGF3γ, are capable of physically interacting in vitro with NF-κB (Fig. 4). Each IRF protein interacts in vitro more strongly with p65-GST than with p50-GST. The functional significance in vivo of this differential interaction is presently unknown. As an additional control, we demonstrated that in vitro translated NF-κB p50 interacted with both p50-GST and p65-GST as previously described, but a truncated NF-κB p50 peptide which lacked the dimerization domain (Franzoso et al., 1993) did not interact with either NF-κB-GST fusion protein, suggesting the specificity of the interactions (data not shown). Collectively, these data indicated that the IRF proteins IRF-2, ICSBP, and ISGF3γ specifically interact in vitro with NF-κB p50 and p65. This provides a general mechanism by which NF-κB and IRF proteins may act in concert to regulate the expression of a variety of cytokine-responsive genes.

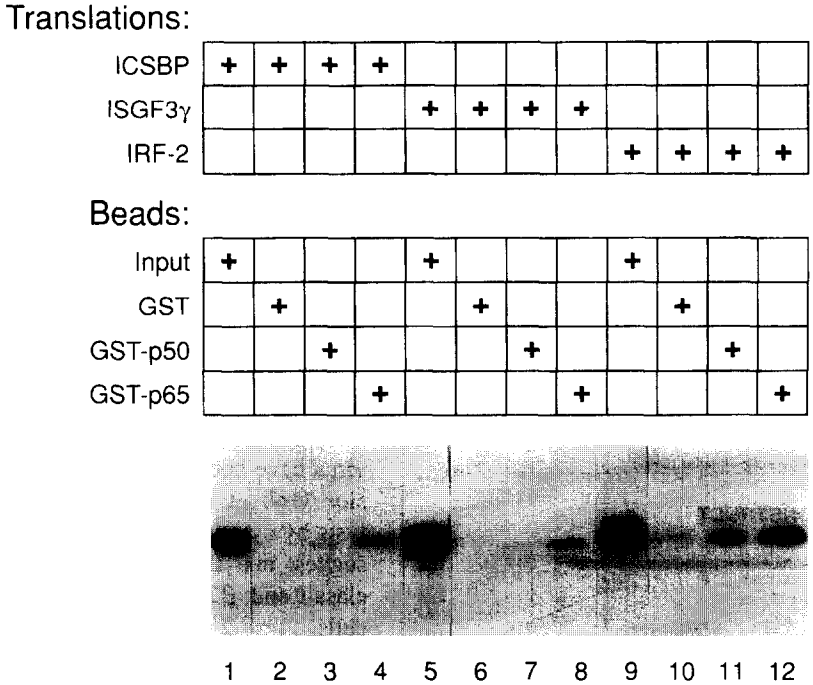

Fig. 4. Interaction of IRF proteins with NF-κB. ^{35}S-labelled in vitro translated ICSBP, ISGF3γ, and IRF-2 (lanes 1, 5, and 9, respectively). The labelled polypeptides shown were incubated in vitro with GST (lanes 2, 6, and 10), or with GST-p50 (lanes 3, 7, and 11) or GST-p65 fusion proteins (lanes 4, 8, and 12) and precipitated with glutathione coated sepharose beads. The translation products shown in lanes 1, 5, and 9 represent 1/10 of the amounts used for GST co-precipitation.

4. Discussion

MHC class I, in association with β2-microglobulin, is essential in the recognition of antigen presenting cells by cytotoxic T lymphocytes. Some neuronal cells have been demonstrated to escape immunesurveillance by cytotoxic T lymphocytes through repression of MHC class I surface expression. IRF-2 functions to repress IFN-stimulated genes in non-neuronal cells (Harada et al., 1989, 1990; Nelson et al., 1993) and thus is a candidate to repress MHC class I and β2-microglobulin gene expression in neuronal cells. We demonstrate that IFNγ treated neuroblastomas produce IRF-2 RNA. We also show that IRF-2 expressed in these cells functions to repress MHC class I and β2-microglobulin promoter activity in these neuroblastoma cells. Thus, IRF-2 may be important in the persistence of viral infection of cells of the central nervous system.

IRF-2 has been shown to inhibit the induction of interferon-stimulated genes by competing with the activator protein IRF-1 for binding to the ISRE of these genes. In order to determine if IRF-2 represses NF-κB induction of MHC class I and β2-microglobulin gene expression by additional molecular mechanisms, we looked for physical interaction between these proteins. We show by GST binding analyses that NF-κB (both p50 and p65) and IRF-2 directly interact which provides a likely additional mechanism by which IRF-2 represses NF-κB activation of these genes. The fact that NF-κB interacts with additional IRF proteins, suggests that physical interaction of these transcription factors are important in the regulation of the expression of a variety of cytokine mediated genes. During preparation of this manuscript, Neish et al. (1995) reported that IRF-1 and NF-κB p50 physically interact. This supports our observations that the remaining IRF family members (IRF-2, ICSBP, and ISGF3γ) physically interact with both NF-κB p50 and NF-κB p65.

Physical interaction of NF-κB and IRF proteins could alter MHC class I and β2-microglobulin gene expression by a variety of mechanisms. For example, interaction of these proteins could effect the affinity with which each protein binds DNA. Alternatively, physical interaction between the proteins could effect their ability to recruit additional enhancer binding proteins or components of the basal transcription machinery. Members of the NF-κB family of proteins have been shown to interact with transcription factors of unshared homology including SP1 (Perkins et al., 1993), as well as CREB ATF and CEBP proteins (Du et al., 1993; Stein et al., 1993). IRF proteins have been documented to physically interact with each other (Bovolenta et al., 1994). The present studies indicate that multiple IRF proteins are capable of interacting with NF-κB (p50 and p65). That IRF-2 represses NF-κB induction of MHC class I and β2-microglobulin gene expression suggests a functional role for this interaction. It would be important to determine whether IRF family members interact with additional non-homologous families of proteins.

NF-κB and ISRE (or virus-inducible) elements have been shown to be important in the regulation of a variety of genes in addition to MHC class I and β2-microglobulin, including IFNβ (Fan and Maniatis, 1989; Fujita et al., 1989) and vascular cell adhesion molecule (VCAM)-1 (Neish et al., 1995) genes. IRF-1 and NF-κB (p50/p65 heterodimer) are important in the activation of IFNβ and VCAM-1 gene expression (Miyamoto et al., 1988; Fujita et al., 1989; Neish et al., 1995).

IRF and NF-κB proteins interact with distinct gene regulatory elements in response to distinct cellular signalling mechanisms. Physical interaction between these transcription factor families provides a mechanism by which these proteins can cooperatively regulate the expression of a variety of genes important in immune responses including MHC class I and β2-microglobulin.

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