Interferon Induction of TAP1
The Phosphatase SHP-1 Regulates Crossover Between the IFN-α/β and the IFN-γ Signal-Transduction Pathways

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Abstract—Interferon (IFN)-γ and IFN-α/β induction of the transporter associated with antigen processing-1 (TAP1) promoter was compared in HeLa cells and endothelial cells (ECs). In HeLa cells, IFN-γ acts through Stat1α/Stat1α homodimers binding to the gamma activating sequence (GAS) and IFN-α/β acts through Stat1/Stat2/p48 binding to the IFN-stimulated response element (ISRE). In ECs, however, IFN-γ and IFN-α/β act through both the GAS and ISRE. The basis of the IFN signaling crossover in ECs was investigated. HeLa and ECs contain similar ratios of Stat1α to Stat2 proteins, and IFN-α/β also activates the same Janus kinases (JAKs) (Jak1 and tyrosine kinase (Tyk) 2 but not Jak2). However, IFN-α/β activates more Stat1α than does IFN-γ in ECs, whereas the reverse occurs in HeLa, and expression of the IFN-α/β receptor-associated phosphatase SHP-1 is much lower in ECs than HeLa cells. Overexpression of SHP-1 in ECs blocks IFN-α/β signaling through GAS, and expression of a dominant negative SHP-1 in HeLa cells permits IFN-α/β signaling through GAS, demonstrating a role for SHP-1 in regulating crossovers between the IFN-α/β and IFN-γ signaling pathways. (Circ Res. 1998;83:815-823.)

Key Words: endothelial cell ■ MHC class I ■ transporter associated with antigen processing (TAP) ■ phosphatase

Major histocompatibility complex (MHC) class I molecules bind peptides and present them on the cell surface in a form that can be recognized by cytotoxic T lymphocytes (CTLs) or natural killer cells. The efficient loading of peptides into MHC class I molecules requires TAP1/TAP2 (transporter associated with antigen processing) heterodimer-mediated translocation of peptides from the cytosol, where proteins are degraded, into the lumen of the endoplasmic reticulum, where HLA class I molecules assemble. Studies of TAP-deficient cell lines, mice with targeted disruptions of TAP genes, and humans with TAP mutations have established that TAP is essential for the expression of MHC class I molecules on the cell surface.

MHC class I molecules are expressed constitutively at low levels by many cells, including vascular endothelial cells (ECs). Expression is increased at sites of inflammation after a delay of a few hours. Increased MHC class I expression increases the efficiency of peptide presentation to CTLs. The inflammatory cytokines interferon (IFN)-α, IFN-β, or IFN-γ increase MHC class I expression in vivo. IFNs induce transcription of MHC class I molecules (called HLA-A, -B, -C in humans) in cultured human umbilical vein ECs and in the human epithelial carcinoma cell line HeLa.

IFN-α, IFN-β (type I IFNs), and IFN-γ (type II IFNs) are thought to activate distinct signal-transduction pathways, leading to different cellular responses (reviewed in Reference 9). IFN-α and -β compete for receptor occupancy whereas IFN-γ binds to a unique receptor. In response to IFN-α/β, the receptor-associated Janus kinases (JAKs) Jak1 and Tyk2 are activated followed by the STAT (signal transducer and activator of transcription) proteins Stat1α, Stat1β (an alternatively spliced form of Stat1), and Stat2. Stat1α or Stat1β forms heterodimers with Stat2, which then associate with a 48-kD protein ISGF3-γ to form Stat1/Stat2/p48 (also known as IFN-stimulated gene factor 3 [ISGF3]). Stat1/Stat2/p48 translocates to the nucleus and binds to the IFN-stimulated response element (ISRE) (AGTTTCNNTTTYCC consensus sequence). In response to IFN-γ, Jak1 and Jak2 are activated followed by Stat1α. Activated Stat1α forms Stat1α/Stat1α homodimers (also called GAF) that bind a GAS (TTCNNAA consensus sequence). Crossovers between the IFN-α/β and IFN-γ signaling pathways have been documented. For example, IFN-γ modulates the IFN-α/β pathway indirectly by increasing the levels of p48, which is said to “prime” the response for stronger activation of Stat1/Stat2/p48 by IFN-α/β. Also, IFN-α–treated human FS2 fibroblasts contain a factor that is indistinguishable from Stat1α/Stat1α in DNaSe (exo III) protection assays. This factor is not detectable, however, in electrophoretic mobility shift assays (EMSAs) of DNA-binding proteins using as probe the gamma activating sequence.13

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sequence (GAS) from the guanylate-binding protein (GBP) gene promoter.18 The molecular basis for this activation has not been established.

Protein phosphatases regulate activation and inactivation of intracellular second-messenger systems by cytokines (reviewed in Reference 19). IFN-α/β signaling requires SHP-2,20 an SH2-containing protein tyrosine phosphatase (also called PTP1D, SHPTP2, Syp)21 that is constitutively associated with the IFN-α/β receptor.20 The phosphatase SHP-1 (also called SHPTP1 and HCP) associates with the IFN-α/β receptor on IFN binding22 and inhibits signaling, perhaps by dephosphorylating Tyk2.21 SHP-2 is expressed ubiquitously, while SHP-1 is expressed at high levels in hematopoietic cells and at lower levels in neural cells,19 malignant colon epithelial cells,25 HeLa cells,26 and ECs (see below).

Both IFN-α/β and IFN-γ induce TAP1 mRNA more rapidly than HLA class I mRNA,27 and IFN-γ increases TAP-dependent peptide transport more rapidly than HLA class I molecule expression.28 IFN-γ–activated Stat1a/Stat1b binds to a GAS in the promoter of TAP129 and the promoter of the transcription factor IRF-1,30 which mediates the delayed response of HLA class I promoter.31 The biological significance of rapid TAP1 induction by IFNs is not known. Elevated concentrations of TAP-supplied peptides in the endoplasmic reticulum may help ensure efficient peptide loading, thereby also limiting expression of “empty” HLA class I molecules that may promote autoimmunity.32 In the present study, the response of the TAP1 promoter to type I IFNs (IFN-α/β) is analyzed and compared in HeLa cells and cultured ECs, a more physiological candidate for antigen presentation to CTLs in vivo.33

Materials and Methods

Cells and Cytokines

HeLa cells were cultured in Dulbecco’s modified Eagle’s medium (GIBCO) supplemented with 10% FBS, 1 mM l-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (all GIBCO). Human ECs were isolated from umbilical veins and cultured on gelatin-coated tissue-culture plastic (Falcon) in Medium 199 containing 20% FBS (both from GIBCO), 50 μg/mL endothelial cell growth factor (Collaborative Biomedical Products), 100 μg/mL porcine heparin (Collaborative Biomedical Products), 100 μg/mL penicillin, and 100 μg/mL streptomycin (GIBCO). ECs were used at passage 2 to 4. Human fibrosarcoma 2FTGH cells and their IFN-unresponsive mutant derivatives U2A (p48−), U3A (Stat1α−), and U6A (Stat2−) were obtained from G.R. Stark (Cleveland Clinic Research Foundation, Cleveland, Ohio). Recombinant IFN-α2b was purchased from Schering. IFN-β (expressed in Escherichia coli; 3×106 U/m) and IFN-γ (expressed in E. coli; 2.5×107 U/mg) were obtained from Biogen Inc. IFN-α or IFN-β was used at 1000 U/mL and IFN-γ at 500 U/mL.

Reporter Gene Constructs, Transfection, and Reporter Assays

The TAP1 promoter-driven reporter constructs TAP-growth hormone (GH) mutant IFN consensus sequence (mICS) (previously called miICS1), miSRE (previously called miICS2), mGAS, and miSRE/mGAS (previously called miICS2/mGAS) have been described.27 The following oligonucleotides were used in EMSAs (listed 5′ to 3′; complement sequence not shown): TAP1 ICS: TCGCTTTCCCCTAAATG (GAS consensus is underlined); TAP1 ISRE: TTTGATTTCGCTTTCCCCTTgATGGCTGAGCTTTCT (ISRE consensus sequence is underlined; small gg mutated from AA of the wild-type GAS sequence). Transient transfections of human umbilical vein ECs were performed using a DEAE-Dextran protocol.34 Two plasmids were transfected: a TAP promoter-reporter construct (6 μg) and SV-β-gal (6 μg), to normalize for transfection efficiency. For SHP-1 studies, 3 plasmids were transfected: a reporter gene (6 μg), SV-β-gal (3 μg) and 3 μg of the expression vector encoding wild-type SHP-1 (pCMV5-SHP1C), a dominant negative SHP-1 mutant (pCMV5-SHP1C [Cys→Ser]) or the empty vector (pCMV5) (described in Reference 20; generously provided by Dr. Andrew Larner, NIH). After 24 hours, transfected cells were replated on gelatin-coated 24-well plates. Forty-eight hours after transfection, cells were treated with IFNs as indicated. Transfections of HeLa and 2FTGH cells were performed using cationic liposomes (Lipofectamine, Gibco) according to the manufacturer’s instructions. DNA (2 μg reporter and 1 μg SV-β-gal) was mixed with Lipofectamine (6 μL) and incubated for 20 minutes at room temperature, then added to each well, along with 2 mL of OptiMEM medium. After transfection overnight, the cells were divided equally into 4 different cultures. For the assay of constitutive and cytokine-induced promoter activity, culture media were harvested after 24 hours. Samples were assayed for hGH using a kit (Nichols Institute). Radioactivity was measured in a gamma counter (5500B, Beckman). Cell lysates were assayed for β-galactosidase using a kit (Promega).

Nuclear Extracts and EMSA

Nuclear extracts were prepared following a modification35 of the procedure of Dignam et al. Briefly, cells (5×10⁶) were harvested by scrape-harvesting into TBS (20 mMol/L Tris, pH 7.2, 0.15 mMol/L NaCl), resuspended in buffer A (0.2 mMol/L HEPES, pH 7.9, 10 mMol/L KCl, 1.5 mMol/L MgCl2, leupeptin and aprotinin each 1 μg/mL, PMSF 0.5 mMol/L, 1 mMol/L orthovanadate, 2 mMol/L pyrophosphate), and incubated 15 minutes on ice. Then 25 μL 2.5% NP-40/buffer A was added, mixed by inversion, and the nuclei pelleted (500g, 4 minutes, 4°C). Nuclear proteins were extracted and analyzed as described previously.36 The antibodies specific for Stat1a and Stat2 were purchased from Santa Cruz Biotech.

Immunoprecipitation and Immunoblotting

Confluent cultures of ECs (three 10-cm plates) or HeLa cells (one 10-cm plate) were treated with IFN-α, IFN-β, or IFN-γ (15 minutes). Cells were washed twice with cold PBS and lysed in 1.5 mL of cold lysis buffer (50 mMol/L Tris-HCl, pH 7.6, 150 mMol/L NaCl, 0.1% Triton X-100, 0.75% Brij 96, 1 mMol/L sodium orthovanadate, 1 mMol/L NaF, 1 mMol/L sodium pyrophosphate, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 2 mMol/L PMSF, 1 mMol/L EDTA) for 20 minutes on ice, then centrifuged (13 000g, 10 minutes). For JAKs, 1 mL lysate was preclarified with 1 μL of normal rabbit serum and 50 μL of GammaBind plus Sepharose beads (PharMacia) with rocking at 4°C overnight. Lysates were then incubated sequentially with JAK-specific antisera (Jak1, Jak2, then Tyk2, 5 μL each) for 2 hours with 50 μL of GammaBind plus Sepharose. For STATs, 200 μL lysate was incubated with 2 μL of antiserum and 50 μL of GammaBind plus Sepharose beads for 2 hours. Immune complexes were collected by centrifugation (13 000g for 10 minutes) and washed 3 to 5 times with lysis buffer. Precipitates were dissolved in SDS-sample buffer (60 μL) and resolved (20 μL) by electrophoresis (6% PAGE for JAKs, 8% PAGE for STATs), immunoblotted (Immobilon, Millipore) with anti-phosphotyrosine antibody 4G10 and detected by chemiluminescence (ECL, Amersham). For detection of Stat1a and Stat2, SHP1, dnSHP1, or SHP2, cell lysates (10 μL) were resolved by SDS-PAGE (8%). Antibodies were purchased from Upstate Biotechnology Inc, except for anti-SHP2 (Transduction Laboratory).

Results

Different Combinations TAP1 Promoter Elements Mediate Induction by IFN-α/β in ECs and HeLa Cells

Both types of IFNs (IFN-α/β and IFN-γ) induce rapid accumulation of TAP1 mRNA in human ECs27 and HeLa cells.
The IFN-α/β response of the TAPI promoter also differs between these two cell types (Figure 1a and 1b). In ECs, the TAPI promoter responds more strongly to IFN-α/β than to IFN-γ, whereas the reverse is observed in HeLa cells. In HeLa cells, mutation of the ISRE abolishes the IFN-α/β response, whereas mutation of the GAS has no effect on the IFN-α/β response (Figure 1a). In ECs, however, the IFN-α/β response is reduced by mutation of either the ISRE or the GAS (Figure 1b), and both elements must be mutated to abolish the response. Therefore, the GAS contributes to the IFN-α/β response of the TAPI promoter in ECs but not in HeLa cells.

**Stat1α, Stat2, and p48 are Essential for the ISRE-Mediated Induction of TAPI by IFN-α/β**

The TAPI ISRE was used as a probe in EMSAs to detect binding proteins in nuclear extracts from IFN-α/β-treated cells. However, no specific binding proteins were detected in either ECs or HeLa cell nuclear extracts (data not shown). In particular, no IRF-1 binding to the ISRE was detected, although IRF-1 does bind the TAPIICS.29 A report that IRF-1 binds the TAP ISRE used a probe that contained an intact ICS flanking the ISRE, and the control oligonucleotide was mutant in both the ISRE and the ICS, so it is unclear whether the detected factor binds to the ISRE or the ICS.27

It has been noted that IFN-α/β–activated transcription factor Stat1/Stat2/p48 (ISGF3) can be detected by EMSA in the nuclear extracts from some cells only if they have been pretreated (primed) with IFN-γ for 24 hours before IFN-α/β treatment.16 Increased Stat1/Stat2/p48 detected after priming correlates with increased ISRE-mediated transcription.16 When control or IFN-γ–primed ECs (Figure 2) or HeLa cells (not shown) are treated with IFN-β for 15 minutes, ISRE-protein complexes are detected in the IFN-γ–primed cells but not in unprimed cells (lane 4 compared with lanes 2 and 3). Competition with excess unlabeled TAP ISRE or the ISG15 ISRE, which binds Stat1/Stat2/p48,13 demonstrates specific binding (lanes 8 and 9). Furthermore, the complex contains Stat1α and Stat2, because formation of the complex is blocked by specific antisera (lanes 5 and 6) but not by normal serum (NRS, lane 7). Similar results were obtained using IFN-α–treated ECs and HeLa cells (not shown). These results suggest that Stat1/Stat2/p48 can bind to the ISRE of TAPI promoter. It remained unclear, however, whether the small amount of Stat1/Stat2/p48 formed in unprimed cells mediates transcriptional activation by IFN-α/β.

To determine whether Stat1α and Stat2 mediate the ISRE-dependent IFN-α/β response of the TAPI promoter, the fibrosarcoma cell line 2TGH and three mutant daughter lines lacking Stat1α (U3A), Stat2 (U6A), or p48 (U2A)38 were tested in transient transfections with TAPI promoter-reporter genes. 2TGH cells resemble HeLa cells in that mutation at the ISRE abolishes the IFN-α/β response of the TAPI promoter (Figure 2b). 2TGH cells differ from HeLa cells (and resemble ECs) in that the IFN-γ response is mediated by the ISRE as well as the GAS. In U3A mutant cells (lacking Stat1α), both IFN-α/β and IFN-γ responses are abolished, indicating that Stat1α is essential for signaling by both types of IFN. In U6A cells (lacking Stat2) and U2A cells (lacking...
p48), the IFN-α/β response of TAP1 promoter is abolished (although the IFN-γ response is intact), confirming that the components of ISGF3 complex (Stat1, Stat2, and p48) participate in the ISRE-dependent transcriptional activation of the TAP1 gene by IFN-α/β.

Although Stat1/Stat2/p48 can be detected by EMSA of nuclear extracts from IFN-β-treated HT1080 fibrosarcoma cells, the derivative 2TGH cell line requires IFN-γ priming to form detectable levels of IFN-β-activated Stat1/Stat2/p48 (Reference 39 and data not shown). Taken together, these results are consistent with the conclusion that Stat1/Stat2/p48 mediates ISRE-dependent IFN-α/β responses in ECs and HeLa cells, despite the fact that it is detected by EMSA only in IFN-γ–primed cells. An alternative explanation, that transfection somehow mimics IFN-γ priming, is not true because transfected cells do not increase their HLA class I expression and remain IFN-β inducible to the same extent as untreated cells (Figure 2c). The simplest interpretation of this finding is that transfection assays are more sensitive than EMSA.

These experiments also reveal that Stat1α but not Stat2 or p48 participates in the IFN-γ response of the TAP1 ISRE, because the response is absent in cells lacking Stat1α (U3A) but intact in cells lines lacking p48 or Stat2 (U2A or U6A). The same conclusion was reached using a different reporter gene (luciferase) and a different transfection protocol (calcium phosphate coprecipitation), while control transfections performed in parallel demonstrated that the IFN-α/β and IFN-γ responses of the HLA class I promoter (HLA-B7) require Stat1, Stat2, and p48 (data not shown). In contrast, it has been shown that the ISRE-dependent IFN-γ response of the ISG54 promoter requires both Stat1 and p48.41

In ECs, IFN-α/β Activates Stat1α/Stat1α Homodimers That Bind the TAP1 GAS

The TAP1 GAS contributes to the IFN-α/β response in ECs but not HeLa cells (Figure 1b). Consistent with the transfection results, IFN-α/β only weakly activates GAS-binding nuclear proteins in HeLa cells, although IFN-γ does so effectively (Figure 3a). In contrast, GAS-binding proteins are detected in IFN-α/β– as well as IFN-γ–treated ECs (Figure 3b), and binding is specifically competed (lane 5). The proteins in the complex were identified as Stat1α (presumably in the form of a Stat1α homodimer, GAF) by antibody EMSA (not shown), as demonstrated previously in IFN-γ–treated HeLa cells.29 In contrast, Stat1α binding to the GAS in the promoter of the GBP gene is not detectable in IFN-α/β–treated FS2 fibroblasts,18 perhaps because the GBP GAS is lower affinity than the TAP1 GAS or because more Stat1α is activated in ECs.

IFN-α/β Activates the Same JAKs in ECs and HeLa Cells

Studies in HeLa cells and other cell types have shown that IFN-γ activates Jak1 and Jak2, leading to the activation of Stat1α and the formation of Stat1α/Stat1α, whereas IFN-α/β activates Jak1 and Tyk2, leading to activation of Stat1 and Stat2 and the formation of Stat1/Stat2/p48 (ISGF3).41 To test whether IFN-α/β activation of Jak2 in ECs could account for the formation of Stat1α/Stat1α, JAK phosphorylation was
assessed by immunoprecipitation with JAK-specific antibodies (Jak1, Jak2, or Tyk2) followed by Western blotting with a phosphotyrosine-specific antibody (Figure 4, upper panels). JAK-specific antibodies confirm comparable loadings of the gel (lower panels). IFN-β activates Jak1 and Tyk2 but not Jak2 in both ECs and HeLa cells. Similar results were obtained using IFN-α (not shown). IFN-γ activates Jak1 and Jak2 but not Tyk2 in both ECs and HeLa cells (Figure 4). These data demonstrate that IFNs activate the same JAKs in ECs and HeLa cells and suggest that IFN-α/β activation of Stat1α/Stat1α in ECs does not result from the activation of Jak2.

IFN-α/β Activates More Stat1α Than Stat2 in ECs But Not in HeLa Cells

If IFN-α/β activated more Stat1α than Stat2 in ECs, then excess activated Stat1α might form homodimers. The levels of Stat1α and Stat2 proteins were found by Western blotting to be comparable in ECs and HeLa cells (Figure 5a). When phosphorylated Stat1α was measured, however, more Stat1α was found to be phosphorylated in response to IFN-α/β than IFN-γ in ECs, whereas the reverse is true in HeLa cells (Figure 5b, upper panel). Phosphorylated Stat2 was strongly induced only by IFN-α/β, and the extent of phosphorylation appeared comparable in ECs and HeLa cells (not shown). In both cell types, similar amounts of Stat2 associate with Stat1 in response to IFN-β (Figure 5b, compare middle with lower panels). These data demonstrate that the ratio of IFN-β–activated Stat1α to Stat2 is much higher in ECs than in HeLa cells (compare lanes 3 and 5 of upper panel with lower panel) and support the suggestion that excess activated Stat1α molecules in IFN-α/β–treated ECs may form homodimers.

IFN-α/β Activates Stat1α More Transiently Than Does IFN-γ in ECs

More Stat1α/Stat1α is activated by IFN-β than by IFN-γ at 15 minutes (Figure 6a, compare lanes 2 and 3), but the levels are similar by 30 minutes (Figure 3), and Stat1α/Stat1α is not detected after 4 hours IFN-β treatment (Figure 6a, lane 4), while it is still detectable after 16 hours of IFN-γ treatment (lane 7). The kinetics of Stat1α phosphorylation was also examined by immunoprecipitation and immunoblotting (Figure 6b and 6c). Consistent with the EMSA observations, IFN-α/β activates rapid but transient phosphorylation of Stat1α (Figure 6b, upper panel, lane 2, 15 minutes; lane 4, 4 hours). In HeLa cells, IFN-β rapidly but only weakly activates Stat1α, and phosphorylated Stat1α is no longer detectable by 1 hour (Figure 6c). Stat2 is probably associated with phosphorylated Stat1α at times after 15 minutes, but the levels of

Figure 3. IFN-α/β activates the GAS-binding Stat1α homodimers in ECs but not in HeLa cells. a. IFN-γ but not IFN-α/β activates Stat1α/Stat1α in HeLa cells. Nuclear extracts from untreated HeLa cells (lane 1) or from HeLa cells treated for 30 minutes with IFN-α (lane 2), IFN-β (lane 3), or IFN-γ (lanes 4 and 5) were incubated with a probe containing the TAP1 GAS (see “Materials and Methods”). Unlabeled GAS competitor was present in 50-fold molar excess (lane 5). b. IFN-α/β, as well as IFN-γ, activates Stat1α/Stat1α in ECs. Nuclear extracts from untreated ECs (lane 1) or ECs treated for 30 minutes with IFN-γ (lane 2), IFN-α (lane 3), or IFN-β (lanes 4 and 5) were incubated with the TAP1 GAS probe. The Stat1α/Stat1α complexes are indicated by arrows.

Figure 4. IFN-β activates Jak1 and Tyk2 but not Jak2 in both HeLa cells and ECs. Cells were treated with IFN-β (10 minutes; lanes 2, 5, and 8), IFN-γ (10 minutes; lanes 3, 6, and 9), or left untreated (lanes 1, 4, and 7). Lysates were immunoprecipitated with JAK-specific antibodies and Western blotted with a phosphotyrosine-specific antibody (labeled pY) or JAK-specific antibodies (labeled protein). IFN-α activates the same JAKs as does IFN-β in both cell types (data not shown).

Figure 5. Stat1α and Stat2 are present in equal amounts, but the ratio of activated Stat1α/Stat2 is much higher in IFN-α/β–treated ECs than in HeLa cells. a. Stat1α and Stat2 are expressed at similar levels in ECs and HeLa cells. Lysates (10 μg) of ECs (lane 1) or HeLa cells (lane 2) were resolved on SDS-PAGE and Western blotted with an antibody specific for Stat1α (top) or Stat2 (bottom). b. IFN-β activates more Stat1α in ECs than in HeLa cells, although it activates similar amounts of Stat2 in both cell types. Cells were left untreated or treated for 15 minutes with IFN-β or IFN-γ (ECs, lanes 1, 3, and 4; HeLa, lanes 2, 5, and 6). Lysates were immunoprecipitated with Stat1α antibody followed by Western blotting with antibodies specific for phosphotyrosine (top), Stat1α (middle), or Stat2 (bottom).
phosphorylated Stat1α drop below the detection limits for phosphotyrosine at later times (1 hour and 4 hours, lanes 3 and 4). The rapid decline of phosphorylated Stat1α raised the possibility that the failure to observe IFN-α/β-activated Stat1α/Stat1α in HeLa cells is caused by a more effective dephosphorylation in this cell type compared with ECs.

The Tyrosine Phosphatase SHP-1 Downregulates IFN-α/β-Mediated Stat1α Activation and GAS-Mediated TAP1 Promoter Activation

The SH2-containing tyrosine phosphatase-1 (SHP-1) regulates the IFN-α/β-stimulated Jak/Stat pathway. Therefore, a higher level of SHP-1 in HeLa cells might account for the lower level of IFN-α/β-activated Stat1α/Stat1α. SHP-1 is indeed expressed at a much higher level in HeLa cells than in ECs, as measured by immunoblotting (Figure 7a). A structurally related phosphatase that is required for IFN-α/β signaling, SHP-2, is expressed at comparable levels in ECs and HeLa cells (Figure 7a, middle panel). Consistent with the explanation that a tyrosine phosphatase controls Stat1α/Stat1α activation in HeLa cells, IFN-β can induce significantly more GAS-binding Stat1α/Stat1α in HeLa cells in the presence of the tyrosine phosphatase inhibitor orthovanadate (van) (Figure 7b).

To test directly the role of SHP-1 in regulating IFN-α/β activation of Stat1α, expression constructs encoding wild-type or mutant SHP-1 were transfected, together with TAP1 promoter-reporter constructs into HeLa cells and ECs (Figure 8). SHP-1 is overexpressed in transfected ECs (Figure 8a, lanes 1 to 3), and the dominant negative SHP-1 mutant does not alter the high, constitutive level of endogenous SHP-1 in HeLa cells (lanes 4 and 5). TAP1 promoter-reporter constructs containing a mutant ISRE and a wild-type or mutant GAS were used to test GAS-mediated transcription without ISRE-mediated effects (Figure 8b). Increased expression of SHP-1 in ECs blocks IFN-β signaling through the GAS, while expression of the dominant negative SHP-1 (dnSHP-1) or transfection with the empty expression vector (vector) have no effect. In contrast, increased expression of SHP-1 in HeLa cells has no effect on the low level of IFN-β-induced, GAS-mediated transcription, while expression of the dominant negative SHP-1 allows IFN-β signaling through the GAS. These data demonstrate that SHP-1 regulates crossover between the IFN-α/β-ISRE and IFN-γ-GAS signaling pathways in HeLa cells and ECs (Figure 8c).

**Discussion**

Rapid induction of TAP1 by IFN-γ in HeLa cells is mediated by activated Stat1α homodimers (GAF) binding to the GAS...
in the TAP1 promoter. However, rapid induction by type I IFNs (IFN-α/β) in HeLa cells is mediated by ISGF3, which is a complex that includes activated Stat proteins (Stat1/Stat2/ISGF3), binding to an ISRE in the TAP1 promoter (Figure 1 and data not shown). These observations are consistent with the known behavior of the two types of IFN: IFN-γ drives transcription of GAS-containing genes and IFN-α/β drives transcription of ISRE-containing genes. Unexpectedly, different combinations of TAP1 promoter elements mediate IFN responses in ECs: specifically, both the GAS and the ISRE contribute to the full IFN-α/β response in ECs. This is because in ECs but not in HeLa cells, IFN-α/β activates Stat1α/Stat1β, which binds to the GAS on the TAP1 promoter. Similar crossovers between the IFN-α/β and IFN-γ signal-transduction pathways have been reported, but no mechanism has been demonstrated.

The TAP1 ISRE is required for the IFN-α/β response of the TAP1 promoter in HeLa cells and contributes to the response in ECs (Figure 1). Although EMSA detection of ISRE-binding Stat1/Stat2/p48 in IFN-α/β-treated ECs and HeLa cells requires IFN-γ priming (Figure 2a), induction of the TAP1 gene or TAP1 promoter by IFN-α/β does not (Figure 1). EMSA detection of Stat1/Stat2/p48 requires IFN-γ priming also in 2TGH cells (not shown). The 2TGH and Stat mutant daughter cell lines demonstrate, however, that Stat1α, Stat2, and p48 are all essential for the ISRE-mediated IFN-α/β response in this cell type (Figure 2b). This analysis supports the conclusion that Stat1/Stat2/p48 mediates IFN-α/β–induced TAP1 expression even in unprimed cells and suggests that the EMSA is less sensitive than transcription assays with promoter-reporter constructs.

The ISRE contributes to the IFN-γ response of the TAP1 gene in ECs and 2TGH but not HeLa cells (Figures 1 and 2b). IFN-γ can induce the ISG54 promoter in 2TGH cells by activating a complex of Stat1α and p48 that lacks Stat2. However, Stat1α but not Stat2 or p48 are involved in the IFN-γ response of the TAP1 ISRE (Figure 2b). It is unclear how the Stat1 could bind to the ISRE without the IRF family member p48. Differences in the TAP1 and ISG54 ISREs may account for different dependencies on p48. IFN-γ does not induce any ISRE-binding complex detectable by EMSA, however, and the identity of the IFN-γ–activated transcription factor(s) binding to this site was not pursued in this study.

IRF1 binding to the TAP1 ICS but not the TAP1 ISRE is detected by EMSA (not shown). The ICS does not contribute to an IFN response in either HeLa cells or ECs (Figure 1), however, suggesting that IRF1 does not contribute to the IFN response in these cells. In contrast, IRF1 knockout mice express lower levels of TAP1 in their lymphocytes, strongly supporting a role for IRF1 in TAP1 expression. Surprisingly, TAP1 is induced by IFNs in IRF1 knockout mice (personal communication, J. Ting, University of North Carolina, 1998). Therefore, IRF1 may promote constitutive TAP1 transcription in some cell types but does not contribute to the IFN response.

In ECs, IFN-α/β induces the TAP1 gene and the TAP1 promoter (Figure 1) more strongly than does IFN-γ, whereas IFN-γ is stronger than IFN-α/β in HeLa cells (Figure 1). The TAP1 GAS contributes to the IFN-α/β response in ECs but not in HeLa cells (Figure 1) and GAS-binding Stat1α/Stat1β are activated by IFN-α/β in ECs but not in HeLa cells (Figure 3). The residual response of the mutant ISRE construct in ECs (mISRE, containing GAS alone, Figure 1b) suggests that IFN-α/β–activated Stat1α/Stat1β contributes to TAP1 induction. Therefore, IFN-α/β activation of both the Stat1/Stat2/p48–ISRE and Stat1α/Stat1α–GAS signal-transduction pathways must account for the stronger induction of TAP1 in ECs, whereas in HeLa cells the Stat1/Stat2/p48–ISRE pathway alone is activated. Stat1α/Stat2 heterodimers bind to a GAS-like site (pIRE) in the IRF-1 promoter in response to IFN-α. This complex was not detected, however, in IFN-α/β–treated ECs by EMSA with a TAP1 GAS probe (data not shown).
suggesting that either the EMSA is not sensitive enough or Stat1α/Stat2 is not involved in GAS-mediated TAP1 induction in response to IFN-α/β in ECs.

The molecular basis of IFN-α/β activation of Stat1α/Stat1α in ECs but not HeLa cells was examined. The pattern of JAK activation does not explain the ability of IFN-α/β to activate Stat1α/Stat1α in ECs (Figure 4). Instead, the data suggest that IFN-α/β activates a much higher ratio of Stat1α to Stat2 in ECs than in HeLa cells (Figure 5). Thus, in addition to associating with Stat2, activated Stat1α may form homodimers in response to IFN-α/β. IFN-α/β activation of Stat1α is more transient than IFN-γ activation of Stat1α in ECs (Figure 6). Similarly, IFN-α activates in fibroblasts a factor called AAF (α[IFN]-activated factor) that is identical to Stat1α homodimers (GAF) in DNA binding and reporter gene assays.17 The activation of AAF by IFN-α is also more transient than that of GAF by IFN-γ.

Inactivation of IFN-activated transcription factors is not fully understood. A nuclear tyrosine phosphatase that acts on both IFN-α/β and IFN-γ–activated Stat1α is unlikely to account for the transience of IFN-α/β–activated Stat1α in ECs because the response to IFN-γ is sustained. The tyrosine phosphatase SHP-1 reversibly associates with the IFN-α/β receptor to downregulate the activities of Jak1 and Stat1α.22 In SHP-1 mutant mice, formation of Stat1α/Stat1α is selectively increased.6 Similarly, much lower levels of SHP-1 in ECs than in HeLa cells correlates with more Stat1α activated by IFN-α/β in ECs than in HeLa cells (Figure 7a). Consistent with this interpretation, IFN-α/β can activate Stat1α/Stat1α in HeLa cells treated with the tyrosine phosphatase inhibitor orthovanadate (Figure 7b). The tyrosine phosphatase SHP-2 is required to initiate IFN-α/β signaling,20 so it is unlikely that orthovanadate stimulates IFN-α/β signaling in HeLa cells by inhibiting this phosphatase. Finally, overexpression of SHP-1 in ECs blocks IFN-β signaling through GAS, producing a phenotype that resembles HeLa cells (Figure 8). Overexpression of a dominant negative SHP-1 (dnSHP1) mutant in HeLa cells allows IFN-β signaling through GAS, confirming the role of SHP-1 in regulating the IFN-β response. In contrast, overexpression of SHP-1 in HeLa cells has been recently shown to increase IFN-γ–activated Stat1, while expression of a dominant negative SHP-1 decreases IFN-γ–activated Stat1.26

In conclusion, ECs differ from HeLa cells in that they use additional pathways for STAT activation in response to IFN-α/β and IFN-γ, involving bidirectional crossovers between the two IFN pathways (Figure 8c). SHP-1 regulates crossover between IFN-α/β and IFN-γ signal-transduction pathways. These cell type–specific differences in IFN signaling may explain why ECs are more responsive than other cell types to IFN in vivo.6

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References


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