Immunity, Vol. 6, 591–600, May, 1997, Copyright ©1997 by Cell Press

Induction of MHC Class I Expression by the MHC Class II Transactivator *CIITA*

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Summary

Major histocompatibility complex (MHC) class I–deficient cell lines were used to demonstrate that the MHC class II transactivator (*CIITA*) can induce surface expression of MHC class I molecules. CIITA induces the promoter of MHC class I heavy chain genes. The site α DNA element is the target for CIITA-induced transactivation of class I. In addition, interferon-γ (IFNγ)–induced MHC class I expression also requires an intact site α. The G3A cell line, which is defective in CIITA induction, does not induce MHC class I antigen and promoter in response to IFNγ. *Trans*-dominant–negative forms of CIITA reduce class I MHC promoter function and surface antigen expression. Collectively, these data argue that *CIITA* has a role in class I MHC gene induction.

Introduction

Major histocompatibility complex (MHC) class I and class II molecules are cell surface glycoproteins that are involved in the antigen presentation arm of the immune response. MHC class II molecules are composed of heterodimeric α and β chains. These molecules, in association with the accessory molecules Invariant chain (Ii) (Cresswell, 1994) and the DM proteins (Denzin and Cresswell, 1995; Sherman et al., 1995; Sloan et al., 1995), facilitate the presentation of predominantly extracellular antigens to CD4$^+$ T lymphocytes. Expression of proteins in the class II MHC pathway is generally restricted to professional antigen-presenting cells, although their expression can be induced by a number of stimuli, the most potent being interferon-γ (IFNγ) (reviewed by Glimcher and Kara, 1992; Ting and Baldwin, 1993; Mach et al., 1996). The molecular regulation of the MHC class II genes has been well studied, and several factors that contribute to constitutive and IFNγ- induced expression have been identified (reviewed by Glimcher and Kara, 1992; Ting and Baldwin, 1993; Mach et al., 1996). However, these factors are not sufficient to reconstitute DRA expression, since mutant cell lines containing all of these proteins still do not express class II MHC genes (Mao et al., 1993). This suggests the existence of a missing link that may be a global regulator of class II expression. The class II transactivator (*CIITA*), identified as a genetic defect of the bare lymphocyte syndrome (BLS), represents such a global regulator.

BLS patients have a pan-deficiency in class II expression and suffer from severe immunodeficiency (reviewed by Mach, 1995; Mach et al., 1996). B cells from one complementation group of BLS have an occupied class II MHC promoter that is indistinguishable from that of B cells expressing class II, as assessed by in vivo footprinting (Kara and Glimcher, 1991). Complementation cloning of the defective gene in this group of BLS led to the identification of *CIITA* (Steimle et al., 1993). This novel gene was analyzed and found to be a global regulator of the class II MHC genes. De novo expression of CIITA facilitates expression of the genes encoding all of the classic MHC class II α and β chains (DR, DP, and DQ) (Steimle et al., 1993), Ii, and the DM proteins (Chang et al., 1994; Chin et al., 1994; Steimle et al., 1994; Chang and Flavell, 1995). An N-terminal domain in the protein can serve as a transcriptional activator when fused to the GAL4 DNA-binding domain (Riley et al., 1995; Zhou and Glimcher, 1995), but CIITA does not have an intrinsic DNA-binding activity (Steimle et al., 1993). Mice with a defective *CIITA* gene modified by homologous recombination have a phenotype similar to that of BLS patients (Chang et al., 1996). The function of *CIITA* in class II MHC expression is currently an area of vigorous investigation; however, the effect of *CIITA* on MHC class I expression has not yet been noted.

MHC class I antigen presentation, in general, functions by presentation of cytosol-derived peptides to CD8$^+$ T lymphocytes. Class I MHC molecules generally have a ubiquitous expression pattern (reviewed by Burke and Ozato, 1989; Tatake and Zeff, 1993). The level of class I MHC proteins can be regulated by many immune cytokines, including the IFNs. The molecular regulation of the MHC class I molecules has been well studied (reviewed by Singer and Maguire, 1990; Ting and Baldwin, 1993). There is a high degree of cross-species conservation of key elements of the class I MHC promoter/enhancer (reviewed by Vallejo and Pease, 1995). Figure 1 shows a schematic diagram of the conserved elements of the class I MHC promoter. The region that extends from −200 to −140 bp is referred to as the class I regulatory complex. Included in this area is a consensus NF-κB binding sequence, the region I enhancer (Shirayoshi et al., 1987), and region II, which binds RXR hormone receptors and mediates the retinoic acid response in some cell types and functions as a low-level enhancer in others (Segars et al., 1993). The IFN consensus sequence (ICS), which contains the IFN response element, functions in the IFN-induced expression of class I genes (Sugita et al., 1987). Additional upstream sites, including silencer and enhancer elements, also play a role in class I MHC regulation (Maguire et al., 1992; Saji et al., 1992). Results from in vivo footprinting of HLA and H-2 genes in the region from −200

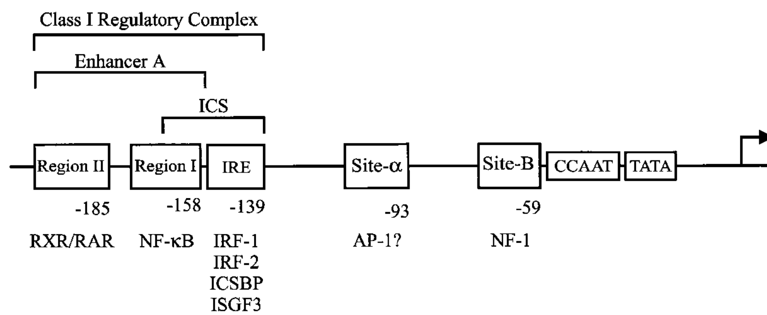

Figure 1. The MHC Class I Promoter

The conserved elements of the class I promoter are indicated by boxes, and the factors known or hypothesized to bind to the elements are presented under their respective sites. The numbers represent the location of the respective sites in the mouse L^d promoter.

to −100 bp identified footprints in region I and in the ICS (Dey et al., 1992). However, the strongest footprints are in site α, a CRE-like sequence involved in class I regulation in some cells but not others (Dey et al., 1992).

Most studies addressing the role of *CIITA* have not noted major effects of CIITA on class I MHC expression. However, a rigorous and systematic investigation has not been done. One report indicates that the ability of IFNγ to stimulate MHC class I expression is attenuated (Mao et al., 1993) in a fibrosarcoma with a known defect in CIITA expression (Chin et al., 1994). Others have shown that CIITA expression (or lack thereof) did not modulate class I expression. However, in none of these reports was a comprehensive evaluation of effect of CIITA on class I expression undertaken.

Following the lead of Mao et al. (1993), we extended studies of CIITA-induced MHC class I expression to a number of cell lines. To assess the effect of CIITA on MHC class I expression, we examined the levels of MHC class I after transducing cells with retrovirus containing *CIITA*. The results show that CIITA enhances the expression of surface MHC class I antigens and transactivates the promoter. Transactivation of the class I MHC promoter by CIITA is specifically mediated by site α. The physiological importance of site α is also observed in the native IFNγ induction of MHC class I promoter. Using various deletion and point mutation constructs of *CIITA*, we identified *trans*-dominant mutants of CIITA that equally decreased class I and II MHC antigens. These studies show that CIITA expression is important in class II antigen expression and in addition may have a significant effect on class I MHC antigens.

Results

Transduction of *CIITA* into Class I MHC Negative Cell Lines Induces Surface Class I Expression

Several studies have shown that de novo expression of CIITA in class II MHC deficient cell lines leads to the induction of all known genes in the class II antigen presentation pathway (Chang et al., 1994; Chin et al., 1994; Steimle et al., 1994; Kern et al., 1995; Chang and Flavell, 1995). In the studies that examined HLA expression, it was reported that the expression of CIITA had no effect on class I MHC molecules (Zhou and Glimcher, 1995; Chang et al., 1996). However, those studies were conducted using cells and cell lines that have high levels of surface class I MHC expression, and it is possible that changes in the levels of these proteins were masked by the high background expression. To assess the role of *CIITA* in class I MHC expression, a retroviral vector expressing human CIITA was transduced into cell lines that express low levels of MHC class I proteins. The resultant stably transduced polyclonal populations were analyzed by flow cytometry using a rat anti-mouse H-2 antibody for murine cell lines and mouse anti-human HLA-A, -B, and -C for human cell lines (Figure 2 and Table 1). The Line1 murine lung carcinoma cell has a very low basal MHC class I expression that is highly inducible by IFNγ (Pulaski et al., 1993). Expression of CIITA in this cell line led to a significant increase in the levels of class I molecules on the cell surface (Figure 2A). Histogram analysis demonstrated a 3-fold increase in the mean channel fluorescence (MCF) (Table 1). Increased class I expression was confirmed by examining the expression of class I and class II on monoclones derived from *CIITA*-transduced Line1 cells. In all cases, clones that had high class II expression had increased class I expression as well (up to a 30-fold induction of class I expression; data not shown).

In a second murine tumor with low class I expression, the melanoma B16-F1 (Chen et al., 1994), CIITA induced class I levels 2.4-fold (Figure 2A and Table 1). However, CIITA did not induce class I MHC expression on the mouse sarcoma SaI, which has high constitutive levels of surface MHC class I (Figure 2A and Table 1) (Ostrand-Rosenberg et al., 1990). These data show that human CIITA can induce MHC class I surface protein expression in murine tumor cells that are deficient in the expression of MHC class I.

These studies were extended to the human cell lines K562 (chronic myelogenous leukemia) and MCF-7 (breast carcinoma). K562 has very low class I MHC expression, which lends to its use as a natural killer cell target (Ramirez et al., 1992), while MCF-7 has modest amounts of HLA on the cell surface (Dolo et al., 1995). As shown in Figure 2B and Table 1, CIITA induced high levels of class I in the K562 cell line, demonstrating an 11.9-fold induction over the vector-transduced polyclonal population. MCF-7 cells have a modest constitutive level of class I MHC, which was not changed by the introduction of *CIITA* (Figure 2B and Table 1). These data demonstrate that CIITA can induce MHC class I proteins on the surfaces of both human and murine class I–deficient cell lines.

CIITA Induces Expression of MHC Class I Promoter–CAT Reporter Constructs

The enhanced cell surface expression of MHC class I in the previous experiments may have resulted either

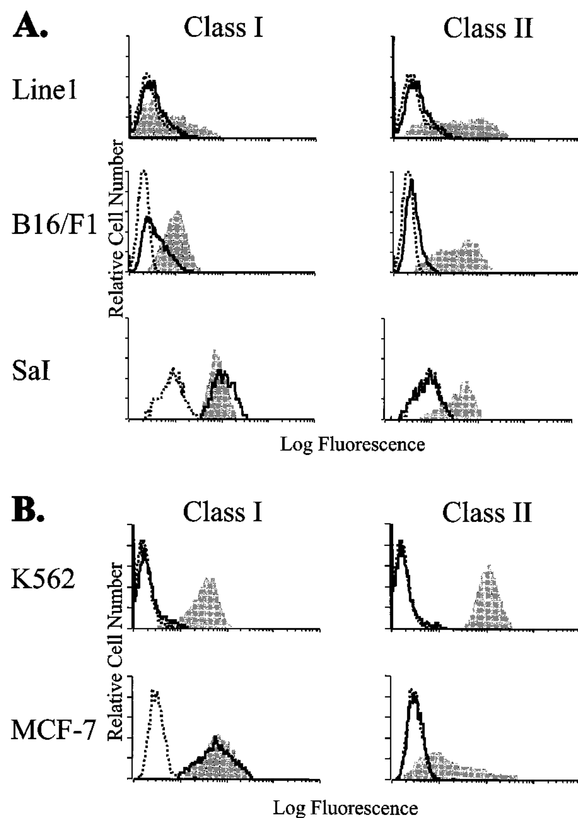

Figure 2. Flow Cytometry Analysis of MHC Class I and Class II Expression on Mouse and Human Tumor Cell Lines Transduced with *CIITA*

A human *CIITA* cDNA was cloned into the retroviral vector LXSNb and retrovirus produced in the packaging cell line PA317. Mouse tumor cell lines (A) or human tumor cell lines (B) were transduced with retrovirus (either empty vector, LXSNb, or *CIITA*-containing vector, LCIITASN) followed by selection in G418. For all cell lines except B16-F1, the resultant polyclonal population was analyzed by flow cytometry. B16-F1 cells had very low class II expression and the polyclonal pool was enriched for class II expression by two rounds of magnetic bead selection. Dotted line, secondary antibody alone control; solid line, cells transduced with vector alone; filled histogram, cells transduced with *CIITA*.

from transcriptional or from posttranscriptional mechanisms. However, since *CIITA* has been identified as a transcriptional transactivator (Riley et al., 1995; Zhou and Glimcher, 1995), we tested the hypothesis that the induction was due to transcriptional enhancement. K562 cells were cotransfected with H-2 L^d-CAT constructs, which have been described previously (Driggers et al., 1992), and either control or *CIITA* expression plasmids. The K562 cell line was selected because it displayed the highest inducible class I expression in response to *CIITA* transduction. The results of these experiments are shown in Figure 3. The *CIITA* expression vector enhanced CAT expression from full-length L^d-CAT1.4k by approximately 8-fold when cotransfected with *CIITA* expression plasmid (Figure 3, row B). The L^d-CAT237 construct, which contains the well-defined region I and ICS enhancers, retained CIITA inducibility (Figure 3, row C). This construct also showed higher basal expression than did L^d-CAT1.4k, which agrees with previous reports of a negative regulatory element in the upstream region (Maguire et al., 1992; Saji et al., 1992). L^d-CAT123 deletes both the region I and region II enhancers as well as the ICS. Although the basal expression of L^d-CAT123 is lowered relative to L^d-CAT237, cotransfection of *CIITA* with L^d-CAT123 enhanced CAT expression to a level similar to the full-length L^d-CAT1.4K construct. The average enhancement was approximately 7.5-fold (Figure 3, row D). In addition, previously defined point mutations in the class I control elements region I and region II also retained the ability to be transactivated by CIITA (data not shown). Further promoter deletion that gives rise to the L^d-CAT60 construct resulted in very low basal activity and loss of CIITA transactivation (Figure 3, row E). The only identifiable elements in L^d-CAT60 are a CCAAT element and a degenerate TATA box (Driggers et al., 1992). These data demonstrate that the site responsible for CIITA inducibility is between −123 and −60 bp in the mouse L^d promoter. CIITA did not enhance transcription from the promoterless CAT construct (Figure 3, row A), nor did CIITA enhance transcription from the pSV2-CAT reporter (data not shown), indicating that the transactivation effect of CIITA is promoter specific.

Site α Is Responsible for CIITA-Induced Class I Expression

Previously, a site between −123 and −60 bp in both the mouse and human class I promoters was shown to exhibit a very strong in vivo footprint (Dey et al., 1992) and this sequence was termed site α. The site was shown to contain a canonical AP-1/CRE–like sequence, although the binding complex was probably not a typical Fos/Jun heterodimer since site α did not efficiently compete for Fos/Jun binding to the AP-1 site from c-*fos*

Table 1. Mean Channel Fluorescence of MHC Class II and Class I Molecules on Retrovirally Transduced Cell Lines

	Class II			Class I		
Cell Line	Vector	CIITA	Fold Induction[a]	Vector	CIITA	Fold Induction[a]
Line1	3.45	52.33	15.2	4.17	12.78	3.1
B16-F1	2.89	34.76	12.0	4.71	11.16	2.4
SaI	9.68	42.97	4.4	121.2	89.54	0.74
K562	2.64	116.41	44.1	3.03	36.63	11.9
MCF-7	4.54	51.15	11.3	88.76	84.84	0.96

The cell lines were transduced with the empty vector (LXSN) or with the CIITA-expressing vector (LCIITASN). Cells were selected with G418 and the resultant polyclonal populations were analyzed on a Becton-Dickenson FACScan. Data were quantitated using the Cyclops program.
[a]Fold induction was calculated as mean channel fluorescence displayed by cells with CIITA divided by mean channel fluorescence of cells with the empty vector.

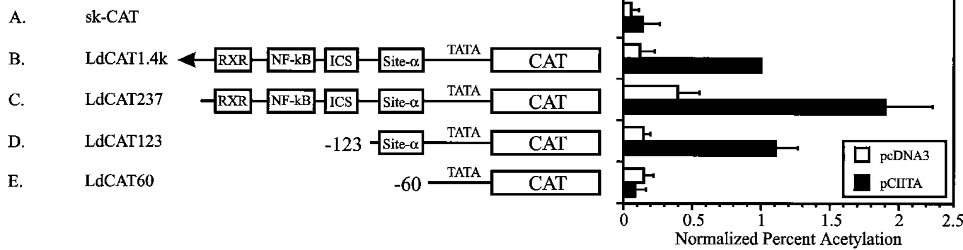

Figure 3. Promoter Deletion Analysis of CIITA-Induced MHC Class I Expression Using the L^d-CAT Promoter

Constructs were electroporated into K562 cells with equimolar amounts of vector control DNA (pcDNA3) or with the *CIITA* expression plasmid (pCIITA). Constructs used were promoterless CAT (A), full-length L^d-CAT1.4k (B), and deletion constructs L^d-CAT237 (C), L^d-CAT123 (D), and L^d-CAT60 (E). CAT activity is normalized to the percentage acetylation for CIITA-induced L^d-CAT1.4k, and error bars represent the standard error of the mean of three separate experiments.

(Dey et al., 1992). To address the specific contribution of site α to CIITA-induced class I transcription, CIITA inducibility of the K^b-CAT construct and that of a mutant construct (K^b-CATΔα) containing a specific 6 bp deletion in site α (Dey et al., 1992) were compared. As shown in Figure 4 (row A), the K^b-CAT construct was strongly induced by CIITA. The K^b-CAT construct with the 6 bp deletion in site α showed enhanced basal CAT expression in the absence of *CIITA* (Figure 4, row B). When K^b-CATΔα was cotransfected with *CIITA*, no enhancement was observed (compare Figure 4, row B). These data show that CIITA absolutely requires site α for induction of MHC class I.

Physiological Tests of CIITA Induction of MHC Class I

The previous data indicate that CIITA induces class I MHC gene expression via site α. If CIITA is to play a physiological role in the IFNγ induction of class I genes, the IFNγ induction of class I MHC promoter should be dependent on the presence of an intact site α. This possibility has not been previously explored. To investigate this scenario, the IFNγ responsive fibrosarcoma line, 2fTGH, was transfected with MHC class I promoter constructs containing either an intact or mutant site α

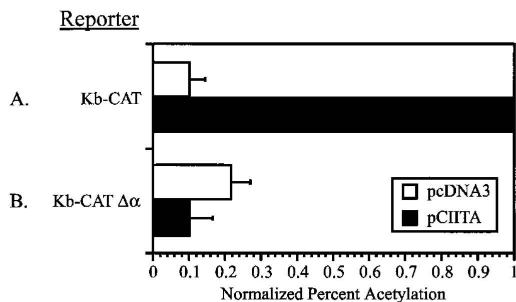

Figure 4. CIITA Activates the K^b-CAT Promoter through Site α

The K^b-CAT (A) and K^b-CATΔα (B) constructs were electroporated into K562 cells with either vector control DNA (pcDNA3) or with the *CIITA* expression plasmid (pCIITA). CAT activity is normalized to the percentage acetylation for CIITA-induced K^b-CAT, and error bars represent the SEM of three separate experiments.

sequence. As shown in Figure 5, IFNγ induced the K^b promoter (lane A). Deletion of site α in the K^b-CATΔα construct resulted in the loss of IFNγ inducibility (Figure 5, lane B). This indicates that in the 2fTGH cell line, site α is an element responsible for IFNγ-induced MHC class I gene expression. Taken together with the previous observation that the CIITA up-regulation of the class I MHC promoter is also mediated by site α, this finding strongly suggests that CIITA is important in the physiological IFNγ induction of class I MHC.

In a second experiment to address the physiological significance of CIITA in IFNγ-induced class I MHC expression, the above- mentioned studies were extended to the G3A cell line, which is a *CIITA*-defective mutant line derived from a sister clone of 2fTGH (Chin et al.,

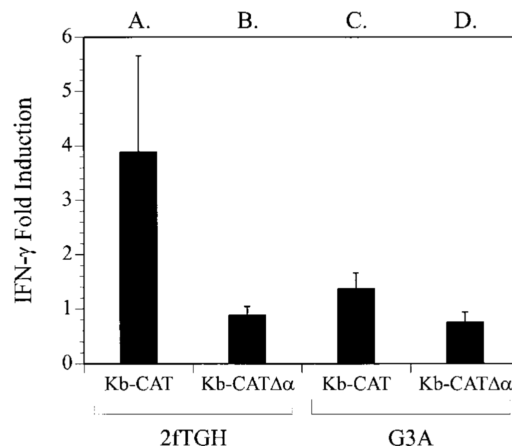

Figure 5. Promoter Activity of MHC Class I Promoters Containing Intact or Mutant Site α Sequences in Response to IFNγ in the 2fTGH and G3A Cell Lines

Cells were transfected with either wild type K^b promoter or the site α–deleted K^b promoter, and at that time IFNγ (300 U/ml) was added to the cultures in half of the sample while the other half of each sample was left untreated. Forty hours later the cells were harvested and assayed for CAT activity. The fold induction is presented as the percentage acetylation of IFNγ-treated cells divided by the percentage acetylation of untreated cells for each construct and cell line. (A) K^b-CAT transfected into 2fTGH cells; (B) K^b-CATΔα transfected into 2fTGH cells; (C) K^b-CAT transfected into G3A cells; and (D) K^b-CATΔα transfected into G3A cells. The error bars represent the standard error of the mean of three separate experiments.

Table 2. Attenuated IFNγ Responsiveness of MHC Class I and Class II Proteins in the 2fTGH and G3A Cell Lines

	Fold Induction[a]	
Cell Line	Class II	Class I
2fTGH	16.6	2.3
G3A	1.4	1.2

Cells were treated with IFNγ (300 U/ml) for 48 hr. They were analyzed by fluorescence-activated cell sorting, and a comparison was made of the MCF of unstimulated versus IFNγ-treated protein levels for each cell line.
[a]Fold induction is the MCF of IFNγ treated cells divided by the MCF of untreated cells. MCF induction of class I in multiple experiments ranged from 1.7- to 3.3-fold in 2fTGH.

1994). The lack of CIITA in this clone is correlated with a lack of IFNγ-enhanced class II MHC expression and attenuation of class I MHC inducibility (Mao et al., 1993). The lack of CIITA expression in this line correlates with the lack of an IFNγ enhancement of the K^b promoter (Figure 5C). In addition, deletion of site α did not influence basal promoter expression or IFNγ inducibility in this cell line (Figure 5D). In a third set of experiments to assess the physiological role of CIITA in the IFNγ induction of class I genes, the expression of class I MHC antigens on 2fTGH and on G3A cells was compared. IFNγ induced class I MHC antigens on the 2fTGH cells, whereas such an induction was not observed for G3A (Table 2). It must be emphasized that although the 2.3-fold induction by IFNγ is modest, by absolute numbers of molecules expressed on the cell surface, it is comparable to that of MHC class II (MCF after IFNγ treatment changed from 2.3 to 48 for class II and from 43 to 99 for class I). This modest induction also agrees with the literature (Sugita et al., 1987; Giuliani et al., 1995). Another laboratory has found that the transfection of *CIITA* into the G3A line enhanced class I expression (Gobin et al., 1997 [*Immunity*, this issue]). These data indicate that the lack of CIITA in the G3A cell line may be the principal reason that G3A has an attenuated ability to induce MHC class I by IFNγ.

Gel Shift Assays with Site α Show No Changes in DNA-Binding Activity in Response to CIITA Expression

The finding that the CIITA-specific induction of MHC class I gene expression mapped precisely to the site α suggests the possible induction of a DNA-binding activity at this site or CIITA transactivation of a protein or proteins already bound to this site. To investigate these possibilities, gel shift experiments were performed using oligonucleotides spanning this sequence (Figure 6). Several DNA-binding complexes were detected in extracts from both Line1 and K562 cells in control and *CIITA*-transduced cells. These include two DNA–protein complexes of similar size in both Line1 and K562 extracts (Figure 6, arrows A and B) and another specific to K562 (Figure 6, arrow C). These complexes were specifically competed by cold competitor DNA (Figure 6, lanes 3, 5, 7, and 9). The pattern of DNA–protein complexes from control cells and *CIITA*-transduced cells was indistinguishable (Figure 6, compare lane 2 to lane 4 and lane 6 to lane 8). However, the possibility cannot be ruled out that CIITA induced changes that were subtle or that were missed by the gel shift assay. Another possible experimental approach would be to supershift the bands using CIITA antisera to reveal whether CIITA is tethered on the DNA–protein complex. However, all attempts to supershift the class II MHC promoter with anti-CIITA antisera have been unsuccessful (data not shown), and hence this strategy was not used for the class I promoter. Nonetheless, these data suggest either that CIITA induces changes that are not detected by the gel shift assay or that CIITA induction through the site α sequence does not result from new protein binding to this site.

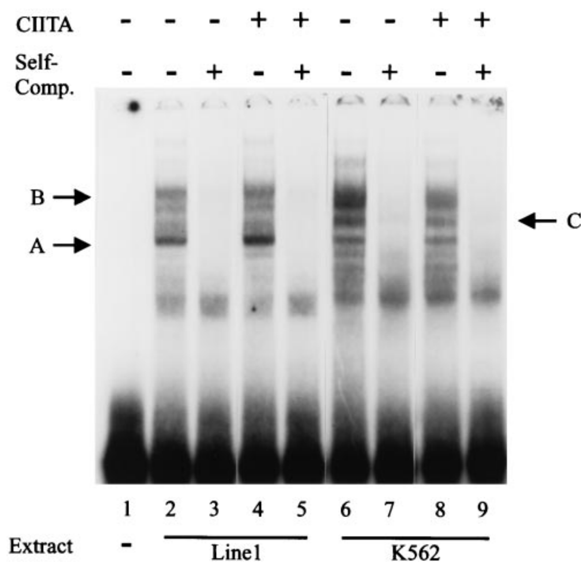

Figure 6. Gel Shift Analysis of Proteins Interacting with Site α in Line1 and K562 Cells with and without *CIITA*

Gels shifts were performed using untransduced Line1 cells (lanes 2 and 3) and K562 cells (lanes 6 and 7) or a *CIITA*-transduced Line1 clone (L1/LCIITSNF6) expressing high levels of MHC class I and class II (lanes 4 and 5) and K562 polyclonal *CIITA*-transduced cells (lanes 8 and 9). A 31 bp probe encompassing the site α sequence was used in gel shift analysis (see Experimental Procedures for sequence). Self-competition used a 200-fold excess of cold competitor site α oligonucleotides (lanes 3, 5, 7, and 9). Arrows A and B indicate gel shift complexes with similar migration patterns between Line1 and K562 cells; arrow C indicates a complex unique to K562 cells.

Trans-Dominant–Negative CIITA Mutants Repress the IFNγ Induction of MHC Class I Expression

We have recently demonstrated that certain mutant forms of CIITA function as *trans*-dominant–negative repressors of class II MHC promoter activation (Chin et al., 1997). In other words, these mutants serve as decoy molecules that titrate out the wild-type CIITA, thus decreasing class II MHC promoter activation. The availability of these mutants suggests an additional approach to examine the physiological contribution of CIITA to MHC class I induction by IFNγ. To identify *trans*-dominant–negative CIITA mutants of class I MHC, the effect of a panel of structural mutants was first tested on class I promoters.

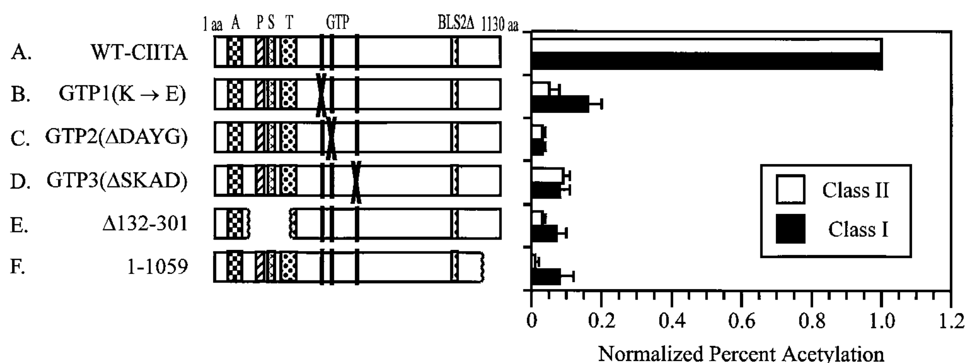

Figure 7. Comparison of the Reporter Activity of MHC Class II and Class I Promoters Induced by Different CIITA Mutant Constructs

CIITA mutant constructs were electroporated into K562 cells with either the class I L^d-CAT1.4k promoter or the class II DRA300CAT promoter. The CAT levels were normalized to that induced by wild-type CIITA for MHC class I and class II, and the error bars reflect the standard error of the mean of four (class I) or three (class II) separate experiments. Expression constructs contained the following *CIITA* sequences: (A) wild type; (B) point mutation in the G1 domain; (C) 4 bp deletion in the G3 motif; (d) 4 bp deletion in the G4 modtif; (E) internal deletion in the proline/serine/threonine-rich domains; and (F) 3′ deletion in the *CIITA* coding sequence. The mutations are indicated by a space or marked by an X. A, P, S, T represent acidic, proline, serine, and threonine-rich domains, respectively. The three black bars under the label GTP represent the G1, G3, and G4 motifs. BLS2Δ is the region deleted in the CIITA BLS complementation group. Open bars, DRA300CAT reporter cotransfected with indicated *CIITA* mutant; filled bars, L^d-CAT1.4k reporter cotransfected with indicated *CIITA* mutant. Normalized percentage acetylation is relative to wild-type CIITA-induced expression levels for class II (open bars) and class I (filled bars).

Previous analysis of CIITA reveals that a domain homologous to the GTP-binding domains of a wide variety of GTP-binding proteins is important in class II MHC activation (Dever et al., 1987). Three motifs are contained within this domain: G1 (the phosphate-binding motif), G3 (the Mg^{2+} binding motif), and G4 (the guanine nucleotide binding motif), according to conventional nomenclature. To assess the contribution of each of these homology motifs to class I MHC activation, site-specific mutations and deletions were generated and cotransfected with either a class II MHC reporter construct (DRA300CAT) as a positive control or a class I MHC reporter construct (L^d-CAT1.4k) (Figure 7, rows A–E). Mutations in all three GTP motifs decreased CIITA induction of both class I and II promoters to background levels (Figure 7, rows B–D). The 5′ region of *CIITA* encodes proline/serine/threonine–rich domains that have been shown to have transactivation function (Riley et al., 1995; Zhou and Glimcher, 1995). When all three domains were deleted (Figure 7, row E), the level of activation was reduced to background levels. Finally, a deletion in the 3′-terminal end of *CIITA* abolished class II and class I induction (Figure 7, row F). These data suggest that the 3′ sequence in the *CIITA* gene is necessary for both MHC class II and class I induction. These data collectively indicate that the domains of CIITA that are necessary for class I MHC induction are inseparable from those involved in class II MHC induction.

These *CIITA* mutants were next tested for *trans*-dominant–negative suppression of class I MHC expression. Three of these mutants, CIITA(Δ132–301), CIITA(1–1059), and GTP2(ΔDAYG), were highly efficient in suppressing IFNγ-induced levels of reporter gene driven by the L^d promoter (81% ± 1.7%, 79% ± 2.6%, and 72% ± 4.0% repression, respectively, compared to empty control plasmid pcDNA3; mean ± standard error of three independent experiments). As an additional and more physiological approach, plasmid vectors expressing these mutant CIITA molecules were stably transfected into the 2fTGH cell line. Cells were treated with IFNγ for 48 hr and then were analyzed by fluorescence-activated cell sorting for surface MHC class II and class I expression, relative to cells with stably integrated pcDNA3 vectors. As shown in Table 3, all three constructs suppressed MHC class II surface expression by 25% to 36%. The suppression is reproducible but incomplete and is most likely due to the low level of mutant CIITA expressed in stable lines (K.-C. C., unpublished data). Similarly, these same constructs repressed MHC class I surface expression 24% to 44%. These constructs did not repress the expression of the class I associated gene TAP1 (K.-C. C., unpublished data). Although the repression was incomplete, it must be viewed in context. Since the response of MHC class II to IFNγ has been shown to be absolutely dependent on CIITA expression, the 25% to 36% repression in this experiment is indicative of the repression that can be expected using this experimental design. The observation that the MHC class I levels are similarly repressed is good evidence that *CIITA* is involved in MHC class I induction during an IFNγ response.

Discussion

The *CIITA* gene has been characterized as a global regulator of genes in the MHC class II antigen presentation pathway (reviewed by Mach et al., 1996). In this role, CIITA can transcriptionally induce all of the genes known to be involved in this pathway, including those encoding class II α and β chains, Ii, and the DM molecules (Steimle et al., 1993; Chang et al., 1994; Chin et al., 1994; Steimle et al., 1995). In the present report we demonstrate that CIITA can induce MHC class I heavy chain in addition to class II expression in cell lines that are low expressors of class I MHC. This effect was observed as an increase in cell surface expression in both mouse and human

Table 3. Dominant-Negative Repression of MHC Class I and Class II Levels in IFNγ-Treated 2fTGH Cells

DNA Construct	Percentage Expression (relative to vector control)	
	Class II	Class I
pCDNA3	100	100
CIITA (Δ132–301)	74	76
CIITA (1–1059)	77	56
GTP2 (ΔDAYG)	64	75

2fTGH cells were stably transfected with the indicated construct. Cells from the polyclonal pool were then treated with IFNγ (300 U/ml) for 48 hr. They were analyzed by fluorescence-activated cell sorting, and a comparison was made of the mean channel fluorescence for each cell line. The absolute levels for the pCDNA3 constructs were 94.0 for class II and 244.7 for class I.

cells and as an activation of both the H-2K^b and H-2L^d promoters. Promoter deletion analysis localized site α as the region that is responsive to CIITA induction.

Three lines of evidence are presented to validate the biological relevance of this observation. First, the class I promoter with a site α deletion did not respond to IFNγ or to CIITA, indicating the involvement of this site in both a CIITA response and a normal IFNγ response. Second, mutant cell lines with a defect in CIITA induction were defective in the IFNγ induction of class I MHC promoter as well as surface antigen. Finally, *trans*-dominant–negative CIITA molecules reduced the expression of surface MHC class I proteins to the same degree as class II MHC proteins. These *trans*-dominant mutants also reduced class I promoter activity. Collectively, these data provide strong biological evidence that *CIITA* is involved in MHC class I gene regulation.

It should be underscored that in contrast to class II MHC promoters, which are completely dependent on CIITA for gene expression, class I MHC promoters are expressed even in the absence of CIITA. However, CIITA is required for optimal induction by IFNγ, and the lack of CIITA correlates with the loss or decrease of IFNγ-induced class I MHC expression. These results do not negate the importance of previous findings that the binding of IFNγ-induced factors to the IFN response element region of class I MHC promoters is important, but simply indicates that the IFNγ induction of class I MHC requires multiple elements, including the site α element, which mediates CIITA function.

On first glance, our finding contradicts previous findings that class I MHC expression appears to be normal in B cell lines from BLS patients lacking *CIITA* and cells from *CIITA*-knockout mice. However, these studies did not examine the cytokine-induced experimental conditions used in this study. Our study also noted the lack of a CIITA effect in some cell lines; most share the characteristics of high basal class I MHC expression. However, analysis of the IFNγ-responsive 2fTGH cell line and the *CIITA*-defective mutant G3A clearly shows a difference in class I MHC promoter activity that is linked to *CIITA*. Therefore, it would be of interest to examine IFNγ-induced class I levels in *CIITA*-deficient patient cell lines. A parallel line of observation has been made for the involvement of IRF-1 in class I MHC expression. Although the IRF-1 gene product is shown to be involved in IFNγ-induced class I MHC gene expression, basal levels of class I MHC gene expresion in cells from IRF-1$^{-/-}$ mice are not decreased relative to that in cells from control mice, even though the IRF gene is constitutively expressed at low levels in the tissues that were examined (Matsuyama et al., 1993; Reis et al., 1994). Nonetheless, the finding in the knockout mice cannot be used to abolish the significance of IRF-1 in IFNγ-induced class I MHC gene expression. A similar argument can be made here for CIITA. IRF-1 and CIITA may represent similar molecules that are IFNγ induced and primarily important in the IFNγ induction of class I promoters.

The mapping and mutagenesis analysis clearly show that CIITA induces MHC class I transcription through the site α sequence. The data show for the first time a pathway that requires site α. This sequence was originally identified as exhibiting a strong in vivo footprint in both splenic and brain cells (Dey et al., 1992). This site is similar to the consensus sequence for the AP-1 site and the cAMP response element (Lee and Masson, 1993). Gel shift analysis with site α probes revealed that the complex formed with this site was eliminated upon competition with the AP-1 site from the c-*fos* promoter; however, the H-2K^b site α did not compete effectively against a consensus AP-1 probe for Jun/Fos binding. This suggests that the makeup of the site α binding complex is different than that of consensus AP-1 (Dey et al., 1992). Previously, the region corresponding to site α was shown to bind to an AP-1–like complex (Israel et al., 1989). Site α also binds to RXR heterodimers (Dey et al., 1992). Defining what is functionally binding and regulating this element is important for understanding how CIITA regulates class I genes. One possible explanation for the lack of class I induction in some cell types is that the makeup of proteins bound to the site α sequence may differ among different cells. For instance, in the Line1 cell lines, the proteins bound may actively interact with CIITA to induce mRNA expression, while in the SaI cell line a different set a factors that do not interact with CIITA may be bound at site α, and hence there is no promoter induction. This possibility is being actively investigated in our laboratory.

The activation of an MHC class I promoter through site α is interesting given that the promoters of the class I and class II genes are divergent. MHC class II promoter activity is critically dependent on the W, X, and Y elements for constitutive expression in B cells and in IFNγ-induced expression in other cells (reviewed by Glimcher and Kara, 1992; Ting and Baldwin, 1993). The class I promoter is dependent on region I, ICS, and in some cases the region II enhancer, in addition to upstream silencers and enhancers. The only sequence previously shown to be involved in IFN induction is the ICS site (reviewed by Burke and Ozato, 1989; Tatake and Zeff, 1993). The MHC class I promoter does not contain sequences homologous to the W or X1, nor does the class II promoter contain an ICS sequence. The X2 box of the class II promoter does contain sequences homologous to site α. The core of the K^b site α sequence (TGACGC) is also the core sequence of the X2 box of the DRA promoter, and the X2 box of DRA is important for CIITA-induced class II expression in B cells (Zhou and Glimcher, 1995). It is possible that a factor(s) binding to

the X2 element in DRA and site α may be the common link through which CIITA transactivates class I and class II MHC promoters.

Experiments were also performed with β_2m and TAP1 promoters and revealed that CIITA did not induce their expression (data not shown). The induction of class I surface expression is interesting given that β_2m and TAP1 are not similarly induced. It is well established that β_2m expression is required for surface expression of class I molecules, as has been demonstrated particularly by the lack of surface H-2 expression in β_2m knockout mice (Zijlstra et al., 1990). It has also been demonstrated that lack of the peptide transporter TAP can also lead to defects in surface class I expression (Restifo et al., 1993; Cromme et al., 1994; Fehling et al., 1994; Keating et al., 1995). It is paradoxical that the induction of MHC heavy chain alone can lead to an increase in cell surface expression of class I. We suggest that cells that are capable of CIITA-induced class I expression express excess TAP and β_2m molecules sufficient for higher class I surface expression, whereas cells that are unresponsive to MHC class I induction by CIITA may not have excess TAP and β_2m to support higher levels of surface expression. Further induction of surface class I expression would necessitate the up-regulation of these auxiliary molecules. This may place a restriction on the ability of CIITA to induce class I expression at the cell surface. Therefore, it is possible that in cells in which we have observed no increased surface class I expression (Sal and MCF-7) may in fact have increased levels of class I messenger RNA and intracellular protein. However, in the absence of increased β_2m, these cells are unable to increase surface protein expression. Another question still to be answered is what effect *CIITA* expression has on other class I and class I–like genes.

These possibilities suggest three situations in which the expression of CIITA would be expected not to influence MHC class I expression. First, in certain cells with very high basal expression, the effect of CIITA may be negligible or difficult to detect. Second, the lack of excess molecules of TAP or β_2m in some cells might not support increased MHC class I surface expression, even though CIITA induces heavy chain gene expression. However, in cells with these accessory molecules in excess, increased class I surface expression would be observed with CIITA induction. Finally, in cells with AP-1 molecules that are not able to interact with CIITA, there would be no enhanced class I mRNA expression and hence no increased surface expression. These possibilities suggest that the ability of CIITA to induce surface class I expression may be very cell specific.

In summary, the data presented in this report indicate that *CIITA* may play a significant role in MHC class I transcription in cells with little or no class I expression. CIITA also activates class I surface expression in 2fTGH cells that have relatively high basal class I expression. Investigation of the mechanism of CIITA-induced MHC class I expression will be important to determine the contribution of *CIITA* to the endogenous antigen presentation pathway. This report demonstrates that *CIITA* plays a greater role in the regulation of genes involved in both antigen presentation pathways than previously believed.

Experimental Procedures

Cell Lines and Culture Conditions

The Sal and Line1 cell lines have been described previously (Ostrand-Rosenberg et al., 1990; Pulaski et al., 1993). The B16-F1 melanoma and the K562 chronic myelogenous leukemia cell lines were obtained from the Lineberger Comprehensive Cancer Center Tissue Culture Facility (Chapel Hill, NC). The MCF-7 breast carcinoma was obtained from the American Type Culture Collection. Sal and B16-F1 were maintained in DMEM (GIBCO-BRL, Gaithersburg, MD) with 7% fetal bovine serum (GIBCO–BRL). The other lines were maintained in RPMI-1640 (GIBCO–BRL) with 7% fetal bovine serum. For IFNγ induction, cells were cultured in 300 U/ml recombinant human IFNγ (Genzyme, Cambridge, MA) for approximately 42 hr.

Construction of Plasmid Constructs and Transduction of Cells

The EcoRI fragment of the FLAG.CIITA8 construct was cloned into the EcoRI site of the LXSNb retroviral plasmid (Olsen and Sechelski, 1995; Chin et al., 1997). The construction of the *CIITA* mutant plasmids is described elsewhere (Chin et al., 1997). Production of retrovirus was done as previously described (Olsen and Sechelski, 1995). In brief, plasmid DNA (either control vector or *CIITA*-containing vector) was transfected into the PA317 helper cell line via calcium phosphate precipitation, and the following day the media were changed. Forty-eight hours after transfection, the supernatant was collected, sterilized by filtration, and stored at -70°C for later use.

Adherent cells were seeded on plates at 1×10^5 cells/35 mm plate on the day before transduction. Suspension cultures in mid–log growth phase were harvested on the day of transduction. Viral supernatant (250 μl) with 8 μg/ml polybrene was added to 1×10^5 cells and incubated for 2 hr at 37°C. After incubation the viral supernatant was aspirated and replaced with fresh growth medium. Two days after transduction, the cells were passaged 1:20 and placed in selection media (400 μg/ml G418). The resultant polyclonal population was then analyzed for class II expression. For the B16-F1 melanoma cell line, analysis of the polyclonal population after *CIITA* transduction revealed that less than 5% of the population stained positive for class II (data not shown). Therefore, the cells were enriched for class II expression by two rounds of magnetic bead selection (Dynal, Oslo, Norway). The antibody used in the selection was 7–16/17 (from Dr. J. F. Frelinger; see next section for antibody specificity).

Flow Cytometry Analysis of MHC Class I and Class II Expression

The antibodies used for these studies were L243 mouse anti-human HLA-DR (hybridoma from ATCC) and mouse anti-human HLA-A, -B, and -C (Pharmingen, San Diego, CA). The following were kindly provided by Dr. J. F. Frelinger: M1/42 (rat anti-mouse H-2), 34–7–23s (anti H-2 K^d/D^d), BP1072.2 (anti I-Eβ/I-Aβ, reactive with haplotypes d, b, p, q, u, and j), 7–16/17 (anti I, reactive with haplotypes p, b, k, q, r, s, and j). Secondary antibodies used were goat anti-mouse IgG–fluorescein isothiocyanate conjugate (Pharmingen) and goat anti-rat IgG–fluorescein isothiocyanate (Sigma, St. Louis, MO).

For flow cytometry, cells in log growth phase were harvested and washed twice with 1× PBS containing 0.1% sodium azide. The cells were resuspended at 1×10^7 cells/ml and 100 μl was used for each sample. The cells were incubated for 30 min with diluted primary antibody (20 μl/sample). The cells were washed three times with 1× PBS–sodium azide and then incubated for 20 min in diluted secondary antibody (20 μl). The cells were washed three times with 1× PBS–sodium azide. The cells were either analyzed immediately or fixed in 2% paraformaldehyde and stored for less than 1 week for analysis.

Flow cytometry was performed on a Becton-Dickinson FACScan (San Jose, CA) using Cyclops software (Cytomation, Fort Collins, CO). Five thousand cells were analyzed for each sample.

Plasmids and Transfection

The L^d-CAT1.4k and its derivatives (Driggers et al., 1992), the K^b-CAT and its derivative (Dey et al., 1992), and the DRA300CAT (Sherman et al., 1987) plasmids have been previously described.

For transfections, K562 cells were in mid–log growth phase. Cells were harvested and resuspended in complete media at a concentration of 1×10^7 cells/ml. Three hundred microliters of this cell suspension was added to an electroporation cuvette (0.4 cm electrode gap, Biorad, Melville, NY) and mixed with the DNA of interest (10 μg of reporter and 10 μg of expression plasmids). Cells were electroporated using the Gene Pulser apparatus (Biorad) at 200 V and 960 μF. Electroporated cells were added to 8 ml of complete media and allowed to recover for approximately 42 hr before harvest for CAT assay. For 2fTGH cells, cells were plated on 10 cm plates at 5×10^5 cells/plate on the day before transfection. Transfection was done via the $CaPO_4$ method as described (Ausebel et al., 1987). Cells were allowed to recover for approximately 42 hr before harvest for CAT assay.

Cell extracts were prepared by freeze–thaw lysis as previously described (Sherman et al., 1989). For CAT assay the protein concentration of each sample was determined by Protein Assay Reagent (Biorad), and equal amounts of protein were used in a given experiment (10–15 μg). CAT assays were performed as previously described (Sherman et al., 1989). The thin-layer chromatography plates were quantitated by phosphor imaging (Molecular Dynamics, Sunnyvale, CA).

Gel Shift Analysis

Nuclear extracts were prepared according to the method of Dignam et al. (1983). Gel shift assays were as previously described (Martin and Weis, 1993). In brief, binding reactions were performed under the following conditions: 12 mM HEPES (pH 7.9), 12% glycerol, 60 mM KCl, 120 μM EDTA, 120 μM PMSF, 300 μM DTT, with 4.5 μg BSA, 2 μg poly (dI·dC), 0.2 pg double-stranded oligonucleotide probe (100,000 cpm, end-labeled), and 25 μg nuclear extract. Reactions were incubated 30 min at 30°C and then run for 2 hr on a 4% native polyacylamide gel (0.5× Tris-borate-EDTA). The oligonucleotides used have been previously described (Dey et al., 1992). The sequence of the H-2K^b site α plus strand was TTC TGT CCG GAC ACT GTT GAC GCG CAG TCA G; the sequence of the minus strand was CTG ACT GCG CGT CAA CAG TGT CCG GAC AGA A.

Acknowledgments

The authors would like to thank Dr. John G. Frelinger and Dr. Suzanne Ostrand-Rosenberg for the gift of cells; Dr. Jeffrey A. Frelinger for the gift of antibodies; and Dr. Kenneth L. Wright for critical review of this manuscript. This work was supported by National Institutes of Health Grants AI29564 and NCI48185. J. P.-Y. T. is a recipient of an American Cancer Society Faculty Award. B. K. M. is supported by National Institutes of Health Individual NRSA F32 CA67480–02.

Received August 7, 1996; revised January 29, 1997.

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