Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11964–11968, December 1992
Immunology

Interferon γ rapidly induces in human monocytes a DNA-binding factor that recognizes the γ response region within the promoter of the gene for the high-affinity Fcγ receptor

KEVIN C. WILSON AND DAVID S. FINBLOOM*

Food and Drug Administration, Center for Biologics Research and Evaluation, Division of Cytokine Biology, 8800 Rockville Pike, Bethesda, MD 20892

Communicated by Henry Metzger, September 15, 1992 (received for review July 21, 1992)

ABSTRACT **Interferon γ (IFN-γ) transcriptionally activates several early-response genes in monocytes that are important for the ultimate phenotype of the activated macrophage. One of these genes is the high-affinity Fc receptor for IgG (FcγRI). Recently, Pearse *et al.* [Pearse, R. N., Feinman, R. & Ravetch, J. V. (1991) *Proc. Natl. Acad. Sci. USA* 88, 11305–11309] defined within the promoter region of the FcγRI gene an element, the γ response region, which was necessary for IFN-γ-induced enhancement of FcγRI. In this report we describe the induction by IFN-γ of a DNA-binding factor, FcRFγ (FcγRI DNA-binding factor, IFN-γ induced), that specifically recognizes the γ response region element. Electrophoretic mobility shift assays (EMSAs) demonstrated the presence of FcRFγ in human monocytes within 1 min after exposure to IFN-γ. On EMSA, FcRFγ consisted of two complexes termed FcRFγ$_1$ and FcRFγ$_2$. The nuclear concentration of FcRFγ rapidly increased, peaked at 15 min, and then fell after 1–2 hr. Dose–response studies revealed (*i*) as little as 0.05 ng of IFN-γ per ml induced FcRFγ, (*ii*) maximum activation occurred at 1 ng/ml, and (*iii*) steady-state levels of FcγRI mRNA closely paralleled that of FcRFγ. Since FcRFγ was activated in cells normally not expressing FcγRI RNA, other regulatory mechanisms must control FcγRI-restricted tissue expression. Activation of FcRFγ by IFN-γ was inhibited by pretreatment with 500 nM staurosporin and 25 μM phenyl arsine oxide. These data suggest that a kinase and possibly a phosphatase activity are required for IFN-γ-induced signaling of FcRFγ in monocytes.**

Interferon γ (IFN-γ), a cytokine secreted from stimulated T cells, is a potent activator of monocytes and macrophages and, therefore, is a critical component for host defense and inflammation (1–3). The diverse effects elicited by IFN-γ are in part due to the induction of various early-response genes. While some of these genes encode for proteins that can act as soluble, secreted mediators of inflammation, such as interleukin 8 (4) and IP-10 (5), others encode receptor proteins that are crucial for immune responsiveness and host defense. An example of the latter is the high-affinity receptor for the Fc region of IgG (FcγRI) (6), which binds monomeric IgG1 with high affinity (7–10). Crosslinking of FcγRI with IgG immune complexes mediates several important biologic actions such as phagocytosis (10), antibody-dependent cellular cytotoxicity (11), and the elaboration of toxic oxygen metabolites (12). Consequently, a potential site of control in the inflammatory cascade is the regulation of FcγRI expression on phagocytes.

IFN-γ binds to high-affinity receptors on the cell surface, thereby initiating a sequence of events that ultimately results in a severalfold enhancement over the basal levels of FcγRI expression (13). Although the portion of the receptor that binds IFN-γ was cloned several years ago (14), the mechanisms implicated in IFN-γ signaling remain unclear. Classical second messengers do not appear to be involved nor does that part of the receptor that binds IFN-γ possess kinase or phosphatase activity. A growing body of evidence indicates that additional component(s) of the receptor are necessary for signal transduction (15–19).

Through the use of promoter deletions in transfection studies, Pearse *et al.* (20) have defined a region of the FcγRI gene 18 base pairs (bp) 5′ to the major initiation start site that is necessary and sufficient for enhanced transcription in the presence of IFN-γ, the γ response region (GRR). Other undefined elements within 1000 bp 5′ to the start site of transcription appear to regulate the basal expression and restricted tissue distribution of the FcγRI. The GRR consists of a 39-bp element that contains motifs found in other IFN-γ-induced genes such as the major histocompatibility complex (MHC) class II gene. Using an electrophoretic mobility shift assay (EMSA) with a GRR probe, we have been able to identify an IFN-γ-induced DNA-binding factor(s) generated within seconds after the binding of IFN-γ to its receptor. The rapid activation of this DNA-binding protein(s) will allow for a more complete understanding of the signaling mechanisms for IFN-γ on monocytes.

MATERIALS AND METHODS

Cells. Human monocytes were purified from leukopacks prepared by leukapheresis of normal volunteers by Ficoll/Hypaque sedimentation followed by countercurrent centrifugal elutriation (21). Cells obtained in this manner are >95% monocytes as determined by histochemical and flow cytometric analysis. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO) supplemented with 10% fetal bovine serum (HyClone), both of which were endotoxin free. Cells were treated with IFN-γ either in suspension at 20–25 × 10^6 cells per ml or adherent on tissue culture dishes at a density of 30–40 × 10^6 cells per 75 cm^2 with similar results. Other cells studied were U937, a monocyte-like, histiocytic lymphoma cell line; BUD8, a primary fibroblast culture; and HeLa, a cervical carcinoma cell line.

IFNs. *Escherichia coli*-derived, recombinant human IFN-γ (2 × 10^7 units/mg) was provided by Genentech. *E. coli*-derived, recombinant human IFN-α 2a (2 × 10^8 units/mg) was provided by Hoffmann–La Roche.

Measurement of FcγRI RNA Expression. Following treatment with IFN-γ, total cellular RNA was isolated using

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EMSA, electrophoretic mobility shift assay; FcγRI, high-affinity Fc receptor for IgG; FcRFγ, FcγRI DNA-binding factor, interferon γ induced; GAS, interferon γ activation sequence; GRR, γ response region of the FcγRI gene; GBP, guanylate-binding protein; IFN, interferon; ISRE, interferon α-stimulated response element; PAO, phenyl arsine oxide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MHC, major histocompatibility complex.

*To whom reprint requests should be addressed.

RNA-zol (Tel-test, Friendswood, TX). Steady-state RNA concentrations were determined using a RNase protection assay (22). Briefly, 5–10 μg of total cellular RNA was hybridized in solution to a ^{32}P-labeled antisense riboprobe for 16 hr at 56°C in 80% formamide. The riboprobe was prepared from a 490-bp *Hin*dIII–*Eco*RI fragment of the FcγRI cDNA (F90) (23) (kindly provided by Brian Seed, Harvard University, Boston) subcloned into the BlueScript plasmid (Stratagene). The plasmid was linearized with *Xho* I, and an *in vitro* transcription was performed in the presence of [α-^{32}P]UTP. The protected fragment (about 500 bp) was separated on a denaturing sequencing gel, which was then subjected to autoradiography. As an internal control for the amount of RNA loaded onto the gel, RNA was simultaneously hybridized to an antisense ^{32}P-labeled probe for the gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (24), which yielded a 300-bp protected fragment.

Nuclear and Cytoplasmic Extracts of Cells. After treatment with IFN-γ, monocytes were immediately cooled by the addition of cold (4°C) phosphate-buffered saline and were placed on ice. Thirty million cells were washed and resuspended into 2 ml of hypotonic buffer A consisting of 10 mM KCl, 20 mM Hepes (pH 7.0), 1 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.1% Triton X-100, 20% glycerol, 2 mM phenylmethylsulfonyl fluoride, aprotinin and leupeptin, each at 5 μg/ml, pepstatin, 2 μg/ml, and 0.3 mM N^{α}-(*p*-tosyl)lysine chloromethyl ketone. Cells were disrupted in a glass Dounce homogenizer (B pestle) with 20 strokes and then centrifuged at 300 × *g* to pellet the nuclei. The supernatant portion was defined as the cytoplasmic extract (0.4–0.6 mg of protein per ml) to which NaCl was added to a final concentration of 0.3 M. The nuclei were then resuspended in 0.3 ml of nuclear extract buffer consisting of buffer A without the Triton X-100 but with 0.3 M NaCl. Nuclear proteins were extracted by repeated, intermittent agitation in a Vortex over a 20-min period on ice. Nuclei were then sedimented at 18,000 × *g*, supernatant was collected, and the extract was stored at −70°C. Protein concentrations of nuclear extracts were between 0.3 and 0.7 mg/ml.

Measurement of DNA-Binding Factors. EMSAs were performed using native 6% polyacrylamide gels in 0.25× Tris/borate/EDTA (pH 8.3) buffer. The ^{32}-P labeled double-stranded oligonucleotide probe consisted of the 39-bp GRR of the FcγRI gene (20): 5′-AGCATGTTTCAAGGATTTGAGATGTATTTCCCAGAAAAG-3′. Probes used to define specificity included the IFN-α-stimulated response element (ISRE) of ISG15 (5′-GATCCATGCCTCGGGAAAGGGAAACCGAAACTGAAGCC-3′) (25), the ISRE and IFN-γ activation sequence (GAS) of the guanylate-binding protein (GBP) gene (5′-AGTACTTTCAGTTTCATATTACTCTAAATC-3′) (26), and Sp1 (5′-GATCCGGGGCGGGGCGGGGCGGGGC-3′) (27). The binding assay was performed by incubating 5 μg of either cytoplasmic (about 150,000 cell equivalents) or nuclear extract (about 1 × 10^6 cell equivalents) with 2 ng of end-labeled GRR probe in the presence of 4 μg of poly(dI·dC) (Pharmacia) in a buffer consisting of 100 mM KCl, 10 mM Tris (pH 7.4), 5 mM $MgCl_2$, 1 mM dithiothreitol, and 10% glycerol for 30 min at room temperature. Separation of free and complexed probe was performed by electrophoresis followed by autoradiography. Quantification of radioactivity within each band was determined using an AMBIS gel radiodetector (AMBIS Systems, San Diego).

RESULTS

IFN-γ Induction of a DNA-Binding Factor(s) that Specifically Recognizes the GRR Region of the FcγRI Promoter. Nuclear extracts obtained from monocytes treated with 10 ng of IFN-γ per ml for 30 min at 37°C were assayed by EMSA for their ability to bind to the GRR of the FcγRI gene (Fig. 1). Compared to untreated cells, cells treated with IFN-γ contained factor(s) that interacted with the ^{32}P-labeled GRR probe, resulting in the electrophoretic retardation of two distinct bands (Fig. 1, lanes 1 and 2), which we refer to as FcRFγ_1 and FcRFγ_2 (FcγRI DNA-binding factor, IFN-γ induced). Nuclear extracts of IFN-γ-treated cells were exposed to ^{32}P-labeled GRR probe in the presence of increasing concentrations of several unrelated, unlabeled oligonucleotides. Specificity of the IFN-γ-induced complexes (FcRFγ) was indicated by the fact that only the GRR oligonucleotide competed. Formation of FcRFγ_1 and FcRFγ_2 was not competitively inhibited by (*i*) the oligonucleotide containing the ISRE from the IFN-α-stimulated gene, ISG15, (*ii*) the oligonucleotide containing an ISRE and GAS element from the IFN-α- or IFN-γ-stimulated gene, GBP, or (*iii*) an oligonucleotide corresponding to a Sp1 site. Additionally, when nuclear extracts of IFN-γ-treated monocytes were exposed to *N*-ethylmaleimide prior to incubation in the binding reaction, the FcRFγ complex remained intact (data not shown). This is in contrast to the IFN-α-induced DNA-binding complex (ISGF3), which is *N*-ethylmaleimide sensitive (28). Therefore, FcRFγ appears to be distinct from those factors that bind to the ISRE and GAS elements. Indeed, treatment of monocytes with IFN-α itself resulted in essentially no induction of FcRFγ (data not shown).

The induction of FcγRI expression by IFN-γ occurs only in cells of myelomonocytic origin (10). Elements distinct from the GRR appear to be responsible for the basal level expression of FcγRI and its restricted tissue distribution (20). Therefore, we examined the ability of two nonmyeloid cells and the monocyte-like cell line U937 to respond to IFN-γ with the production of FcRFγ (Fig. 2*B*) and FcγRI mRNA (Fig. 2*A*). Monocytes readily responded to IFN-γ by the activation of FcRFγ followed by a marked increase in steady-state levels of mRNA. FcRFγ was pronounced in extracts from U937 cells, but accumulation of RNA was considerably slower than that observed in monocytes. In contrast, al-

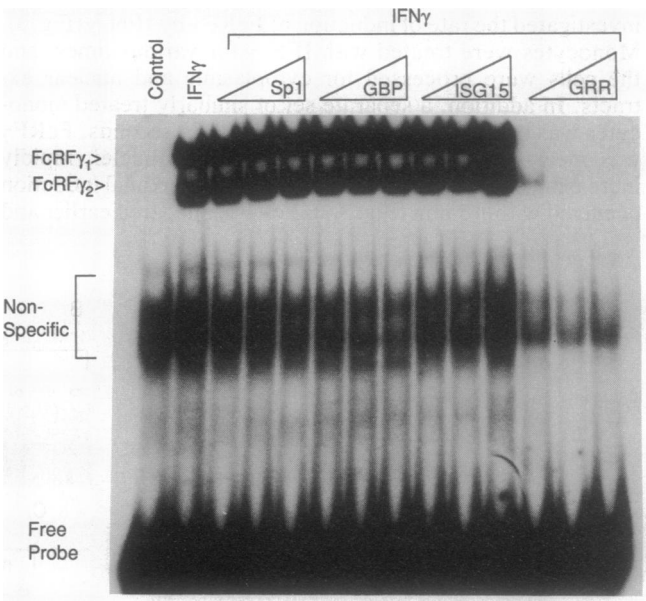

FIG. 1. Induction and specificity of binding of FcRFγ in monocytes. Monocytes were treated with 10 ng of IFN-γ per ml for 30 min at 37°C, and nuclear extracts were prepared and allowed to bind ^{32}P-labeled GRR (^{32}P-GRR) in the presence of excess competitive oligonucleotides. The DNA–protein interaction was then assayed by EMSA followed by autoradiography. Each group of three lanes represents increasing molar excess (25-, 50-, and 100-fold) of competitor over the ^{32}P-GRR probe.

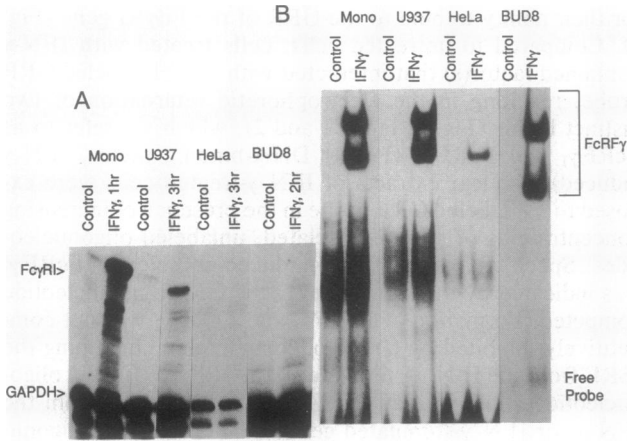

FIG. 2. Induction of FcRFγ by IFN-γ occurs in cells where FcγRI mRNA is not induced. Primary cultures of monocytes (Mono) or fibroblasts and cell lines were exposed to 10 ng of IFN-γ per ml for 30 min at 37°C prior to preparation of nuclear extracts or for 1–3 hr prior to preparation of total cellular RNA. (*A*) RNase protection assay of total cellular RNA hybridized to a FcγRI and GAPDH ^{32}P-labeled antisense probe. All samples were analyzed on the same gel, which became folded during processing. FcγRI has identical mobility for all samples. (*B*) EMSA using ^{32}P-GRR. For each panel control cells received no IFN-γ. The EMSA for the BUD8 was electrophoresed for a longer time.

though primary fibroblasts (BUD8) and the carcinoma cell line HeLa responded to IFN-γ with the activation of FcRFγ, there was no evidence of RNA expression. Although the FcRFγ$_1$ signal is low in the HeLa cells in Fig. 2*B*, subsequent experiments demonstrated an EMSA pattern equivalent to that of monocytes. This result was consistent with the implication by Pearse *et al.* (20) that restricted tissue distribution of FcγRI is regulated by sequences distinct from the GRR. This also demonstrated that signaling through the IFN-γ receptor was intact in HeLa and BUD8 cells despite the absence of induced transcription.

Kinetics of the Induction of FcRFγ by IFN-γ. We next investigated the rate of induction of FcRFγ by IFN-γ (Fig. 3). Monocytes were treated with IFN-γ for various times, and the cells were processed for cytoplasmic and nuclear extracts. In addition, a separate set of similarly treated monocytes was used as a source of RNA. Within seconds, FcRFγ was measurable within the cytoplasm and nuclei, rapidly increasing in concentration such that near maximal induction occurred within 5 min (Fig. 3*A*). FcRFγ$_2$ appeared earlier and increased in concentration three to four times more rapidly during the first 60 sec than did FcRFγ$_1$. Both complexes, however, were maximally induced within 3–5 min. This was observed in the cytoplasmic and nuclear extracts. However, the cytoplasmic extracts contained ≈5-fold more FcRFγ when calculated on a per cell basis (see *Materials and Methods*). The rapid induction of FcRFγ correlated well with the observed increase in steady-state levels of RNA for the FcγRI (Fig. 3*C*). Within 30 min there was an obvious increase in the level of RNA, which increased rapidly over the 2-hr time period of measurement.

We had previously shown in U937 cells that a 15-min pulse was sufficient for the ultimate up-regulation of FcγRI on the cell surface (29). Longer kinetic studies with IFN-γ continuously present demonstrated that maximal concentrations of nuclear FcRFγ occurred within 15–20 min and then began to decline after 30–60 min (Fig. 3*B*). When monocytes were pulsed with IFN-γ for 15 min, washed free of any unbound IFN-γ, and continued in culture, there was a relatively rapid decline in the concentration of nuclear FcRFγ such that by 2–3 hr post-pulse there was essentially none present (data not shown).

Dose–Response Induction of FcRFγ by IFN-γ. We had previously demonstrated that for optimal FcγRI expression at the cell surface only 10–20% receptor occupancy was necessary (29). This observation was even more apparent when evaluating the activation of FcRFγ (Fig. 4). Maximal induction of FcRFγ occurred at about 1 ng/ml (Fig. 4*A*), which corresponded to about 10% receptor occupancy (about 100 receptors) in monocytes (D.S.F., unpublished data). Half-maximal responsiveness occurred at 0.4 ng/ml (Fig. 4*C*). FcRFγ induction was observed at concentrations of IFN-γ as low as 0.05 ng/ml (1 unit/ml). At this concentration, monocytes, which express about 1000 IFN-γ receptors per cell, would have <10 receptors occupied. Clearly, ligation of very few receptors is required to transduce the FcγRI signal. The induction of FcγRI mRNA closely approximated that of FcRFγ (Fig. 4*B*). At 1 hr, mRNA expression increased at 0.05 ng/ml, and maximal expression occurred at 0.5–1.0 ng/ml.

To obtain information regarding the mechanisms of activation of FcRFγ by IFN-γ, monocytes were pretreated with various kinase and phosphatase inhibitors prior to exposure to IFN-γ (Fig. 5). There was no effect on FcRFγ generation by either genistein (30 μg/ml), a tyrosine kinase inhibitor, or vanadate (1 mM), a tyrosine phosphatase inhibitor. In contrast, pretreatment with staurosporin for 15 min at 37°C at a concentration (500 nM) that inhibits several different kinases (30) resulted in marked suppression of activation of FcRFγ.

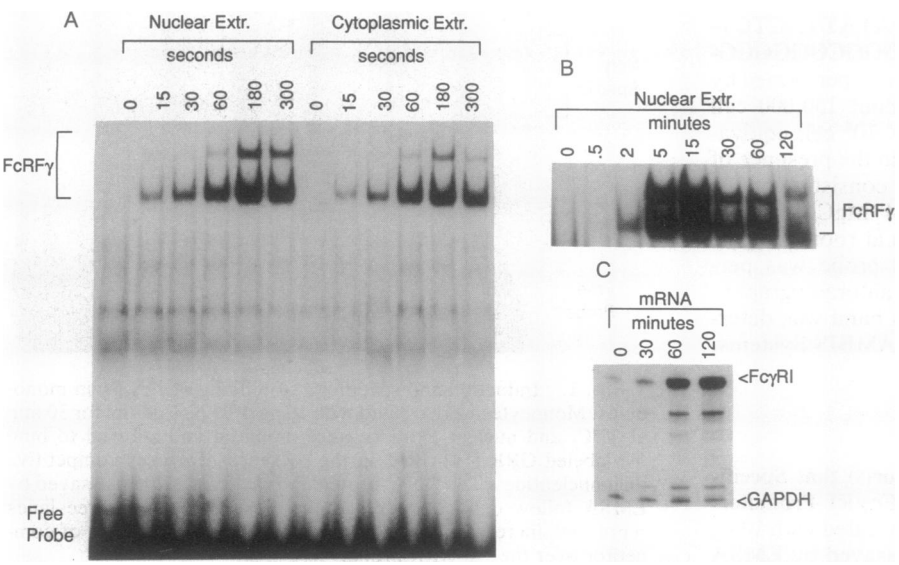

FIG. 3. Kinetics of the induction of FcRFγ and FcγRI mRNA by IFN-γ. Monocytes were exposed to IFN-γ for various times and processed for nuclear and cytoplasmic extracts (Extr.) or total cellular RNA. (*A*) Monocytes were treated 10 ng of IFN-γ per ml for up to 5 min. Nuclear and cytoplasmic extracts were prepared as described in the text and assayed by EMSA. (*B*) EMSA of nuclear extracts prepared from monocytes from another donor which were treated with 10 ng of IFN-γ per ml for up to 2 hr. Extracts were prepared from nuclei that were centrifuged through a phthalate oil cushion to minimize contaminating cytoplasm and membranes. (*C*) RNase protection assay of mRNA from cells treated with 1 ng of IFN-γ per ml.

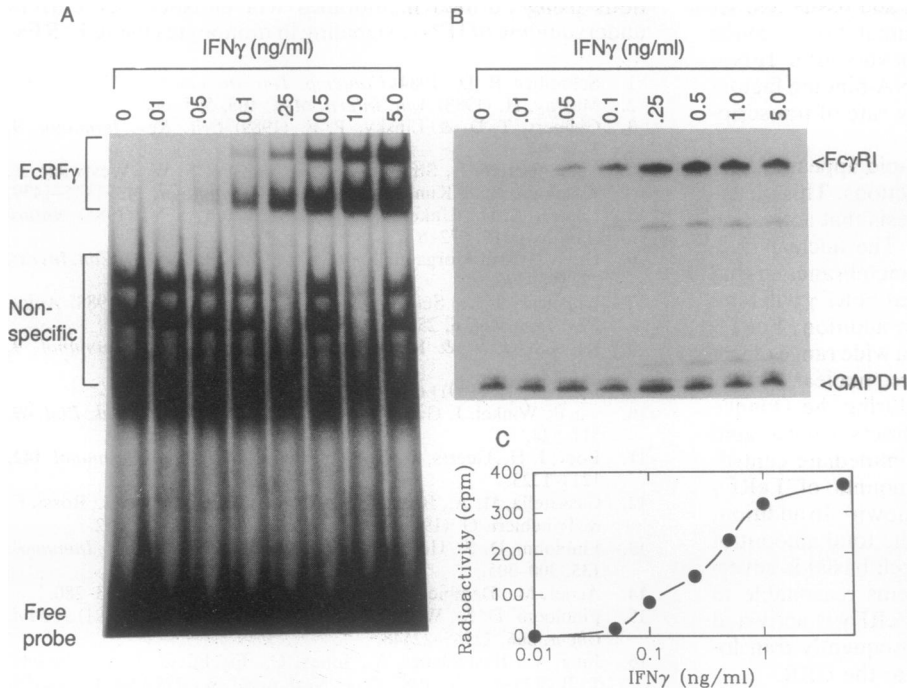

FIG. 4. Dose–response study of induction of FcRFγ by IFN-γ. Monocytes were exposed to various doses of IFN-γ and then processed for either nuclear extracts or total cellular RNA. (*A*) EMSA of nuclear extracts prepared from monocytes treated with increasing doses of IFN-γ for 30 min at 37°C. (*B*) RNase protection of monocytes exposed to increasing dose of IFN-γ for 1 hr at 37°C. (*C*) Graphic representation of radiodetector scan quantification of FcRFγ.

In addition, PAO, a cell-permeable trivalent arsenical, which has been shown to inhibit insulin-stimulated serine phosphorylation of pp24 and pp240 (31) and phosphotyrosine dephosphorylation of pp15 (32) in adipocytes, was a potent inhibitor of FcRFγ activation. PAO had no direct effect on the binding of FcRFγ to the GRR element (data not shown).

DISCUSSION

In this report we describe a DNA-binding factor that upon treatment of monocytes with IFN-γ binds to the GRR of the FcγRI gene. FcRFγ recognizes neither DNA elements required for the formation of the IFN-α transcription complex that binds to ISRE sequences nor the element required for the binding of the IFN-γ activation factor to the GAS element of the GBP gene. These observations are consistent with the absence of the predominantly IFN-α-specific, ISRE element and the IFN-γ-specific, GAS element within the GRR of the FcγRI gene. In addition, FcRFγ is *N*-ethylmaleimide resistant, unlike the IFN-α transcription complex ISGF3 (28). Within the GRR are motifs, such as an H and X box and a γ-IRE, which are necessary for induction of IFN-γ-specific genes such as the MHC class II antigens HLA-DR and HLA-DQ. Whether or not the protein(s) comprising the FcRFγ is the same as those that bind to and subsequently induce transcription of the MHC class II genes remains to be determined. Available data suggest that substantially slower kinetics exist for the induction of DNA-binding proteins that recognize the promoter of MHC class II genes (33) and that the induction of these genes is cycloheximide sensitive (34). This contrasts to the activation of FcRFγ, which occurs seconds after receptor occupancy and minutes prior to the appearance of increased levels of FcγRI mRNA.

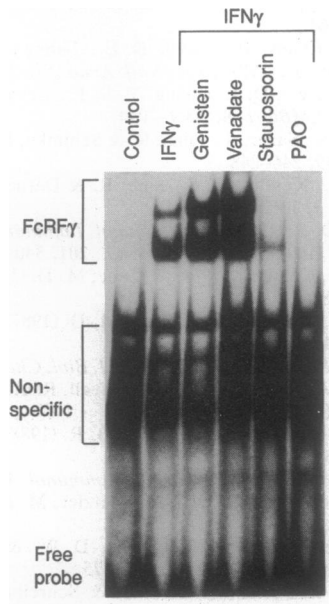

FIG. 5. Effect of kinase and phosphatase inhibitors on the formation of FcRFγ. Monocytes were preincubated at 37°C for 15 min with either genistein (30 μg/ml), vanadate (1 mM), staurosporin (500 nM), or phenyl arsine oxide (PAO, 25 μM) prior to exposure to 10 ng of IFN-γ per ml for 15 min. Cells were then washed, nuclear extracts were prepared, and FcRFγ activity was evaluated by EMSA. Cell viability as determined by trypan blue exclusion was unaltered during the treatments.

FcRFγ consists of two shifted complexes detected by EMSA. Although stable for 24 hr at 4°C, both complexes degrade at room temperature with no evidence that FcRFγ_2 is a breakdown product of FcRFγ_1 (data not shown). Data from preliminary purification of FcRFγ complexes suggest that FcRFγ_2 consists of a 40- to 50-kDa protein, whereas FcRFγ_1 consists of a complex of FcRFγ_2 and another protein.

Unlike some IFN-induced genes, such as the GBP and MHC class I genes, which exhibit enhanced expression following exposure to IFN-α and IFN-γ, the FcγRI gene responds essentially only to IFN-γ. Exposure of monocytes to IFN-α results in the formation of only a very weak complex whose migration is slightly faster than that of FcRFγ. Interestingly, IFN-γ also induces the appearance of FcRFγ in primary fibroblasts and the carcinoma cell line HeLa. However, in neither cell does IFN-γ transcriptionally activate the FcγRI gene. This observation is compatible with the fact that IFN-γ signaling is intact in HeLa and BUD8 cells. However, additional control of transcription of the FcγRI gene probably occurs at sites along the promoter that are distinct from

the GRR and involved in basal expression and tissue restriction (20). Inasmuch as FcγRI RNA accumulation is much slower in U937 cells compared to monocytes after IFN-γ exposure, this also suggests that other DNA-binding factors and elements within the gene influence the rate of transcription.

Following IFN-γ exposure, there is a rapid appearance of FcRFγ in the cytoplasmic and nuclear fractions. This observation is not inconsistent with the hypothesis that activation of FcRFγ occurs outside of the nucleus. The nuclear fractions visibly contain portions of attached membrane, so that if FcRFγ is membrane associated, activated FcRFγ will tend also to separate along with the nuclei. In addition, FcRFγ elutes from the nuclear compartment over a wide range of salt concentration (data not shown). Therefore, nuclear FcRFγ may elute into the cytoplasmic fraction during the Dounce procedure. Preparation of cytoplasmic extracts using a rapid (<1 min) Dounce technique followed by immediate centrifugation of nuclei reveals substantial amounts of FcRFγ within the cytoplasmic extracts (data not shown). In addition, since early after stimulation with IFN-γ the total amount of cytoplasmic FcRFγ (calculated on a per cell basis) is severalfold greater than nuclear FcRFγ, it seems reasonable to implicate a sequence of events in which FcRFγ is activated on or near the plasma membrane and subsequently translocates to the nucleus where upon it binds to the GRR.

The dose–response studies reinforce our previous finding (29) that minimal receptor occupancy is required for IFN-γ to enhance FcγRI expression. IFN-γ begins to induce FcRFγ at concentrations of 0.05 ng/ml (<10 molecules of IFN-γ bound per cell). The enhancement of RNA levels for the FcγRI clearly parallels the activation of FcRFγ by IFN-γ. This finding further supports the contention that the activation of FcRFγ is necessary for the IFN-γ-induced enhancement of FcγRI expression. As observed earlier with FcγRI expression at the surface, only a minority of the receptors needs to be occupied to achieve maximal signal transduction. Optimal expression of FcRFγ and mRNA occurs between 0.5 and 1 ng/ml. This corresponds to a receptor occupancy on monocytes of about 10%.

The mechanisms of signal transduction for IFN-γ are still controversial. It appears that some genes are activated by IFN-γ through pathways that are sensitive to protein kinase C inhibitors (35). Other IFN-γ-induced genes display no sensitivity to these inhibitors (36). Phosphorylation of the receptor on serines and threonines may play a role, but these data are only correlative (37, 38). Recent data suggest that the distal third of the cytoplasmic region of the IFN-γ receptor is necessary for IFN-γ-induced MHC class I expression (39). Whether or not tyrosines within this region are phosphorylated or even dephosphorylated in response to IFN-γ is as yet undefined. Since the IFN-γ receptor has a multicomponent structure with at least one protein component encoded for on chromosome 21 (15–19), the precise role these components play in transducing the signal across the membrane remains to be determined. Using concentrations of staurosporin that inhibit several classes of protein kinases (30), we can prevent IFN-γ-induced formation of FcRFγ (Fig. 5). In addition, PAO treatment results in marked inhibition of the induction of FcRFγ. The precise protein targets for PAO action are not precisely defined. It is clear that PAO can inhibit membrane protein tyrosine phosphatases such as CD45 (40). PAO also affects enzymes insensitive to vanadate (41). Alternatively, PAO has been observed to block insulin-induced protein phosphorylation on serines (31) and insulin-induced p21ras activation (42). These data are compatible with the requirement of at least a kinase and possibly a phosphatase activity for signaling of FcRFγ by IFN-γ in monocytes. Investigations using purified membranes will enhance our current understanding of IFN-γ signaling in monocytes using FcRFγ.

1. Schreiber, R. D. (1984) *Contemp. Top. Immunobiol.* **13**, 171–198.
2. Murray, H. (1988) *Ann. Intern. Med.* **108**, 595–608.
3. Geppert, T. D. & Lipsky, P. E. (1989) *Crit. Rev. Immunol.* **9**, 313–362.
4. Standiford, T. J., Strieter, R. M., Chensue, S. W., Westwick, J., Kasahara, K. & Kunkel, S. L. (1990) *J. Immunol.* **145**, 1435–1439.
5. Luster, A. D., Unkeless, J. C. & Ravetch, J. V. (1985) *Nature (London)* **315**, 672–676.
6. Guyre, P. M., Morgannelli, P. M. & Miller, R. (1983) *J. Clin. Invest.* **72**, 393–396.
7. Unkeless, J. C., Scigliano, E. & Freedman, V. H. (1988) *Annu. Rev. Immunol.* **6**, 251–281.
8. Ravetch, J. V. & Kinet, J.-P. (1991) *Annu. Rev. Immunol.* **9**, 457–492.
9. Metzger, H. (1991) *Curr. Opin. Immunol.* **3**, 40–46.
10. van de Winkel, J. G. J. & Anderson, C. L. (1991) *J. Leuk. Biol.* **49**, 511–524.
11. Boot, J. H., Geerts, M. E. & Aarden, L. A. (1989) *J. Immunol.* **142**, 1217–1223.
12. Cassatella, M. A., Bazzoni, F., Calzetti, F., Gausparri, I., Rossi, F. & Trinchieri, G. (1991) *J. Biol. Chem.* **266**, 22079–22082.
13. Finbloom, D. S., Hoover, D. L. & Wahl, L. M. (1985) *J. Immunol.* **135**, 300–305.
14. Aguet, M., Dembic, Z. & Merlin, G. (1988) *Cell* **55**, 273–280.
15. Finbloom, D. S., Wahl, L. M. & Winestock, K. D. (1991) *J. Biol. Chem.* **266**, 22545–22548.
16. Jung, V., Rashidbaigi, A., Jones, C., Fischfield, J. A., Showns, T. B. & Pestka, S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4151–4155.
17. Hemmi, S., Merlin, G. & Aguet, M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2737–2741.
18. Gibbs, V. C., Williams, S. T., Gray, P. W., Schreiber, R. D., Pennica, D., Rice, G. & Goeddel, D. V. (1991) *Mol. Cell. Biol.* **11**, 5860–5866.
19. Bono, M. R., Benech, P., Couillin, P., Alcaide-Loridan, C., Grisard, M. C., Jouin, H., Fischer, D. G. & Fellow, M. (1989) *Somat. Cell Mol. Genet.* **15**, 513–523.
20. Pearse, R. N., Feinman, R. & Ravetch, J. V. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11305–11309.
21. Gerrard, T. L., Jurgensen, C. H. & Fauci, A. S. (1983) *Cell. Immunol.* **82**, 394–402.
22. Akai, H. & Larner, A. C. (1989) *J. Biol. Chem.* **264**, 3252–3255.
23. Allen, J. M. & Seed, B. (1989) *Science* **243**, 378–381.
24. Piechaczyk, M., Blanchard, J. M., Marty, L., Dani, C., Panabieres, F., El Sabouty, S., Fort, P. & Jeanteur, P. (1984) *Nucleic Acids Res.* **12**, 6951–6963.
25. Reich, N., Evans, B., Levy, D. E., Fahey, D., Knight, E. & Darnell, J. E., Jr. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6394–6398.
26. Decker, T., Lew, D. J., Cheng, Y.-S. E., Levy, D. E. & Darnell, J. E. (1989) *EMBO J.* **8**, 2009–2014.
27. Dynan, W. S., Sazer, S., Tijan, R. & Schimke, R. T. (1986) *Nature (London)* **319**, 246–248.
28. Levy, D. E., Kessler, D. S., Pine, R. & Darnell, J. E., Jr. (1989) *Genes Dev.* **3**, 1362–1371.
29. Finbloom, D. S. (1988) *Clin. Immunol. Immunopathol.* **47**, 93–105.
30. Tatsuya, T. (1991) *Methods Enzymol.* **201**, 340–347.
31. Frost, S. C., Kohanski, R. A. & Lane, M. D. (1987) *J. Biol. Chem.* **262**, 9872–9876.
32. Bernier, M., Laird, D. M. & Lane, M. D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1844–1848.
33. Barr, C. L. & Saunders, G. F. (1991) *J. Biol. Chem.* **266**, 3475–3481.
34. Blanar, M. A., Boettger, E. C. & Flavell, R. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4672–4676.
35. Fan, X.-D., Goldberg, M. & Bloom, B. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5122–5125.
36. Celada, A. & Maki, R. A. (1991) *J. Immunol.* **146**, 114–120.
37. Mao, C., Merlin, G., Ballotti, R., Metzler, M. & Aguet, M. (1990) *J. Immunol.* **145**, 4257–4264.
38. Khurana-Hershey, G. K., McCourt, D. W. & Schreiber, R. D. (1990) *J. Biol. Chem.* **266**, 17868–17875.
39. Farrar, M. A., Fernandez-Luna, J. & Schreiber, R. D. (1991) *J. Biol. Chem.* **266**, 19626–19635.
40. Garcia-Morales, P., Minami, Y., Luong, E., Klausner, R. D. & Samelson, L. E. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9255–9259.
41. Liao, K., Hoffman, R. D. & Lane, M. D. (1991) *J. Biol. Chem.* **266**, 6544–6553.
42. Medema, R. H., Burgering, B. M. & Bos, J. L. (1991) *J. Biol. Chem.* **266**, 21186–21189.