

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.

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).

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 μ g 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 condition were described previously (2). Sequences of oligonucleotides for primers used in PCR amplification are as follows: Ii sense, TCCCAAGCCTGTGAGCAAGATG; Ii antisense, CCAGTTCCAGTGACTCTTTTCG; DMB sense, TCCTTCAACAAGGATCTGCTG; DMB antisense, CTTCCTCCACGTGATAGTCAC. 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 10^6 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,

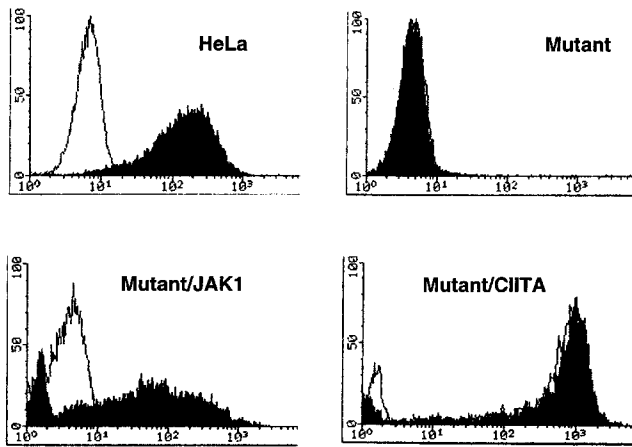


Figure 1. Cell surface expression of MHC class II molecules. Cells were untreated (*open profile*) or treated (*shaded profile*) with human IFN- γ at 500 U/ml for 2 d and analyzed by flow cytometry for the expression of HLA-DR.

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. 2 *a*, 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. 2 *a*). This may reflect additional transcriptional con-

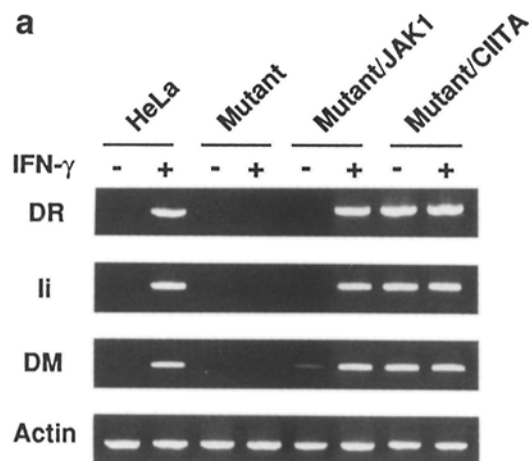


Figure 2. The expression of HLA-DR, Ii, and HLA-DM molecules by CIITA. (*a*) The expression of HLA-DR, Ii, and HLA-DM genes in mutant cells and in transfected cells with either JAK1 or CIITA. Cells were harvested after 2 d of incubation either with (+) or without (-) human IFN- γ at 500 U/ml in the medium. The data are representative of three independent analyses. (*b*) Expression of HLA-DM β protein in mutant cells and mutant cells transfected with CIITA. Whole cell lysates from cells were subjected to SDS-PAGE, transferred to nitrocellulose filter, and probed with an antiserum to human HLA-DM β subunit. Raji cells are MHC class II positive human B lymphoblastoid cells.

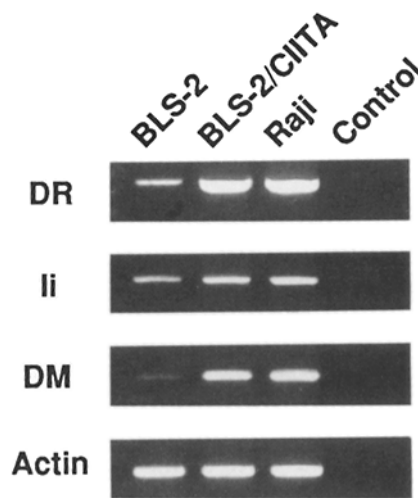


Figure 3. The expression of HLA-DR, Ii, and HLA-DM genes in BLS-2 cells and BLS-2 cells transfected with CIITA. Control lane has the same components as others except that it does not have the first-strand product. The data are representative of three independent analyses.

trols 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. 2 *b*, lanes 3–6). In contrast, HeLa cells show normal inducible expression of HLA-DM protein (Fig. 2 *b*, 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 induced 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 transfected 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, therefore, 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 transactivation 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.

We thank Drs. Joseph Fontes and Matija Peterlin for providing cDNA for CIITA, Dr. Peter Cresswell for providing HLA-DM antibody and critical reading of the manuscript. We also thank Dr. Soon-Cheol Hong for the helpful discussion and reading of the manuscript.

This work was supported by the Howard Hughes Medical Institute. C.-H. Chang is a Howard Hughes Medical Institute associate and Richard Flavell is a Howard Hughes Medical Institute investigator.

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Received for publication 30 September 1994.

References

1. Steimle, V., L.A. Otten, M. Zufferey, and B. Mach. 1993. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). *Cell*. 75:135-146.
2. Chang, C.-H., J. Fontes, M. Peterlin, and R.A. Flavell. 1994. Class II transactivator (CIITA) is sufficient for the inducible expression of major histocompatibility complex class II genes. *J. Exp. Med.* 180:1367-1374.
3. Steimle, V., C.-A. Siegrist, A. Mottet, B. Lisowska-Grospierre, and B. Mach. 1994. Regulation of MHC class II expression by interferon- γ mediated by the transactivator gene CIITA. *Science (Wash. DC)*. 265:106-109.
4. Kelly, A.P., J.J. Monaco, S. Cho, and J. Trowdale. 1991. A new human HLA class II-related locus, DM. *Nature (Lond.)*. 353:571-576.
5. Fling, S.P., B. Arp, and D. Pious. 1994. HLA-DMA and -DMB genes are both required for MHC class II/peptide complex formation in antigen-presenting cells. *Nature (Lond.)*. 368:554-558.
6. Morris, P., J. Shaman, M. Attaya, M. Amaya, S. Goodman, C. Bergman, J.J. Monaco, and E. Mellins. 1994. An essential role of HLA-DM in antigen presentation by class II major histocompatibility molecules. *Nature (Lond.)*. 368:551-554.
7. Cresswell, P. 1994. Getting peptides into MHC class II molecules. *Current Biology*. 4:541-543.
8. Glimcher, L.H., and C.J. Kara. 1992. Sequences and factors: a guide to MHC class II transcription. *Annu. Rev. Immunol.* 10:13-49.
9. Loh, J.E., C.-H. Chang, W.L. Fodor, and R.A. Flavell. 1992. Dissection of the interferon- γ -MHC class II signal transduction pathway reveals that type I and type II interferon systems share common signaling component(s). *EMBO (Eur. Mol. Biol. Organ.) J.* 11:1351-1363.
10. Loh, J.E., C. Schindler, A. Ziemiecki, A.G. Harpur, A.F. Wilks, and R.A. Flavell. 1994. Mutant cell lines unresponsive to interferon- α/β , and - γ are defective in tyrosine phosphorylation of ISGF-3 α components. *Mol. Cell. Biol.* 14:2170-2179.