HLA-G Transactivation by cAMP-response Element-binding Protein (CREB)

AN ALTERNATIVE TRANSACTIVATION PATHWAY TO THE CONSERVED MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) CLASS I REGULATORY ROUTES

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The expression of HLA-G in extravillous cytotrophoblasts coincides with a general lack of classical major histocompatibility complex (MHC) class I expression in this tissue. This differential expression of HLA-G and classical HLA class I molecules in trophoblasts suggests a tight transcriptional control of MHC class I genes. Transactivation of the classical MHC class I genes is mediated by two groups of juxtaposed cis-acting elements that can be viewed as regulatory modules. Both modules are divergent in HLA-G, rendering this gene unresponsive to NF-κB, IRF1, and class II transactivator (CIITA)-mediated induction pathways. In this study, we searched for alternative regulatory elements in the 1438-bp HLA-G promoter region. HLA-G was not responsive to interferon-α (IFNα), IFNβ, or IFNγ, despite the presence of an upstream ISRE binding IRF1 in vitro. However, the HLA-G promoter contains three CRE/TRE elements with binding affinity for CREB and CBP in vitro. In transient transfection assays, it was shown that HLA-G transactivation is regulated by CREB, CREB-binding protein (CBP), and p300. Moreover, immunohistochemical analysis demonstrated that HLA-G is co-expressed with CREB and CBP in extravillous cytotrophoblasts, revealing the in vivo relevance of this transactivation pathway. This implies a unique regulation of HLA-G transcription among the MHC class I genes.

The classical HLA class I molecules (HLA-A, HLA-B, and HLA-C) are highly polymorphic and are ubiquitously expressed on most somatic cells (reviewed in Ref. 1). They are essential in the immune response as they present antigen-derived peptides to cytotoxic T-lymphocytes and are important in protection against natural killer cell-mediated cytotoxicity. The nonclassical HLA class I molecules HLA-E, HLA-F, and HLA-G have a limited polymorphism and display a restricted expression pattern (reviewed in Refs. 1–3). Among the nonclassical HLA class I molecules, HLA-F and HLA-G have been shown to be able to present peptide (3, 4), whereas HLA-E is predominantly expressed as an empty intracellular molecule (5). Although there is increasing evidence that nonclassical class I molecules could be important in protection against natural killer cell-mediated responses (2, 3), their exact role in T cell-mediated immune responses is still under debate. Additionally, HLA-G has been attributed alternative functional properties, such as interaction with and down-regulation of the function of CD4- and CD8-positive T cells (6–10). Among the MHC class I molecules, the expression of HLA-G is particularly restricted and is found in few tissues, including extravillous cytotrophoblast cells (3, 11). Coinciding with the expression of HLA-G in this tissue is a general lack of classical MHC class I expression (3, 5). Together, this is proposed to be of importance for materno-fetal tolerance (2, 3, 11–13). The differential expression of HLA-G and classical HLA class I molecules in trophoblasts suggests a tight transcriptional control, which favors the expression of HLA-G and represses the expression of the classical class I genes HLA-A and HLA-B.

Transcriptional control of classical MHC class I genes is mediated by conserved cis-acting regulatory elements in the proximal promoter region. These regulatory elements include enhancer A, the ISRE, and the SXY module (14). Enhancer A and the ISRE mediate the constitutive and cytokine-induced expression of MHC class I (15, 16). The recently identified SXY module is important in the constitutive and CIITA-mediated transactivation (17). These classical regulatory elements are divergent in HLA-G, rendering this gene unresponsive to NF-κB, IRF1, and CIITA-mediated induction pathways (18).

In this study, we investigated the regulation of HLA-G transactivation and searched for alternative regulatory pathways. We assessed the capacity of putative regulatory elements in the promoter of HLA-G to bind transcription factors and to mediate transcriptional regulation of HLA-G. This search lead to the identification of three CRE/TRE elements, which bind proteins of the CREB/ATF and Fos/Jun families of transcription factors. CREB1 was able to stimulate HLA-G promoter activity in vitro, and the coactivators CBP and p300 enhanced this effect, re...
vealing that HLA-G is under the transcriptional control of the CREB/ATF family of proteins.

MATERIALS AND METHODS
Cell Culture—The trophoblast-derived cell lines JEG-3 and JAR, the teratocarcinoma cell line Tera-2, the cervical carcinoma HeLa (American Type Culture Collection, Manassas, VA), the Burkitt lymphoma cell line Raji, and the T leukemia cell line Jurkat were grown in Iscove's modified Dulbecco's medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (Invitrogen), penicillin (100 international units/ml), and streptomycin (100 µg/ml).

Reagents and Expression Vectors—Luciferase reporter plasmids used were generated by cloning genomic promoter fragments into pGL3-Basic (Promega, Madison, WI). These constructs contain, respectively, a 1438-bp promoter fragment of HLA-G (pGL3-G1500), a 302-bp β2m PCR-generated promoter fragment (pGL3-β2m), and a 269-bp Asp7AsaAlII HLA-B7 promoter fragment (pGL3-HLA-B). The mutant promoter constructs of HLA-G were generated by overlap extension PCR and are based on pGL3-G1500. In the mutant promoter constructs, the putative CRE/TRE site(s) (underlined) were disrupted by inserting a SmoI site or related sequence (bold). The most upstream putative CRE/TRE site (CRE, 1306) was flanked by an additional putative CRE/TRE site (both underlined). Therefore, in pGL3-Gm1, both CRE and CRE2 were mutated, changing the CRE, 1306 from 5'-AGAAATGACACACTCTGACTCATAGTAGCAGG-3' to 5'-GAGAGAAAACCCGACCCTGGAGGCAAGCC-3'. The CRE/TRE sites were fixed with a 10% methanol and 10% acetic acid solution.

Transient Transfection—Adherent cells were transfected by the calcium phosphate precipitate method with a DNA precipitate of 1 µg of pGL3 reporter plasmid, 1 or 0.5 µg of expression vector, and 0.1 µg of Renilla luciferase control plasmid (pRL-actin) per well. The cells were harvested 3 days after transfection. Luciferase activity was determined by using a luminesimeter (Molecular Devices, Menlo Park, CA) and corrected for transfection efficiency with the Renilla luciferase activity values.

Preparation of Nuclear Extracts and Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared as described previously (19). The expression vector pSG5-Rc/RSV-CREB1 and pECCE/RSV-ATF1, Rc/RSV-CREB2, and pECCE/RSV-ATF2, Rc/RSV-CREB3, and pECCE/RSV-ATF3 (all from Santa Cruz Biotechnology Inc., Santa Cruz, CA) except the following: CREB1 antibody (a kind gift of Dr. J. M. Boss; 20), CREB/ATF (sc-270), ATF1 (sc-243), CREB1 (sc-271), CREB2 (sc-272), CREB3 (sc-273), and mouse IRF1 (sc-640) (all from Santa Cruz Biotechnology Inc., Santa Cruz, CA, except the first CREB1 antibody).

Chromatin Immunoprecipitation (ChIP) Assay—Formaldehyde-crosslinked chromatin was prepared from 2 x 10^7 cells as described (20). The chromatin suspension was precipitated with 5 µg of Ab and pulled-down with anti-rabbit and anti-mouse Ig-coupled magnetic beads (Dynal, Oslo, Norway) or with protein A-Sepharose beads (Amersham Biosciences). After several wash steps, the samples were analyzed by PCR using the primers specific for the promoter region of HLA-G around CRE-C1500 (GF,-1461, 5'-TGTCATAGGCTGCTATTCTAGAGGTTCCTCAAACGAGTGGGACCC-3' and GR-1500, 5'-GCTCCTTTTCCTACCATGCTCCGCTCCAGCCCCCTTCC-3'). The amplified PCR product is 250 bp.

Immunohistochemistry—Human placenta tissue sections were prepared short after cesarean section delivery and snap-frozen in liquid nitrogen (tissue material kindly provided by Dr. C. A. Van Meir, Department of Obstetrics and Gynecology, Leiden University Medical Center, Leiden, The Netherlands). Tissue sections were stored at −80 °C. Serial 5-µm-thick cryostat sections of placenta tissue were cut at −25 °C and collected on gelatin-coated glass slides. Tissue sections were dried overnight at 50 °C, fixed in acetone for 10 min, and blocked with 40% human serum and 3% bovine serum albumin in phosphate-buffered saline for 30 min. Next, the sections were incubated with primary Abs against keratin-7 (OVTL1/320; IgG1, 1:1000, DAKO, Carpintera, CA), HLA-G (G233, IgG2a, 1:200, kindly provided by Dr. J. W. Loke and Dr. A. King), CREB/ATF (sc-570, 4 µg/ml, Santa Cruz Biotechnology), and CBP (sc-7300, 4 µg/ml, Santa Cruz Biotechnology) for 30 min. The Abs DAK-G01 (IgG1; 1:50) and DAK-G05 (IgG2a, 1:1000, DAKO) were used as isotype controls. After three washes with phosphate-buffered saline, the tissue sections were further incubated with labeled polymer conjugated to goat anti-mouse IgG (Envision + System, DAKO) for 30 min. Tissue sections were washed three times and incubated with 0.5 mg/ml 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma) in 50 mM Tris HCl, pH 7.6, containing 0.03% H2O2 for 5 min to give a brown-colored reaction product. The sections were counterstained with hematoxylin.

RESULTS
Promoter Structure and Regulatory Sites of HLA-G—Our previous studies have shown that the classical regulatory elements found in other MHC class I proximal promoter regions, such as enhancer A, the ISRE, and SXY module, were not intact in the HLA-G promoter. Since HLA-G lacked these conserved regulatory regions in its proximal promoter region, we searched for putative regulatory elements in the extended promoter region of HLA-G that could provide for alternative transcriptional control mechanisms.

IFN Regulatory Sites in the HLA-G Promoter—A computer-aided search in the promoter region first led to the identification of one putative ISRE and two putative GAS sites. The two putative GAS sites (designated GAS, 1302, and GAS, 955) did not show any detectable STAT1 binding after IFNγ stimulation in JEG-3 nuclear extracts, whereas STAT1 binding to a consensus STAT1 binding site was readily detectable (data not shown). In contrast, the putative ISRE site (designated ISRE, 754) showed binding of IRF1 using nuclear extracts from IFNγ-stimulated JEG-3 cells, but no binding of IRF2, IRF3, and STAT1 was detected (Fig. 1A). This IRF1 binding to ISRE, 754 raised the possibility of this site to mediate IFNγ-induced transactivation of HLA-G. However, in transient transfection assays, no effect has been detected of IFNγ on HLA-G promoter activity in JEG-3 cells (Fig. 1B). Similarly, induction of HLA-G promoter activity by IFNα and IFNβ was negligible in JEG-3 cells (Fig. 1B). Previously, a repression of ISRE-mediated induction by IFNγ was detected in trophoblast-derived cell lines (18). Since this trophoblast-specific repression could be the cause of the repressed IFN induction of HLA-G in JEG-3 cells, we also tested IFN responses in Tera-2 cells, in which classical MHC class I genes are readily induced by IFNγ (16). However, also in this cell type, the promoter activity of HLA-G by IFNβ or IFNγ was not induced at greater than 2-fold (Fig. 1C) despite a strong induction of HLA-B and β2m promoter activity by these IFNs. This shows that these ISRE and GAS boxes do not play a significant role in HLA-G transactivation.
HLA-G Transactivation by CREB

The most upstream of the three sequences (CRE$_{1380}$) was flanked by a second potential CRE/TRE site. Interestingly, this double CRE/TRE site (CRE$_{1380}$) was positioned in the region shown previously to be important for the tissue-specific expression of HLA-G in transgenic mice (21).

We first tested the three CRE/TRE sites (CRE$_{1380}$, CRE$_{930}$, and CRE$_{770}$) for binding activity of proteins of the CREB/ATF and Fos/Jun families of transcription factors in vitro. The most upstream CRE/TRE site (CRE$_{1380}$) showed binding of complexes containing CREB1, ATF1, and c-Jun (Fig. 2). The second CRE/TRE site (CRE$_{930}$) predominantly showed binding of a complex that contained predominantly CREB1, whereas the third CRE/TRE site (CRE$_{770}$) showed binding of CREB1 and ATF1 (Fig. 2). This implies that the CRE/TRE sites bind different heterodimers, which could be formed between CREB1, ATF1, and c-Jun, and that CREB1 was the predominant factor in the complexes binding to these sites.

To establish the in vivo binding of CREB/ATF and Fos/Jun proteins, we performed a ChIP assay. Using this assay, immunoprecipitation of cross-linked JEG-3 DNA with Abs recognizing CREB/ATF, CREB, c-Fos, or c-Jun showed the in vivo binding of CREB1 and c-Jun to the upstream of the HLA-G promoter region around CRE$_{1380}$ in JEG-3 cells (Fig. 3). However, this binding appeared not to be tissue-specific because in vivo CREB binding to the HLA-G promoter was also detected in Tera-2 and Raji cells (data not shown).

**CREB Regulates HLA-G Promoter Activity**—The contribution of these CRE/TRE sites to HLA-G transactivation was tested in a reporter gene assay using a construct encompassing the 1438-bp promoter region of HLA-G. Both CREB1 and ATF1 could enhance HLA-G promoter activity (Fig. 4A). However, co-transfection of CREB1 with ATF1 could not further enhance transactivation (Fig. 4A). Interestingly, HLA-B promoter activity was not significantly induced by CREB1 in JEG-3 cells (data not shown). Co-transfection of CREB1 with the coactivators CBP and p300 also enhanced CREB1-induced transactivation of HLA-G (Fig. 4B). Neither c-Fos nor c-Jun could significantly stimulate promoter activity of HLA-G (data not shown). CREB1 could also enhance HLA-G promoter activity in Tera-2 cells, indicating that for this CREB-mediated transactivation, no trophoblast-specific transcription factors are required (data not shown).

The role of CREB in the transactivation of HLA-G was further investigated by testing the effect of ICER, which can repress CREB-mediated transcription (22). In transient co-transfection experiments, ICER strongly inhibited the CREB-induced transactivation of HLA-G (Fig. 4C). At higher concentrations (1–8 μg/well), ICER was also able to reduce the basal promoter activity of HLA-G (88–28% of normal; data not shown).

To investigate the importance of the individual CRE/TRE sites in the transactivation of HLA-G, we introduced mutations in the CRE/TRE sites CRE$_{1380}$, CRE$_{930}$, and CRE$_{770}$. Transient transfection assays of these modified HLA-G constructs, bearing a mutation in the individual CRE/TRE sites (pGL3-Gm1, pGL3-Gm2, and pGL3-Gm3) or in all three CRE/TRE sites (pGL3-Gm1–2–3), revealed that mutation in only one of the three CRE sites resulted only in a strong reduction of transactivation, whereas mutation of all three CRE sites abrogated the ability of CREB to enhance the transactivation of HLA-G (Fig. 4D). Furthermore, the basal levels of HLA-G transactivation of the mutation constructs were reduced to a various extent, being the lowest for the HLA-G construct with CRE$_{1380}$ mutated (pGL3-Gm1; Fig. 4D). This indicates that the contribution of CRE$_{1380}$ was most important followed by CRE$_{930}$ and CRE$_{770}$. Similar results were observed in other
cell lines, indicating that the importance of the three CRE/TRE sites for the basal level of promoter activity was ubiquitous (data not shown). Together, this demonstrates that the three CRE/TRE sites in the promoter region of \textit{HLA-G} are important for the CREB-mediated transactivation of \textit{HLA-G}.

\textbf{HLA-G Is Co-expressed with CREB and CBP in Extravillous Cytotrophoblasts}—The promoter binding and regulation of \textit{HLA-G} by CREB in the trophoblast cell line JEG-3 inferred co-expression of HLA-G with its regulating transcription factors in trophoblast tissue. To investigate the physiological relevance of this transactivation pathway, we tested for the \textit{in situ} expression of CREB, CBP, and HLA-G by immunohistochemical analysis. In caesarian section-derived placenta tissue, the extravillous cytotrophoblast cells were identified on the basis of their morphology and their strong staining for keratin-7 (data not shown). These extravillous cytotrophoblasts also stained strongly for HLA-G, particularly on the cell membrane (Fig. 5B). This expression of HLA-G in the cells coincided with the predominantly nuclear staining of CREB/ATF and CBP (Fig. 5, C and D). The \textit{in situ} nuclear localization of CREB/ATF and CBP indicates that these transcription factors could fulfil their role in \textit{HLA-G} transactivation in trophoblast cells.

\textbf{DISCUSSION}

Detailed studies have shown that HLA-G is found in extravillous cytotrophoblasts and few other tissues. The limited tissue distribution suggests that HLA-G has specialized immunological functions during pregnancy (3, 11, 23).

Among the MHC class I molecules, the regulation of HLA-G is not well understood. HLA-G is not regulated by the transactivation pathways that govern the expression of the classical MHC class I molecules (Fig. 6). Conserved \textit{cis}-acting regulatory elements that are known to govern the transcription of the classical class I molecules, such as enhancer A, the ISRE, and the SXY module, diverge significantly in HLA-G (18). The two putative H\textsubscript{9260}B sites of enhancer A of HLA-G have been shown to bind predominantly the p50 homodimer of NF-H\textsubscript{9260}B using nuclear extract of B cells (15). However, in trophoblast cells, there is no (basal) NF-H\textsubscript{9260}B expression, and it is barely induced by tumor necrosis factor-H\textsubscript{9251}. Furthermore, p50 homodimers do not possess transactivation properties, which explains the lack of response to tumor necrosis factor-H\textsubscript{9251} and the lack of activation by cotransfected NF-H\textsubscript{9260}B in reporter gene assays. In addition, the most upstream of the two H\textsubscript{9260}B sites (H\textsubscript{9260}B2) has been found to bind also Sp1 (15). The directly flanking and downstream positioned ISRE region is partly deleted and has no binding affinity for IRF1, IRF2, p48, or STAT1 (16). However, a probe encompassing this ISRE region was able to bind Sp1. It is

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possible that Sp1 acting through these sites could contribute to the basal level of HLA-G transactivation. The SXY module of HLA-G is also different from the consensus sequences of the X1, X2, and Y boxes. Although others have detected a weak binding of RFX to the X1 box of HLA-G (24), our efforts to determine binding of protein complexes to the SXY module with electrophoretic mobility shift assay favoring multiprotein complex formation did not reveal any significant binding of the RFX/H18528 CREB/ATF/H18528 NFY complex, such as that observed for classical MHC class I genes (19). This explains the lack of induction of HLA-G by CIITA (14, 18).

Fig. 4. The HLA-G promoter is regulated by CREB1, ATF1, and the coactivators CBP and p300. A, transient transfection of the HLA-G reporter construct pGL3–1500 with CREB1 and ATF1 expression vectors (1 μg of each) in JEG-3 cells showing the induced promoter activity by CREB1 and ATF1. RLU, relative light units. B, transient transfection of HLA-G reporter construct pGL3–1500 with CREB1 (0.5 μg) and CBP/p300 expression vectors (1 μg of each) in JEG-3 cells showing the further enhanced promoter activity by the coactivators. C, transient transfection of HLA-G reporter construct pGL3–1500 with CREB1 (1 μg) and ICER expression vectors (0.5 μg) in JEG-3 cells showing repression of CREB enhanced promoter activity by ICER. D, transient transfection of HLA-G reporter constructs bearing mutations in CRE–1380 (pGL3-Gm1), CRE–920 (pGL3-Gm2), or CRE–775 (pGL3-Gm3) or in all three CRE/TRE sites (pGL3-Gm1–2–3) with CREB expression vector (1 μg) in JEG-3 cells. The luciferase activity values were normalized with the Renilla luciferase activity values and are expressed as mean ± S.D. of n = 4. The induction ratios are indicated above the histogram.

Fig. 5. Co-localization of HLA-G and its regulatory transcription factors CREB and CBP in extravillous cytotrophoblasts. Immunohistochemical staining of keratin-19 positive (not shown) population of extravillous cytotrophoblasts showing the cell surface expression of HLA-G (B), the nuclear staining of CREB/ATF (C), and CBP (D). Isotype staining served as control (A).

Fig. 6. Schematic representation of the promoter of HLA-G. Three functional CRE/TRE sites located in the upstream promoter region of HLA-G are binding sites for CREB1, ATF1, and c-Jun. Although the ISRE in HLA-G is able to bind IRF1, this site has no clear role in HLA-G transactivation. The classical regulatory elements, such as the enhancer A (enhA), ISRE, and SXY module, deviate from those found in the promoters of classical MHC class I genes (e.g., HLA-B). This results in the more restricted binding of NF-κB p50 homodimers and Sp1 in the enhancer A region.
There is still much debate as to whether HLA-G is responsive to IFN. Previous reports have indicated that HLA-G is weakly induced by IFNα and IFNβ (25). In this study, we identified one putative ISRE and two putative GAS sites. Indeed, the putative ISRE site (ISRE$_{-74}$) showed binding of IRF1 using nuclear extracts from IFNγ-stimulated JEG-3 cells. However, no STAT1 binding was detected to the two putative GAS sites (GAS$_{-1020}$ and GAS$_{-985}$), which is in line with findings by others (26). The binding of IRF1 to the ISRE$_{-74}$ raised the possibility that IFNγ could induce transactivation of HLA-G. However, in transient transfection assays, no effect was detected of IFNγ on HLA-G promoter activity in JEG-3 cells. Similarly, induction of HLA-G promoter activity by IFNα and IFNβ was negligible in JEG-3 cells. Also in Tera-2 cells, the promoter activity of HLA-G was never induced more strongly than 2-fold by IFNβ or IFNγ, which was in contrast to the strong induction of the promoter activity of HLA-B and β2m by these IFNs. We therefore concluded that the extended promoter of HLA-G is not significantly responsive to stimulation by IFN.

In our search for alternative regulatory pathways that control the transcription of HLA-G, we identified three CRE/TRE elements in the promoter region of HLA-G (Fig. 6). Using the trophoblast-derived cell line JEG-3, the sites bound CREB1, ATF1, and c-Jun in vitro. The binding of different proteins of the CREB/ATF and FoxO/jun families of proteins allows the formation of various dimers. However, the precise composition of the protein complexes binding to the promoter is not certain, and the participation of other transcription factors cannot be excluded. Furthermore, in ChIP assays, CREB1 and c-Jun were found associated to the upstream promoter region containing CRE$_{-1380}$ in vivo. Previously, this region has been associated with the tissue-specific expression of HLA-G in transgenic mice and was shown to display protein binding activity (21, 27).

Our data show that HLA-G transactivation is regulated by CREB since CREB augmented promoter activity and ICER reduced CREB-mediated promoter activity in reporter assays. In addition, the activation by CREB was further enhanced by overexpression of the coactivators CBP/p300. Furthermore, mutational analysis revealed the importance of all three CRE/TRE sites in the regulation of HLA-G transcription by CREB and the basal level of promoter activity of HLA-G. CREB1 could also enhance HLA-G promoter activity in non-trophoblast cell lines, indicating that for this CREB-mediated transactivation, no trophoblast-specific transcription factors are required. However, HLA-B promoter activity was not significantly induced by CREB1 in JEG-3 cells, suggesting a differential regulation of HLA-G versus classical MHC class I promoters in trophoblasts.

CREB is thought to be an important factor for the expression of genes in trophoblasts and during differentiation. Similar to HLA-G, the α subunit of glycoprotein hormone is regulated by multiple sites including CRE (28–30). However, the tissue-specific expression of the α subunit of glycoprotein hormone was controlled by an additional regulatory element mediating the trophoblast-specific expression that is not found in the HLA-G promoter. Moreover, CREB/ATF proteins are ubiquitously expressed transcription factors, and ChIP analysis revealed the binding of CREB to the HLA-G promoter in several cell lines not expressing HLA-G. Therefore, they are not likely to account for tissue-specific expression, although it cannot be excluded that a difference between trophoblasts and other cells in the composition of the CREB/ATF dimer complex binding the CRE sites contributes to the trophoblast-specific expression (31, 32). Therefore, it remains to be determined whether a specific regulatory site or transcription factor(s) is responsible for the trophoblast-specific expression of HLA-G. It could also be envisaged that more general mechanisms play a role in the tissue-specific expression or silencing of HLA-G, such as promoter methylation. Indeed, treatment with 5-azaacytidine brought about expression of HLA-G in several cell lines lacking HLA-G expression (JAR, HeLa) as detected by reverse transcriptase-PCR, whereas in other cell lines, no induction of HLA-G expression was observed. However, in vitro promoter methylation of the pGL3-G1438 reporter construct only resulted in a weak reduction of promoter activity and of CREB-induced transactivation. Moreover, in several cell lines lacking HLA-G expression (Tera-2, Jurkat), no methylation of the HLA-G promoter was observed. This suggests that, although it may contribute, promoter methylation is certainly not the sole mechanism securing a tissue-specific expression of HLA-G (33). Another general epigenetic mechanism that could play a role in the trophoblast-specific expression of HLA-G is the recruitment of histone deacetylase activity. As stated before, the HLA-G promoter contains two CRE sites in enhancer A, which display binding affinity specifically for p50 homodimers (15). Since the p50 subunit of NF-kB does not possess transactivating activity and NF-kB sites are present in most cell types but not in trophoblast cells, the functional relevance of this binding in non-trophoblast cells has remained unclear. However, a recent study has attributed a role for p50 to establish transcriptional silencing through its recruitment of HDAC1 (34). It could be speculated that in non-trophoblast cells, p50 homodimer binding to the CRE sites of HLA-G recruits HDAC1, which represses HLA-G transcription and as such contributes to the tissue-specific expression of HLA-G.

Taken together, multiple CRE/TRE sites in the promoter region of HLA-G mediate its regulation by CREB/ATF factors. This is the first report of a regulatory pathway acting through the 1438-bp promoter region of HLA-G.

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