Methylation of Class II trans-Activator Promoter IV: A Novel Mechanism of MHC Class II Gene Control

Ann C. Morris, Wendi E. Spangler and Jeremy M. Boss

*J Immunol* 2000; 164:4143-4149; doi: 10.4049/jimmunol.164.8.4143

http://www.jimmunol.org/content/164/8/4143

---

**References**

This article cites 57 articles, 32 of which you can access for free at:

http://www.jimmunol.org/content/164/8/4143.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Inhibition of class II trans-activator (CIITA) expression prevents embryonic trophoblast cells from up-regulating MHC class II genes in response to IFN-γ. This is thought to be one mechanism of maternal tolerance to the fetal allograft. The CIITA gene is regulated by four distinct promoters; promoter III directs constitutive (B cell) expression, and promoter IV regulates IFN-γ-inducible expression. Using in vivo genomic footprinting, promoter-reporter analysis, Southern blot analysis, and RT-PCR, we have examined the cause of CIITA silencing in a trophoblast-derived cell line. We report here that methylation of promoter IV DNA at CpG sites in Jar cells prevents promoter occupancy and IFN-γ-inducible transcription. The inhibition of CpG methylation in Jar cells by treatment with 5-aza-2'-deoxycytidine restores IFN-γ-inducibility to CIITA. This is the first description of an epigenetic mechanism involved in regulation of CIITA and MHC class II gene expression. The Journal of Immunology, 2000, 164: 4143–4149.

The MHC class II molecules are α/β heterodimers that participate in the adaptive arm of the immune response by presenting endogenously derived antigenic peptides to CD4+ T cells. Although the normal pattern of expression of MHC class II genes is restricted to APCs, thymic epithelium, and B cells (1, 2), class II induction can occur on most cell types through exposure to various cytokines, the most potent of which is IFN-γ (1, 3–5). Expression of MHC class II on cells other than APCs can aid in the initiation of an acute immune response. However, aberrant expression of class II in inappropriate tissues, such as in cases of autoimmune disease, can have deleterious consequences. It is therefore crucial that the timing and location of expression of class II molecules be carefully controlled.

The genes encoding the three MHC class II isotypes, HLA-DR, -DQ, and –DP, as well as the HLA-DM and invariant chain genes are all coordinately regulated at the transcriptional level by a set of conserved cis-acting promoter elements termed the W, X1, X2, and Y boxes (reviewed in Refs. 2 and 6). The X1 and X2 boxes are both necessary and sufficient to direct class II expression in B cells (7, 8). They are bound by the regulatory factor X (RFX)3 and cAMP response element binding protein (CREB), respectively (9–11). The Y box is required for maximal class II expression and is bound by the heterotrimERIC factor NF-Y (12). The factors that bind the W box have not been well characterized. Although RFX and CREB are both necessary for promoter activity, their presence is not sufficient to activate gene expression. The other essential component of this system is the class II trans-activator (CIITA), a non-DNA-binding protein (13–15). CIITA expression correlates directly with MHC class II expression and is induced by IFN-γ in a time frame that precedes MHC class II gene expression (16–19). Cells negative for CIITA are also MHC class II negative. Thus, CIITA expression functions as a molecular switch for MHC class II gene regulation.

Transcription of CIITA is regulated by four distinct promoters that direct the transcription of four separate first exons spliced to a common second exon (20). Promoter I is involved in dendritic cell expression. The function of promoter II is unknown. Promoter III drives constitutive expression of CIITA, such as that observed in B cells, and promoter IV controls the IFN-γ-inducible expression of CIITA seen in most other cell types (20). There has also been a report that promoter III contributes to IFN-γ-inducible CIITA expression (21).

The inhibition of expression of MHC class II genes on fetal trophoblast cells is one of the numerous mechanisms that have been proposed to explain the phenomenon of maternal-fetal tolerance during pregnancy (22–31), whereby the maternal immune system fails to react to placental tissues expressing both maternal and paternal genes. Not only do fetal trophoblasts lack constitutive expression of class II, they also resist induction of class II mRNA when exposed to IFN-γ (although other IFN-γ-inducible genes are still responsive) (32–34). There is evidence that inhibition of class II gene expression on trophoblast cells is important for pregnancy outcome, as in some cases of chronic villitis of unestablished etiology (a condition associated with recurrent spontaneous abortions) class II expression is observed in the inflamed regions of the placenta (35, 36). We and others have recently shown that CIITA gene expression is absent and unable to be induced by IFN-γ treatment in trophoblast-derived cell lines (34, 37). Introduction of a CIITA expression plasmid into these cell lines restores MHC class II gene expression at both the mRNA and protein levels, indicating that inhibition of CIITA expression prevents class II transcription in this cell type. The mechanism of CIITA transcription inhibition in these cells is unknown.

There is considerable evidence that methylation of CpG dinucleotides can negatively affect transcription, either directly, by preventing transcription factor access to the DNA, or indirectly, by
recruiting repressor molecules that bind methylated CpGs (reviewed in Ref. 38). In mammals, DNA methylation has been shown to be indispensable for development, as mice homozygous for a mutant DNA methyltransferase gene fail to develop past midgestation (39). In many cases, re-expression of genes shut down by methylation can be achieved by exposure to 5-aza-2’-deoxycytidine (5AC), an inhibitor of DNA methyltransferase (40).

In this report we have examined the cause of CIITA silencing in trophoblast-derived chorioncarcinoma cells. Our results suggest that CIITA expression is prevented due to a failure to assemble regulatory factors at both CIITA promoters III and IV, but that the factors needed for CIITA transcription are present and can activate reporter gene expression. We present evidence suggesting that the absence of factor binding at pIV is caused by methylation of promoter IV DNA.

Materials and Methods

Cells and cell culture

Raji, a B cell line derived from a patient with Burkitt’s lymphoma (41), was grown in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 5% FCS (HyClone, Logan, UT) and 5% bovine calf serum (HyClone). A431 (CRL-1555, American Type Culture Collection, Manassas, VA), a human vulvar epidermoid cancer cell line, was grown in DMEM (Mediatech, Washington, DC) supplemented with 10% bovine calf serum. Jar (HTB-144, American Type Culture Collection), a chorioncarcinoma cell line, was grown in RPMI 1640 medium supplemented with 10% FCS. JEG-3 (HTB-36, American Type Culture Collection), another chorioncarcinoma cell line, was grown in DMEM supplemented with 10% FCS. All cell culture medium was supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100 U/ml streptomycin (Life Technologies). For some experiments, 5AC (Sigma, St. Louis, MO) was added to cells to a final concentration of 0.75–1 µM. Cells were incubated in 5AC for 3–7 days, at which time the drug was washed out and grown in fresh medium with or without 200–500 U/ml IFN-γ (Biogen, Cambridge, MA) for the indicated time.

In vivo genomic footprinting (IVGF)

IVGF was conducted as previously described (42, 43). Where indicated, cells were treated with IFN-γ for 8–24 h. The oligonucleotide sequences of the first-strand, PCR, and extension primers were as follows: pIII coding strand, 5’-TCCCTCTACCACTTTTAACTTCCCCC, 5’-TGCTTCTTAG ACGGCAGACC CGC, and 5’-CTGGCAAGCCGGAAGGTGAGGAC; pIII noncoding strand, 5’-GAAGGTTGGCCATATTGCGAGCT, 5’-CAACCTGG TGAGACCTGCACTGA, and 5’-GTGCTCCCCACAGACGTTCGTG CCAAC; pII coding strand, 5’-CTCGTGGTGGCTCCTCCCT, 5’-GCC GGAAGTCTGGTCGACCT, and 5’-AGTCTGTGGCAGCTGGTCCG TCGGTT; and pIV noncoding strand, 5’-AGAAGAAAACAGAGAC CACCCAGG, 5’-GGACTTGGCACTGACATTGGCCCAAG, and 5’- CATCTGGCAAGTGCTTCCCTTGCTCCT.

DNA constructions, transient transfections, and reporter assays

P3CIITA.CAT was constructed from pCAT-Basic (Promega, Madison, WI) and contains 320 bp of CIITA promoter III cloned upstream of the chloramphenicol acetyltransferase (CAT) gene. Similarly, p4CIITA.CAT contains 393 bp of CIITA promoter IV cloned upstream of CAT, and p3-p4CIITA.CAT contains promoters III and IV, as well as the genomic sequence between the two, cloned upstream of CAT.

Seven hundred picomoles of the CIITA promoter-reporter constructs and 2 µg of the luciferase control vector pGL3 (Promega) were cotransfected into Jar or JEG-3 cells by electroporation as described previously (44). Cells were harvested 2 days after transfection. CAT expression was determined by ELISA (Roche, Indianapolis, IN) according to the manufacturer’s instructions. Luciferase activity was measured on a FB12 Lumino-meter (Zylux, Maryville, TN) and was used to normalize the transfections for transcription efficiency. The results shown are the average of three independent transfections.

Southern blot analysis

Genomic DNA was isolated from Raji, A431, Jar, and JEG-3 cells as described previously (45). Five micrograms of genomic DNA was digested overnight with 10 U/µg of the indicated restriction enzyme. The digests were loaded onto a 1% agarose gel and transferred to nylon membrane. The membrane was then hybridized to a radiolabeled probe containing 393 bp of promoter IV. Bands were visualized by autoradiography or on a Molecular Dynamics Storm 860 PhosphorImager (Sunnyvale, CA).

RT-PCR analysis

Total cytoplasmic RNA was isolated from cells using the NuSpectro P-40 lysis method, and RT-PCR was conducted as described previously (34). For the CIITA and DRA RT-PCR, 1.5 µg of RNA was used per reaction. For the GAPDH RT-PCR, 0.5 µg of RNA was used. PCR for 30 s at 94°C, 30 s at 55–60°C (depending on the gene), and 1 min at 72°C for the indicated number of cycles was performed. RT-PCR products were visualized on 1–1.5% agarose gels. The sequences of the PCR primers used are as follows: GAPDH5’, 5’-CCATGGGGAAGGTGAGGAC; GAPDH3’, 5’-GAGAGTGGGTGTCGCAAGTCTGTGTTTGAAGTC; HLA-DRA5’, 5’-CGACAAGTTACCCACCAACAGT; HLA-DRA3’, 5’-CAGGAAAAAG GCAATAGCAGG; CIITA-17, 5’-CCACCTTGATGACCCAGTTGAC; and CIITA-38, 5’-CGTTGACAGTGATCCACT.

In vitro methylation

In vitro methylation reactions included 20 µg of plasmid DNA, 5 mM S-adenosylmethionine, and 8 U of SsoI (or water for mock reactions). Methylation was conducted at 37°C for 8 h. Reactions were then extracted with phenol/chloroform and precipitated with ethanol. Plasmid methylation was confirmed by restriction digestion with the methylation-sensitive enzyme HpaII.

Results

Factor assembly at CIITA promoters III and IV does not occur in trophoblast cells

Jar chorioncarcinoma cells were chosen for analysis because these cells showed the greatest response to transfected CIITA, were easy to grow in the laboratory, and, as shown below, can be induced under certain conditions to express MHC class II genes. Jar cells have been shown to be representative of some fetoplacental trophoblast cells and have been used as a model of these cells in many studies (30, 32, 46–48). JEG-3, another chorioncarcinoma cell line (30, 46, 47, 49), was used in some of the analyses as well.

Previous work demonstrated that CIITA expression in trophoblast cells is blocked at the transcriptional level (34, 37). To determine the molecular mechanism responsible for this block, transcription factor assembly at the CIITA promoter was examined by IVGF. As described above, CIITA is expressed from multiple promoters. PIIR is responsible for constitutive expression in B cells, and pIV is primarily responsible for IFN-γ-inducible expression. Both regulatory regions are correlated to the start site transcription of their respective first exons. The in vivo footprints of genomic DNA isolated from Raji B cells (express CIITA constitutively), A431 epithelial cells with or without IFN-γ (express CIITA after stimulation with IFN-γ), and Jar cells with or without IFN-γ (do not express CIITA under either condition) were compared. Both promoters III and IV were examined, because although pIIIR has mostly been associated with constitutive expression of CIITA in B cells, there is evidence suggesting that PIIR also contributes to IFN-γ-inducible expression (21).

IVGF analysis of CIITA pIIIR in Raji B cells revealed two regions of factor occupancy, which we have designated IIA and IIIB, centered at −138 and −57, respectively, from the pIIIR transcription start site (Fig. 1). The IIB region also displayed a constitutively hypersensitive G on the coding strand in both Raji and A431 cells. The IIA and IIIB sites as well as a third site centered at −23 designated IICC and possibly extending to −14, became protected in A431 cells upon treatment with IFN-γ, supporting the results and conclusions of Piskurich et al. (21) that elements of promoter III contribute to IFN-γ-inducible, as well as B cell expression of, CIITA. However, these same regions remain unoccupied in Jar cells treated with IFN-γ, suggesting that the factors that bind these sites are either absent or unable to access the promoter.
in this trophoblast cell line. Recently, Ghosh et al. (50) identified several regions of in vivo occupancy within pIII that were important for B cell expression. Three of these regions, 2142 to 2133, 266 to 256, and 227 to 218, correspond to the sites we have identified. Thus, our results confirm those of Ghosh et al. with B cells and also indicate that these sites may be important for IFN-γ induction of CIITA as well.

In CIITA pIV, three sites were previously shown to be required for IFN-γ induction: a GAS element centered at 2137, which binds the factor STAT1, an adjacent E box, to which the factor USF-1 binds, and an IRF-1 site centered at 260 (20, 51). IVGF analysis of the STAT1/E box region of pIV showed no protection in untreated A431 or Jar cells, suggesting that this region is inactive when the gene is off. However, upon IFN-γ treatment of A431 cells, two protected Gs and a hypersensitive site were observed (Fig. 2). The hypersensitive G lies just 3‘ of the E box, one protected G is within the E box, and the other protected G is in between the E box and the GAS element. This pattern is similar to that described previously for pIV by Piskurich et al. (21). No protection was observed on the noncoding strand for promoter IV (data not shown). Furthermore, no protection or hypersensitivity was observed at the promoter IV sites in DNA isolated from Jar cells treated with IFN-γ, indicating that CIITA transcription is prevented in these cells due to an absence of factor assembly at the promoter.

The factors required for transcription of CIITA type IV are present in Jar cells

The absence of a footprint at promoters III and IV of Jar cells could be due either to a lack of expression of the necessary transcription factors in this cell type or to their inability to access promoter DNA. To distinguish between these possibilities, reporter constructs were created that contained either promoter III, promoter IV, or the contiguous DNA containing both promoters III and IV, cloned upstream of a promoterless CAT gene. The constructs were transiently cotransfected with a constitutive luciferase reporter construct into Jar cells. The transfected cells were left untreated or were treated 24 h later with IFN-γ for 16 h. The activity of the CAT reporter gene was determined (Fig. 3). Although the cells that were transfected with the pIII construct (p3CIITA.CAT) showed no induction of CAT activity relative to untreated controls, cells transfected with either the pIV (p4CIITA.CAT) or the contiguous pIII-pIV (p3-p4fullCIITA.CAT) construct showed an increase in CAT activity upon IFN-γ stimulation. The fold induction of the construct containing only pIV was

![Image](http://www.jimmunol.org/)

**FIGURE 1.** Factor assembly does not occur on CIITA promoter III in trophoblasts. IVGF for the promoter III region of the CIITA gene in various cell types is shown for the coding strand (A) and the noncoding strand (B). Lanes marked with a V indicate in vitro methylated DNA. ○, Protected Gs; ●, hypersensitive sites. The dashed line at IIIIC in B indicates a weak protection. The results shown are representative of at least three independent experiments. The genomic sequence of the region examined by IVGF is depicted in C, with the darker boxes indicating regions of strong protection and the lighter box indicating the weak protection at IIIIC.
The GAS and USF-1 elements are occupied in vivo in A431 cells treated with IFN-γ, but not in untreated A431, Raji, or Jar cells. IVGF of the coding strand for the promoter IV region of CIITA is shown, along with a sequence summary of the region. ○, Protection; ●, hypersensitivity. The in vitro methylated DNA lane is marked with a V.

FIGURE 2.

CIITA promoter IV is methylated near the transcription start site in trophoblasts

Because the factors necessary for transcription of CIITA pIV are present in trophoblasts but do not bind the promoter DNA, we investigated the possibility that occupancy of the regulatory regions is prevented by promoter methylation at CpG dinucleotides. Several reports have implicated methylation in the down-regulation of transcription (38, 40, 52, 53). Although CIITA pIII does not contain an appreciable number of CpG dinucleotides, pIV has several that are centered around the transcription start site. To examine the methylation status of CIITA pIV, genomic DNA was prepared from Raji, A431, and Jar cells; digested with the methylation-sensitive restriction enzymes XbaI and SacII or BssHII; and analyzed by Southern blot with a probe specific for pIV (Fig. 4). In Raji cells and A431 cells, XbaI digestion alone yields a 4-kb band (top arrow), whereas complete digestion with XbaI and SacII or BssHII releases a 1-kb band (bottom arrow). The region covered by the probe is indicated under the schematic map of the CIITA promoters.

FIGURE 3.

FIGURE 4.

CpG sites in pIV are methylated in Jar cells, but not in Raji or A431 cells. Genomic DNA from Raji, A431, untreated Jar cells, and Jar cells treated with 5AC was digested with either XbaI alone or XbaI plus SacII or BssHII. Digested DNA was analyzed by Southern blot as described in Materials and Methods. XbaI digestion alone yields an 4-kb band (top arrow), whereas complete digestion with XbaI and SacII or BssHII releases a 1-kb band (bottom arrow). The region covered by the probe is indicated under the schematic map of the CIITA promoters.

two promoters may be regulated differently within the same cell type.

Because the factors necessary for transcription of CIITA pIV are present in trophoblasts but do not bind the promoter DNA, we investigated the possibility that occupancy of the regulatory regions is prevented by promoter methylation at CpG dinucleotides. Several reports have implicated methylation in the down-regulation of transcription (38, 40, 52, 53). Although CIITA pIII does not contain an appreciable number of CpG dinucleotides, pIV has several that are centered around the transcription start site. To examine the methylation status of CIITA pIV, genomic DNA was prepared from Raji, A431, and Jar cells; digested with the methylation-sensitive restriction enzymes SacII and BssHII; and analyzed by Southern blot with a probe specific for pIV (Fig. 4). In Raji cells and A431 cells, SacII and BssHII were able to digest the genomic DNA and produce a pattern representative of nonmethylated DNA. In Jar cells, however, the two sites were not cut, suggesting that the enzyme recognition sequences are methylated in this cell type. To confirm this interpretation, Jar cells were treated with 5AC, an inhibitor of DNA methylation. Exposure of Jar cells...
for 72 h to 5AC rendered them sensitive to digestion with \( \text{SssIII} \), suggesting that at least these two sites are differentially methylated in Jar cells compared with other cell types. A similar observation was made for JEG-3 cells (data not shown).

**Exposure of Jar cells to 5AC restores IFN-\( \gamma \)-inducible expression of CIITA**

The treatment of cells with 5AC has been used in several systems to allow the re-expression of a gene that has been silenced by methylation (reviewed in Ref. 40). Thus, to examine the relevance of pIV methylation to CIITA expression, RNA was prepared from methylation-sensitive enzyme \( \text{HpaII} \). Thus, in vivo factor occupancy cannot be verified.

**In vitro methylation inhibits pIV promoter activity**

To confirm that the re-expression of CIITA observed after 5AC treatment is due to a direct effect at CIITA pIV, reporter constructs methylated in vitro were transiently transfected into Jar cells and assayed for expression. Plasmids were incubated with \( \text{SssI} \) methylase (meRSV CAT and me-p4CIITA.CAT) or with water (RSV CAT and p4CIITA.CAT). Methylation of the constructs was verified by incubation with \( \text{Ncol} \) alone (N) or with \( \text{Ncol} \) and the methylation-sensitive enzyme \( \text{HpaII} \) (N/H). A representative digest is shown. B. Methylated or unmethylated constructions were transfected into Jar cells, and cells transfected with either p4CIITA.CAT or me-p4CIITA.CAT were treated with IFN-\( \gamma \) 24 h after transfection. CAT activity was measured by ELISA 48 h after transfection. The data represent the result of three independent transfections.

**Discussion**

The results of this study indicate that expression of CIITA in Jar cells is inhibited due to a lack of binding of transcription factors at the CIITA promoter upon exposure to IFN-\( \gamma \). This inhibition is probably due to the methylation of CpG dinucleotides within the pIV promoter, as this region, which is responsible for activation of the CIITA gene by IFN-\( \gamma \), was methylated, and inhibition of methylation led to re-expression of the CIITA gene. Moreover, methylated pIV DNA was unable to stimulate expression in a transient transfection assay. Thus, these results provide evidence for epigenetic regulation of CIITA and suggest a novel mechanism for the control of CIITA and ultimately MHC class II genes.

IVGF analysis revealed that sites in pIV that have been shown previously to be required for IFN-\( \gamma \)-inducible expression of CIITA were unoccupied in Jar cells. In addition, sites were found in promoter III that showed differential occupation in CIITA-expressing cells and in Jar cells. A recent report by Ghosh et al. (50) showed that two sites in promoter III, which they designated ARE-1 and ARE-2, were critical for transcriptional activity. They also found several other sites of protein/DNA interaction in pIII, a region designated site A (−27 to −18). However, site A was not required...
for transcription from the pIII promoter. Sites IIIA, IIIB, and IIIC, identified by the IVGF analysis presented here, are located at the same positions as ARE-1, ARE-2, and site A, respectively. Thus, our results confirm the findings of Ghosh et al. and further suggest a role for these regions in IFN-γ regulation of CIITA.

Transient transfection assays demonstrated that pIV is capable of driving transcription of a reporter gene in Jar cells, but pH1 is not. This suggested that the transcription factors that act at pH1 are present in Jar cells and can bind to naked DNA, but are prevented from reaching their target sites on the endogenous gene. One interpretation of this result is that transcriptional silencing is achieved through an epigenetic mechanism. This result is also consistent with earlier findings that STAT-1 and IRF-1, both of which are required for transcription from pIV (20, 51), are expressed in Jar cells. The fact that the pH1 reporter construct was inactive suggests that in this case the regulatory factors that act at promoter III are either missing from Jar cells or are unable to activate transcription. This also explains why the pH1–pIV construct did not show increased CAT activity over pIV alone.

Southern blot and RT-PCR analysis suggested that the lack of basal and IFN-γ-inducible CIITA expression in Jar trophoblasts is due to selective methylation of pH1. We also examined the methylation status of pH1 in another choriocarcinoma cell line, JEG-3, and obtained similar results (data not shown). If methylation of the CIITA promoter also occurs in primary trophoblast cells, this suggests at least one mechanism that may explain maternal tolerance at the fetal-maternal interface. Some studies have examined the affect of chronic exposure to 5AC on class II expression and embryo viability in mice and rats (54–56), and the results have varied. Although Athanassakis-Vassilaidis et al. (54) found that 5AC induced class II expression in the placenta and that this correlated with fetal loss, Gustafsson et al. (55) and Yuan et al. (56) found no evidence of increased class II expression on trophoblast cells after 5AC treatment. However, in none of these studies was the methylation status of the CIITA gene examined either before or after 5AC treatment, so it is unclear whether CIITA re-expression (which is activation for MHC class II gene expression) was achieved.

In addition to the absence of class II, trophoblast cells do not express classical MHC class I molecules, and this is also thought to play a role in maternal tolerance to the fetal allograft. MHC class I genes have been shown to be regulated in part by CIITA through its action at the site α region of the class I promoter (57). Site α is part of a X1, X2, Y box module that is homologous to the MHC class II X-Y box region. Jar cells treated with 5AC re-express class I (58), and it is therefore tempting to speculate that methylation of CIITA may be at least partially responsible for the class I-negative phenotype of these cells. Recent findings that transfection of trophoblast cells with CIITA restores class I expression (49) support this hypothesis. The methylation status of MHC class I genes in trophoblastic cell lines was examined by Guillaudeux et al. (59) and was found to vary depending on the cell line examined. It was therefore concluded that methylation did not play a role in inhibition of class I gene expression in trophoblasts. It is possible, however, that methylation could play a role in class I expression by acting through inhibition of CIITA gene expression rather than at the class I loci themselves.

This is the first time that methylation has been demonstrated to regulate the expression of CIITA, and it suggests a way in which one might experimentally control class I for therapeutic intervention. Future studies will explore whether methylation is specific for cells of trophoblast origin or if it is a global mechanism for regulation of CIITA in class II-negative tissues and during development.

Acknowledgments

We thank Dr. M. Brown for critical reading of the manuscript.

References


