A Specific Interferon (IFN)-stimulated Response Element of the Distal HLA-G Promoter Binds IFN-regulatory Factor 1 and Mediates Enhancement of This Nonclassical Class I Gene by IFN-β*

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Type I interferons display a broad range of immunomodulatory functions. Interferon β increases gene expression at the transcriptional level through binding of factors to the interferon-stimulated response element (ISRE) within the promoters of interferon-inducible genes, such as HLA class I. Despite mutation of the class I ISRE sequence within the nonclassical HLA-G class I gene promoter, we show that interferon β enhances both transcription and cell surface expression of HLA-G in trophoblasts and amniotic and thymic epithelial cells that selectively express it in vivo. Deletion and mutagenesis analysis of a putative interferon-regulatory factor (IRF)-1 binding site within the HLA-G promoter show that HLA-G transactivation is mediated through an ISRE sequence 746 base pairs upstream from ATG, which is distinct from the interferon-responsive element described within proximal classical class I gene promoters. Electrophoretic mobility shift analysis and supershift analysis further demonstrate that interferon-responsive transcription factors, including IRF-1, specifically bind to the HLA-G ISRE. Our results provide evidence that IRF-1 binding to a functional ISRE within the HLA-G promoter mediates interferon β-induced expression of the HLA-G gene. These observations are of general interest considering the implication of HLA-G in mechanisms of immune escape involved in fetal-maternal tolerance and other immune privilege situations.

HLA-G is defined as a nonclassical HLA class I antigen (1, 2) that was originally found to be restrictively expressed in the human placenta, where it is thought to play a role in maternal tolerance of the fetal semilignaft. HLA-G expression was further characterized on several placentatcell types such as extravillous cytotoxophoblasts, amniocytes, or endothelial cells of choriocarcin vessels and also on subsets of thymic epithelial cells (3). HLA-G expression allows down-regulation of both NK and T lymphocyte cytolytic functions (4) through interaction with killing inhibitory receptors, namely p49/KIR2DL4, ILT2, and ILT4 (5). The capacity of HLA-G leader peptides to stabilize surface expression of nonclassical HLA-E antigens also indirectly contributes to modulate cytolytic activity mediated by the widely expressed CD94/NKG2 receptors (6). HLA-G associates with a wide array of nonamer peptides (7) and binds to the CD8 T-cell coreceptor (8, 9). Other immunomodulatory roles, such as its capacity to elicit a T-cell receptor-restricted response in transgenic mice (10), to trigger apoptosis of activated T and NK cells bearing the CD8 molecule (11), to impair NK cell migration (12), and to modulate cytokine (13, 14) or the function of B or myelomonocytic cells bearing killing inhibitory receptors, have also been evoked. Tissue-specific patterns of HLA-G expression and their frequent alteration in pathological situations such as pregnancy disorders (15), viral infections (16–19), tumors (20–24), or transplantation (25) may medulate the mounting of an efficient immune response and thus provide an additional mechanism to escape immune surveillance.

Interferons are classified in a family of related cytokines, type I (IFN-α, -β, -ω, and -γ) and type II (IFN-γ) interferons, which mediate diverse functions including antiviral, antiprofenerative, and immunomodulatory activities. Type I interferons are produced by many cell types (e.g. macrophages, T cells, keratinocytes, and Langerhans cells) in response to viral or bacterial infection and tumors. They display pleiotropic effects on the immune system, including stimulation of NK cells and macrophage activation, T-cell activation and survival, and up-regulation of various genes such as IFN-γ and major histocompatibility complex class II (26). The cascade of events that yield to IFN type I induction involves activation of Jak/STAT transduction pathways and transactivation of inducible gene promoter through the ISRE regulatory element, a binding site for ISGF3 (IFN-stimulated gene factor 3) or IRFs (27, 28). After binding to its receptor, IFN type I induces translocation of activated STAT1 and STAT2 into the nucleus and formation of a heterotrimeric complex containing p48/ISGF3γ (ISGF3). Among transcription factors belonging to the IRF family, IRF-1

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5. The abbreviations used are: NK, natural killer; AEC, amniotic epithelial cell; TEC, thymic epithelial cell; IRF, interferon regulatory factor; ISRE, interferon-stimulated response element; GAS, interferon γ-activated site; IFN, interferon; EMSA, electrophoretic mobility shift assay; STAT, signal transducers and activators of transcription; mAb, monoclonal antibody; RT-PCR, reverse transcription-polymerase chain reaction; PCR, polymerase chain reaction; kb, kilobase; bp, base pair(s); mfi, mean of fluorescence intensity; ISGF3, IFN-stimulated gene factor 3; RPA, RNase protection assay.
and IFR-2 are secondary IFN response factors that interact mainly with ISRE to activate or repress target promoter activity (29).

Another well-characterized IFN cis-acting regulatory element is the ISN-y activation site (GAS), which rather mediates the immediate response of several genes to IFN-y (30).

The pattern of HLA-G expression is tightly regulated due to cell-specific transcriptional control, but the regulatory pathways controlling its tissue-specific transactivation remain to be established. HLA-G gene expression can be activated by interleukin 10 (31), glucocorticoids, or stress treatment (32) independently of classical HLA class I genes or by IFNs (33–36), leukemia virus reverse transcriptase (Life Technologies, Inc.). PCR products were amplified using sense 5′-GGAAAAGGAGCAAGCG-3′ and antisense HLA-GA.3U (5′-TGAAACAGACTGAGGAAGGCAA-3′) primers. HLA-G5 isoforms were specifically amplified using 5′-ATGACGGC (44) and irrelevant IR 5′-TGAGAGGGACGGAGG-3′ primers. β-Actin was coamplified during the last 16 cycles for 20 min. Double-stranded oligonucleotides containing wild type or mutated ISRE sites were used as competitors.

**MATERIALS AND METHODS**

**Cells, Tissues, and Primary Cultures**—The JEG-3 choriocarcinoma cell line was purchased from American Type Culture Collection. The LTK-2 cell line was derived from an independent proliferative clone of thymic epithelial cells transformed by the SV40LT oncogene (40). Cell lines were maintained as described previously (41). Thymic fragments were collected and subcultured into Primaria 24-well plates (Becton Dickinson, San Jose, CA).

First trimester trophoblasts were obtained from voluntarily interrupted normal pregnancies at 5–7 weeks of gestation (with local ethic committee approval).

Fetus-surrounding membranes (amnionchorion) were obtained from whole term placenta after normal delivery. Chorionic membrane was stripped from the amnion by scraping. Amnion epithelial cells (AECs) were prepared according to Hammer et al. (42). Amnion membrane was minced into small pieces and dissociated by two rounds of 1-h trypsinization in phosphate-buffered saline, 0.05% trypsin (Difco, Detroit, MI), and 40 units/ml DNase and washing. The resulting amnion epithelial cells were grown in Dulbecco’s modified Eagle’s medium: Ham’s F-12 (Life Technologies, Inc.) supplemented with 10% fetal calf serum, glutamine, and antibiotics for 6–12 days.

Cell and trophoblast explants were stimulated with human recombinant IFN-β (1000 units/ml; PeproTech EC, London, United Kingdom) for 2–72 h as further stated or not stimulated.

**Flow Cytometry Analysis**—The following mAbs were used: 87G (IgG2a anti-HLA-G α chains associated with β2-microglobulin), W6/32 (IgG2a anti-HLA class I α chains associated with β2-microglobulin (Sigma)), B1.23.2 (IgG2b anti-HLA-B and -C α chains associated with β2-microglobulin), and TP25.99 (IgG1 anti-HLA class I except HLA-G; kindly provided by Soldano Ferrone). Cells were labeled by sequential incubations with mouse-specific primary mAbs and secondary goat anti-mouse phycoerythrin-conjugated F(ab′)2 fragments in phosphate-buffered saline and 2% fetal calf serum for 30 min at 4 °C. After further washing, cells were fixed and analyzed on a Becton Dickinson Facs Vantage. Controls were stained with an isotype-matched irrelevant antibody.

**Reverse Transcription-PCR Analysis**—Total RNA was extracted using the RNA NOW reagent (Biogentex Inc., Seabrook, TX) according to the manufacturer’s instructions and controlled by electrophoresis in a 1.5% agarose-denaturing gel. Complementary DNAs were prepared from total RNA using oligo(dT)12–18 primers and Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.). PCR amplification of the cDNA preparation was carried out in a thermal cycler (PerkinElmer Life Sciences, Norwalk, CT). Pan HLA-G transcripts were amplified using sense HLA-G.257 (5′-GGAAAAGGAGCAAGCG-3′) and antisense HLA-GA.3U (5′-TGAAACAGACTGAGGAAGGCAA-3′) primers. HLA-G5 isoforms were specifically amplified using 5′-ATGACGGC (44) and irrelevant IR 5′-TGAGAGGGACGGAGG-3′ primers. β-Actin was coamplified during the last 16 cycles as a semiquantitative control of total CDNA (CLONTECH). PCR products were size-fractionated by electrophoresis on a 1.5% agarose gel, blotted onto nylon membranes (Hybond N+; Amersham Pharmacia Biotech), and hybridized with 32P-labeled HLA-GR, HLA-G14F, and β-Actin specific probes as described previously (32).

**RNase Protection Assay (RPA)**—Single-stranded radiolabeled RNA probes were synthesized using the MAXIScript in vitro transcription kit (Ambion, Austin, TX) with T7 RNA polymerase and [α-32P]CTP as described previously (31). The HLA-G template was prepared by PCR amplification of a genomic fragment in the 3′-untranslated region with G-1089F forward primer (5′-CCCTTGTGACTCAAGAAC) and T7 promoter containing reverse primer T7G.1250R (5′-GGATCCTAATACGACTCACTATAGGGAGGTTATAGCTCAGTGGCCCAC). Cyclophilin standard template was obtained from Ambion. Protection of HLA-G transcripts was carried out using a HybSpeed RPA kit (Ambion). 5 μg of total RNA were hybridized with 5 x 106 cpm of HLA-G and cyclophilin riboprobes for 10 min in 10 μl of hybridization buffer and digested for an additional 30 min with RNase T1 (Ambion) at 37 °C. Radioactive riboprobes and fragments were precipitated and separated on a 5% acrylamide (19:1) gel. The gel was dried and exposed to a molecular imager (Bio-Rad) for quantification.

**Reporter Constructs**—The 1.4-kb fragment of the HLA-G promoter was prepared from PCR amplification of a JEG-3 genomic DNA region (nucleotide −1435 to the ATG) using primers sense −1438F and antisense −148F, which is expressed independently of classical HLA class I genes or by IFNs (33–36). The gel was run on a 1% agarose gel and the 1.4-kb fragment was excised and purified.

**One-step ISRE-GAS reporter vector**—For further study, a construct was made by replacing the HLA-G promoter of the one-step ISRE-GAS reporter vector (ProMega) with the HLA-G promoter to generate the plasmid-one step ISRE/GAS (hs-vector). This vector was transfected into LTK-2 cells, and luciferase activity was measured in duplicate or divided by the mean of at least three independent experiments. For reporter assays, 1 μg of pGL3-firefly luciferase reporter construct and 10 ng of pRL-TK Renilla luciferase vector (Promega) as an internal control for transfection using the Exgen 5000 reagent (Euromedex, Souffelweyersheim, France) according to the manufacturer’s instructions. On the next day, cells were fed with culture medium alone or supplemented with IFN-β. Firefly and Renilla luciferase activities of precleared cell extracts (10 μl) were measured 20 h after transfection by injection of the Dual Luciferase Reporter assay system (Promega) using a luminometer (EG and G Berthold). Firefly luciferase activity values (relative light units/μl) were either measured in duplicate or divided by the Renilla luciferase activity values to correct for transfection efficiency and expressed as a mean of at least three independent experiments.

**Nuclear Extracts and Gel Mobility Shift Assays**—Nuclear extracts and gel mobility shift assays were carried as described previously (43). Nuclear extracts (2 μl) were incubated with a radiolabeled double-stranded oligonucleotide probe (1–2 ng; 106 cpm), a 200-fold molar excess of competitor oligonucleotides, and 2 μg of poly(dI-dC) in binding buffer for 20 min. Double-stranded oligonucleotides containing wild type or mutated ISREs were synthesized from HLA-G promoter by T7 polymerase and annealed to luciferase substrates (50 μl) provided in the Dual Luciferase Reporter assay system (Promega) using a luminometer.
RESULTS

IFN-β Enhances HLA-G and Classical HLA Class I Cell Surface Expression in Trophoblast, Amnion, and Thymus-derived Cells—Primary cultured cell types expressing HLA-G in vivo were chosen as a model to study HLA-G modulation by IFN-β. Human AECs and TECs were derived from term placenta amnions and surgically removed pediatric thymuses. These primary cells maintain HLA-G expression ex vivo, although a down-regulation of the level of cell surface HLA-G antigens occurring throughout the culture led to a weak steady-state level of HLA-G cell surface antigens at the time of analysis (45). A trophoblast-derived choriocarcinoma cell line (JEG-3) and a thymic epithelial-derived cell line (LT-TEC2) were also included in the study. LT-TEC2, which is derived by SV40 transformation of TEC primary culture, shares several features with primary cells (40).

Cells were left untreated or subjected to a 48-h IFN-β treatment and analyzed in flow cytometry experiments. HLA-G as well as classical HLA class I surface expression was significantly induced on amniotic and thymic primary cultures as assayed by the enhancement of the mean of fluorescence intensity (mfi) after IFN-β stimulation (Fig. 1A). IFN-β-mediated induction was confirmed using several primary cultures derived from distinct individuals (Fig. 1B). Lower levels of induction were also observed in the trophoblast-derived JEG-3 cell line (Fig. 1B). Fold levels of HLA-G antigen surface induction varied from 3 to 1.5 times, depending on the cell lineage (Fig. 1B), and were higher in primary cultures expressing low (TECs) or intermediate (AECs) levels of steady-state HLA-G antigens due to a decreased HLA-G expression resulting from an 8–15-day culture. We also show that, like primary TECs, IFN-β-treated LT-TEC2 cells retain the capacity to up-regulate HLA-G as well as major histocompatibility complex class I antigens (data not shown).

IFN-β Enhances the Levels of HLA-G Transcripts in Trophoblast, Amnion, and Thymus-derived Cells—To further characterize mechanisms involved in IFN-β-mediated HLA-G surface induction, we investigated the effect of this cytokine on HLA-G transcript levels by RT-PCR (Fig. 2, A and B) and RNase protection assay (Fig. 2B). Pan HLA-G primers specific for exon 2- and 3′-untranslated region-containing transcripts were used to amplify all HLA-G isoforms (Fig. 2A, top panel), whereas HLA-G primer sets located in exon 3 and intron 4 were used to specifically detect HLA-G5 soluble isoforms (Fig. 2A, middle panel). We show that all HLA-G transcripts, including those encoding membrane-bound and soluble HLA-G5 isoforms, are significantly enhanced after IFN-β treatment in both primary cultured amniotic and thymic cells. Similar RT-PCR results were reproducibly obtained using primary TEC and AEC cultures derived from several individuals (Fig. 2A). IFN-β-mediated enhancement of HLA-G messengers was also observed in treated first trimester trophoblast explants (Fig. 2B). Concomitant protection of HLA-G- and cyclophilin-specific transcripts from RNase degradation, measured by a RNase protection assay, allowed us to further quantify the level of IFN-β induction to a 3.5-fold enhancement of HLA-G messages in trophoblast explants (Fig. 2B, right panel).

IFN-β Transactivation of the HLA-G Promoter Is Mediated through an Upstream ISRE—HLA-G gene transcription is up-regulated in response to IFN-β, despite the disruption of enhancer A/ISRE/site α-regulatory sequences known to be the target of IFN response factors within the classical HLA class I gene promoter. We thus investigated whether cis-regulatory elements mediating HLA-G transcriptional activation by IFN-β could be identified within a 1.4-kb region of the HLA-G promoter.

Computer search and manual sequence analysis based on sequence homologies led us to identify two putative ISRE sites within the HLA-G promoter. The upstream one, located at −746 bp from the ATG, beside a GAS element (46), is highly homologous to the consensus ISRE (Fig. 3). The other one, lying downstream at −380 bp, is less conserved.

To assess the functionality of these sequences, we analyzed HLA-G promoter activity in JEG-3 and LT-TEC2 cells. Fragments of the 1.4-kb HLA-G promoter were subcloned upstream of the firefly luciferase reporter gene into the promoterless pGL3 basic vector. Extracts from transfected cells subjected or not to a 20-h IFN-β treatment were analyzed for luciferase activity. To discriminate between both ISRE putative sites, the activity of the 1.4-kb HLA-G promoter fragment construct (−1400) containing both ISREs was compared with that of a
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FIG. 2. IFN-β enhances the levels of HLA-G transcripts. A, representative RT-PCR analysis performed on amniotic and thymic epithelial primary cultures left unstimulated or stimulated with IFN-β (1000 units/ml) for 24 h. All HLA-G transcripts were detected using the pan HLA-G primer set (G.257 and GA.32) and GR probe (top panel). The HLA-G5-specific primer set (G.526 and GI4b) and GI4 probe were used to detect HLA-G5 soluble isoforms (middle panel). β-Actin was amplified as a control (bottom panel). B, representative RT-PCR and RPA analysis performed on first trimester trophoblast explants stimulated or not stimulated with IFN-β (1000 units/ml) for 72 h. Total RNA was extracted and either included in RT-PCR analysis (left panel) or protected from RNase degradation with HLA-G and cyclophilin 32P-labeled riboprobes in RPA analysis (right panel).

FIG. 3. Localization of putative ISRE and GAS interferon-responsive sites within the HLA-G promoter sequence. Arrows indicate the orientation of ISRE and GAS (boxed areas), with regard to the consensus ISRE and GAS described in the literature (GAS cons. and ISRE cons.). Filled squares identify residues mutated to disrupt the ISRE site of the sequence of the mutated HLA-G promoter/pGL3 construct used in the luciferase activity assays and the ISRE and GAS of the mutated HLA-G oligonucleotides (ISREMUT/GAS, ISRE/GASmut) used in EMSA (coding strand only).

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shorter construct (~500) with the upstream ISRE deleted and retaining solely the downstream ISRE (Fig. 4).

We show that the activity of the ~1400 construct exhibits a 3-fold enhancement upon IFN-β induction in TECs, demonstrating that the 1.4-kb promoter region is responsive to IFN-β. Enhancement of promoter activity was much weaker but reproducible (1.4 ± 0.4) in JEG-3 choriocarcinoma cells, in accordance with the very weak cytokine-mediated transcriptional activation of HLA-G (33, 36, 47) and classical HLA class I (43) described previously in this cell line. In contrast, promoter activity of a deleted fragment (~500) lacking the upstream putative site was not affected by IFN treatment in both cell types (Fig. 4), showing that deletion of the upstream ISRE impaired transcriptional activation of the 1.4-kb HLA-G promoter fragment. Functionality of the upstream ISRE was further analyzed by directed mutagenesis. No enhancement of HLA-G promoter activity was observed using a construct in which the upstream ISRE site is mutated within the 1.4-kb promoter fragment (Fig. 4), suggesting that deleterious muta-
ISRE motif, whereas the GAS motif or surrounding nucleotides within the HLA-G ISRE/GAS probe are unlikely to interfere or cooperate in the binding of these factors to the ISRE of the HLA-G promoter. To identify the nuclear proteins that bind to the HLA-G ISRE, supershift experiments were conducted. JEG-3 and LT-TEC2 nuclear extracts were incubated with antibodies specific for DNA-binding factors involved in transactivation of IFN-β-inducible genes before the addition of the HLA-G ISRE/GAS probe. We thus identified the presence of IRF-1 within the C1 complex interacting specifically with the HLA-G ISRE (Fig. 5B). As expected from published data, IRF-1 binds transiently to the ISRE in response to IFN-β treatment.

**Fig. 4.** A functional ISRE mediates IFN-β transactivation of the HLA-G promoter. Functional analysis of HLA-G promoter was conducted using 1.4-kb wild type (−1400) and ISRE mutated (−1400mut) or deleted (−500) HLA-G promoter fragments subcloned into the firefly luciferase promoterless pGL3 basic vector. Numbers indicate the position relative to ATG (+1). Discontinuous boxes represent wild type (−166) or mutated (−746) nonfunctional ISRE. JEG-3 and LT-TEC2 cells were transiently transfected and subjected to a 20-h IFN-β treatment (1000 units/ml) or left untreated. Firefly luciferase activity (pGL3 construct) was normalized to Renilla luciferase activity (pRL-TK vector) measured in the same sample. Results are expressed as fold induction where the normalized luciferase activity of IFN-β-treated cells is shown relative to that of untreated cells. Values represent the average of at least four independent experiments ± S.E.

**Fig. 5.** Binding of nuclear proteins to the HLA-G ISRE in response to IFN-β. Representative EMSA performed using nuclear extracts from JEG-3, trophoblasts, AECs, or LT-TEC2 cells stimulated with IFN-β for different time periods and the HLA-G ISRE/GAS probe (Fig. 3). A, unlabeled homologous (ISRE/GAS), ISRE-mutated (ISREmut/GAS), GAS-mutated (ISRE/GASmut), or irrelevant (IR) competitors were used in 100–200-fold molar excess. C1 and C2 complexes bound specifically to the HLA-G ISRE/GAS probe are indicated by an arrow. B, supershift experiments. IRF-1 rabbit polyclonal antibodies supershift (*) an IRF-1-containing complex bound to the HLA-G ISRE/GAS probe (C1). A rabbit polyclonal serum serves as nonspecific control (IR).
because IRF-1 shifted complexes can be detected 2 h (Fig. 5B, top panel) or 4 h (Fig. 5B, bottom panel) after induction, whereas they are not observed within untreated extracts or after a 24-h treatment (Fig. 5B; data not shown).

DISCUSSION

A previous study reported the ability of IFN-β to enhance levels of HLA-G surface antigens, but these data remained preliminary because they were only reported on transfected mouse fibroblasts. Although IFN-β was identified as a potent inductor of HLA-G transcription in other cell types (33–35), the regulatory pathways yielding to this transactivation were not elucidated. Indeed, little is known about sequences and factors which most of the conserved regulatory DNA elements involved in the constitutive and cytokine-induced expression of the HLA class I genes are disrupted and nonfunctional.

Our findings represent the first evidence of HLA-G modulation and overall IFN-β-mediated up-regulation of HLA class I cell surface expression in amnion and thymic epithelial cells expressing HLA-G. We also show that IFN-β-mediated enhancement of the level of HLA-G surface antigens was comparable to that of classical HLA class I surface antigens and was correlated to an overall enhancement of the levels of HLA-G transcripts. The extent of up-regulation of HLA-G transcript levels we observed in IFN-β-treated primary cells, cell lines, and trophoblast tissue was in agreement with the 2–2.5-fold enhancement reported previously and may not be attributed to increased stability of mRNA but rather to stimulation of the HLA-G transcription rate (35). Our results confirm that IFN-β is a potent inducer of HLA-G transcription, which may explain the effect observed at the surface of HLA-G-expressing cells, although a posttranscriptional effect of IFN-β on HLA-G enhancement cannot be excluded.

We further elucidated part of the regulatory mechanisms involved in IFN-β induction of HLA-G transcription. We have identified a functional ISRE at position −746 bp from the ATG within the distal HLA-G promoter, and we demonstrate that this site is necessary to confer IFN-β transactivation of the 1.4-kb HLA-G gene promoter fragment. This ISRE does not appear to be conserved among other classical HLA class I promoters and could represent a locus-specific pathway of IFN-β-mediated induction of the nonclassical HLA-G class I antigen. We also demonstrate that the GAS element, lying downstream of the ISRE, does not play a role in IFN-β transactivation of HLA-G.

Previous studies on the transactivating effect of the ISRE element upon IFN stimulation point out that both enhancer A and site α are crucial for maximal induction of HLA class I genes through the ISRE (37, 39). The nonclassical HLA-E also contains a specific IFN-γ response region constituted of two adjacent cis-acting regulatory elements, both of which are required to mediate the full response to IFN-γ (49). No adjacent cooperative sequence seems to be required for full induction by IFN-β through the functional ISRE within the HLA-G promoter, which identifies the particular nature of IFN-β-mediated HLA-G induction. The level of IFN-β-mediated induction of HLA-G surface antigens was quite well correlated to the level of HLA-G promoter transactivation through the −746 ISRE and was similar to that reported using a transfected HLA-G 6.0-kb fragment containing the 1.4-kb promoter fragment and 3′ sequences (35). Nevertheless, we cannot exclude the presence of other functional IFN-responsive sites within the HLA-G gene.

HLA-G expression can be induced by IFN-α and IFN-γ in transfected mouse fibroblasts (35). Whether the functional ISRE is able to mediate transcriptional activation of HLA-G upon both type I IFNs remains to be verified. Interestingly, we have previously reported a lack of activation of the 1.4-kb HLA-G promoter fragment in IFN-γ-treated JEG-3 or in IFN-γ-treated Tera-2 cells (36, 37) that displayed a 3-fold enhancement of HLA-G promoter activation upon treatment with IFN-β (data not shown). This could suggest that the ISRE mediating the IFN-β response within the HLA-G promoter is not involved in IFN-γ transactivation of HLA-G or requires additional cooperative regulatory elements. These results point out that differential and overlapping mechanisms, including several regulatory pathways and cooperative interactions between cellular factors involved in the specific response to IFNs, regulate IFN-α, -β, and -γ transactivation of HLA-G and other HLA class I genes.

We further provide evidence that IRF-1 binds to the functional ISRE within the HLA-G promoter in response to IFN-β and may thus represent a key factor in the regulation of the HLA-G gene in trophoblast, amnion, and thymus-derived cells. Accordingly, IFN-1 is described as the principal transcription factor binding to the ISRE within the major histocompatibility complex class I promoter (49). Supershift analysis failed to identify other known ISRE-binding proteins such as IRF-2, p48, or STAT1α. An altered activity of the p48/ISGF3′ subunit of the ISGF3 factor has been reported in trophoblast cells (47). STAT1-containing complexes binding to HLA class I ISREs were not detected in IFN-γ-treated HEK293 cells, unlike such GAS-binding complexes (38), suggesting that ISRE-protein complexes would be barely detectable in supershift experiments.

In addition to playing essential roles in IFN responses, IFN-1 proteins are involved in other regulatory processes, i.e. cell cycle regulation, tumor suppression, oncocytic activities, apoptosis, and development and function of immune effector cells (50, 51). Interleukin 2 and interleukin 12 have been shown to directly induce IRF-1 gene expression in human T and NK cells (52). Whether these cytokines that promote cell-mediated immune response could also enhance HLA-G expression through binding of IFN-1 to ISRE should be further investigated in immunocompetent cells.

Type I interferons are highly involved in the innate and specific host protective response, such as the T-cell IFN-γ response to viral infection (53–55). Facing this, viruses have evolved numerous strategies to subvert host defenses, including modulation of major histocompatibility complex class I expression or mimicking host cell genes such as IRF-1 or I.LT2 (56). Alteration of HLA-G or locus-specific class I gene expression may also interfere in the balance between escape from cytotoxic T lymphocytes and maintenance of protection from NK cells (16–19, 57). Given that IFN-β is produced in virally infected trophoblast and amnion (58, 59), specific pathways of HLA-G modulation could permit infected cells to block NK and T-cell responses, as seen during maternal-fetal transmission of human cytomegalovirus (60) or human immunodeficiency virus. Alternatively, lysis of infected material could be triggered by CD94/NKG2C receptors through HLA-E surface expression or by HLA-G-restricted T-cell Receptor and thus prevent pathogen spreading from the placental cells to the fetus. IFN-β-mediated HLA-G up-regulation in TECs could also affect selection of the thymic repertoire.

Type I interferons are used in the treatment of several human pathologies such as infectious diseases, multiple sclerosis, or tumors. Whether the nonclassical HLA-G molecule is up-regulated during IFN treatment or plays a role in favoring or impairing immune responses to such therapeutic approaches also remains an interesting issue. Indeed, analysis of aberrant expression of HLA-G combined with loss of classical HLA mol-
ecules on melanoma cells could have important practical implications for the selection of patients likely not to benefit from interferon α therapy (61).

Our results point out that IFN-β-mediated enhancement of HLA-G expression may occur as a way to refine modulation of immune responses during pregnancy, thymic involution, or IFN-β treatment of autoimmune diseases and tumor progression.

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A Specific Interferon (IFN)-stimulated Response Element of the Distal HLA-G Promoter Binds IFN-regulatory Factor 1 and Mediates Enhancement of This Nonclassical Class I Gene by IFN-β

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