Interferon-γ and Lipopolysaccharide Induce Mouse Guanylate-Binding Protein 3 (mGBP3) Expression in the Murine Macrophage Cell Line RAW264.7

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Mouse guanylate-binding protein 3 (mGBP3) is a 71-kDa GTPase which belongs to GTP-binding protein family. The present study showed that the expression of mGBP3 transcript was readily induced in a dose dependent fashion in the macrophage cell line RAW264.7 treated with either interferon-γ (IFN-γ) or lipopolysaccharide (LPS). The expression of mGBP3 protein was also apparent by 4 and 6 h after the treatment of cells with IFN-γ (100 U/ml) or LPS (1 μg/ml), and remained at plateau for at least 24 h. Cycloheximide (10 μg/ml) had no effect on the IFN-γ- or LPS-induced mGBP3 expression, suggesting that the mGBP3 induction did not require further protein synthesis. Interestingly, a protein kinase C (PKC) inhibitor staurosporine (50 nM) abolished the induction of mGBP3 expression by LPS, but not by IFN-γ. These findings suggest that mGBP3 may be involved in the macrophage activation process and both IFN-γ and LPS induce the mGBP3 expression through distinct signal transduction pathways.

Key words : Guanylate-binding protein 3, Gene expression, Interferon-γ, Lipopolysaccharide, Macrophage activation, Staurosporine, AG490

INTRODUCTION

Macrophages play essential roles in inflammation and immunity. The treatment of macrophages with interferon-γ (IFN-γ) induces a number of genes of which protein products are involved in the regulation of macrophage function (Darnell *et al.*, 1994; Sorace *et al.*, 1995; Sano *et al.*, 1997). One of INF-γ-response gene products includes a family of guanylate-binding proteins (GBPs) which belongs to the GTP-binding protein superfamily (Han *et al.*, 1998; Cheng *et al.*, 1991; Wynn *et al.*, 1991; Asundi *et al.*, 1994; Schwemmle *et al.*, 1994; Strehlow *et al.*, 1994). To date, three structurally related mammalian GBPs have been identified as IFN-γ-inducible genes in human : GBP1 (Cheng *et al.*, 1991), GBP2 (Cheng *et al.*, 1991), and a partial sequence of GBP3 (Strehlow *et al.*, 1994), in mouse : GBP1/MAG1 (Cheng *et al.*, 1991; Wynn *et al.*, 1991), MAG2 (Wynn *et al.*, 1991), GBP3 (Han *et al.*, 1998) and in the other species (Asundi *et al.*, 1994; Schwemmle *et al.*, 1994). All members of GBPs share a high degree of sequence homology and have only the first two elements of three GTP-binding consensus elements, GXXXGK(S/T), DXXG, and (N/T)KXD, present in typical GTP-binding proteins (Bourne *et al.*, 1991; Manavalan *et al.*, 1995). Nevertheless, GBPs exhibit the binding specificity to guanine nucleotides and GTPase activities with a variety of catalytic properties.

We have previously cloned and characterized the 71-kDa mGBP3 which is highly expressed in the erythroid progenitor cells isolated from mouse spleen infected with Friend virus (Han *et al.*, 1998; Park *et al.*, 1993). Moreover, in macrophage cell line RAW 264.7, mGBP3 gene expression is ordinarily undetectable in unstimulated cells, but is readily induced after the treatment with IFN-γ (Han *et al.*, 1998). Recombinant mGBP3 exhibits an intrinsic GTPase activity that hydrolyzes GTP to GDP (Han *et al.*, 1998) in a manner similar to the kinetic property seen in hGBP2 and chicken GBP (Schwemmle *et al.*, 1996; Neun *et al.*, 1996). In contrast, hGBP1 apparently hydrolyzes GTP predominantly to GMP (Schwemmle *et al.*, 1994). Both GBP1 and GBP2 have at their carboxyl termini an isoprenylation/methylation modification motif, CAAX, at which posttranslational modification promotes the ability of proteins to associate with their target components (e.g., plasma membranes) (Clarke, 1992; Marshall *et al.*, 1993). Both hGBP1 and rat p67 GBP have been reported to be isoprenylated and localized in the plasma membranes (Asundi *et al.*,

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1994; Schwemmle *et al.*, 1994). In contrast, we have found that mGBP3 lacks the CAAX motif at its carboxyl terminus and appears to be mainly localized to the cytosol of cells (Han *et al.*, 1998), suggesting the differential function of mGBP3. However, the exact physiological role of GBPs remains to be elucidated.

The activation of macrophage results in enormous production of cytokines and other inflammatory mediators including interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α), prostanoids, leukotrienes, and nitric oxide (NO) (Reimann *et al.*, 1994; Novogrodsky *et al.*, 1994; Adams *et al.*, 1984). These factors have been implicated in mediating cytolytic activity of macrophages to lyse tumor cells and microorganisms (Fidler *et al.*, 1988; Fidler, 1985). In addition, NO is believe to cause apoptosis of the activated macrophages (Meßmer *et al.*, 1996). IFN-γ and lipopolysaccharide (LPS) are major macrophage activating factors. They are similar but different in other respects related to the macrophage activation. For example, IFN-γ binding to the cell surface results in an activation of protein tyrosine kinases (PTK) such as JAK1 and JAK2, followed by phosphorylation of transcription factors, STAT1. The phosphorylated STAT1 is translocated to the nucleus and in turn activates gene transcription (Briken *et al.*, 1995; Foster, 1997; Shuai *et al.*, 1992; Harada *et al.*, 1994). On the other hand, LPS forms a complex with the cell-surface protein CD14, a glycophosphatidylinositol-anchored protein, which is necessary for the initiation of the intracellular signal transduction. It is well established that protein kinase C (PKC) plays a crucial role in the modulation of NO production and the subsequent induction of apoptosis in macrophages treated with LPS (Harada *et al.*, 1994; Barber *et al.*, 1995; Fujihara *et al.*, 1994; Nonaka *et al.*, 1998). In addition, upon stimulation of macrophages with LPS results in phosphorylation of a variety of cellular proteins including src-related tyrosine kinases, G-proteins, and mitogen-activated protein (MAP) kinases (Reimann *et al.*, 1994; Novogrodsky *et al.*, 1994; Barber *et al.*, 1995; Dong *et al.*, 1993; Schroeder *et al.*, 1997; Sands *et al.*, 1994). It is now evident that IFN-γ and LPS elicit the activation of distinct intracellular signal transduction pathways, which converge on the downstream in the modulation of macrophage activation and apoptosis. Thus, the identification of convergent downstream signaling molecules commonly shared by IFN-γ and LPS activation are of particular interest.

In this paper, the regulation of mGBP3 expression was determined during macrophage activation. The mGBP3 expression was rapidly induced without requiring protein synthesis in RAW264.7 cells by either IFN-γ or LPS. A nonselective PKC inhibitor staurosporine inhibited the mGBP3 expression by LPS but not by IFN-γ, suggesting that IFN-γ and LPS induced the mGBP3 expression through different signal transduction pathways.

MATERIALS AND METHODS

Materials

Recombinant mouse interferon-γ, LPS (*Escherichia coli* serotype 0127:B8), and cycloheximide were purchased from Sigma (St. Louis, Missouri, U.S.A.). Staurosporine and AG490 were obtained from Calbiochem (La Jolla, California, U.S.A.). Rabbit polyclonal anti-mGBP3 peptide antibody was raised against mGBP3 peptide residue 513-541 as described previously (Han *et al.*, 1998) and purified by affinity chromatography.

Cell culture

The mouse macrophage cell line RAW264.7 was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin in an atmosphere of 5% CO_2. RAW264.7 cells at 5×10^6 cells in 60-mm plates were plated a day before the experiments. Cells were then treated with the fresh culture medium containing macrophage activators and inhibitors as described below. After incubation, cells were washed with PBS, harvested, and lysed for transcript and protein analysis

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Expression levels of mGBP3 and β-actin transcripts were determined by RT-PCR as described previously (Han *et al.*, 1998). Briefly, two micrograms of total RNA isolated from RAW264.7 cells were reverse transcribed for 1 h at 37°C with random primers. PCR amplification was performed for 25 cycles with the following parameters: 94°C for 45 sec, 56°C 1 min, and 72°C for 1.5 min. Primers used for PCR were as follows: for mGBP3, 5'TGGAGGCACCCATTTGTCTGGTG-3' and 5'GACAAAGGTGCTGCTCAGAAGCACAG-3'; for β-actin, 5'TCCTATGTGGGTGACGAGGC-3' and 5'-CATGGCTGGGGTGTTGAAGG-3'. The PCR products were separated by electrophoresis in 1.5% agarose gels and visualized by ethidium bromide staining.

Western blot

RAW264.7 cells were cultured for various time points as indicated. Cells were harvested in lysis buffer (10 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, and 2 mM EDTA) containing 2 mM PMSF and Protease Inhibitor Cocktail (Boehringer Mannheim, Indiana Polis, Indiana, U.S.A.). Protein samples were prepared by sonication for 6 sec and centrifuge at 10,000×g for 10 min. Protein concentration was determined by a

coomassie protein assay kit (Pierce, Rackford, Illinois, U.S.A.). Protein samples (50 μg/lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membrane, and preincubated with TTBS (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 80) containing 5% dry milk. The blocked membrane was subsequently incubated with rabbit polyclonal anti-mGBP3 antibody (2 μg/ml), peroxidase-conjugated goat anti-rabbit IgG, and then visualized with an enhanced chemiluminescence system (Pierce).

Nitrite assay

Nitrate and nitrite concentration of the culture supernatants was determined as described (Green *et al.*, 1982). Briefly, an equal volume of 1% sulfonilamide in 5% H_3PO_4 was mixed with 0.1% n-1-naphthylethylenediamine before use. The culture supernatants (50 μl/well) were mixed with an equal volume of the reagent in 96-well plates and incubated at room temperature for 10 min. Absorbance was determined at 540 nm with a microplate reader. Sodium nitrite was used to obtain a standard curve.

RESULTS AND DISCUSSION

mGBP3 expression in RWA264.7 cells induced by IFN-γ or LPS

We have previously demonstrated that mGBP3 is inducible in RAW264.7 cells in response to IFN-γ (Han *et al.*, 1998). In the present study, we explored further the regulation of mGBP3 expression during macrophage activation. Since RAW264.7 cells are known to be activated by IFN-γ and LPS, we determined whether both factors could induce mGBP3 expression. RAW264.7 cells were treated with various concentrations of either IFN-γ or LPS for 4 h, and expression levels of mGBP3 transcript were analyzed by RT-PCR method as described previously (Han *et al.*, 1998). As shown in Fig. 1, expression level of the constitutively expressed β-actin transcript was similar in all RNA preparations, indicating that an equal amount of total RNA per sample was used in RT-PCR. In keeping with previous results (Han *et al.*, 1998), the expression of mGBP3 transcript was undetectable in unstimulated macrophages. However, the mGBP3 transcript expression was induced in a dose dependent manner in macrophages stimulated with IFN-γ or LPS. The induction of mGBP3 transcript was saturated when cells were treated with 100 U/ml IFN-γ or 100 ng/ml LPS. Thus, the mGBP3 gene expression can be induced in macrophages by LPS as well as IFN-γ.

Kinetics of mGBP3 expression during macrophage activation

To examine kinetics of the mGBP3 expression during macrophage activation, RAW264.7 cells were treated with IFN-γ or LPS for various time points. Cells were then harvested for the analysis of the expression levels of mGBP3 transcript and protein. When cells were treated with IFN-γ the expression level of mGBP3 transcript steadily increased to a maximum at 4 h and then remained about the same levels for at least 24 h of culture (Fig. 2). To determine the protein expression levels of mGBP3, protein samples were analyzed by SDS-PAGE and Western blotting using the polyclonal anti-mGBP3 antibody (Fig. 2). In

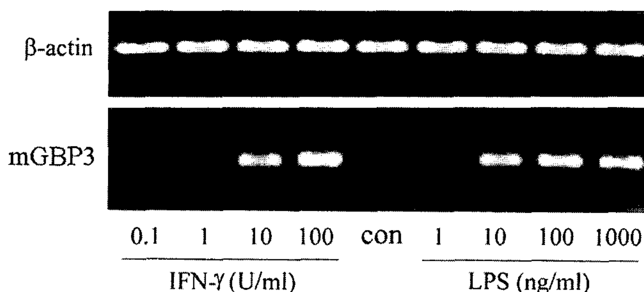

Fig. 1. Dose response of mGBP3 transcript induction in RAW264.7 cells. RAW264.7 cells were incubated for 4 h in medium only (con), or with IFN-γ (0.1, 1, 10, or 100 U/ml), or LPS (1, 10, 100, or 1000 ng/ml). After incubation, cells were washed with PBS and harvested in cell lysis buffer for RNA extraction. Total RNA samples were reverse transcribed and amplified for 25 cycles by PCR as described in Materials and Methods. PCR products were separated by electrophoresis in 1.5% agarose gels and stained with ethidium bromide. *Upper panel*: β-actin transcript, *lower panel*: mGBP3 transcript. Data are representative of two similar experiments.

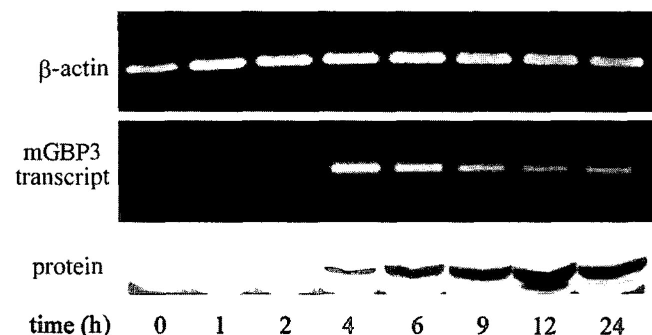

Fig. 2. Time course of mGBP3 transcript expression in IFN-γ-stimulated RAW264.7 cells. RAW264.7 cells were cultured in medium containing 100 U/ml IFN-γ. After incubation for a number of time points as indicated, cells were harvested for analysis. *Top panel*: RT-PCR analysis of β-actin transcript expression; *middle panel*: RT-PCR analysis of mGBP3 transcript expression, *bottom panel*: Western blot analysis of mGBP3 protein expression. Protein samples (50 μg/lane) were separated by SDS-PAGE and immunoblot using polyclonal anti-mGBP3 peptide antibody. Data are representative of two similar experiments.

agreement with the previous results (Han *et al.*, 1998), the polyclonal anti-mGBP3 antibody detected an approximately 70-kDa protein corresponding to the expected molecular weight of mGBP3. The mGBP3 protein expression remained at an almost undetectable level until 2 h after IFN-γ treatment and then increased greatly between 6 and 9 h. Its expression levels were then sustained at plateau for at least 24 h after macrophage activation in response to IFN-γ. Similar results were obtained when macrophages were stimulated with LPS as shown in Fig. 3. These data indicated that the induction of mGBP3 expression resulted in a delayed but prolonged maintenance of the protein during macrophage activation by IFN-γ or LPS. The rat p67 GBP is also shown to be induced in a similar fashion in rat bone marrow-derived macrophages stimulated with IFN-γ or LPS (Vestal *et al.*, 1996). In contrast, we have previously reported that the mGBP3 transcript expression is transiently induced and almost undetectable 12 h after the culture of erythroid progenitors treated with erythropoietin (Han *et al.*, 1998). We suspected that protein synthesis is required in macrophages for the mGBP3 induction by either IFN-γ or LPS. For example, the expression of interferon-regulatory factor-1 (IRF-1) is shown to be required for the induction of hGBP1 gene in fibroblasts (Nicolet *et al.*, 1994). We observed that in RAW264.7 cells, IRF-1 transcript was almost undetectable in unstimulated cells, but readily induced by IFN-γ or LPS with a time course similar to that of mGBP3 expression (data not shown). Moreover, the protein synthesis inhibitor cycloheximide had no effect on the mGBP3 induction by either IFN-γ or LPS as shown Fig. 4. These data suggest that

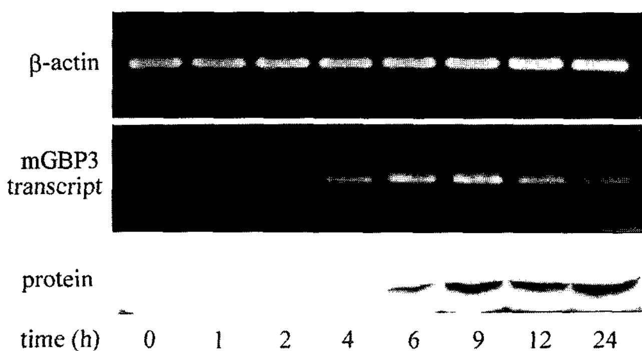

Fig. 3. Time course of mGBP3 transcript expression in LPS-stimulated RAW264.7 cells. RAW264.7 cells were cultured in medium containing 1 μg/ml LPS. After incubation for a number of time points as indicated, cells were harvested for analysis. *Top panel*: RT-PCR analysis of β-actin transcript expression; *middle panel*: RT-PCR analysis of mGBP3 transcript expression, *bottom panel*: Western blot analysis of mGBP3 protein expression. Protein samples (50 μg/lane) were separated by SDS-PAGE and immunoblot using polyclonal anti-mGBP3 peptide antibody. Data are representative of two similar experiments.

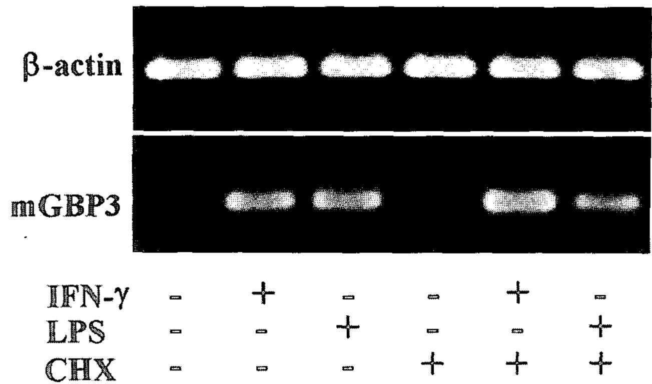

Fig. 4. Effect of cycloheximide on mGBP3 gene induction in RAW264.7 cells. RAW264.7 cells were treated with medium only, or with 100 U/ml IFN-γ, or 1 μg/ml LPS in the presence or absence of 10 μg/ml cycloheximide. Expression of β-actin (*upper panel*) and mGBP3 (*lower panel*) transcripts was analyzed by RT-PCR as described in Fig. 1.

mGBP3 is indeed an immediate-early gene of which induction does not require further protein synthesis.

Regulation of mGBP3 expression by PKC signal transduction

Fig. 5A shows effect of staurosporine and AG490 on the mGBP3 expression. The mGBP3 protein expression was undetectable in unstimulated cells, whereas it was markedly expressed in cells treated with IFN-γ or LPS as also seen Fig. 1. The nonspecific PKC inhibitor staurosporine at a concentration of 50 nM completely abolished the LPS-induced but not IFN-γ-induced mGBP3 expression. To determine the inhibitory effects of the protein kinase inhibitors on macrophage activation, NO production as a marker for macrophage activation in culture medium was also assayed (Fig. 5B). In keeping with previous results (Schroeder *et al.*, 1997; Jun *et al.*, 1994), NO production was significantly increased in macrophages treated with IFN-γ or LPS. Staurosporine inhibited the NO production induced by LPS by 51±1%. These findings indicated that the LPS-induced NO production and mGBP3 expression were more susceptible to staurosporine compared with IFN-γ. Furthermore, staurosporine at a higher concentration (1 μM) completely inhibited the NO production as well as mGBP3 expression induced by IFN-γ or LPS (data not shown). These data suggest that staurosporine-sensitive PKC signaling pathways may be, in part, involved in the induction of mGBP3 expression. However, since multiple isoforms of PKC exist and staurosporine is a nonselective PKC inhibitor, further study is required to clarify PKC isoforms responsible for the mGBP3 induction.

To explore the involvement of PTK signal transduction pathway in mGBP3 induction, we used the

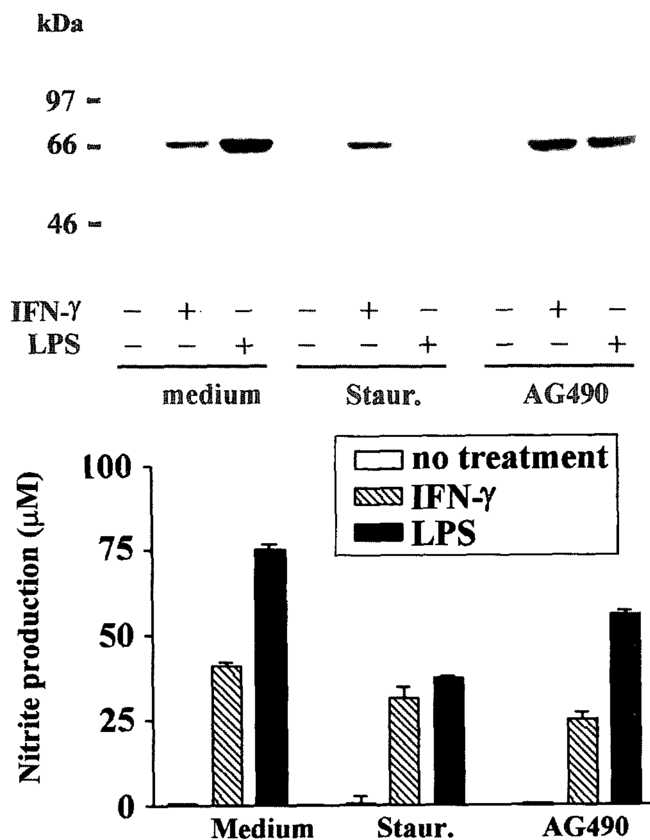

Fig. 5. Effect of staurosporine and AG490 on the mGBP3 expression in RAW264.7 cells. RAW264.7 cells at 1×10^7 cells in 60-mm plates were plated 18 h before activation. Cells were incubated with medium only, or with 100 U/ml IFN-γ, or 1 μg/ml LPS in the presence of protein kinases inhibitors indicated for 24 h. The inhibitors used were 50 nM staurosporine (Staur.) and 50 μM AG490. (A) Western blot analysis of mGBP3 expression. After incubation, cells were harvested and protein samples (50 μg/lane) were analyzed by SDS-PAGE and Western blotting using anti-mGBP3 antibody. (B) Nitrite assay. Nitrite concentration in the culture supernatants was assayed as described in Materials and Methods. Data indicate mean±SD of two experiments in duplicated samples.

selective PTK inhibitor, tyrphostin AG490. It has previously been shown that AG490 selectively blocks JAK2 activity and causes a retardation of leukemic cell growth by inducing apoptosis (Wynn *et al.*, 1991). In RAW264.7 cells, the treatment with AG490 at a concentration of 50 μM resulted in an inhibition of NO production induced by IFN-γ and LPS by 39± 1% and 25±5%, respectively (Fig. 5B). However, AG 490 at a concentration of 50 μM had no significant inhibitory effect on the IFN-γ- or LPS-induced mGBP3 expression (Fig. 5A). These data indicate that the signal transduction through JAK is necessary for the NO production, whereas the induction of mGBP3 expression is apparently independent on the JAK-activated pathway.

Although a growing number of GBP proteins have been identified thus far, the physiological function of these proteins remains to be elucidated. However, it has been shown that GBPs is implicated in the macrophage activation. All GBPs identified to date are IFN-γ inducible. The induction of mGBP1 expression is correlated with the cytolytic capability in macrophages. For example, mGBP1 is able to be induced by IFN-γ and LPS in the cytolytic macrophages RAW264.7, but not in the non-cytolytic cell line WEHI-3 (Wynn *et al.*, 1991; Jun *et al.*, 1994). In addition, GTP-binding proteins are involved in regulation of a variety of fundamental cellular functions including signal transduction, protein synthesis, protein targeting, antiviral activity, and cell motility (Dickey *et al.*, 1993). It is conceivable that overexpression of IFN-γ-inducible GTP-binding proteins such as GBP family proteins may function by altering the intracellular GTP utility competitively with other GTP-binding proteins. Further studies on the molecular mechanisms at which GBPs exert their function to mediate actions of IFN-γ and LPS would be necessary.

In summary, the present study demonstrates that mGBP3 is an immediate-early gene in response to IFN-γ as well as LPS. Multiple signal transduction pathways, including at least staurosporine-sensitive PKC, appear to be involved in the intracellular signaling cascades leading to mGBP3 expression upon macrophage stimulation.

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REFERENCES CITED

Adams, D. O. and Hamilton, T. A. The cell biology of macrophage activation. *Annu. Rev. Immunol.* 2, 283-318 (1984).

Asundi, V. K., Stahl, R.C., Showalter, L., Conner, K. J. and Carey, D. J., Molecular cloning and characterization of an isoprenylated 67 kDa protein. *Biochim. Biophys. Act.* 1217, 257-265 (1994).

Barber, S. A., Perera, P.-Y., McNally, R. and Vogel, S. N., The serine/threonine phosphatase inhibitor, calyculin A, inhibits and dissociates macrophage responses to lipopolysaccharide. *J. Immunol.* 155, 1404-1410 (1995).

Bourne, H. R., Sanders, D. A. and McCormick, F., The GTPase superfamily: conserved structure and molecular mechanism. *Nature* 349, 117-127 (1991).

Briken, V., Ruffner, H., Schultz, U., Schwarz, A., Reis, L. F., Strehlow, I., Deker, T. and Staeheli, P. Interferon regulatory factor 1 is required for mouse Gbp gene activation by gamma interferon. *Mol. Cell Biol.* 15, 975-982 (1995).

Cheng, Y-S. E., Patterson, C. E. and Staeheli, P. 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 (1991).

Clarke, S., Protein isoprenylation and methylation at carboxyl terminal cysteine residues. *Annu. Rev. Biochem.* 61, 355-386 (1992).

Darnell, J. E., Jr., Kerr, I. M. and Stark, G. R., JAK-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 264, 1415-1421 (1994).

Dickey, B. F. and Birnbaumer, L. (Eds) GTPase in Biology, Springer, Berlin, 1991.

Dong, Z., Qi, X., Xie, K. and Fidler, I. J., Protein tyrosine kinase inhibitors decrease induction of nitric oxide synthase activity in lipopolysaccharide-responsive and lipopolysaccharide-nonresponsive murine macrophages. *J. Immunol.* 151, 2717-2724 (1993).

Fidler, I. J. Macrophages and metastasis- a biological approach to cancer therapy. *Cancer Res.* 45, 4714-4726 (1985).

Fidler, I. J. and Schroit, A. J. Recognition and destruction of neoplastic cells by activated macrophages: discrimination of altered self. *Biochim Biophys. Acta* 948, 151-173 (1988).

Foster, G. R. Interferons in host defence. *Semin. Liver Dis.* 17, 287-295 (1997).

Fujihara, M., Connolly, N., Ito, N. and Suzuki, T. Properties of protein kinase isoforms (beta II, epsilon, and zeta) in a macrophage cell line (J774) and their roles in LPS-induced nitric oxide production. *J. Immunol.* 152, 1898-1906 (1994).

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S. and Tannenbaum, S. R., Anaylsis of nitrate, nitrite, and [^{15}N]nitrate in biological fluids. *Anal. Biochem.* 126, 131-138 (1982).

Han, B. H., Park, D. J., Lim, R. W., Im, J. H. and Kim, H. D., Cloning, expression, and characterization of a novel guanylate-binding protein3, mGBP3 in the murine erythroid progenitor cells. *Biochim. Biophys. Act.* 1384, 373-386 (1998).

Harada, H., Takahashi, E.-I., Itoh, S., Harada, K., Hori, T.-A. and Taniguchi, T. Structure and regulation of the human interferon regulatory factor 1 (IRF-1) and IRF-2 genes: implications for a gene network in the interferon system. *Mol. Cell. Biol.* 14, 1500-1509 (1994).

Jun, C.-D., Choi, B.-M., Ryu, H., Um, J.-Y., Kwak, H.-J., Lee, B.-S., Paik, S.-G., Kim, H.-M. and Chung, H.-T. Synergistic cooperation between phorbol esters and IFN-γ for induction of nitric oxide synthesis in murine peritioneal macrophages. *J. Immunol.* 153, 3684-3690 (1994).

Manavalan, P., Dearborn, D. G., McPherson, J. M. and Smith, A. E. Sequence homologies between nucleotide binding regions of CFTR and G-proteins suggest structural and functional similarities. *FEBS Lett.* 366, 87-91 (1995).

Marshall, C. J., Protein prenylation: a mediator of protein-protein interactions. *Science* 259, 1865-1866 (1993).

Meßmer, U. K., Reed, J. C. and Brune, B. Bcl-2 protects macrophages from nitric oxide-induced apoptosis. *J. Biol. Chem.* 271, 20192-20197 (1996).

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

Nicolet, C. M. and Paulock, D. M. Promoter analysis of an interferon-inducible gene associated with macrophage activation. *J. Immunol.* 152, 153-162 (1994).

Nonaka, K., Ishisaki, A, Muro, M., Kato, S., Oido, M., Nakashima, K., Kowashi, Y. and Nishihara, T., Possible involvement of protein kinase C in apoptotic cell death of macrophages infected with Actinobacillus actinomycetemcomitans. *FEMS Microbiol. Lett.* 159, 247-254 (1998).

Novogrodsky, A., Vanichkin, A., Patya, M., Gazit, A., Osherov, N. and Levitzki, A., Prevention of lipopolysaccharide-induced lethal toxicity by tyrosine kinase inhibitors. *Science* 264, 1319-1322 (1994).

Park, D. J., Lim, R. W. and Kim, H. D., Rapid induction of mouse virus-like (VL30) element transcripts by erythropoietin in murine progenitor cells. *Blood* 82, 77-83 (1993).

Reimann, T., Buscher, D., Hipskind, R. A., Krautwald, S., Lohmann-Matthes, M.-L. and Baccarini, M., Lipopolysaccharide induces activation of the Raf-1/MAP kinase pathway. A putative role for Raf-1 in the induction of the IL-1 beta and the TNF-alpha genes. *J. Immunol.* 153, 5740-5749 (1994).

Sands, W. A., Clark, J. S. and Liew, F. Y. The role of a phosphatidylcholine-specific phospholipase C in the production of diacylglycerol for nitric oxide synthesis in macrophages activated by IFN-gamma and LPS. *Biochem. Biophys. Res. Commun.* 199, 461-466 (1994).

Sano, H., Hirai, M., Saito, H., Nakashima, I., and Isobe, K.-I., A nitric oxide-releasing reagent, S-nitroso-N-acetylpenicillamine, enhances the expression of superoxide dismutases mRNA in the murine macrophage cell line RAW264.7. *Immunol.* 92, 118-122 (1997).

Schroeder, R. A., DeltaTorre, A. and Kuo, P. C., CD 14-dependent mechanism for endotoxin-mediated nitric oxide synthesis in murine macrophages. *Am. J. Physiol.* 273, C1030-C1039 (1997).

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

Schwemmle, M., Kaspers, B., Irion, A., Staeheli, P. and Schultz, U., Chicken guanylate-binding protein,

conservation of GTPase activity and induction by cytokines. *J. Biol. Chem.* 271, 10304-10308 (1996).
Shuai, K., Schindler, C., Prezioso, V. R. and Darnell, J. E. Jr., Activation of transcription by IFN-gamma: tyrosine phosphorylation of a 91-kD DNA binding protein. *Science* 258, 1808-1812 (1992).
Sorace, J. M., Johnson, R. J., Howard, D. L. and Drysdale, B. E., Identification of an endotoxin and IFN-inducible cDNA: possible identification of a novel protein family. *J Leukoc. Biol.* 58, 477-484 (1995).
Strehlow, I., Lohmann-Matthes, M. -L. and Decker, T., The interferon-inducible GBP1 gene: structure and mapping to human chromosome 1. *Gene* 144, 295-299 (1994).
Vestal, D. J., Buss, J. E., Kelner, G. S., Maciejewski, D., Asundi, V. K. and Maki, R. A., Rat p67 GBP is induced by interferon-γ and isoprenoid-modified in macrophages. *Biochem. Biophys. Res. Commun.* 224, 528-534 (1996).
Wynn, T. A., Nicolet, C. M. and Paulnock, D. M. Identification and characterization of a new gene family induced during macrophage activation *J. Immunol.* 147, 4384-4392 (1991).