Virology **256,** 8–14 (1999)
Article ID viro.1999.9614, available online at http://www.idealibrary.com on IDEAL®

Interferon-Induced Guanylate Binding Protein-1 (GBP-1) Mediates an Antiviral Effect against Vesicular Stomatitis Virus and Encephalomyocarditis Virus

Sylvia L. Anderson, Jill M. Carton, Jing Lou,[1] Lily Xing,[2] and Berish Y. Rubin[3]

Department of Biological Sciences, Fordham University, Bronx, New York 10458

Received September 14, 1998; returned to author for revision December 1, 1998; accepted January 21, 1999

A cDNA encoding the human guanylate binding protein-1 (hGBP-1) was expressed in HeLa cells using a constitutive expression vector. Stably transfected clones expressing hGBP-1 exhibited resistance to the cytopathic effect mediated by both vesicular stomatitis virus (VSV) and encephalomyocarditis virus (EMCV) and produced less viral progeny than control cells following infection with these viruses. To study the role hGBP-1 plays in the IFN-mediated antiviral effect, cells were stably transfected with a construct expressing antisense RNA for hGBP-1. VSV infection of IFN-α-treated antisense RNA-expressing cells produced an amount of virus comparable to that produced in the parental cell line, while EMCV infection of the IFN-α-treated transfected cells and VSV and EMCV infection of the IFN-γ-treated transfected cells produced far more virus than was produced in the parental cell line. These results demonstrate that GBP-1 mediates an antiviral effect against VSV and EMCV and plays a role in the IFN-mediated antiviral response against these viruses.

INTRODUCTION

The interferons (IFNs) are a family of regulatory molecules, which, after binding to cell surface receptors of responsive cells, are capable of mediating an antiviral state, an antiproliferative effect, and a variety of immune responses (Lengyel, 1982; Pestka *et al.,* 1987; Johnson, 1987; Staeheli, 1990; Sen and Lengyel, 1992). The establishment of the antiviral effect by the IFNs is dependent on *de novo* RNA and protein synthesis (Friedman and Sonnabend, 1965; Lockart, 1964; Taylor, 1964). Extensive characterization of IFN-treated cells has revealed the IFN-mediated induction of new RNAs and proteins. Elucidation of the role these proteins play in the IFN-mediated antiviral effect has in part been accomplished by specifically modulating the expression of particular IFN-induced gene products and then characterizing the antiviral responses of these cells. Through such studies, investigators have demonstrated that: (1) murine Mx1 confers resistance to influenza virus (Staeheli *et al.,* 1986), Thogoto virus (Haller *et al.,* 1995), and Dhori virus (Thimme *et al.,* 1995); (2) functional murine Mx2 confers resistance to vesicular stomatitis virus (VSV) (Zurcher *et al.,* 1992); (3) human MxA protein confers resistance to influenza virus and VSV (Pavlovic *et al.,* 1990), measles virus (Schnorr *et al.,* 1993), and Thogoto virus (Frese *et al.,* 1995; Thimme *et al.,* 1995); (4) human double-stranded RNA-activated p68 kinase (PKR) confers resistance to encephalomyocarditis virus (EMCV) (Meurs *et al.,* 1992); and (5) human 2′-5′-oligoadenylate synthetase (OAS) confers resistance to Mengo virus and EMCV (Chebath *et al.,* 1987; Coccia *et al.,* 1990). Recent studies employing homologous recombination have enabled the generation of mice devoid of a functional PKR. Antiviral studies performed in these animals reveal that PKR plays a critical role in the antiviral effect mediated against EMCV following injection with poly(IC) or IFN-γ (Yang *et al.,* 1995). These findings are consistent with those observed in cells constitutively expressing PKR. The role other IFN-induced gene products play in IFN-mediated antiviral effects remains to be elucidated.

The IFN-induced guanylate binding proteins (GBPs) represent a family of proteins whose role in IFN-mediated biological effects is not understood. There are reports of at least two forms of the IFN-induced GBPs in human and murine cells (Cheng *et al.,* 1991; Wynn *et al.,* 1991). The IFN-induced GBPs exhibit significant amino acid sequence homology among themselves, have an affinity for guanylate, and function as GTPases (Cheng *et al.,* 1983; Schwemmle and Staeheli, 1994; Neun *et al.,* 1996). These proteins contain the first two motifs but lack the third motif of the tripartite consensus sequence reported by Dever and co-workers to be present in nine GTP-binding protein families (Dever *et al.,* 1987; Dever and Merrick, 1989). To investigate the biological activity of the IFN-induced GBPs, we have generated and characterized cells constitutively expressing the human IFN-induced GBP-1 (hGBP-1) and cells producing a reduced

[1] Present address: LifeGen Inc., 15 Womans Hill Court, Building E, Frederick, MD 21701.

[2] Present address: Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850.

[3] To whom correspondence and reprint requests should be addressed. Fax: (718) 817-3645. E-mail: rubin@murray.fordham.edu.

0042-6822/99 $30.00

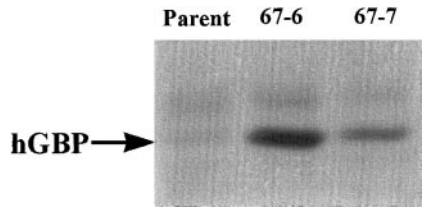

FIG. 1. Immunoprecipitation of hGBP-1 from cells stably transfected with the hGBP-1 producing pBactneo vector. The parental HeLa cell line and two G418-resistant clones termed 67-6 and 67-7 that were stably transfected with the constitutive hGBP-1 producing pBactneo vector were incubated in the presence of [^{35}S]methionine for 18 h at which time the cells were lysed and immunoprecipitations employing a monoclonal antibody to hGBP-1 were performed as described (Rubin *et al.,* 1988a).

amount of GBPs in response to IFN treatment as a result of the expression of an antisense RNA to hGBP-1. The biological responsiveness of these cells is presented herein.

RESULTS

Isolation of a full-length cDNA for hGBP-1

A λgt10 cDNA library prepared using mRNA isolated from IFN-γ-treated FS_4 cells was screened with a partial cDNA fragment for hGBP-1 isolated by Margolis-Nunno *et al.* (1990). A λgt10 clone containing a full-length cDNA insert of approximately 2 kb that has >99% nucleotide sequence homology with the reported sequence of hGBP-1 (Locus HUMGBP1) was isolated (Cheng *et al.,* 1991).

Generation of cells overexpressing hGBP-1

The cDNA for hGBP-1 was incorporated into the pBactneo vector in which constitutive expression of the insert is driven by the β-actin promoter (Reis *et al.,* 1992) in an orientation that resulted in the expression of hGBP-1 RNA. Transfection of HeLa cells with this plasmid resulted in the generation of clones resistant to G418. The ability of these cells to constitutively synthesize hGBP-1 was determined by immunoprecipitation using monoclonal antibody developed against and capable of recognizing hGBP-1. As can be seen in Fig. 1, two clones, termed 67-6 and 67-7, that constitutively express hGBP-1 were identified.

Replication of virus in cells constitutively expressing hGBP-1

The ability of virus to replicate in cells constitutively expressing hGBP-1 was first studied by assessing the cytopathic effect of VSV and EMCV on these cells. The parental cell line and clones 67-6 and 67-7 were infected with either VSV or EMCV at a multiplicity of infection (m.o.i.) of 1 as described under Materials and Methods. Eighteen hours later the culture medium was removed and the cytopathic effect of virus infection was examined by staining the wells with crystal violet. As can be seen in Fig. 2, the hGBP-1 producing cells exhibited far less VSV- and EMCV-mediated cytopathic effect than was observed in the parental cell line.

An assessment of the ability of virus to replicate in these cells was further examined by performing virus yield assays. For this study, cultures of the parental cell line as well as the hGBP-1 expressing clones were infected with VSV or EMCV and the virus yield was determined as described under Materials and Methods. Figure 3, which presents data generated in four experiments performed on clones 67-6 and 67-7, reveals that constitutive expression of hGBP-1 results in an approximately 50% reduction in the amount of VSV and EMCV produced by these cells. To further study the antiviral response observed in cells expressing hGBP-1, we infected cells transiently transfected either with the pBactneo vector alone or with the pBactneo vector producing hGBP-1 mRNA with VSV or EMCV and assessed the virus yield generated in these cells. A similar inhibition of both VSV and EMCV replication was observed (data not shown). In experiments performed using the GBP sense-producing constructs, parallel cultures were prepared in which the number of cells present at the time of infection was determined. No significant difference in the number of cells in the different cell cultures was detected.

Generation of cells producing a reduced amount of hGBP-1 as a result of expression of antisense RNA for hGBP-1

As the above studies indicate that the expression of hGBP-1 mediates an antiviral response in cells, we studied the role hGBP-1 plays in the IFN-mediated antiviral response. For this study, hGBP-1 cDNA was incorporated into the pBactneo vector in an orientation that resulted in

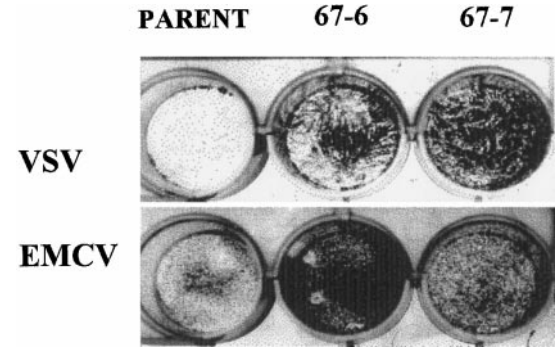

FIG. 2. Examination of the cytopathic effect of VSV and EMCV in cells constitutively producing hGBP-1. HeLa cells and cells of clones 67-6 and 67-7 were seeded in 24-well plates at a density of 10^5 cells/well and allowed to incubate at 37°C for 18 h at which time the wells were infected with either VSV or EMCV at an m.o.i. of 1. Eighteen hours later, the culture medium present in the wells was removed, and the cells were washed twice with PBS and then stained with 20% ethanol containing 10% crystal violet.

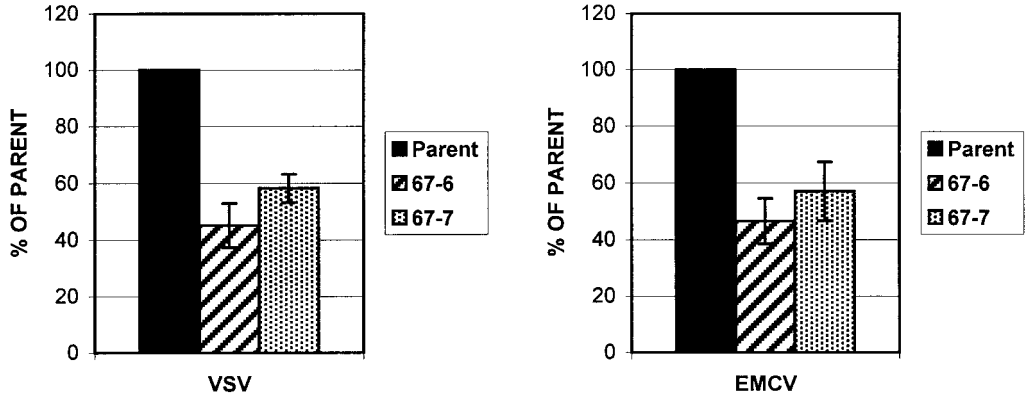

FIG. 3. Virus production in cells constitutively producing hGBP-1. HeLa cells and cells of clones 67-6 and 67-7 were seeded in 24-well plates at a density of 10^5 cells/well and allowed to incubate at 37°C for 18 h at which time they were infected with either VSV or EMCV at an m.o.i. of 1. Following an 18-h incubation period, the virus yields were determined as described under Materials and Methods. Results presented reflect the relative virus yields of the two clones constitutively expressing hGBP-1 compared to the parental cell line. The results are given as the means ± SD ($n = 4$).

the production of antisense hGBP-1 RNA. This construct was introduced into HeLa cells and cells isolated due to their resistance to G418 were assessed for their ability to produce hGBP-1 in response to IFN treatment. Two clones, termed 67-2-AS and 67-4A-AS, were identified as producing hGBP-1 antisense RNA and less hGBP-1 protein in response to IFN-γ treatment than was produced in the parental cell line (Fig. 4).

IFN-mediated antiviral response in cells inducing in response to IFN treatment a reduced amount of hGBP-1 as a result of transfection with a construct constitutively expressing antisense RNA to hGBP-1

To examine the role hGBP-1 plays in the IFN-mediated antiviral state, cells producing a reduced amount of hGBP-1 as a result of constitutive expression of antisense RNA to hGBP-1 were treated with no IFN or with IFN-α or IFN-γ for 18 h and then infected with either VSV or EMCV at an m.o.i. of 1. The cells were harvested 18 h later and virus yields were determined as described under Materials and Methods. As can be seen in Fig. 5, the reduced production of hGBP-1 in IFN-α-treated cells had little effect on the replication of VSV, while the virus-inhibiting effect of IFN-α treatment on EMCV and the virus-inhibiting effect of IFN-γ treatment on both VSV and EMCV were greatly reduced in cells producing a reduced amount of hGBP-1.

As the generation of double-stranded RNA in cells producing both the sense and the antisense forms of an RNA might interfere with the action of IFN-induced PKR (Schneider *et al.,* 1985; Kitajewski *et al.,* 1986; Katze *et al.,* 1987), it was important to control for any effects mediated by the generation of double-stranded RNA. To accomplish this, the IFN-mediated antiviral effect was exam-

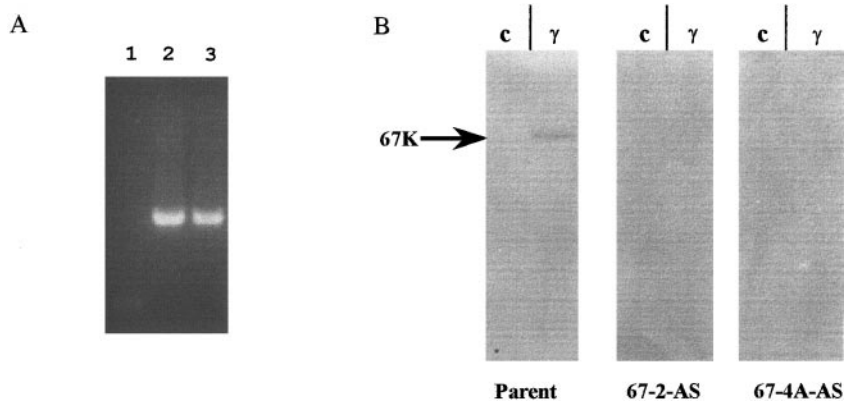

FIG. 4. RT-PCR (A) and Western blot analysis (B) of cells stably transfected with a vector containing the hGBP-1 cDNA in the antisense orientation relative to the vector promoter. (A) 50 ng of DNase-treated total RNA isolated from the parental HeLa cell line (1) and two G418-resistant clones, 67-2-AS (2) and 67-4A-AS (3), stably transfected with a construct expressing antisense hGBP-1 RNA, was analyzed for the presence of antisense hGBP-1 RNA as described under Materials and Methods. RT-PCR samples were run on a 0.8% ethidium bromide-containing agarose gel and photographed under UV light. (B) Lysates prepared from the parental HeLa cell line or from two hGBP-1 antisense RNA-producing clones that had been treated for 18 h with 300 U/ml IFN-γ were subjected to Western blot analysis as described under Materials and Methods.

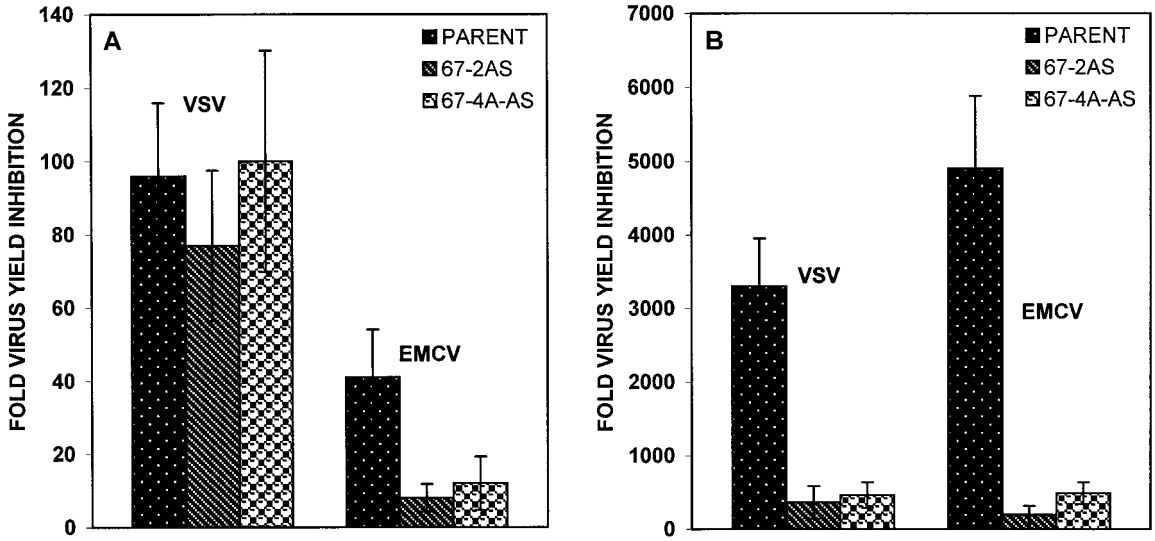

FIG. 5. Virus production in IFN-treated cells constitutively producing antisense RNA to the hGBP-1 mRNA. HeLa cells and cells of clones 67-2-AS and 67-4A-AS were seeded in 24-well plates at a density of 5×10^4 cells and allowed to incubate at 37°C for 24 h at which time the cells were incubated for 18 h in the presence of 300 U/ml of IFN-α or IFN-γ. These cultures were then infected with either VSV or EMCV at an m.o.i. of 1. Following an 18-h incubation period, the cultures were harvested and the virus yields determined as described under Materials and Methods. Results presented reflect the fold reduction in virus titers in the presence of IFN-α (A) or IFN-γ (B) in the respective cell lines. The results are given as the means $\pm$ SD ($n = 3$).

ined in cells transfected with two constructs such that they produced both sense and antisense RNA to β-galactosidase. These cells exhibited an IFN-mediated antiviral response comparable to that of the parental cell line (data not shown). This observation, taken together with the observation that PKR expression does not mediate an inhibition of VSV, suggests that the observations made in the hGBP-1 antisense RNA producing cells result from the reduced amount of GBP produced in these cells.

DISCUSSION

Since the dependence of the IFN-mediated antiviral response on *de novo* RNA and protein synthesis was observed, investigators have studied the IFN-mediated induction of numerous proteins and RNAs. Through (1) the generation of cells constitutively expressing IFN-induced gene products, (2) the generation of dominant negative mutants blocking IFN-induced enzymatic pathways, and (3) the generation of mice incapable of producing IFN-inducible gene products, scientists have gained further insight into the role some of these gene products play in IFN-mediated effects.

To examine the role that IFN-induced GBP-1 plays in the IFN-mediated antiviral effect, we generated stably and transiently transfected cells constitutively producing hGBP-1 and cells inducing, in response to IFN treatment, a reduced amount of hGBP-1 as a result of having been transfected with a vector constitutively producing an antisense RNA to hGBP-1. Constitutive expression of hGBP-1 in stably transfected cells results in an inhibition of the VSV- and EMCV-mediated cytopathic effects and results in a modest but reproducible reduction in the amount of VSV and EMCV produced by these cells. Cells transiently transfected with the hGBP-1 producing construct show a similar reduction in the amount of VSV and EMCV progeny produced. Taken together, these results demonstrate that the expression of GBP-1 in the absence of IFN treatment results in an inhibition of viral replication.

To examine the role hGBP-1 plays in the IFN-mediated antiviral effect, we generated cells constitutively producing antisense RNA to hGBP-1 that induced a reduced amount of hGBP-1 in response to IFN treatment. Infection of these IFN-treated cells revealed that they produce a weaker IFN-α-mediated antiviral effect against EMCV and a weaker antiviral effect against both VSV and EMCV in IFN-γ-treated cells than is observed in the parental cell line. These findings are consistent with those made in cells constitutively producing hGBP-1. In light of the preferential induction of MxA in IFN-α-treated cells and in light of the role MxA plays in the inhibition of VSV (Pavlovic *et al.,* 1990), and the minimal induction of GBP-1 observed in IFN-α-treated cells (Rubin *et al.,* 1988b), it is perhaps not surprising that the production of antisense RNA to hGBP-1 had no effect on the ability of IFN-α to inhibit VSV. Conversely, in IFN-γ-treated cells in which there is minimal IFN-mediated MxA induction but a strong induction of GBP-1 (Rubin *et al.,* 1988b), the inhibition of GBP-1 production was sufficient to partially abrogate the IFN-induced antiviral activity against VSV. The reduced antiviral effect observed in the IFN-α- and

IFN-γ-treated cells infected with EMCV, which is not inhibited by MxA expression (Pavlovic *et al.,* 1990), suggests that MxA and GBP-1 may serve as nonredundant effectors of IFN-mediated antiviral activities.

While early experiments suggested that IFN-α is a more potent inhibitor of VSV and EMCV than is IFN-γ (Rubin and Gupta, 1980), subsequent studies have revealed that the degree of responsiveness to the different IFNs is dependent on the cell type being studied and that even clonal isolates of a single cell line can differ in their antiviral responsiveness to either IFN-α or IFN-γ (Kumar *et al.,* 1987). Examination of the data presented in Fig. 5 reveals that in the HeLa cell line we employed in this study, IFN-γ mediates a more potent antiviral effect against VSV and EMCV than does IFN-α.

As the antisense RNA produced in the cells we studied would likely interact with other members of the IFN-induced GBP family, it is possible that the effect observed in cells producing the antisense RNA for GBP-1 might be due to an inhibition of the synthesis of GBP-1 as well as other members of this gene family.

Since the production of double-stranded RNA has been observed to interfere with the action of the IFN-induced PKR (Schneider *et al.,* 1985; Kitajewski *et al.,* 1986; Katze *et al.,* 1987) and might through other unknown mechanisms interfere with the antiviral action of the IFNs, it was necessary to assess whether the production of double-stranded RNA in the antisense RNA-producing cells might explain the reduced IFN-mediated antiviral response observed in these cells. For these studies cells generated that produced both sense and antisense RNA for β-galactosidase were treated with IFN and the antiviral response of these cells was assessed. The simultaneous production of both the sense and the antisense RNAs had no effect on the IFN-mediated antiviral effect.

With these findings we establish that constitutive expression of hGBP-1 results in an inhibition of the replication of VSV and EMCV and that the reduced ability of IFN-treated cells to produce GBP-1 results in an elevated production of viral progeny. These findings provide the first evidence that GBP-1 plays a critical role in the inhibition of VSV and EMCV replication. Interestingly while both MxA and GBP-1 exhibit GTPase activity, their virus specificity differs, as GBP-1 is capable of inhibiting the replication of both VSV and EMCV while MxA is incapable of inhibiting the replication of EMCV (Pavlovic *et al.,* 1990). The observed ability of GBP-1 to mediate an antiviral effect may in part explain the results of Pine (1992) and Kimura *et al.* (1994), who report that modulation of expression of IRF-1, which results in an alteration in the levels of various gene products including GBP-1, also results in a corresponding effect on the replication of VSV and EMCV.

MATERIALS AND METHODS

Materials

FS_4 cells and the pBactneo vector were kindly provided by J. Vilcek. HeLa cells were obtained from the American Type Culture Collection. Recombinant human IFN-α and IFN-γ were purchased from Collaborative Research and Promega Corp., respectively. Recombinant mouse IFN-α and IFN-γ were purchased from PBL Biomedical Laboratories. Radioisotopes were purchased from NEN Life Science Products.

RNA preparation

Total RNA was prepared using the RNAqueous kit purchased from Ambion.

cDNA library construction

A λgt10 cDNA library was constructed from RNA isolated from IFN-γ-treated FS_4 cells using the Riboclone cDNA Synthesis System, *Eco*RI Adaptor Ligation System, and Packagene Lambda DNA Packaging System according to the manufacturer's directions (Promega Corp.).

Transfection of HeLa cells

Dishes (60 mm) containing approximately 5×10^5 cells were washed with serum-free medium and then incubated for 24 h in serum-free medium to which a mixture of 5 μg of DNA and 10 μl Transfectam (Promega Corp.) was added. For the generation of stable transfectants, the cells were trypsinized the next day and transferred to culture dishes with medium containing G418. G418-resistant colonies were harvested and immediately subcloned by serial dilution. For the generation of transiently transfected cells, cells were trypsinized the day after transfection, placed in new wells, and allowed to recover for 24 h in culture medium before virus experiments were performed.

Immunoprecipitations

Immunoprecipitations on cell extracts containing equal amounts of radioactivity were performed as described (Rubin *et al.,* 1988a) using a monoclonal antibody generated against hGBP-1.

Western blot analysis

Cell lysates were prepared, fractionated, and characterized by Western blot analysis using a monoclonal antibody to hGBP-1 as described (Margolis-Nunno *et al.,* 1990).

Infection with virus and determination of virus yield

Cells seeded in 24-well plates (5×10^4 cells/well) were challenged with VSV or EMCV at a m.o.i. of 1. Virus adsorption was for 1 h at 37°C, and then the cells were

washed and incubated for 18 h at 37°C. The cultures were then frozen and thawed three times and the virus yields were determined as described (Rubin and Gupta, 1980). Alternatively, cells were stained with 10% crystal violet in a 20% ethanol solution immediately following an 18-h incubation period.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

RT-PCR was performed using the GeneAmp RNA PCR kit (Perkin–Elmer) according to the manufacturer's directions. All RNA samples upon which RT-PCR was performed were digested extensively with DNase; experiments performed demonstrated that no PCR products were generated from these RNA preparations in the absence of the reverse transcriptase. For the detection of the antisense RNA for GBP-1, we generated a 1170-bp RT-PCR product from 50 ng of total RNA using primer 5′-TGACCTACGTCAATGCCATC-3′ for first-strand synthesis and added primer 5′-GTCTACTGCTGGTCATCTGG-3′ for second-strand synthesis and subsequent amplification.

ACKNOWLEDGMENT

This work was supported in part by Public Health Service Grant CA-38661 from the National Cancer Institute.

REFERENCES

Chebath, J., Benech, P., Revel, M., and Vigneron, M. (1987). Constitutive expression of (2′-5′) oligo A synthetase confers resistance to picornavirus infection. *Nature* **330,** 587–588.

Cheng, Y. S., Colonno, R. J., and Yin, F. H. (1983). Interferon induction of fibroblast proteins with guanylate binding activity. *J. Biol. Chem.* **258,** 7746–7750.

Cheng, Y. S., Patterson, C. E., and Staeheli, P. (1991). Interferon-induced guanylate-binding proteins lack an N(T)KXD consensus motif and bind GMP in addition to GDP and GTP. *Mol. Cell. Biol.* **11,** 4717–4725.

Coccia, E. M., Romeo, G., Nissim, A., Marziali, G., Albertini, R., Affabris, E., Battistini, A., Fiorucci, G., Orsatti, R., Rossi, G. B., and Chebath, J. (1990). A full-length murine 2-5A synthetase cDNA transfected in NIH-3T3 cells impairs EMCV but not VSV replication. *Virology* **179,** 228–233.

Dever, T. E., Glynias, M. J., and Merrick, W. C. (1987). GTP-binding domain: Three consensus sequence elements with distinct spacing. *Proc. Natl. Acad. Sci. USA* **4,** 1814–1818.

Dever, T. E., and Merrick, W. C. (1989) *In* "The Guanine-Nucleotide Binding Proteins" (L. Bosch, B. Kraal, and A. Parmeggiani, Eds.), Plenum, New York.

Frese, M., Kochs, G., Meier-Dieter, U., Siebler, J., and Haller, O. (1995). Human MxA protein inhibits tick-borne Thogoto virus but not Dhori virus. *J. Virol.* **69,** 3904–3909.

Friedman, R. M., and Sonnabend, J. A. (1965). Inhibition of interferon action by puromycin. *J. Immunol.* **95,** 696–703.

Haller, O., Frese, M., Rost, D., Nuttall, P. A., and Kochs, G. (1995). Tick-borne Thogoto virus infection in mice is inhibited by the orthomyxovirus resistance gene product Mx1. *J. Virol.* **69,** 2596–2601.

Johnson, H. M. (1987) *In* "Mechanisms of Interferon Actions, Vol. II" (L. M. Pfeffer, Ed.), CRC Press, Boca Raton, FL.

Katze, M. G., DeCorato, D., Safer, B., Galabru, J., and Hovanessian, A. G. (1987). Adenovirus VAI RNA complexes with the 68 000 M_r protein kinase to regulate its autophosphorylation and activity. *EMBO J.* **6,** 689–697.

Kimura, T., Nakayama, K., Penninger, J., Kitagawa, M., Harada, H., Matsuyama, T., Tanaka, N., Kamijo, R., Vilcek, J., Mak, T. W., and Taniguchi, T. (1994). Involvement of the IRF-1 transcription factor in antiviral responses to interferons. *Science* **264,** 1921–1924.

Kitajewski, J., Schneider, R. J., Safer, B., Munemitsu, S. M., Samuel, C. E., Thimmappaya, B., and Shenk, T. (1986). Adenovirus VAI RNA antagonizes the antiviral action of interferon by preventing activation of the interferon-induced eIF-2 alpha kinase. *Cell* **45,** 195–200.

Kumar, R., Tiwari, R. K., Kusari, J., and Sen, G. C. (1987). Clonal derivatives of the RD-114 cell line differ in their antiviral and gene-inducing responses to interferons. *J. Virol.* **61,** 2727–2732.

Lengyel, P. (1982). Biochemistry of interferons and their actions. *Annu. Rev. Biochem.* **51,** 251–282.

Lockart, R. Z., Jr. (1964). The necessity of cellular RNA and protein synthesis for viral inhibition from interferon. *Biochem. Biophys. Res. Commun.* **15,** 513–518.

Margolis-Nunno, H., Rubin, B. Y., Anderson, S. L., Lunn, R. M., Roy, S. N., Dizik, M., and Sen, G. C. (1990). Isolation of cDNA clones for the interferon-induced 67,000-dalton protein: Direct induction of a family of mRNAs by human interferon-alpha and interferon-gamma. *J. Interferon Res.* **10,** 309–319.

Meurs, E. F., Watanabe, Y., Kadereit, S., Barber, G. N., Katze, M. G., Chong, K., Williams, B. R., and Hovanessian, A. G. (1992). Constitutive expression of human double-stranded RNA-activated p68 kinase in murine cells mediates phosphorylation of eukaryotic initiation factor 2 and partial resistance to encephalomyocarditis virus growth. *J. Virol.* **66,** 5804–5814.

Neun, R., Richter, M. F., Staeheli, P., and Schwemmle, M. (1996). GTPase properties of the interferon-induced human guanylate-binding protein 2. *FEBS Lett.* **390,** 69–72.

Pavlovic, J., Zurcher, T., Haller, O., and Staeheli, P. (1990). Resistance to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. *J. Virol.* **64,** 3370–3375.

Pestka, S., Langer, J. A., Zoon, K. C., and Samuel, C. E. (1987). Interferons and their actions. *Annu. Rev. Biochem.* **56,** 727–777.

Pine, R. (1992). Constitutive expression of an ISGF2/IRF1 transgene leads to interferon-independent activation of interferon-inducible genes and resistance to virus infection. *J. Virol.* **66,** 4470–4478.

Reis, L. F., Harada, H., Wolchok, J. D., Taniguchi, T., and Vilcek, J. (1992). Critical role of a common transcription factor, IRF-1, in the regulation of IFN-β and IFN-inducible genes. *EMBO J.* **11,** 185–193.

Rubin, B. Y., and Gupta, S. L. (1980). Differential efficacies of human type I and type II interferons as antiviral and antiproliferative agents. *Proc. Natl. Acad. Sci. USA* **77,** 5928–5932.

Rubin, B. Y., Anderson, S. L., Lunn, R. M., Hellermann, G. R., Richardson, N. K., and Smith, L. J. (1988a). Production of a monoclonal antibody directed against an interferon-induced 56,000-dalton protein and its use in the study of this protein. *J. Virol.* **62,** 1875–1880.

Rubin, B. Y., Anderson, S. L., Lunn, R. M., Richardson, N. K., Hellermann, G. R., Smith, L. J., and Old, L. J. (1988b). Tumor necrosis factor and IFN induce a common set of proteins. *J. Immunol.* **141,** 1180–1184.

Schneider, R. J., Safer, B., Munemitsu, S. M., Samuel, C. E., and Shenk, T. (1985). Adenovirus VAI RNA prevents phosphorylation of the eukaryotic initiation factor 2 alpha subunit subsequent to infection. *Proc. Natl. Acad. Sci. USA* **82,** 4321–4325.

Schnorr, J. J., Schneider-Schaulies, S., Simon-Jodicke, A., Pavlovic, J., Horisberger, M. A., and ter Meulen, V. (1993). MxA-dependent inhibition of measles virus glycoprotein synthesis in a stably transfected human monocytic cell line. *J. Virol.* **67,** 4760–4768.

Schwemmle, M., and Staeheli, P. (1994). The interferon-induced 67-kDa guanylate-binding protein (hGBP1) is a GTPase that converts GTP to GMP. *J. Biol. Chem.* **269,** 11299–11305.

Sen, G. C., and Lengyel, P. (1992). The interferon system. A bird's eye view of its biochemistry. *J. Biol. Chem.* **267,** 5017–5020.

Staeheli, P. (1990). Interferon-induced proteins and the antiviral state. *Adv. Virus Res.* **38,** 147–200.

Staeheli, P., Haller, O., Boll, W., Lindenmann, J., and Weissmann, C. (1986). Mx protein: Constitutive expression in 3T3 cells transformed with cloned Mx cDNA confers selective resistance to influenza virus. *Cell* **44,** 147–158.

Taylor, J. (1964). Inhibition of interferon action by actinomycin. *Biochem. Biophys. Res. Commun.* **14,** 447–451.

Thimme, R., Frese, M., Kochs, G., and Haller, O. (1995). Mx1 but not MxA confers resistance against tick-borne Dhori virus in mice. *Virology* **211,** 296–301.

Wynn, T. A., Nicolet, C. M., and Paulnock, D. M. (1991). Identification and characterization of a new gene family induced during macrophage activation. *J. Immunol.* **147,** 4384–4392.

Yang, Y. L., Reis, L. F., Pavlovic, J., Aguzzi, A., Schafer, R., Kumar, A., Williams, B. R., Aguet, M., and Weissmann, C. (1995). Deficient signaling in mice devoid of double-stranded RNA-dependent protein kinase. *EMBO J.* **14,** 6095–6106.

Zurcher, T., Pavlovic, J., and Staeheli, P. (1992). Mouse Mx2 protein inhibits vesicular stomatitis virus but not influenza virus. *Virology* **187,** 796–800.