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Inhibition of Alpha Interferon but Not Gamma Interferon Signal Transduction by Phorbol Esters Is Mediated by a Tyrosine Phosphatase

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Previous studies have indicated that the expression of viral oncoproteins, cell transformation, or phorbol ester treatment of cells can inhibit alpha/beta interferon (IFN-α/β)-induced gene expression. The mechanisms by which these promoters of cell growth exert their inhibitory effects vary, but in most instances they involve a disruption of the IFN-α/β-induced transcription complex ISGF3 such that the DNA-binding component of this complex (the 48-kDa ISGF3γ protein) does not bind to the interferon-stimulated response element (ISRE). In this report, we demonstrate that phorbol ester treatment of human peripheral blood monocytes dramatically inhibits activation of IFN-α/β-stimulated early response genes but by a mechanism which does not involve abrogation of the ISRE binding of ISGF3γ. Phorbol ester treatment of monocytes inhibited IFNα-stimulated tyrosine phosphorylation of the transcription factors Stat1α, Stat2, and Stat3 and of the tyrosine kinase Tyk2 but had no effect on IFN-γ activation of Stat1α. IFNα-stimulated tyrosine phosphorylation of Jak1 and the α subunit of the IFN-α receptor were unaffected by phorbol 12-myristate 13-acetate (PMA). Moreover, PMA caused the dephosphorylation of Tyk2 but not of Jak1, which was activated by IFN. Pretreatment of cells with vanadate prevented the effects of PMA with regard to PMA-induced Tyk2 dephosphorylation. These observations suggest that PMA exerts its inhibitory effects by activation of a tyrosine phosphatase which selectively regulates Tyk2 but not Jak1 activity.

Rapid activation of cellular genes by exposure of cells to interferons utilizes a signaling pathway which requires tyrosine phosphorylation of a family of transcription factors termed signal transducers and activators of transcription (Stat proteins) (15, 20). The Janus family of tyrosine kinases are also integral components of this signaling cascade (15). Tyrosine phosphorylation of Stat proteins enables them to translocate to the nucleus and bind to enhancers with conserved sequences located within the promoters of responsive genes. Two classes of enhancers which are necessary and sufficient for activation of genes by alpha/beta or gamma interferon (IFN-α/β and IFN-γ) have been characterized. One is termed the interferon-stimulated response element (ISRE), and the other is the gamma interferon activation sequence (GAS). Either enhancer can be activated by IFN-α/β, while IFN-γ-stimulated genes utilize primarily the GAS element (20). Tyrosine-phosphorylated Stat1 and Stat2 interact with the ISRE primarily through their association with ISGF3γ, a 48-kDa DNA-binding protein which is a member of the IFN regulatory factor family of DNA-binding proteins (32). The complex which contains these three components has been termed ISGF3 (21). Stat1 and other members of this family of transcription factors (excluding Stat2) interact with GAS-like sequences in the absence of ISGF3γ (13, 35).

Inhibition of IFN-stimulated gene expression has been shown to occur under a variety of circumstances related to enhanced cellular growth and viral infection. The expression of several viral oncoproteins, including adenovirus E1A, human papillomavirus E6/E7, and the Epstein-Barr virus nuclear antigen 2 inhibits the activation of IFN-α/β-stimulated genes (1, 11, 16, 17). Many transformed cell lines also display severely diminished IFN-α/β-stimulated formation of ISGF3 and expression of ISGF3-regulated genes (25).

Several viruses that mediate inhibition of IFN activation of cellular genes have been described (1, 3, 11, 16, 17, 29). In the case of infection with vaccinia virus, a soluble IFN receptor which interferes with the actions of IFN is expressed (3, 29). Expression of the Epstein-Barr virus nuclear antigen 2 gene inhibits IFN-α-induced expression of ISRE-dependent genes but has no effect on IFN-α-induced ISGF3 formation (17). In several other cases thus far examined, inhibition of IFN-α/β-stimulated genes involves the expression of a cellular competitor(s) which interacts with ISGF3γ such that it cannot form a stable complex with Stat1 and Stat2 (1, 11, 16, 17, 24, 25). In fact, in several cervical cancer cell lines, the activity of this competitor, termed TKO, can be assayed in vitro by its ability to disrupt preformed ISGF3 and to bind ISGF3γ directly (25). Phorbol ester treatment of primary human fibroblasts results in inhibition of IFN-α-induced gene expression by disrupting the ISGF3γ-Stat protein interactions (24). Because the inhibitory effects of phorbol 12-myristate 13-acetate (PMA) with regard to IFN-stimulated gene expression were cell specific and were most pronounced in primary cells (2), we decided to examine the actions of this growth promoter in primary peripheral blood monocytes. In these cells, PMA caused dramatic inhibition of IFN-α/β-induced gene expression by selectively enhancing the dephosphorylation of the Tyk2 kinase.

MATERIALS AND METHODS

Cells and reagents. Human monocytes were purified (>95%) by leukapheresis of normal volunteers followed by Ficoll-Hypaque sedimentation and counter-

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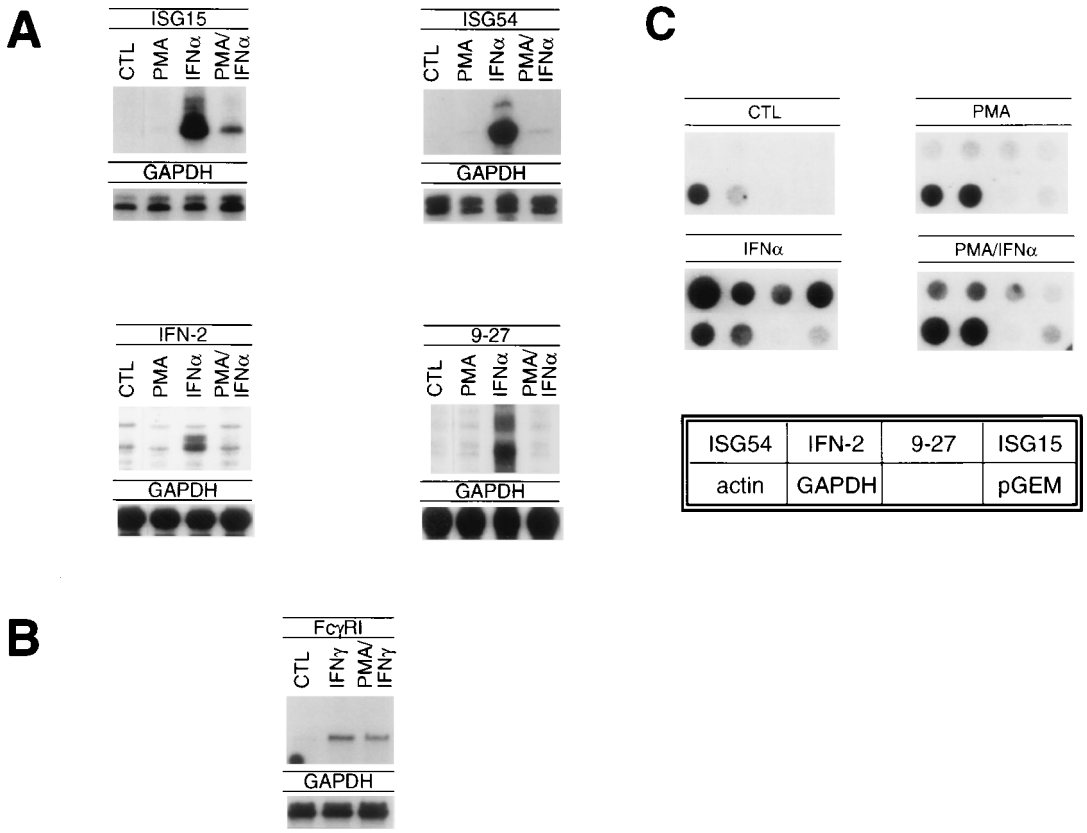

FIG. 1. Phorbol ester treatment of monocytes inhibits IFN-α- but not IFN-γ-stimulated gene expression. Primary peripheral blood monocytes were isolated from normal volunteers as described previously (35) and incubated with 10^{-8} M PMA for 1 h at 37°C, at which time either IFN-α (10^3 U/ml) or IFN-γ (10 ng/ml) was added to some of the samples for an additional 1 h. (A) Total cellular RNA was isolated and hybridized with antisense riboprobes corresponding to several known IFN-α-induced genes including ISG15 (27), ISG54 (2), IFN-2 (2), and 9-27 (28). CTL, control. (B) The IFN-γ induced RNA for the high-affinity receptor for Fcγ. A riboprobe corresponding to glyceraldehyde phosphate-3-dehydrogenase was included in each hybridization (in panels A and B) to ensure that equal amounts of RNA were analyzed. (C) Nuclear run-on assays were performed on monocytes incubated with either PMA or IFN-α plus PMA as in panel A. Isolated nuclear RNA was hybridized to nitrocellulose filters containing the indicated cDNAs. The intensity of the autoradiographic signal reflects the transcription rate of the gene at the time the nuclei were isolated.

current centrifugal elutriation. All monoclonal antibodies were obtained from Transduction Laboratories. 4G10 agarose beads were purchased from UBI.

Whole-cell extracts. Cells were diluted with ice-cold phosphate-buffered saline (PBS) and centrifuged at 1,500 × *g* for 10 min at 4°C. The cells were washed with PBS and resuspended in 200 μl of lysis buffer (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.4], 1 mM vanadate, 150 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonic acid, 1 mM dithiothreitol).

EMSA. Electrophoretic mobility shift assays (EMSAs) were performed with whole-cell extracts prepared with Triton-containing buffers as described previously (19, 35). Probes, consisting of the gamma response region (GRR) found within the promoter of the FcγRI gene (5′ AGCATGTTTCAAGGATTTGAG ATGTATTTCCCAGAAAAG 3′) and the ISRE of the ISG15 gene (5′ GATCC ATGCCTCGGGAAAGGGAAACCGAAACTGAAGCC 3′), were end labeled with polynucleotide kinase and [γ-^{32}P]ATP.

Immunoprecipitations. Cell lysates (350 μg) prepared with Triton X-100-containing buffers were incubated with anti-Tyk2, anti-Stat1, anti-Stat2, or anti-Stat3 antiserum for 2 h. The preparation and characterization of these antiserum have been previously described (4, 5). Antibodies to the IFN-α/β receptor α subunit (31) were generated against the extracellular domain by immunizing mice with CHO cells expressing a high concentration of cell surface IFN-α/β receptor α subunit. The antibodies were screened with IFN-α/β receptor α-subunit fusion protein and also by cell surface binding, using fluorescence-activated cell sorter analysis (4, 5). Immune complexes were collected after each immunoprecipitation on protein G beads, resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (8% polyacrylamide), immunoblotted with monoclonal antiphosphotyrosine antibody 4G10 (UBI), and detected by enhanced chemiluminescence. In some experiments, tyrosine-phosphorylated proteins were immunoprecipitated with antiphosphotyrosine antibody (4G10) coupled to agarose beads (UBI). Immunoprecipitated proteins were eluted from 4G10 agarose by incubation with 100 mM phenylphosphate, and eluted material was resolved by SDS-PAGE and transferred to Immobilon. Blots were probed with monoclonal antibodies specific for either Tyk2, Jak1, Stat1, Stat2, or Stat3, all purchased from Transduction Labs.

Measurement of Cell Surface IFNα receptors. Recombinant IFNα-2b (Schering Corp., Kenilworth, N.J.) was radioiodinated to high specific activity with Bolton Hunter reagent. Specific activity was determined by an antiviral assay. Binding of cells to ^{125}I-labeled recombinant IFNα-2b was performed as described previously (9). Scatchard analysis was carried out with bound and free ligands.

RESULTS

The phorbol ester PMA inhibits IFN-α-stimulated early response genes in peripheral blood monocytes. Previous studies have demonstrated that in some cells, PMA treatment results in the inhibition of IFN-α-stimulated 54-kDa gene (ISG54) expression (2, 24). The effects of PMA were cell as well as gene specific but were most prominent in primary fibroblasts (2). To determine whether PMA modulated IFN-α signaling in other primary cells, we examined the actions of this growth promoter in primary human monocytes. Monocytes are very sensitive to the effects of phorbol esters in that they promote cell attachment and phagocytosis, both of which may modulate the actions of IFNs. RNA was prepared from monocytes which had been left untreated or were treated with PMA in the presence or absence of IFN-α, and the induction of several IFN-α-induced RNAs was analyzed by RNase protection assays (Fig.

1A). In contrast to primary human fibroblasts, in which PMA treatment of cells inhibited ISG54 but not ISG15 RNA (24), in primary human monocytes, PMA inhibited IFN-α-stimulated expression of several IFN-α-induced RNAs including ISG54, ISG15, 9-27, and IFN-2. However, under the same conditions, IFN-γ-stimulated expression of FcγR1 RNA was unaffected, suggesting that the effects of PMA were specific for the IFN-α/β signaling cascade (Fig. 1B). To determine whether PMA treatment of monocytes was affecting IFN-α-stimulated transcription of these early response genes rather than modifying the RNA half-life, nuclear run-on assays were performed (Fig. 1C). Monocytes were incubated with PMA for 1 h prior to the addition of IFN-α for another 45 min. Nuclei were isolated and incubated in the presence of [α-^{32}P]UTP, and isolated nuclear RNA was hybridized to nitrocellulose filters containing the indicated cDNA probes. The intensity of the autoradiographic signal is a reflection of the transcription rate of a given gene at the time the nuclei were isolated (8). PMA inhibited by 80 to 90% the transcription of all IFN-α-stimulated genes examined while the effects on actin and glyceraldehyde phosphate-3-dehydrogenase transcription were insignificant (Fig. 1C).

PMA treatment of monocytes does not alter IFN-α binding to its receptor. One possible explanation of the inhibitory actions of PMA on IFN-α activation of early response genes is that PMA treatment of monocytes decreases the number or affinity of IFN-α cell surface receptors. To explore this possibility, receptor-binding assays were performed with ^{125}I-labeled IFN-α2b; and the receptor number and affinity were determined by Scatchard analysis. Peripheral blood monocytes contain approximately 625 receptors per cell with an apparent affinity of 6.2×10^{10} for IFN-α2b. Incubation of monocytes with PMA for 60 min prior to performing the binding assay had no significant effect on either the number (575) or affinity (7.1×10^{10}) of IFN-α receptors for their ligand. These results rule out the possibility that a simple change in IFN-α cell surface receptors can account for the inhibitory actions of PMA.

IFN-α-induced formation of ISGF3 and complexes that bind GAS enhancers are decreased in PMA-treated monocytes. Five of the signaling components besides the receptor that are required for IFN-α/β activation of early response genes include the tyrosine kinases Tyk2 and Jak1 and the transcription factors Stat1, Stat2, and Stat3. Each of these proteins is rapidly tyrosine phosphorylated as a result of IFN-α/β binding to its receptor. Tyrosine phosphorylation of the Stat proteins allows them to form dimers and translocate to the nucleus, where they bind to specific enhancers (GAS and ISRE) within the promoters of IFN-α/β-activated genes (20). While Stat1 and Stat3 bind to both GAS-like and ISRE enhancers, Stat2 binds only to the ISRE (20). To initially determine whether PMA inhibited IFN-α/β-stimulated binding to the GAS and ISRE enhancers, EMSAs were performed with oligonucleotide probes corresponding to the ISRE of the ISG15 gene and the GRR of the FcγR1 enhancer. PMA treatment of monocytes prior to the addition of IFN-α clearly inhibited formation of the IFN-α-induced complex which bound to the GRR (Fig. 2A) and also formation of the ISGF3 transcription complex that bound to the ISRE (Fig. 2B). This finding excluded ISGF3γ as the primary target for PMA, because ISGF3γ is required for the formation of ISGF3 but not for the GRR-binding complex (14). Interestingly, IFN-γ-induced tyrosine phosphorylation of Stat1 and its binding to the GRR were not affected by PMA treatment of the same monocytes (Fig. 2A, lanes 6 and 7).

IFN-α-stimulated tyrosine phosphorylation of Stat1α, Stat2, and Stat3 is inhibited in monocytes incubated with PMA. To examine directly whether PMA treatment of monocytes affected IFN-α-stimulated tyrosine phosphorylation of the Stat proteins, monocytes were incubated with or without PMA prior to the addition of IFN-α for 30 min. Cell lysates were prepared and immunoprecipitated with antiserum specific for Stat1α, Stat2, or Stat3. Immunoprecipitated proteins were resolved by SDS-PAGE and transferred to Immobilon. Immunoblotting was then performed with antiphosphotyrosine antibodies (Fig. 3). PMA treatment of monocytes inhibited

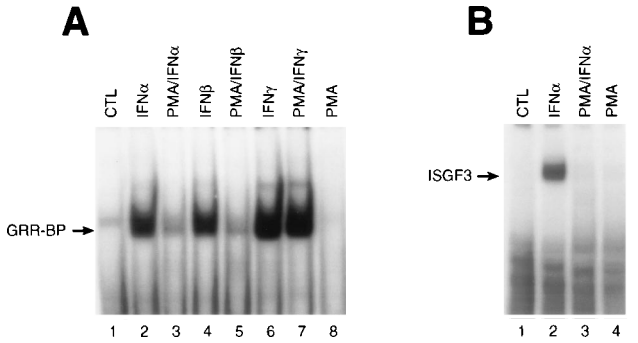

FIG. 2. Incubation of monocytes with PMA inhibits IFN-α-induced DNA binding to the GRR of the high-affinity FcγR1 enhancer and to the ISRE. Peripheral blood monocytes were incubated with PMA as in Fig. 1 for 1 h prior to the addition of either IFN-α, IFN-β, or IFN-γ for 15 min. Whole-cell extracts were prepared, and EMSAs were performed to assay for binding to the GRR enhancer (A) or for ISGF3 formation (B) by using a probe corresponding to the ISRE of the ISG15 gene (24). Samples treated with only PMA were incubated for a total of 75 min. CTL, control. GRR-BP indicates the IFN-induced complex which binds to the GRR.

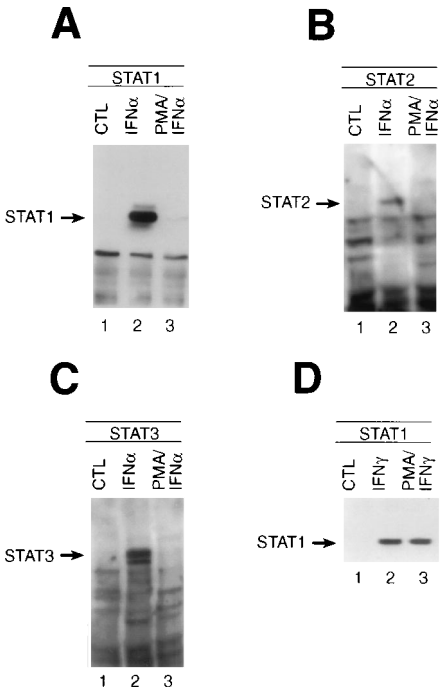

FIG. 3. Incubation of monocytes with PMA inhibits IFN-α-stimulated tyrosine phosphorylation of Stat1α, Stat2, and Stat3. Peripheral blood monocytes were incubated with or without PMA for 1 h prior to the addition of IFN-α or IFN-γ for 30 min as described in the legend to Fig. 2. Cell extracts were prepared, and Stat1α (A), Stat2 (B), and Stat3 (C) were immunoprecipitated from the extracts, resolved by SDS-PAGE, and transferred to Immobilon. For samples treated with IFN-γ only (D), tyrosine phosphorylation of Stat1 was analyzed, since this is the primary or only Stat activated by this cytokine. The resulting blots were probed with 4G10 antiphosphotyrosine antibody. CTL, control.

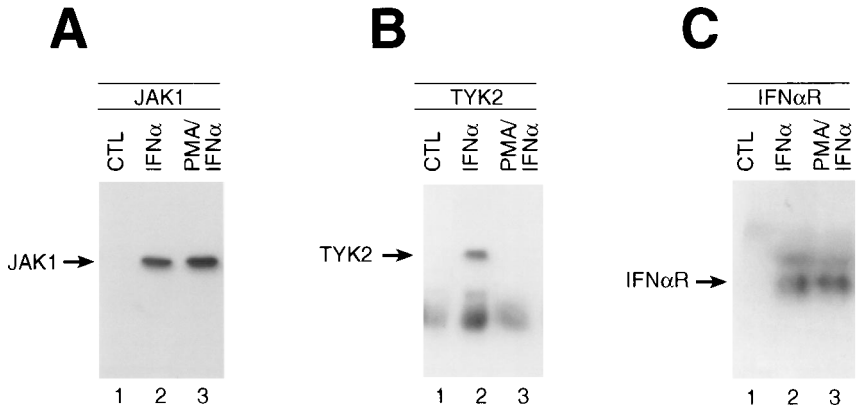

FIG. 4. Incubation of monocytes with PMA inhibits IFN-α-stimulated tyrosine phosphorylation of Tyk2 but not of Jak1 or the α subunit of the IFN-α/β receptor (IFNαR). Peripheral blood monocytes were incubated as described in the legend to Fig. 2, and cell extracts were prepared. (A and B) The extracts were subjected to immunoprecipitation with 4G10 antiphosphotyrosine antibody, and the subsequent blots were probed for the presence of either Jak1 (A) or Tyk2 (B). (C) The extracts were directly precipitated with monoclonal antibody to the α subunit of the IFN-α/β receptor, and the resulting blot was probed with antiphosphotyrosine antibody (4G10).

IFN-α-stimulated tyrosine phosphorylation of all three Stat proteins (Fig. 3), whereas IFN-γ activation of Stat1α was not affected (Fig. 3D). The blots were reprobed with their respective Stat-specific antisera to ensure that equal amounts of Stat protein were present in each sample (data not shown).

PMA inhibits IFNα-stimulated tyrosine phosphorylation of Tyk2 but not Jak1 and the α subunit of the IFN-α/β receptor. The observation that PMA inhibited IFN-α-stimulated tyrosine phosphorylation of Stat1α, Stat2, and Stat3 suggested that it affects a signaling component(s) upstream of these transcription factors. Both the Tyk2 and Jak1 tyrosine kinases are required for IFN-α-stimulated gene expression, and activation of these kinases by IFN-α treatment of cells induces their tyrosine phosphorylation (22, 33). To examine whether PMA affected IFN-α-induced tyrosine phosphorylation of Tyk2 and Jak1, we incubated monocytes with or without PMA for 30 min prior to the addition of IFN-α for 10 min. Cell lysates were prepared and incubated with antiphosphotyrosine antibody. Immunoprecipitated proteins were resolved by SDS-PAGE, blotted, and probed for the presence of either Tyk2 or Jak1 (Fig. 4A and B). Although IFN-α-stimulated tyrosine phosphorylation of Jak1 was unaffected by treatment with PMA, there was virtually complete inhibition of IFN-α-induced tyrosine phosphorylation of Tyk2. Direct immunoprecipitations of Jak1 and Tyk2 followed by antiphosphotyrosine blotting revealed similar results (data not shown).

The α subunit of the IFNα/β receptor has also been demonstrated to be rapidly tyrosine phosphorylated as a result of exposure of cells to IFN-α or IFN-β (26). Since Tyk2 but not Jak1 appeared to be a target for the PMA-induced inhibition of this signaling cascade, this observation allowed us to examine whether tyrosine phosphorylation of the α subunit of the receptor paralleled the actions of PMA with regard to Tyk2. Monocytes were incubated with PMA (Fig. 4A and B), and extracts were immunoprecipitated with a monoclonal antibody to the α subunit of the IFN-α/β receptor. The subsequent blots were probed with antiphosphotyrosine antibody (Fig. 4C). It is evident from these results that while IFN-α induced the tyrosine phosphorylation of this subunit of the receptor, PMA had no inhibitory effect on this event. This finding suggested that tyrosine phosphorylation of the α subunit of the receptor in primary monocytes may be more directly linked to activation of Jak1 rather than of Tyk2.

Evidence for the role of a PMA-activated tyrosine phosphatase which targets phosphorylated Tyk2. From the results presented above, it appeared that PMA treatment of peripheral blood monocytes selectively inhibited IFN-α-stimulated tyrosine phosphorylation of Tyk2. Two possible mechanisms could account for the actions of PMA. Either PMA treatment of monocytes inactivates the kinase which is responsible for tyrosine phosphorylation of Tyk2, or PMA activates a tyrosine phosphatase (protein tyrosine phosphatase [PTPase]) which dephosphorylates this kinase. If a tyrosine phosphatase were activated by PMA treatment of monocytes, incubation of cells with the tyrosine phosphatase inhibitor vanadate should reverse the inhibitory effects of PMA. Monocytes were incubated for 30 min with vanadate prior to the addition of PMA for 30 min. IFN-α was then added to the cells for a further 15 min prior to the preparation of cell extracts. EMSAs were performed with the GRR enhancer, or antiphosphotyrosine immunoprecipitates were probed for Tyk2 (Fig. 5). Preincubation of monocytes with vanadate prevented most of the inhibition by PMA of IFN-α-stimulated formation of the GRR-binding complex (Fig. 5A, lanes 2 and 6). In other experiments, vanadate completely blocked the actions of PMA and also enabled Tyk2 to be tyrosine phosphorylated in the presence of PMA and IFN-α (Fig. 5B, lanes 5, 6, and 8). Vanadate treatment of cells in the absence of PMA or IFN-α had no effect on either formation of the GRR-binding complex (Fig. 5A, lane 5) or tyrosine phosphorylation of Tyk2 (Fig. 5B, lane 3).

If the inhibitory actions of PMA were mediated through the activation of a tyrosine phosphatase which targeted Tyk2 specifically, PMA treatment of cells should also stimulate the dephosphorylation of Tyk2 that is already tyrosine phosphorylated after IFN-α treatment. To address this possibility, we incubated monocytes with IFN-α for 10 min to allow Tyk2 to become tyrosine phosphorylated (Fig. 5B, lane 5). PMA was then added to the cells in the continuous presence of IFN-α for an additional 10 min prior to preparation of cell lysates. Addition of PMA to cells which had previously been exposed to IFN-α induced the dephosphorylation of Tyk2 on tyrosine (compare lanes 5 and 7), as did the addition of PMA prior to IFN-α (lane 6). In a separate aliquot from these samples, Tyk2 was directly immunoprecipitated and probed for Tyk2 by an immunoblot (Fig. 5B, lower panel). Equal amounts of Tyk2 protein were present in all samples, ruling out the possibility

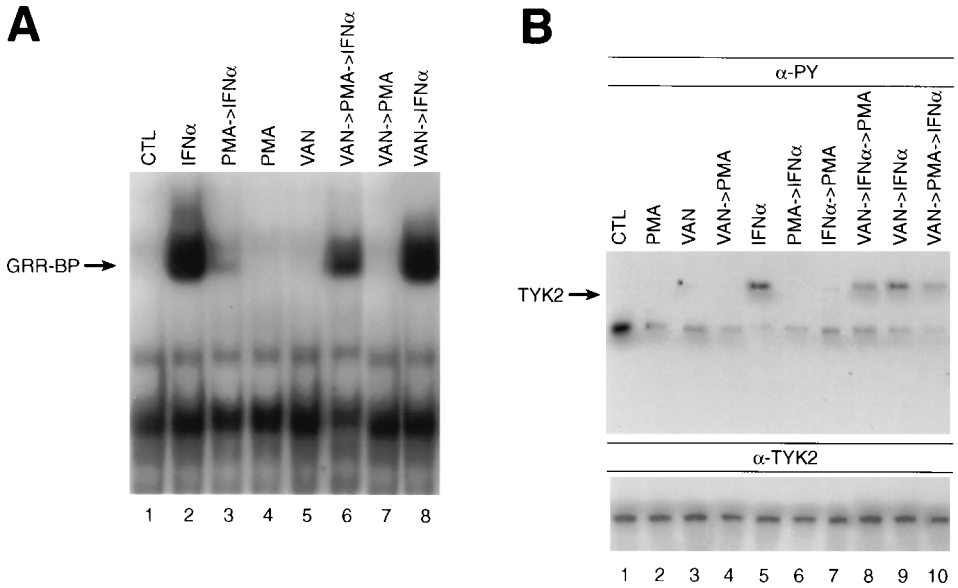

FIG. 5. PMA stimulates a PTPase activity in monocytes which targets Tyk2. (A) Vanadate reverses PMA inhibition of IFN-α-stimulated binding to the GRR enhancer. Monocytes were incubated with 1 mM vanadate at 22°C (lanes 5 through 8) for 30 min. PMA (lanes 3, 4, 6, and 7) was then added to some samples, and all samples were incubated at 37°C for 1 h. IFN-α (lanes 2, 3, 6, and 8) was then added for 15 min, cell extracts were prepared, and EMSAs were performed with the GRR oligonucleotide probe as described in the legend to Fig. 2. (B) Vanadate reverses PMA inhibition of IFN-α-stimulated tyrosine phosphorylation of Tyk2, and PMA stimulates the dephosphorylation of tyrosine-phosphorylated Tyk2. Monocytes were incubated with vanadate, PMA, and/or IFN-α as in panel A. The incubation time with IFN-α was 20 min. In lane 7, PMA was added to the culture 10 min after IFN-α and the incubation with both reagents was continued for an additional 10 min. After the indicated treatments, cell extracts were prepared and immunoprecipitated with 4G10 antiphosphotyrosine antibody (α-PY). The resulting blot was probed with Tyk2 monoclonal antibody as in Fig. 4B. In the lower part of panel B, separate aliquots were immunoprecipitated with anti-Tyk2 antiserum and the immunoprecipitates were resolved by SDS-PAGE. The resulting blot was probed for the presence of Tyk2 by using a Tyk2 monoclonal antibody (Transduction Labs).

that incubation of monocytes with PMA induced a selective degradation of the protein. Rather, these results indicate that PMA is activating a tyrosine phosphatase that dephosphorylates Tyk2 such that the Stat proteins can not be activated and transcription of IFN-α-stimulated early response genes is blocked.

DISCUSSION

Modulation of growth factor and cytokine-regulated signaling cascades by viruses and oncogenic proteins is a well-known phenomenon. For IFN-α or IFN-β activation of early response genes, a common control point used by adenovirus E1A, HPV E6/7, or phorbol esters is the disruption of the interaction of the 48-kDa ISRE-binding protein, ISGF3γ, with activated Stat transcription factors (1, 11, 16). The results of these studies clearly indicate that phorbol ester treatment of primary human peripheral blood monocytes selectively inhibits IFN-α/β activation of the Jak/Stat pathway. In contrast to the other mechanisms, PMA exerts its inhibitory effects in monocytes by inducing a tyrosine phosphatase activity that targets Tyk2 but has no effects on IFN-α-stimulated tyrosine phosphorylation of either Jak1 or the α subunit of the IFN-α/β receptor. There is recent evidence that tyrosine phosphorylation of Tyk2 by IFN-α treatment of cells is not required for some aspects of IFN-α signaling (34). That study showed that a kinase-inactive Tyk2 transfected into a cell line lacking Tyk2 could rescue cell growth under selective pressure, presumably via an ISGF3-independent pathway. However, IFN-α treatment of cells expressing the kinase-inactive Tyk2 failed to stimulate the tyrosine phosphorylation of Stat1 and Stat2 (34). These results are in agreement with those presented here, in which tyrosine phosphorylation of Tyk2 occurs in parallel with IFN-α-induced expression of early response genes and tyrosine phosphorylation of the Stat proteins.

The mechanisms by which PMA inhibits IFN-α-induced gene expression by stimulating a PTPase activity which dephosphorylates Tyk2 are unresolved. Presumably, either the PTPase or a PMA-activated kinase which regulates the PTPase activity is very cell specific, since the dramatic inhibitory actions of this tumor promoter are restricted to primary monocytes and have thus far not been observed in any cell lines of monocytic origin. Although it is still unclear whether PMA is exerting its actions by stimulating a protein kinase C isoform, we have observed that the protein kinase C inhibitor bisindolylmaleimide reverses the effects of PMA (data not shown), a finding which is consistent with this possibility. Previous studies have implicated the role of tyrosine phosphatases as both positive (6, 12, 30) and negative (4, 13) regulators of IFN-α/β-stimulated activation of the Jak/Stat pathway. However, the PMA-stimulated PTPase activity described here appears to be unique. Although PTPases are clearly important modulators of cell growth and growth factor-mediated signaling cascades, few instances in which the activities of PTPases are known to be selective have been described. One such PTPase is the SH2 domain-containing PTPase, PTP1C. This PTPase has recently been implicated in down regulation of both erythropoietin and IFN-α activation of Jak2 and Jak1, respectively (4, 18). Its activity has been shown to be enhanced by treatment of HL60 cells with phorbol esters (36). PTP1C therefore seemed to be one candidate for a PMA-stimulated PTPase which could inhibit IFN-α/β-stimulated Tyk2 activity in primary monocytes. However, in the studies examining the role of PTP1C in IFN-α/β activation of the Jak/Stat pathway, it appears that this PTPase seems to selectively inactivate Jak1, with only modest or no effects on IFN-α/β-stimulated Tyk2 tyrosine phosphor-

ylation (4). In addition, experiments with macrophages from motheaten mice, which do not express PTP1C, indicate that PMA still is able to inhibit IFN-α/β stimulation of the Stat activation in the absence of PTP1C (data not shown). Three other PTPases which are regulated by serine phosphorylation and/or PMA treatment of cells include PTP1D, PTP-PEST, and receptor protein-tyrosine phosphatase α (RPTPα) (7, 10, 23). The first two PTPases are inactivated by PMA treatment of cells, whereas the third (RPTPα) is widely expressed and PMA augments its activity in several cell types (7). However, since its expression is ubiquitous, RPTPα remains a possible but relatively unlikely candidate to stimulate selective dephosphorylation of Tyk2 in monocytes. Therefore, the identity of the PTPase that is being regulated by PMA in these cells and the mechanism by which this PTPase displays substrate specificity for Tyk2 remain to be determined.

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