Activation of Signal Transducer and Activator of Transcription 1 (STAT1) Is Not Sufficient for the Induction of STAT1-dependent Genes in Endothelial Cells

COMPARISON OF INTERFERON-γ AND ONCOSTATIN M®

Keyvan Mahboubi and Jordan S. Pober‡

From the Interdepartmental Program in Vascular Biology and Transplantation, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

We compared human endothelial cell (EC) responses to interferon-γ (IFNγ) and oncostatin M (OnM), cytokines that utilize Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling. Both cytokines cause phosphorylation of Tyr residue 701 and Ser residue 727 of STAT1, as shown by immunoblotting. Both activate DNA binding of STAT1 homodimers, shown by electrophoretic mobility shift assay. However, only IFNγ increases expression of three STAT1-dependent gene products examined, namely transporter associated with antigen processing-1 (TAP1), interferon regulatory factor-1 (IRF1), and class I major histocompatibility complex (MHC) protein, as demonstrated by immunoblotting. Only IFNγ increases TAP1 transcription assessed by reporter gene assay. OnM pretreatment or co-treatment does not inhibit IFNγ responses. Interestingly, IFNγ activation of STAT1 is considerably more long-lived than that produced by OnM. To determine whether duration is functionally significant, we transduced EC with a chimeric receptor containing antigen-processing-1 (TAP1), interferon regulatory factor-1 (IRF1), and class I major histocompatibility complex (MHC) protein, as demonstrated by immunoblotting. Only IFNγ increases STAT1 activation that is comparable in magnitude and duration to that caused by IFNγ, but still fails to induce TAP1, IRF1, or class I MHC molecules. OnM also activates STAT1 but not transcription of STAT1-dependent genes in HepG2 cells. Transient transfection of HepG2 cells with a STAT-defective mouse IFNγ receptor failed to complement the OnM STAT signal. We conclude that STAT1 activation is necessary but not sufficient for induction of transcription of IFNγ-responsive genes. However, signals provided by IFNγ other than STAT1 activation cannot be provided in trans to complement the response to OnM.

Vascular endothelial cells (EC) are the principal cellular targets of many pro- and anti-inflammatory cytokines. They are unusually sensitive to activation by interferon-γ (IFNγ), showing IFNγ-dependent expression of class I and class II major histocompatibility complex (MHC) molecules under basal conditions, when most other cell types are unresponsive (1–3). A previous study from our laboratory suggested that this sensitivity may be attributed to the fact that EC are relatively deficient in expression of Src homology-containing phosophatase-1 (SHIP-1) which normally dampens signaling by IFNγ (4). The predominant signaling pathway activated by IFNγ involves Janus kinases (JAKs), namely JAK1 and JAK2, and signal transducer and activator of transcription-1 (STAT1) (5–8). Binding of IFNγ to IFNγ receptor-1 (IFNGR1) in the presence of IFNγ receptor-2 (IFNGR2) results in receptor clustering followed by rapid phosphorylation of the intracellular region of the IFNGR1 at Tyr residue 440 by receptor-associated JAKs (7). Cytoplasmic STAT1 proteins then bind to this phosphorylated Tyr residue in IFNGR1 and subsequently become phosphorylated themselves at Tyr residue 701 by receptor-associated JAKs. The tyrosine-phosphorylated STAT1 proteins dissociate from the receptor and form a homodimer which translocates to the nucleus and binds to specific γ-activated sequence (GAS) elements (TTCCNNNGAA consensus sequence) in the enhancers of IFNγ-inducible genes, stimulating their transcription (9, 10). The ability of STAT1 to activate gene transcription may additionally depend on phosphorylation of Ser residue 727, possibly through the actions of a mitogen-activated protein kinase (MAPK) family member (11–13).

Many cell types, including EC, respond to IFNγ by rapidly increasing the expression of TAP1 and IRF1 (14, 15). Both responses are STAT1-dependent and involve binding of STAT1 homodimers to GAS elements in the 5′-flanking regions of these genes (4, 16, 17). TAP proteins contribute to the assembly and peptide loading of nascent class I MHC molecules (18, 19), while IRF1 mediates the delayed transcription of class I MHC molecules as well as sustained transcription of TAP proteins (15, 16, 20). The coordinated up-regulation of these proteins by IFNγ enhances the capacity of these cells to present foreign peptides bound to class I MHC molecules to cytolytic CD8+ T cells (21). In this way, IFNγ contributes to immune-mediated
OnM Activates STAT1 but Not STAT1-dependent Genes

host defense. Whereas IFNγ is considered to be pro-inflammatory, interleukin-6 (IL-6)-type cytokines are often immunoregulatory or cytoprotective, reducing inflammation and host injury (22–24). The members of this cytokine family which include IL-6, IL-1, leukemia-inhibitory factor, ciliary neurotrophic factor, cardiotrophin-1, and oncostatin M (OnM), utilize a common cytokine receptor signaling subunit, called gp130, which is expressed on the surface of target cells (25). Typically, IL-6-type cytokines associate with a cytokine-binding α chain that, upon ligand binding, heterodimerizes with gp130 and promotes receptor clustering. Receptor-associated JAKs (JAK1, JAK2, and TYK2) then phosphorylate gp130 on several intracellular tyrosine residues, providing docking sites for adapters and transcription factors. Four tyrosine residues in the intracellular region of gp130 (Tyr-767, Tyr-814, Tyr-905, and Tyr-915) contribute to the activation of STAT3 and two of these tyrosine residues (Tyr-905 and Tyr-915) also contribute to the activation of STAT1 (26, 27). Phosphorylation of Tyr residue 759 leads to recruitment and activation tyrosine phosphatase SHP-2 (28–32). SHP-2 both down-regulates JAK-STAT signaling through its tyrosine phosphatase activity and acts as a gp130-associated adapter protein, binding Grb2 and initiating the activation of a MAPK pathway independently of its tyrosine phosphatase activity (29, 33). The mutation of Tyr residue 759 to Phe in gp130 prevents activation of MAPK (27, 29, 33). It also causes prolonged STAT3 and STAT1 activation and enhances the induction of STAT3-dependent genes such as α2-macroglobulin (α2M) in human hepatoma HepG2 cells (29–32, 34).

We have previously described the effects of IL-11 on human umbilical vein EC (HUVEC) (35, 36). In these experiments, we used OnM as a positive control for activation of STAT1 and STAT3. The levels of STAT1 phosphorylation on Tyr residue 701 induced by treatment with OnM were striking, as assessed by immunoblotting, yet there was no evidence of increased expression of class I MHC molecules. This observation led us to examine more carefully whether OnM can increase the expression of IFNγ-inducible STAT1-dependent proteins in HUVEC. We find that although OnM is a potent activator of STAT1, it cannot activate STAT1-dependent genes. These findings suggest that IFNγ must have some other, yet unidentified, activities that are necessary for STAT1-dependent gene expression.

EXPERIMENTAL PROCEDURES

Reagents and Cells—Recombinant human OnM was purchased from R&D Systems (Minneapolis, MN). Recombinant human IFNγ (0.32 × 10⁷ units/mg), recombinant mouse IFNγ, and recombinant human platelet-derived growth factor BB (PDGFBB) were purchased from Upstate Biotechnology (Lake Placid, NY). Fibroblast growth factor-1, commonly called endothelial cell growth supplement, was obtained from Collaborative Research/Becton Dickinson (Bedford, MA) and used in conjunction with porcine intestinal heparin (Sigma). Polybrene, phenol blue, 20% glycerol) and heating the mixture in a boiling water bath for 3 min. 20 µg of protein were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Immobilon P, Millipore, Bedford, MA) by electrophoresis. After blocking with Tris-buffered saline Tween (10 mM Tris-Cl, pH 8.0, 0.150 mM NaCl, 0.05% Tween 20) containing 5% nonfat milk for 1 h at room temperature, the membranes were incubated with blocking solution containing the indicated antibody at 4°C. Membranes were washed and incubated with a horseradish peroxidase-conjugated detecting reagent specific for phosphotyrosine-phosphothreonine-p42/p44 MAPK were purchased from Cell Signaling Technology, Inc. Beverly, MA). Rabbit polyclonal Abs to phospho-tyrosine phosphoratin (Ser/Thr), and mouse monoclonal antibody (Ab) to β-actin were also obtained from Sigma. Rabbit polyclonal Abs to STAT1, to phosphoSTAT1, to phosphoSTAT3, and to phospho-HEK293 cells were transiently transfected using Targetfect F-2 and peptide enhancer reagents according to the manufacturer's instructions (Targeting Systems, Santee, CA). Transient transfection of HUVEC was performed using a DEAE-dextran protocol as described previously (39). HepG2 cells were transiently transfected using Targetfect F-2 and peptide enhancer reagents according to the manufacturer's instructions (Targeting Systems, Santee, CA).

Promoter Reporter Gene Assays—To assess the induction of TAPI-GH promoter-reporter gene construct, culture medium was assayed for GH using a solid phase sandwich radioimmunoassay as described by the manufacturer (Nichols Institute, San Juan Capistrano, CA). Radioactivity was measured in a γ-counter (20/20 series, Iso-Data Inc., Palatine, IL). Cell lysates were assayed for renilla luciferase activity using a Promega reporter assay system (Promega, Madison, WI) and measured using a Berthold model LB9501 luminometer (Schwarzwald, Germany). GH values in ng/ml were normalized to luciferase values in relative light units (RLU) to control for transfection efficiency. To assess the induction of α2M-luciferase promoter-reporter gene construct, cell lysates were assayed for renilla luciferase activity using a dual Promega reporter assay system (Promega).
and measured as described above. Firefly luciferase activity in RLU was normalized to Renilla luciferase values in RLU to control for transfection efficiency.

Nuclear Extractions and Electrophoretic Mobility Shift Assay (EMSA)

Nuclear extracts were a modification of the procedure described by Diamant et al. (40). Briefly, cells (3 × 10^6) were harvested by scraping into PBS, resuspended into 200 μl of buffer A (10 mM HEPES (pH 8.0), 10 mM KC1, 100 μM EDTA, 1 mM dithiothreitol) supplemented with the mixture of protease and phosphatase inhibitors used for immunoblotting and incubated 10 min on ice. Cells were lysed by the addition of 15 μl of 1% Nonidet P-40 and incubated on ice for 5 min, vortexed for 10 s, and the nuclei pelleted by centrifugation (500 g, 2 min, 4 °C). The nuclei pellets were washed with 100 μl of buffer A, resuspended in 50 μl of extraction buffer C (20 mM HEPES (pH 8.0), 400 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol) supplemented with protease and phosphatase inhibitors as above, and incubated for 15 min at 4 °C on rocking platform, the tubes were then centrifuged (20 min at 11,000 × g, 4 °C) to remove debris from the extract. Protein concentrations of nuclear extracts were determined to be 1 to 2 μg/μl against a BSA standard using a Bio-Rad assay kit (Bio-Rad, Hercules, CA). A final concentration of 10% glycerol was added to the extracts and nuclear extracts were either used immediately or stored at −80 °C.

The following oligonucleotides were used in EMSAs (listed 5′ to 3′: complement sequence are not shown): sis-inducible element of the c-Fos promoter (笋SE): GTCGAACCTTCTGGTAAATC (41); IRF1 promoter (笋IRF1): GTGATTTCCCCGAAATGACG (17). Briefly, complementary oligonucleotides were annealed, labeled with [γ-32P]ATP (3000 Ci/mmol, Amersham Biosciences, Inc., Arlington Heights, IL) with T4 polynucleotide kinase (New England Biolabs Inc., Beverly, MA), and separated from unincorporated nucleotides over a Sephadex G-25 spin column (Amersham Biosciences, Inc.). Nuclear extracts (10 μl) containing 4 μg of protein were incubated at room temperature for 20 min with 10 μl of EMSA mixture (2 μg of poly(dI-dC), 1 μM dithiothreitol, 5 μg/ml BSA, 20 mM Tris-HCl (pH 7.5), 0.5 M NaCl, 2 μM EDTA, 10% glycerol, 1 μl of probe). Protein-DNA complexes were separated by electrophoresis on a 4% polyacrylamide gel containing 10% glycerol in a Tris glycine-EDTA buffer (9.25:1:0.25). Nuclear extracts were either used immediately or stored at −80 °C.

RESULTS

OnM Does Not Increase the Expression of IRF1 or TAPI in HUVEC—We have previously shown that OnM and IFNγ each stimulate phosphorylation of STAT1 Tyr residue 701 in a dose-dependent manner in HUVEC (4, 35), as detected by immunoblotting with Ab specific for this tyrosine-phosphorylated form of STAT1 (Tyr-701), although these responses were not compared with each other. However, FACS analysis showed that IFNγ but not OnM increased expression of class I MHC molecules. We initiated the present study to further examine this discrepancy. IRF1 and TAPI are two IFNγ-inducible proteins whose rapid induction is mediated via STAT1-dependent mechanisms (4, 17). Both are necessary for class I transcription (20) and surface expression (18). We first compared the effects of IFNγ and OnM on the expression of IRF1 and TAPI proteins.

A round of infection was carried out with 15 μl of virus stock containing 10% glycerol (three or four virus stocks were used for each condition). Virus stock were used in the presence of Polybrene (8 μg/ml) and puromycin-resistant cells were used to condition medium, as shown in Fig. 1B. There was no activity of TAPI promoter in unstimulated HUVEC (Fig. 1B, closed bars). TAPI promoter activity was significantly increased by IFNγ (Fig. 1B, closed bars) as reported previously (16), whereas pOγH plasmid was unresponsive to IFNγ (Fig. 1B, open bars). However, the TAPI promoter did not display a transcriptional response to OnM (Fig. 1B, open bars). These findings are consistent with the failure of OnM to increase TAPI protein expression.

Next, we examined whether inhibitory signals generated through gp130 interfere with the transcriptional activity of STAT1 homodimers. To do so, we tested the effects of OnM on tyrosine-mediated STAT1 phosphorylation and on the induction of STAT1-dependent proteins. In these experiments IFNγ and OnM each stimulate tyrosine phosphorylation of STAT1 and addition of OnM did not reduce the IFNγ response (Fig. 1C). Six hours and 48 h after addition of IFNγ, there was a significant induction of IRF1 and class I MHC protein heavy chain, respectively (Fig. 1C). OnM did not reduce the IFNγ-mediated induction of TAPI or class I MHC protein heavy chain (Fig. 1C). We also observed that OnM did not reduce IFNγ-mediated induction of TAPI promoter activity (Fig. 1B, open bars).
Similarly, pretreatment of HUVEC with OnM for various times (15 min, 1 h, and 4 h) did not inhibit phosphorylation of STAT1 stimulated by IFNγ/H9253 (Fig. 1D). IFNγ-mediated induction of IRF1 and class I MHC protein heavy chain also were not inhibited by preincubation with OnM (Fig. 1D). Thus the failure of OnM to induce these proteins despite phosphorylation of STAT1 on Tyr residue 701 does not appear to involve an inhibitory signal.

**OnM Induces Tyrosine Phosphorylation, Serine Phosphorylation, Nuclear Translocation, and DNA Binding Activity of STAT1 Homodimers**—Tyrosine phosphorylation of STAT1 on Tyr residue 701 is necessary for dimerization and subsequent translocation of STAT1 homodimers to the nucleus where they bind to specific promoter elements in target genes and increase the rate of transcription. However, it may not be sufficient to cause these responses. We therefore compared the effects of OnM and IFNγ on STAT1 activation in greater detail, beginning with the dose response and the time course of STAT1 phosphorylation in HUVEC in response to these two cytokines.

No tyrosine phosphorylation of STAT1 was detected in unstimulated HUVEC by immunoblot analysis using Ab to phosphotyrosine STAT1 (Fig. 2A). After 30 min of cytokine treatment, tyrosine phosphorylation of STAT1 was induced by 0.62 ng/ml OnM and increased, in a dose dependent manner, to maximal levels at 10 ng/ml (Fig. 2A). A dose response to IFNγ was observed over a similar range (Fig. 2A), but at all concentrations tested, OnM stimulated a greater level of STAT1 phosphorylation on Tyr residue 701 than did IFNγ. Phosphotyrosine STAT1 levels were significantly increased after 15 min of treatment with IFNγ and the levels stayed elevated for up to 150 min (Fig. 2B). In contrast, OnM induced rapid tyrosine phosphorylation of STAT1 but phosphotyrosine STAT1 was no longer detectable after 1 h of stimulation with OnM (Fig. 2B).
While phosphorylation on Tyr residue 701 is necessary for STAT1 dimerization, for nuclear translocation, and for DNA binding in response to IFNγ, full transcriptional activation by STAT1 is obtained only when Ser residue 727 in the transcriptional activation domain is also phosphorylated (11–13). Therefore, we determined whether OnM causes serine phosphorylation of STAT1. HUVEC were stimulated with either IFNγ or OnM for various times and cell lysates were subjected to immunoblot analysis with an antibody specific to the serine phosphorylated form of STAT1 (Ser-727) (Fig. 2). Increased serine phosphorylation of STAT1 was detected in the nucleus in the absence of cytokine (Fig. 3A, control). At early times, there was a significant amount of phosphoserine STAT1 in the unstimulated cells (Fig. 2C). There was a small amount of phosphoserine STAT1 in the unstimulated cells (Fig. 2, control). Increased serine phosphorylation of STAT1 after addition of IFNγ was seen as early as 30 min and remained elevated for 90 min. OnM also increased serine phosphorylation of STAT1 but this increase largely disappeared by 60 min (Fig. 2C). Thus, similar to tyrosine phosphorylation of STAT1, serine phosphorylation of STAT1 by OnM was more transient than that caused by IFNγ.

We next examined whether the tyrosine phosphorylation of STAT1 by OnM is associated with nuclear translocation of STAT1 homodimers. HUVEC were stimulated with either IFNγ or OnM for various times. Nuclear extracts were subjected to immunoblot analysis with an antibody specific to the tyrosine-phosphorylated form of STAT1 (Fig. 3A, upper panel), the serine-phosphorylated form of STAT1 (Fig. 3A, middle panel), or the tyrosine-phosphorylated form of STAT3 (Fig. 3A, lower panel). No phosphorylated STAT1 or STAT3 proteins were detected in the nucleus in the absence of cytokine (Fig. 3A, control). Strong phosphotyrosine and phosphoserine STAT1 signals were seen at 30, 60, and 120 min after stimulation with IFNγ. IFNγ did not induce phosphorylation of STAT3 in HUVEC (Fig. 3A, lower panel). At early times, there was a significant amount of phosphotyrosine and phosphoserine STAT1 and phosphotyrosine and phosphoserine STAT3 in the nuclear extracts prepared from OnM-treated cells (Fig. 3A), typically exceeding that seen with IFNγ treatment. However, phosphorylated STAT1 signal completely disappeared at 60 min after stimulation with OnM and phosphotyrosine and phosphoserine STAT3 largely disappeared by 60 min (Fig. 3A), consistent with the kinetics observed with whole cell lysates (Fig. 2).

To assess the activation of DNA binding of STAT1 induced by OnM, the same nuclear extracts examined by immunoblotting were used in EMSA with a labeled sis-inducible element (m67SIE) derived from the c-Fos promoter region, a probe known to bind both STAT1 and STAT3 with high affinity (41). Nuclear extracts collected after 30 min of stimulation with IFNγ generated a single DNA binding complex (Fig. 3B). This DNA binding activity was maintained through 120 min stimulation with IFNγ (Fig. 3B). In contrast, three major DNA complexes were formed from nuclear extracts prepared 30 min after addition of OnM and all three complexes were no longer detected in extracts collected after 60 min of treatment (Fig. 3B). These three major complexes, corresponding to SIF-A, -B, and -C, have been described previously and shown to be composed of STAT1 homodimers, STAT1/STAT3 heterodimers, and STAT1 homodimers, respectively (26, 43). To confirm the presence of STAT1 in the complexes induced by IFNγ or OnM treatments of HUVEC, experiments were performed with antibodies reactive with STAT1 (Fig. 3B) or control Abs. Preincubation of nuclear extracts with anti-STAT1 Ab resulted in the complete elimination of SIF-A and SIF-B complexes without affecting the formation of SIF-C (data not shown). Nuclear extracts prepared from cells stimulated with IFNγ or OnM confer binding of STAT1 homodimers to GAS elements of IRF1 (Fig. 3C) and TAP1 (data not shown) promoter region. Preincubation of nuclear extracts with anti-STAT1 Ab resulted in the complete elimination of DNA complexes formed by extracts from either IFNγ- or OnM-treated cells (Fig. 3C). These results confirm the presence of STAT1 homodimers in the DNA binding complexes formed from nuclear extracts of OnM-treated HUVEC, and the overall analysis suggests that OnM differs from IFNγ in that STAT1 activation in response to OnM is much more transient than that produced by IFNγ.

FIG. 3. OnM-induced STAT complexes are transient. A, HUVEC were incubated with IFNγ (40 ng/ml) or OnM (10 ng/ml) for various times. Nuclear extracts were prepared and analyzed for tyrosine phosphorylation of STAT1 (P-STAT1, Tyr-701), serine phosphorylation of STAT1 (P-STAT1, Ser-727), and tyrosine phosphorylation of STAT3 (P-STAT3, Tyr-705) by immunoblotting as described under “Experimental Procedures.” B and C, HUVEC were incubated with IFNγ (40 ng/ml) or OnM (10 ng/ml) for indicated times (panel B) or 30 min (panel C). Nuclear extracts were analyzed for STAT factor binding by using m67SIE probe (panels B and C) and IRF1-GAS probe (panel C) as described under “Experimental Procedures.” Data are from one of two experiments with similar outcome.
tated so that it cannot interact with SHP-2 has resulted in more prolonged STAT activation (29–32). Since the effects of transduction of mutated gp130 into HUVEC could not be readily analyzed due to the presence of endogenous wild type gp130, we created a PDGFRβ-gp130 chimeric receptor, in which the extracellular domains of PDGFRβ were combined with the transmembrane and intracellular domains of gp130. This chimeric receptor allowed us to turn on the gp130 signaling pathway independently of endogenous gp130 receptors by addition of PDGFBB, taking advantage of the fact that HUVEC lack PDGF receptors. We stably transduced HUVEC with retroviruses encoding either EGFP, PDGFRβ-gp130(Y759), or PDGFRβ-gp130(Y759F) as described under “Experimental Procedures.” A. HUVEC were trypsinized, stained for PDGFRβ using specific anti-PDGFRβ Ab and analyzed by FACS. B. Aliquots of total cell lysates from transduced HUVEC were resolved on SDS-PAGE and immunoblotted with specific anti-PDGFRβ Ab. Lysates from human fibroblast were used as a positive control. Data are from one of two independent sets of transductants with similar outcome.

We next tested the function of these chimeric receptors. Cytokines that signal through gp130 cause threonine/tyrosine phosphorylation of p42 and p44 MAPK in a manner that depends upon docking of SHP-2 (29, 30, 32). Therefore, the function of the chimeric receptor proteins was assessed by the PDGFBB phosphorylation of p42 and p44 MAPK proteins in PDGFRβ-gp130-transduced cells, as well as by PDGFBB-induced STAT1 phosphorylation. Both PDGFRβ-gp130(Y759) and PDGFRβ-gp130(Y759F)-transduced cells respond to OnM by increasing tyrosine phosphorylation of STAT1 and threonine/tyrosine phosphorylation of p42 and p44 MAPK that was indistinguishable from the control EGFP cells (data not shown). Thus, transduction did not appear to inhibit the endogenous response to OnM. To assess the potential effects of Y759F mutation on signaling, we compared time-dependent changes of STAT1 and p42 and p44 MAPK phosphorylation in PDGFRβ-gp130(Y759) and PDGFRβ-gp130(Y759F) cells in response to PDGFBB. Essentially the same time course of STAT1 (Fig. 5, upper panel) and p42 and p44 MAPK phosphorylation (Fig. 5, middle panel) was detected in PDGFRβ-gp130(Y759) in response to PDGFBB as observed for OnM. However, tyrosine phosphorylation of STAT1 after addition of PDGFBB remained elevated for up to 120 min in PDGFRβ-gp130(Y759F) cells (Fig. 5, upper panel). Moreover, treatment with PDGFBB did not elicit phosphorylation of p42 and p44 MAPK in PDGFRβ-gp130(Y759F) cells (Fig. 5, middle panel). These results are consistent with previous findings that preventing the recruitment of SHP-2 by the Y759F mutation in gp130 both prolongs the activation of STAT proteins and prevents p42 and p44 MAPK activation (29, 32).

Next, we assessed the effects of the Y759F mutation in gp130 on the induction of IFNγ-responsive gene products. First, we compared the effects of PDGFBB on STAT1 to those in response to OnM in PDGFRβ-gp130 transduced cells. After 30 min of cytokine treatment, tyrosine phosphorylation of STAT1 was induced by 2.5 and 10 ng/ml IFNγ and PDGFBB, respectively, in both PDGFRβ-gp130(Y759) and PDGFRβ-gp130(Y759F) cells (Fig. 6, upper panel). Maximum tyrosine phosphorylation of STAT1 was obtained with 40 and 10 ng/ml PDGFBB in PDGFRβ-gp130(Y759) and PDGFRβ-gp130(Y759F) cells, respectively. We next compared the time course of STAT1 phosphorylation between IFNγ and PDGFBB (Fig. 7A, upper panel). IFNγ-induced tyrosine phosphorylation of STAT1 which remained elevated for 3 h in both PDGFRβ-gp130(Y759) and PDGFRβ-gp130(Y759F) cells (Fig. 7A, upper panel). As shown above (Fig. 5), increased tyrosine phosphorylation of STAT1 (Fig. 7A, upper panel) and p42/p44 MAPK (Fig. 7A, middle panel) after

**Fig. 4. Expression of PDGFRβ-gp130 chimeric receptor on HUVEC.** HUVEC were stably transduced with retroviruses encoding either EGFP, PDGFRβ-gp130(Y759), or PDGFRβ-gp130(Y759F) as described under “Experimental Procedures.” A. HUVEC were trypsinized, stained for PDGFRβ using specific anti-PDGFRβ Ab and analyzed by FACS. B. Aliquots of total cell lysates from transduced HUVEC were resolved on SDS-PAGE and immunoblotted with specific anti-PDGFRβ Ab. Lysates from human fibroblast were used as a positive control. Data are from one of two independent sets of transductants with similar outcome.

**Fig. 5. Tyrosine phosphorylation of STAT1 and p42/p44 MAPK through PDGFRβ-gp130 chimeric receptor in HUVEC.** HUVEC were stably transduced with retroviruses encoding either PDGFRβ-gp130(Y759) or PDGFRβ-gp130(Y759F) as described under “Experimental Procedures.” Cells were washed and incubated with M199 for 4 h prior to the addition of cytokines. Cells were incubated with OnM (10 ng/ml) or PDGFBB (30 ng/ml) for various times as indicated and analyzed for tyrosine phosphorylation of STAT1 (P-STAT1, Tyr-701), p42/p44 MAPK phosphorylation (P-p42/p44, Thr202/Tyr204), and total STAT1 by immunoblotting as described under “Experimental Procedures.” Data are from one of two independent sets of transductants with similar outcome.

**Fig. 6.** Time course of STAT1 and p42/p44 MAPK phosphorylation in PDGFRβ-gp130(Y759) and PDGFRβ-gp130(Y759F)-transduced HUVEC. Data are from one of two independent sets of transductants with similar outcome.
OnM Activates STAT1 but Does Not STAT1-dependent Genes

addition of PDGFBB was transient and largely gone by 60 min in PDGFRβ-gp130(Y759) cells. However, tyrosine phosphorylation of STAT1 remained elevated for up to 3 h after stimulation with PDGFBB in PDGFRβ-gp130(Y759F) cells, which was indistinguishable from the time course observed in response to IFNγ (Fig. 7, upper panel). As expected, PDGFBB did not phosphorylate p42 and p44 MAPK in these cells (Fig. 7A, middle panel). Nevertheless, PDGFBB caused a rapid and prolonged serine phosphorylation of STAT1 in PDGFRβ-gp130(Y759F) cells (Fig. 7B, second panel).

Finally, we examined the induction of IFNγ-responsive gene products by PDGFBB and IFNγ in both PDGFRβ-gp130(Y759) and PDGFRβ-gp130(Y759F) cells. After 9 h of cytokine treatment, IRF1 and TAP1 protein expression were induced to the same levels by IFNγ in both PDGFRβ-gp130(Y759) and PDGFRβ-gp130(Y759F) cells (Fig. 7C). Although the time course of STAT1 phosphorylation induced by IFNγ and PDGFBB was similar in PDGFRβ-gp130(Y759F) cells, PDGFBB did not stimulate the expression of IRF1, TAP1, or class I MHC protein heavy chain in these cells (Fig. 7C). Thus, we conclude that transient phosphorylation of STAT1 induced by cytokines that signal through gp130 cannot explain why these cytokines do not increase transcription of STAT1-dependent genes in HUVEC.

OnM Activates STAT1 but Does Not Increase the Expression of IRF1 or TAP1 in HepG2 Cells—The STAT3 pathway mediates up-regulation of α2M in HepG2 cells by OnM and by other cytokines that signal through gp130. It has not been shown if STAT1 activation, which also occurs in these cells, is functional. To determine whether our results with HUVEC can be generalized to other cell types, we examined the responses of HepG2 cells to OnM and IFNγ. OnM and IFNγ each increases tyrosine phosphorylation of STAT1 in HepG2 cells (Fig. 8A). As expected, there was a significant increase in IRF1 and TAP1 protein expression in response to IFNγ in HepG2 cells (Fig. 8A). However, consistent with our HUVEC results, OnM did not increase either IRF1 or TAP1 protein expression in these cells (Fig. 8A). Furthermore, when the TAPI promoter-reporter construct was transiently transfected into HepG2 cells (Fig. 8B), TAPI promoter activity was significantly increased by IFNγ whereas the TAPI1 promoter did not display a transcriptional response to OnM (Fig. 8B). As a positive control α2M promoter activity was increased in response to OnM in HepG2 cells (Fig. 8C). Therefore, OnM increased the transcription of STAT3-dependent genes, but it did not stimulate the transcription of STAT1-dependent genes in HepG2 cells. We have also observed that IFNγ but not OnM increases IRF1 expression in coronary artery smooth muscle cells.2 We conclude that the inability of OnM to activate STAT1 but not induce expression of

FIG. 6. Dose-dependent tyrosine phosphorylation of STAT1 by IFNγ and PDGFBB. HUVEC were stably transduced with retroviruses encoding either PDGFRβ-gp130(Y759) or PDGFRβ-gp130(Y759F) as described under “Experimental Procedures.” Cells were washed and incubated with M199 media for 4 h prior to incubating with various doses of IFNγ or PDGFBB for 30 min, and analyzed for tyrosine phosphorylation of STAT1 (P-STAT1, Tyr-701) and total STAT1 by immunoblotting as described under “Experimental Procedures.” Data are from one of two independent sets of transductants with similar outcome.

FIG. 7. Prolonged activation of STAT1 is not sufficient for the induction of IRF1, TAP1, and class I MHC. HUVEC were stably transduced with retroviruses encoding either PDGFRβ-gp130(Y759) or PDGFRβ-gp130(Y759F) transduced cells were incubated with either IFNγ (40 ng/ml) or PDGFBB (50 ng/ml) for various times as indicated. Cells were analyzed for tyrosine phosphorylation of STAT1 (P-STAT1, Tyr-701), p42/p44 MAPK phosphorylation (P-p42/p44, Thr202/Tyr204), serine phosphorylation of STAT1 (P-STAT1, Ser-727), Tyr-701 phosphorylation of STAT1 (P-STAT1, Tyr-701), and total STAT1 by immunoblotting as described under “Experimental Procedures.” C, PDGFRβ-gp130(Y759) and PDGFRβ-gp130(Y759F) transduced cells were incubated with either IFNγ (40 ng/ml) or PDGFBB (50 ng/ml) for 6 and 48 h. Samples harvested after 6 h of stimulation were analyzed for IRF1 and TAP1 protein expression using immunoblotting as described under “Experimental Procedures.” Samples harvested after 48 h of stimulation were analyzed for class I MHC protein heavy chain using immunoblotting as described under “Experimental Procedures.” Data are from one of two independent sets of transductants with similar outcome.

HUVEC were stably transduced with retroviruses encoding either PDGFRβ-gp130(Y759) or PDGFRβ-gp130(Y759F) as described under “Experimental Procedures.” Cells were washed and incubated with M199 media for 4 h prior to incubating with various doses of IFNγ or PDGFBB for 30 min, and analyzed for tyrosine phosphorylation of STAT1 (P-STAT1, Tyr-701), p42/p44 MAPK phosphorylation (P-p42/p44, Thr202/Tyr204), serine phosphorylation of STAT1 (P-STAT1, Ser-727), Tyr-701 phosphorylation of STAT1 (P-STAT1, Tyr-701), and total STAT1 by immunoblotting as described under “Experimental Procedures.” C, PDGFRβ-gp130(Y759) and PDGFRβ-gp130(Y759F) transduced cells were incubated with either IFNγ (40 ng/ml) or PDGFBB (50 ng/ml) for 6 and 48 h. Samples harvested after 6 h of stimulation were analyzed for IRF1 and TAP1 protein expression using immunoblotting as described under “Experimental Procedures.” Samples harvested after 48 h of stimulation were analyzed for class I MHC protein heavy chain using immunoblotting as described under “Experimental Procedures.” Data are from one of two independent sets of transductants with similar outcome.
STAT1-dependent genes is not restricted to HUVEC.

IFNγ Does Not Complement OnM STAT1 Activation—So far, we have demonstrated that STAT1 activation via gp130 is not sufficient for the induction of STAT1-dependent genes. We next tested whether additional signals might be provided by IFNγ to complement the actions of OnM. We transiently transfected HepG2 cells with either mouse IFNGR2 plus wild type mouse IFNGR1(Y440) or mouse IFNGR2 plus mutant IFNGR1(Y440F) containing the mutation of the IFNGR1 chain that prevents STAT1 binding (Fig. 9). Surface expression of IFNGR1(Y440) and mutant IFNGR1(Y440F) was quantitated by indirect immunofluorescence followed by FACS analysis and indicated that both IFNGR1(Y440) and IFNGR1(Y440F) were expressed to a similar level (data not shown). Like untransfected cells, HepG2 cells transfected with IFNGR2 plus IFNGR1(Y440) or IFNGR1(Y440F) showed responsiveness to human IFNγ but not to OnM, assessed by TAP1 promoter-reporter gene assay (Fig. 9A) and show responsiveness to OnM, assessed using a STAT3-dependent α2M-luciferase promoter-reporter gene assay (Fig. 9B). As predicted, HepG2 cells transfected with IFNGR2 plus wild type IFNGR1 acquire responsiveness to mouse IFNγ (measured by the TAP1-GH promoter-reporter assay) whereas cells transfected with IFNGR2 plus mutated IFNGR1(Y440F) do not (Fig. 9A). Significantly, mouse IFNγ treatment of cells expressing mutated receptor did not complement the OnM response (Fig. 9A). In these experiments, we did observe that OnM partially reduced mouse IFNγ-mediated induction of TAP1 promoter activity in HepG2 cells transfected with IFNGR2 plus IFNGR1(Y440) (Fig. 9A). OnM also partially reduced human IFNγ-mediated induction of TAP1 promoter activity in HepG2 cells. However, OnM did not diminish human IFNγ-mediated induction of IFNγ and TAP1 protein in HepG2 cells and we conclude that the observed inhibition is restricted to promoter-reporter genes and unlikely to be of biological significance.

DISCUSSION

In the present study we examined whether activation of the STAT1 pathway in vascular EC by OnM could result in the induction of STAT1-dependent gene products similar to those seen in response to IFNγ. We have found that while OnM induces serine and tyrosine phosphorylation of STAT1, as well as nuclear translocation and DNA binding by STAT1 homodimers at levels comparable with those induced by IFNγ, OnM does not lead to the induction of IFNγ-responsive STAT1-dependent gene products such as IRF1, TAP1, and class I MHC in HUVEC. We noted that STAT1 phosphorylation in response to OnM is more transient than that caused by IFNγ. However, transient phosphorylation of STAT1 is not the reason why OnM does not induce the expression of IFNγ-responsive gene products. This conclusion is based on use of a chimeric PDGFRα-gp130 receptor mutated to prevent SHP-2 binding. In HUVEC transduced with such a receptor, PDGFB leads to prolonged tyrosine and serine phosphorylation of STAT1 comparable in duration to IFNγ responses, yet still does not cause the induction of IRF1, TAP1, and class I MHC protein heavy chain. Since OnM does not inhibit IFNγ responses, we conclude that the activation of STAT1 is not sufficient for the induction of IFNγ-responsive STAT1-dependent gene products in HUVEC.

IFNγ acts on many cell types, including EC, to up-regulate the transcription of class I MHC molecules and related genes such as TAP1 and IRF1 (14, 15, 17). STAT1 is required for these IFNγ-dependent responses as first shown by use of STAT1 negative mutant cell lines (17, 44, 45). The biological importance of STAT1 in mediating these IFNγ responses has since been confirmed in the studies of mice that lack an intact STAT1 gene (5, 6). Specifically, targeted disruption of the mouse STAT1 gene results in compromised innate immunity to viral disease and absence of induction of immunomodulatory proteins, such as class I MHC and class II MHC molecules (5, 6). Although, these studies indicated that STAT1 is required for the induction of many IFNγ-dependent genes, they do not establish that STAT1 is sufficient. Very recently, it has been observed that some IFNγ responses are retained in the absence of STAT1, implying that IFNγ can activate additional signal transduction pathways (46, 47). For example, it was observed that in macrophages derived from STAT1-deficient mice, IFNγ can induce changes in the expression of a large number of genes and that this STAT1-independent response still requires the presence of IFNγ receptor and JAK1 (46). The existence of a STAT1-independent signal(s) raises the possibility that such signals may be necessary, albeit not sufficient, for induction of STAT1-dependent genes.

The TAP1 promoter contains κB, SP1, IFN-consensus se-
OnM Activates STAT1 but Not STAT1-dependent Genes

quence, GAS, and IFN-stimulated response elements. IFN-consensus sequence, IFN-stimulated response element, and GAS bind to STAT1 homodimers in response to IFN-γ. Min et al. (4) demonstrated that mutation of either GAS or the IFN-stimulated response element partially reduced the IFN-γ response and that both elements must be mutated to abolish the IFN-γ response in EC. In the experiments using TAP1-GH reporter construct reported here, we demonstrated that the TAP1 promoter did not display a transcriptional response to OnM. Therefore, we concluded that STAT1 activation by OnM is not sufficient to activate the transcription of TAP1 gene.

A possible explanation for our findings is that STAT1 activation by IFN-γ is qualitatively different from that induced by OnM. A recent study by Mowen et al. (57) showed that in addition to phosphorylation of the Tyr and Ser residues, arginine methylation of STAT1 is also required for IFNα/β-induced transcription of STAT1-dependent genes. It is not known if IFN-γ similarly causes arginine methylation. However, it is formally possible that the lack of response to OnM is due to the inability of this cytokine to induce arginine methylation of STAT1 in HUVEC. This possibility will require further investigation.

Transient transfection of HepG2 cells with a STAT-defective mouse IFNGR1 failed to complement the OnM STAT signal. This finding suggested that signals provided by IFN-γ other than STAT1 activation cannot be provided in trans to complement the response to OnM. An alternative explanation is that STAT-defective mouse IFNGR1 is not only unable to activate STAT1, but is also not able to activate signals provided by IFN-γ other than STAT1 activation. In other words, Tyr-440 of IFNGR1 may mediate signals other than STAT1 activation and our experiments do not formally exclude this possibility.

Several lines of evidence have indicated that EC may be an important cellular target of OnM. In vitro, OnM has been reported to stimulate polymorphonuclear leukocyte transmigration through confluent monolayers of HUVEC (49), release of IL-6 (50) and endothelin-1 (51), and expression of P-selectin (49, 52), E-selectin (49), intracellular adhesion molecule-1 (49), and vascular cell adhesion molecule-1 (49). However, despite these reports, the activities of OnM in vivo are primarily anti-inflammatory and there is no evidence of adhesion molecule induction on EC in vivo (24). Human EC have been shown to express a high affinity cell-surface receptor for OnM (50), presumably OnMR, and it has been reported that Ab to gp130 inhibits the induction of E-selectin induced by OnM (49). We have recently found that HUVEC can respond to human leukemia-inhibitory factor by activation of STAT and MAPK pathways, raising the possibility that OnM utilizes both gp130- and gp100-dependent receptors in complex EC cell type. There is also evidence that OnM uses a tyrosine phosphorylation signal transduction pathway in human EC involving the activation of the p62-IIK kinase, and that this tyrosine kinase may lead to the induction of IL-6 (53). Finally, although OnM is not a mitogen for EC, OnM is the major autocrine growth factor for Kaposi’s sarcoma cells that are thought to be of EC origin (54, 55).

The functional role of protein-tyrosine phosphatase SHP-2 in signal transduction in hematopoietic cells has been assessed indirectly by preventing recruitment of SHP-2 to gp130 or by over-expressing enzymatically inactive SHP-2 mutants (29, 31, 32). The data indicated that activation of SHP-2 via gp130 is not required for the induction of STAT3-dependent genes but it is required for the MAPK activation (29–32). Our observations are concordant with the conclusion that the binding of SHP-2 to tyrosine 759 on the cytoplasmic tail of gp130 negatively regulates STAT1 as well as STAT3 and positively regulates MAPK activation. They also suggest that the p42 and p44 MAPK are not required and probably not involved in gp130-induced STAT1 serine phosphorylation in HUVEC, extending previous observations indicating the p42 and p44 MAPK-dependent and -independent pathways involved in serine phosphorylation of STAT3 in response to gp130 activation (56). The contribution of other MAPKs to the serine phosphorylation of STAT1 in response to gp130 activation as well as identity of the serine kinase activated by IFN-γ remain to be determined.

Recently, Kerr and co-workers (48) demonstrated that a chimeric receptor comprising the external region of erythropoietin receptor, the transmembrane and JAK-binding domains of gp130, and a 7-amino acid STAT1 recruitment motif (Y440) from IFNGR1, efficiently mediates an IFN-γ-like response. However, the receptor comprising the external region of erythropoietin receptor, the transmembrane and full-length intracellular domains of gp130 did not mediate such a response (48). These results, together with those reported here, seem to show that the specific STAT1 binding sequence of the IFNGR1 is essential for STAT1 signaling, albeit not for STAT1 phosphorylation. This raises the probability that additional covalent modifications of this transcription factor beyond tyrosine and serine phosphorylation are needed for transcriptional activities.

In summary, our analysis of the response to OnM has led to the conclusion that sustained STAT1 activation is insufficient to activate STAT1-dependent genes in HUVEC. It is as yet unclear what additional signals might be provided by IFN-γ but not OnM that are necessary for STAT1-dependent responses.

Acknowledgments—We thank Louise Benson, Gwen Davis, and Lisa Gras for excellent technical assistance in cell culture. We also thank Dr. D. R. J. Fulton for excellent technical advice in making the expression vectors for PGDFRα-gp130 chimeric receptors and Dr. D. R. Johnson for critical comments.

REFERENCES

1. Goes, N., Urmson, J., Arnold, D., Ramassar, V., and Halloran, P. F. (1995) J. Immunol. 155, 4559–4566
2. Goes, N., Urmson, J., Hobart, M., and Halloran, P. F. (1996) Transplantation 62, 1889–1894
3. Tellides, G., Terek, D. A., Kirkiles-Smith, N. C., Kim, R. W., Wilson, J. H., Schechner, J. S., Lorber, M. I., and Pober, J. S. (2000) Nature 403, 207–211
4. Min, W., Pober, J. S., and Johnson, D. R. (1998) Circ. Res. 83, 815–823
5. Meraz, M. A., White, J. M., Sheehan, K. C., Bach, E. A., Rodig, S. J., Daghe, A. S., Kaplan, D. H., Riley, J. K., Greenlund, A. C., Campbell, D., Carver-Moore, K., Dubois, R., Clark, R., Aguett, M., and Schreiber, R. D. (1996) Cell 84, 431–442
6. Durbin, J. E., Hackenmiller, R., Simon, M. C., and Levy, D. E. (1996) Cell 84, 543–550
7. Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H., and Schreiber, R. D. (1998) Annu. Rev. Biochem. 67, 227–264
8. Bremberg, J., and Darnell, J. E. (2000) Oncogene 19, 2468–2473
9. Ramana, C. V., Chatterjee-Kishore, M., Nguyen, H., and Stark, G. R. (2000) Oncogene 19, 2619–2627
10. Seidel, H. M., Milosco, L. H., Lamb, P., Darnell, J. E. J., Stein, R. B., and Rosen, J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3041–3045
11. Decker, D., and Kovarik, P. (2000) Oncogene 19, 2628–2637
12. Goh, K. C., Hague, S. J., Williams, B. (1999) EMBO J. 18, 5601–5608
13. Zhu, X., Wen, Z., Xu, L. Z., and Darnell, J. E. J. (1997) Mol. Cell. Biol. 17, 6618–6625
14. Epperson, D. E., Arnold, D., Spies, T., Cresswell, P., Pober, J. S., and Johnson, D. R. (1992) J. Immunol. 149, 3297–3301
15. Johnson, D. R., and Pober, J. S. (1994) Mol. Cell. Biol. 14, 1322–1325
16. Min, W., Pober, J. S., and Johnson, D. R. (1996) J. Immunol. 156, 3174–3183
17. Li, X., Leung, S., Qureshi, S., Darnell, J. E. J., and Stark, G. R. (1996) J. Biol. Chem. 271, 5790–5794
18. Cox, J. H., Yewdell, J. W., Eisenlohr, L. C., Johnson, P. R., and Bennink, J. R. (1990) Science 247, 715–718
19. Ma, W., Lehner, P. J., Cresswell, P., Pober, J. S., and Johnson, D. R. (1997) Biol. Chem. 272, 16516–16520
20. N Operator, V., Ramassar, V., Goes, N., Urmson, J., and Halloran, P. F. (1997) J. Immunol. 158, 4260–4269
21. Johnson, D. R., and Pober, J. S. (1994) Mol. Cell. Biol. 14, 1322–1325
22. Trepicchio, W. L., Bozza, M., Pedneault, G., and Dorner, A. J. (1996) Oncogene 15, 2627–2633
23. Qu, B. S., Pfeiffer, C. J., and Keith, J. C., Jr. (1996) Dig. Dis. Sci. 41, 1675–1680
24. Wallace, P. M., MacMaster, J. F., Rouleau, K. A., Brown, T. J., Loy, K. J., Donaldson, K. L., and Wahl, A. F. (1999) J. Immunol. 162, 5547–5555
25. Bravo, J., and Heath, J. K. (2000) EMBO J. 19, 2099–2111

Downloaded from jbc.asm.org on July 1, 2018
OnM Activates STAT1 but Not STAT1-dependent Genes

26. Gerhartz, C., Heesel, B., Saesse, J., Hemmann, U., Landgraf, C., Schneider-Mergener, J., horn, F., Heinrich, P. C., and Graeve, L. (1996) J. Biol. Chem. 271, 12991–12998
27. Fukada, T., Hibi, M., Yamanaka, Y., Takahashi-Tezuka, M., Fujitani, Y., Yamaguchi, T., Nakajima, K., and Hirano, T. (1996) Immunity 5, 449–460
28. Schmitz, J., Weissbach, M., Haan, S., Heinrich, P. C., and Schaper, F. (2000) J. Biol. Chem. 275, 12848–12856
29. Kim, H., and Baumann, H. (1999) Mol. Cell. Biol. 19, 5326–5338
30. Terstegen, L., Gatsios, P., Bode, J. G., Schaper, F., Heinrich, P. C., and Graeve, L. (2000) J. Biol. Chem. 275, 18810–18817
31. Schaper, F., Gondo, C., Eck, M., Schmitz, J., Weissenbach, M., Haan, S., Heinrich, P. C., and Schaper, F. (1998) Biochem. J. 335, 557–650
32. Kim, H., Hawley, T. S., Hawley, R. G., and Baumann, H. (1998) Mol. Cell. Biol. 18, 1525–1533
33. Takahashi-Tezuka, M., Yoshida, Y., Fukada, T., Ohtani, T., Yamanaka, Y., Nishida, K., Nakajima, K., Hibi, M., and Hirano, T. (1998) Mol. Cell. Biol. 18, 4109–4117
34. Anhuf, D., Weissenbach, M., Schmitz, J., Sobota, R., Hermanns, H. M., Radtke, S., Linnenmann, S., Behrmann, I., Heinrich, P. C., and Schaper, F. (2000) J. Immunol. 165, 2535–2543
35. Mahboubi, K., Li, F., Plescia, J., Kirkiles-Smith, N. C., Mesri, M., Du, Y., Carroll, J. M., Elias, J. A., Altieri, D. C., and Pober, J. S. (2001) J. Immunol. 166, 3837–3846
36. Mahboubi, K., Pfister, J., Kirkiles-Smith, N. C., Mesri, M., Du, Y., Carroll, J. M., Elias, J. A., Altieri, D. C., and Pober, J. S. (2001) Lab. Invest. 81, 327–334
37. Gimbrone, M. A., Jr. (1976) Proc. Hemostasis Thromb. 3, 1–28
38. Thornton, S. C., Mueller, S. N., and Levine, E. M. (1983) Science 222, 623–625
39. Karmann, K., Min, W., Fanslow, W. C., and Pober, J. S. (1996) J. Exp. Med. 184, 173–182
40. Dignam, J. D., Martin, P. L., Shabtay, B. S., and Roeder, R. G. (1983) Methods Enzymol. 101, 582–598
41. Wagner, B. J., Hayes, T. R., Hoban, C. J., and Cochran, B. H. (1990) EMBO J. 9, 4477–4484
42. Zheng, L., Dengler, T. J., Kluger, M. S., Madison, L. A., Schechner, J. S., Maher, S. E., Pober, J. S., and Bothwell, A. L. (2000) J. Immunol. 164, 4665–4671
43. Stephens, J. M., Lumpkin, S. J., and Fishman, J. B. (1998) J. Biol. Chem. 273, 31408–31416
44. McKendry, R., John, J., Flavell, D., Muller, M., Kerr, I. M., and Stark, G. R. (1991) Proc. Natl. Acad. Sci. 88, 11455–11459
45. John, J., McKendry, R., Pellegrini, S., Flavell, D., Kerr, I. M., and Stark, G. R. (1991) Mol. Cell. Biol. 11, 4189–4195
46. Gil, M. P., Bohn, E., O’Gain, A. K., Ramana, C. V., Levine, B., Stark, G. R., Virgin, H. W., and Schreiber, R. D. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 6680–6685
47. Ramana, C. V., Gil, M. P., Han, Y., Ransohoff, R. M., Schreiber, R. D., and Stark, G. R. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 6674–6679
48. Strebel, B., Arulampalam, V., Ichihara, H., Newman, S. J., Claak, J. F., Watling, D., Costa-Pereira, A. P., Schaper, F., Behrmann, I., Sheehan, K. C., Schreiber, R. D., Horn, F., Heinrich, P. C., and Kerr, I. M. (2001) EMBO J. 20, 5431–5442
49. Modur, V., Feldhaus, M. J., Weyrich, A. S., Jicha, D. L., Prescott, S. M., Zimmerman, G. A., and McIntyre, T. R. (1997) J. Clin. Invest. 100, 158–166
50. Brown, T. J., Rowe, J. M., Liu, J. W., and Shoyab, M. (1991) J. Immunol. 147, 2175–2180
51. Sajjannana, O., Nyman, T., Pacek, P., and Frykquist, F. (1998) Am. J. Physiol. 275, H662–H667
52. Yao, L., Fan, J., Setianti, H., Patel, K. D., and McEver, R. P. (1996) J. Exp. Med. 184, 81–92
53. Schieve, G. L., Kallestad, J. C., Brown, T. J., Ledbetter, J. A., and Linsley, P. S. (1992) J. Immunol. 149, 1676–1682
54. Aamaral, M. C., Miles, S., Kumar, G., and Nel, A. E. (1993) J. Clin. Invest. 92, 848–857
55. Cai, J., Gill, P. S., Massad, B., Chandranosma, P., Jung, B., Stave, R. E., and Schreiber, R. D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6508–6516
56. Chung, J., Uchida, E., Grammer, T. C., and Blenis, J. (1997) J. Biol. Chem. 272, 14524–14528
57. Mowen, D. A., Wang, J., Zhu, W., Schratz, B. T., Shuai, K., Herschman, H. R., and David, M. (2001) Cell 104, 731–741
Activation of Signal Transducer and Activator of Transcription 1 (STAT1) Is Not Sufficient for the Induction of STAT1-dependent Genes in Endothelial Cells: COMPARISON OF INTERFERON-γ AND ONCOSTATIN M

Keyvan Mahboubi and Jordan S. Pober

J. Biol. Chem. 2002, 277:8012-8021.
doi: 10.1074/jbc.M107542200 originally published online January 3, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M107542200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 57 references, 38 of which can be accessed free at http://www.jbc.org/content/277/10/8012.full.html#ref-list-1