Class II Transactivator Regulates the Expression of Multiple Genes Involved in Antigen Presentation

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Summary
CIITA (a major histocompatibility complex [MHC] class II transactivator) has been shown to be required for the expression of MHC class II genes in both B cells and interferon-γ-inducible cells. Here we demonstrate that CIITA not only activates MHC class II genes but also genes required for antigen presentation. Mutant HeLa cells, defective in the expression of classic MHC class II genes, invariant chain, and the newly described human histocompatibility leukocyte antigen-DM genes, were used to study the role of CIITA in the regulation of these genes. Upon transfection with CIITA cDNA, the mutant cells expressed all three genes, suggesting that CIITA is a global regulator for the expression of genes involved in antigen presentation.

Materials and Methods

Cell Culture. All cell lines used were maintained in DMEM supplemented with 10% fetal bovine serum and 2 mM glutamine.

Human IFN-γ (gift from Biogen, Cambridge, MA) was used at a concentration of 500 U/ml.

Transfections. Mutant cells were cotransfected with CIITA cDNA or JAK1 cDNA along with the hygromycin gene via the calcium phosphate method. 2 d after transfection, cells were split 1:2 into medium containing 400 µg of hygromycin (Calbiochem Corp., La Jolla, CA). BLS-2 cells were transiently transfected with either control DNA or CIITA cDNA. Cells were harvested 2 d after transfection and RNA was analyzed.

Flow Cytometry Analysis. Cells were stained for HLA-DR surface expression with L243, and secondary staining was performed with PE-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL). The stained cells were analyzed with FACStar (Becton Dickinson & Co., Mountain View, CA).

PCR. RNA preparation, cDNA synthesis, and PCR conditions were described previously (2). Sequences of oligonucleotides for primers used in PCR amplification are as follows: Ii sense, TCCCAAGCCTGTGAGCAAGATG; Ii antisense, CAGTTCCA-GTGACTCTTTCG; DMB sense, TCCTTCAACAAGGATCTGCTG; DMB antisense, CTTCCTCACTGTAGTCAC. The primer sequences for HLA-DR and actin genes were as described (2). Procedures used for lysis, electrophoresis, and blotting were described previously (10). Each lane contained 10⁶ cell equivalent amount of protein.

Results and Discussion

The mutant HeLa cells that we used are defective in the tyrosine kinase JAK1, which is necessary for IFN signaling. Consequently they do not express most IFN-γ-inducible genes, including class II genes (9). It has been shown that the transfection of JAK1 cDNA can rescue the IFN-γ-responsive phenotype (10; Fig. 1). In addition, we have also demonstrated that the transfection of CIITA cDNA activates the expression of MHC class II genes even before IFN-γ induction, suggesting that CIITA is both necessary and sufficient for the expression of MHC class II genes, and furthermore, class II transactivator (CIITA) has initially been isolated and characterized as a transcriptional coactivator for the expression of MHC class II genes (1). CIITA is also necessary and sufficient for the IFN-γ-inducible expression of class II genes (2, 3). The expression of CIITA itself is IFN-γ inducible, and its induction does not require de novo protein synthesis (2, 3). We have also demonstrated that the tyrosine kinase JAK1 is required for the transcription of the CIITA gene after IFN-γ stimulation (2). Although CIITA activates the transcription of class II genes, it does not seem to have DNA-binding activity, suggesting that it may act through other DNA-binding proteins (1).

To serve as APC to CD4 T cells, cells must express not only MHC class II molecules on the cell surface, but also genes necessary for antigen processing and presentation, including the invariant chain (Ii) and the newly described HLA-DM molecules, which are distant evolutionary homologs of HLA-D genes (4–7). The promoter structure and the regulation of transcription of the MHC class II genes and the Ii genes are similar, and they share common regulatory cis-acting DNA elements (8). Although the regulation of HLA-DM is not yet well understood, the promoter structure of DM genes is similar to that of class II genes, suggesting that they may share common regulatory factors for their expression (4). In addition, the expression of HLA-DM is restricted to APC and it is also IFN-γ inducible. Therefore, it is reasonable to speculate that CIITA may regulate not only the classic MHC class II genes but also Ii and DM genes. To test this hypothesis, we used mutant cells which were isolated and characterized previously (9).
that the constitutive expression of CIITA can bypass the requirement for the tyrosine kinase JAK1 (2; Fig. 1). Therefore, we used these mutant cells to examine whether CIITA plays a role in the regulation of genes involved in antigen presentation. As shown in Fig. 2a, mutant HeLa cells, E2A4, do not express HLA-DR, Ii, or HLA-DM genes even after IFN-γ treatment. However, CIITA-transfected mutant cells show a high basal level of transcripts for both Ii and HLA-DM before IFN-γ treatment, and as expected, the level is not further enhanced after IFN-γ treatment because CIITA cDNA is expressed under the control of a constitutive promoter in these transfectants. Ii and HLA-DM transcripts in wild-type HeLa cells and JAK1-transfected mutant cells are inducible upon IFN-γ stimulation. There is, however, a basal level of HLA-DM expression in uninduced wild-type and JAK1-transfected mutant HeLa cells that is not detectable in the mutant cells, suggesting that JAK1 is also required for this (Fig. 2a). This may reflect additional transcriptional controls used for HLA-DM but not Ii or HLA-DR, or alternatively there is a low basal level of CIITA, and HLA-DM may have a greater sensitivity to transactivation.

To determine the level of HLA-DM protein, Western blot analysis was performed. In agreement with the RNA data, mutant HeLa cells have no detectable HLA-DM protein, but cells transfected with CIITA cDNA show constitutive-level HLA-DM protein (Fig. 2b, lanes 3-6). In contrast, HeLa cells show normal inducible expression of HLA-DM protein (Fig. 2b, lanes 1 and 2).

We also tested the effect of CIITA on the expression of both Ii and HLA-DM genes with BLS-2 cells. BLS-2 cells have been shown to have a defective CIITA gene, which results in the lack of MHC class II gene expression (1). As predicted from the HeLa cell results, HLA-DM expression is increased drastically in cells transfected with CIITA cDNA. Ii tran-
scripts, however, show a basal level, which is somewhat in-
duced after transfection with CIITA (Fig. 3). Similarly, a low
basal level of HLA-DR transcripts are present in BLS-2 cells,
although the level is greatly increased in BLS-2 cells trans-
fected with the CIITA gene or human B cells (Fig. 3).

We have shown here that CIITA regulates not only MHC
class II genes but also the Ii and HLA-DM genes, which are
absolutely required for antigen presentation. CIITA, there-
fore, seems to have a global role in the regulation of genes
that participate in antigen presentation. It is not clear why
CIITA-defective BLS-2 cells have residual-level transcripts for
HLA-DR, Ii, and HLA-DM genes. It is possible that the
mutated form of CIITA molecule in BLS-2 cells may have
residual transactivational function, but the efficiency of trans-
activation is in any event greatly reduced. Our study with
mutant HeLa cells, however, clearly demonstrates that the
expression of Ii and HLA-DM genes in addition to HLA-
DR genes is coregulated by CIITA upon IFN-γ induction.

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