Dendritic Cell-specific MHC Class II Transactivator Contains a Caspase Recruitment Domain That Confers Potent Transactivation Activity*

Received for publication, February 9, 2001, and in revised form, March 9, 2001
Published, JBC Papers in Press, March 13, 2001, DOI 10.1074/jbc.M101295200

Kevin Nickerson‡‡, Tyler J. Sisk‡‡, Naohiro Inohara§§, Christina S. K. Yee†, Jennifer Kennell‖, Min-Chul Cho¶¶, P. Joseph Yannie II‡‡, Gabriel Núñez§§, and Cheong-Hee Chang†††

From the Departments of ‡‡Microbiology and Immunology, §§Pathology and Comprehensive Cancer Center, ‖Cellular and Molecular Biology, ‡‡‡Biology, and §§Surgery, University of Michigan Medical School, Ann Arbor, Michigan 48109

The MHC class II transactivator (CIITA) is a critical transcription factor that regulates genes involved in antigen presentation function. At least three functional forms of CIITA gene products are transcribed from three different promoters. The CIITA gene expressed in dendritic cells (DC-CIITA) has a unique first exon encoding an extended N-terminal region of CIITA. Here, we show that the N-terminus of DC-CIITA has high homology to a caspase recruitment domain (CARD) found in components of apoptosis and nuclear factor-κB signaling pathways. However, DC-CIITA does not regulate cell death, nor does it induce nuclear factor-κB activity. Instead, DC-CIITA is transcriptionally a more potent activator of the MHC class II gene than the form expressed in B cells. A single amino acid substitution in the CARD of DC-CIITA, predicted to disrupt CARD-CARD interactions, diminished the transactivation potential of DC-CIITA. These results indicate that the CARD in the context of CIITA serves as a regulatory domain for transcriptional activity and may function to selectively enhance MHC class II gene expression in dendritic cells.

The MHC1 class II transactivator (CIITA) was initially identified as a critical transcription factor that is required for both constitutive and interferon-γ-inducible expression of MHC class II genes (1–3). CIITA also activates other genes involved in antigen presentation such as the invariant chain and the HLA-DM genes (4, 5). The significance of CIITA function in vivo was further confirmed by the generation of CIITA−/− mice that display a phenotype similar to patients with severe combined immunodeficiency disease, the bare lymphocyte syndrome (6, 7). CIITA also acts as a negative regulator for genes expressed in CD4 T cells including the interleukin-4 and Fasl genes (8–10). Therefore, CIITA has dual functions, acting as an activator or a repressor depending on the target promoter.

Four different isoforms of human CIITA transcripts have been identified (11). Each isoform is transcribed from a separate promoter that confers expression specificity. Thus, the first exon of each isoform is unique, but the rest of the exons are shared by all isoforms. Forms I and III are constitutively expressed primarily in dendritic cells (DCs) and B cells, respectively (11). The interferon-γ-inducible form of CIITA (Form IV) is transcribed by the last promoter (11). The second isoform is poorly characterized, and the specificity of its expression is unknown. The B cell form of CIITA (B-CIITA) has been extensively studied for transactivation function (12), but the function of the form unique to dendritic cells (DC-CIITA) remains unknown.

Studies using B-CIITA have demonstrated that CIITA contains four domains shared by all isoforms: the acidic domain, proline-serine/threonine-rich domain, a centrally located nucleotide binding domain, and leucine-rich repeats (1). Each domain serves a unique function, and all domains are required for proper transactivation of the MHC class II gene (12–17).

The caspase recruitment domain (CARD) was first identified as a peptide module present in the promodomains of upstream caspases and adapter molecules such as CED-4/Apaf-1 and RAIDD that mediates the recruitment of caspases via homophilic CARD-CARD interactions (18). More recently, the CARDs have been found in other molecules including Nod1 and RICK that are components of NF-κB signaling pathways (19). The association of the CARD of Nod1 with the corresponding CARD of RICK induces the dimerization of RICK, which in turn activates NF-κB (19). Thus, the CARD functions as an effector domain that mediates specific homophilic interactions with downstream molecules to activate diverse signaling events.

Nod1, a cytosolic protein with homology to plant disease-resistant R gene products and Apaf-1, shares a strikingly similar domain organization with CIITA (19) (Fig. 1D). Both Nod1 and CIITA have N-terminal effector domains, a centrally located nucleotide binding domain, and leucine-rich repeats at the C terminus. The effector domain of Nod1 is the CARD that is not present in the CIITA expressed in B cells. Here, we report that the first exon of DC-CIITA encodes a CARD. We found that DC-CIITA containing the CARD is a more potent transactivator for MHC class II expression than B-CIITA and that the CARD is required for enhanced transactivation activity. Unlike other CARD-containing proteins, however, DC-CIITA...
FIG. 1. Characterization of the CIITA isoform expressed in DCs. A, the exon organization of the CIITA gene. The numbers in boxes indicate exons, and the arrows show the positions of the primers used for reverse transcription PCR in B. The diagram was not drawn to scale. Exons beyond the fourth one are not shown. B, DC-CIITA is expressed in DCs. The primer located at the first exon of DC or B cell type shown in A was used in conjunction with the primer at the fourth exon to detect the transcripts. The 5′ PCR primer used to detect the common exons is located at the second exon as illustrated in A. The dilution factors shown for the actin transcripts refer to first strand templates used for PCR reactions. C, sequence comparison of CIITA CARD with CARDs of members of the caspase family. Alignment of CARDs with the following GenBank™/EBI accession numbers is shown. Human (h) and mouse (m) dendritic cell-type CIITA (AF000092 and AF100719), Nod1 (AF113925), RICK (AF027706), ARC (AF043244), RAIDD (U79115), caspase-2 (U13021), Ced-3 (L29052), Ced-4 (X69016), caspase-9 (U56390), Apaf-1 (AF013263), and c-IAP-1 (L49431) are shown. The residues identical and similar to those of Nod1 are shown by (A) and (F), respectively. The putative helices, H1a to H6, are shown based on the three-dimensional structure of the CARD of Apaf-1 (23). D, comparison of the domain organization of DC-CIITA and Nod1. A, acidic; P/S/T, proline/serine/threonine rich; NBD, nucleotide binding domain; LRR, leucine-rich repeats.

MATERIALS AND METHODS

Plasmid Constructs—To clone the first exon of the dendritic cell form of CIITA, PCR was performed using human genomic DNA and the primers 5′-EcoRI-FLAG-DC (5′-CTGGAATTCTAGGACTACAAAGAGCAGTACATTAACTCCAGGCATCCTG-3′) and the 3′ fourth exon (5′-GTCCTTGCTCAGGCCCTC-3′). To facilitate the cloning and detection by Western blot, an EcoRI site and a FLAG epitope tag were introduced within the 5′ primer. The PCR product was digested with EcoRI and SacI, followed by ligation with an fragment encompassing the second through the last exon of full-length B-CIITA (1). The expression vector for CARD itself was produced by inserting the second through the last exon of full-length B-CIITA (1). The MHC class II promoter-driven luciferase construct contains 2 K promoter-driven luciferase (NF-κB-luciferase) and wild-type B cell isoform of CIITA were as described (8, 9). Full-length Nod1, the CARD of Nod1, and the NF-κB promoter-driven luciferase (NF-κB-luciferase) have been described (19).

Cell Culture—The human kidney cell line 293T was maintained in Click’s medium with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 10−5 M 2-mercaptoethanol. Cells were grown at 37 °C with 5% CO2. R22.2.5 cells were maintained in RPMI 1640 medium containing the same supplements as for the 293T cells. Human dendritic cells were prepared as described (20).
The First Exon of the CIITA Gene Expressed in DCs Encodes a CARD—Because the difference among the three isoforms of CIITA lies in the N termini (11), we studied the first exon unique to CIITA that is expressed in DCs in detail. We first compared CIITA transcripts from Raji B lymphoblastoid cells with DCs prepared from human peripheral blood. To distinguish the different isoforms of CIITA transcripts, we used the 5′ primer specific for the first exon unique to DC- or B-CIITA transcripts (Fig. 1A). The 5′ primer recognizing the second exon was used to detect the common exon. The 3′ primer from the fourth exon was used for all reactions. Consistent with the previous report (11), transcripts containing the B cell-specific first exon were present in both B and DCs (Fig. 1B). However, the DC-specific first exon was detected in dendritic cells, not in B cells.

To further characterize the N-terminal 94-amino acid region that is unique to DC-CIITA, we searched the public databases to identify peptides with homology to this region. The search revealed that the peptide encoded by the first exon of DC-CIITA has significant homology to CARDs found in members of the caspase family, as well as those found in caspase activators and NF-κB signaling molecules such as Nod1 and RICK (Fig. 1C). The predicted CARD of DC-CIITA has a total of six α helices (shaded boxes) that are a hallmark of CARDs (23). Furthermore, the residues most conserved among the CARDs of various molecules are also conserved in DC-CIITA. Of particular interest, the CARD of Nod1 has the highest homology with CIITA CARD (25% identity, 43% similarity; Fig. 1D). Both DC-CIITA and Nod1 have the CARD at the N terminus, the nucleotide binding domain in the middle, and the leucine-rich

**Transfection and Luciferase Assays**—The transfection of 293T cells was performed using the calcium phosphate method. For the activation of the MHC class II promoter, 1 × 10⁵ cells were plated in 12-well plates. Each well of cells was transfected with 100 ng of the E−luciferase reporter to 293T cells (left panel) or RJ2.2.5 cells (right panel). The fold induction was calculated the same way as described in the legend to Fig. 2. The results shown are from three independent experiments. B, dose-dependent activation of the MHC class II promoter by different isoforms of CIITA. 1.5 ng of E−luciferase reporter and 0.5 ng of cytomegalovirus-β-galactosidase were co-transfected with the indicated amount of the CIITA expression vector. The error bar for B-CIITA is too small to be shown. C, the same cell lysate used in B was used for the Western blot using the anti-CIITA antibody to determine the level of CIITA protein. 25 µg of cell lysate was loaded. D, nuclear (N) and cytoplasmic (C) fractions were prepared from 293T cells that were transfected with DC- or B-CIITA. 10 (B-CIITA) and 30 µg (DC-CIITA) of protein were loaded.

**RESULTS AND DISCUSSION**

**Flow Cytometric Analysis**—Cells were suspended in cold 1× phosphate-buffered saline supplemented with 1% fetal bovine serum. Staining was performed in the same buffer. L243 antibody was used to examine MHC class II. Flow cytometry was performed using FACSscan.

**Western Blot**—Nuclear and cytoplasmic fractionation (21) and Western blots were performed as described without any modification (9). An anti-CIITA antibody was used to detect endogenous CIITA (22).

**Sequence Alignment**—CARD homology in DC-CIITA was predicted and confirmed by two independent methods: ISREC ProfileScan, maintained by the Swiss Institute for Experimental Cancer Research, and PSI-BLAST, maintained by the National Center for Biotechnology Information.
repeat at the C terminus. Thus, DC-CIITA and Nod1 share similar domain organization.

**DC-CIITA Activates the MHC Class II but Not the NF-κB Promoter—**Nod1 promotes caspase-9-induced apoptosis and induces NF-κB activity (19). Because of the high homology between DC-CIITA and Nod1, we asked whether DC-CIITA acts as a regulator of apoptosis and/or NF-κB activation. To test this, we co-transfected 293T cells with the expression vector for DC-CIITA or Nod1 along with the NF-κB-luciferase reporter construct. The cytomegalovirus promoter-driven β-galactosidase was co-transfected to monitor transfection efficiency. As a control, we tested the activation of the MHC class II promoter. As expected, Nod1 activated the NF-κB but not the MHC class II promoter (Fig. 2). Conversely, DC-CIITA did not activate NF-κB but did activate the MHC class II promoter. In addition, the expression of DC-CIITA did not induce apoptosis when overexpressed in 293T cells. DC-CIITA but not Nod1 also activated the MHC class II promoter in R22.2.5 B cells that have a defect in the endogenous CIITA gene (24) (see below). These data suggest that the activities of DC-CIITA and Nod1 are distinct and that DC-CIITA does not participate in NF-κB activation.

**DC-CIITA Is a More Potent Activator than B-CIITA—**We then compared the transactivation activities of DC-CIITA and B-CIITA using the Eo-luciferase reporter. When DC-CIITA was co-transfected into 293T cells, the luciferase activity was greater than that from cells transfected with B-CIITA (Fig. 3A, left panel). DC-CIITA also showed a higher activity in R22.2.5 cells, indicating that DC-CIITA is a more potent activator than B-CIITA (Fig. 3A, right panel).

We wanted to examine the transactivation ability of the two isoforms in more detail. To do this, an increasing amount of each CIITA expression vector was co-transfected with a constant amount of the Eo-luciferase reporter. As shown in Fig. 3B, the induction of the MHC class II promoter by DC-CIITA was greater than by B-CIITA at any given concentration of DNA. However, this was not caused by more DC-CIITA protein being expressed in cells because DC-CIITA was expressed at a lower level than B-CIITA upon transfection (Fig. 3C). We next tested whether the protein levels of DC-CIITA in the nucleus are greater than those of B-CIITA, which may result in higher transactivation. When nuclear fractions of the two forms of CIITA were compared, the level of DC-CIITA was much lower than B-CIITA (Fig. 3D). In addition, the relative distribution of DC-CIITA between the two compartments was comparable with that of B-CIITA.

Because the above data were generated using transient assays, we tested whether DC-CIITA is also more potent in activating the endogenous MHC class II gene. We stably transfected DC-CIITA or B-CIITA to R22.2.5 cells and compared the levels of CIITA proteins and MHC class II on the cell surface. When the same amounts of cellular protein from two independent clones of B-CIITA- or DC-CIITA-transfected cells were analyzed, DC-CIITA proteins were barely detectable (Fig. 4A). Despite a lower level of DC-CIITA protein, the levels of MHC class II on the cell surface were comparable between DC-CIITA- and B-CIITA-expressing cells (Fig. 4B).

We next examined the levels of endogenous CIITA proteins in primary DCs that express higher levels of surface MHC class II than B cells. To do this, we prepared total cell lysates from Raji and human DCs and compared endogenous CIITA proteins by immunoblotting with the anti-CIITA antibody. Consistent with the data from transfection studies (Fig. 3C), the levels of DC-CIITA protein were lower than those of B-CIITA (Fig. 4C). Taken together, the results indicate that the transactivation potential of DC-CIITA is greater than that of B-CIITA.

---

K. Nickerson, T. J. Sisk, N. Inohara, G. Núñez, and C.-H. Chang, unpublished data.

---

**Fig. 5.** The presence of the CARD confers the higher transactivation activity of CIITA. The Eo-luciferase construct was co-transfected with the expression vector for the wild-type DC-CIITA, L27Q, or B-CIITA to 293T (left panel) and R22.2.5 cells (right panel). The DNA amount used for the transfection was described under “Materials and Methods.” 25 µg of total cell lysate from cells transfected with the wild-type (WT) or L27Q was used for the Western blot shown in the inset of the left panel. The anti-CIITA antibody was used for the blot. B, the CARD itself can inhibit DC-CIITA transactivation function. 293T cells were transfected with 1.5 µg of Eo-luciferase, 15 ng of the CIITA expression vector, and 1.5 µg of CARD or CARD(L27Q). *, p < 0.001 versus control (n = 5).
The CARD of DC-CIITA Confers Higher Transactivation Activity—We reasoned that the higher potential of DC-CIITA as a transactivator might be caused by the presence of the CARD. The conserved leucine residue at position 27 in the CARD of RAIDD is critical because mutation of this residue resulted in a loss of function (25). Similarly, the corresponding mutation of the CARD of Nod1 abolished its ability to interact with RICK or activate NF-κB (19). Therefore, we generated a mutant of DC-CIITA by substituting the conserved leucine with glutamine at position 27 (L27Q) to determine whether a functional CARD in DC-CIITA is required for enhanced transcriptional activity.

We transfected the mutant and compared its transcriptional potential to that of wild-type DC-CIITA and B-CIITA. As shown in Fig. 5A, the luciferase activity of the cells transfected with the L27Q mutant was equivalent to that of B-CIITA, suggesting that the additional activity of DC-CIITA is caused by the CARD. The level of L27Q protein was equivalent to that of the wild-type DC-CIITA (Fig. 5A).

The CARD is known to interact with other CARD-containing proteins through homophilic CARD-CARD association (18, 23, 26–29). If the CARD is responsible for a higher transactivation potential of DC-CIITA, then the CARD by itself might inhibit DC-CIITA function by interfering with the interaction of the CARD of CIITA with the CARD of a partner. To test this, we generated a construct expressing the wild-type CARD itself or the point mutant of the CARD with the L27Q amino acid substitution. We co-expressed wild-type or mutant CARD in 293T cells with DC-CIITA or B-CIITA in the presence of the Exo-luciferase reporter. The wild-type, but not CARD(L27Q), reduced the transactivation potential of DC-CIITA (Fig. 5C, lanes 1–3). The inhibition was specific for DC-CIITA because wild-type CARD did not affect the ability of B-CIITA to transactivate the MHC class II promoter (Fig. 5C, lanes 4–6).

The CARD is a critical domain mediating protein-protein interactions and is known to be present in proteins in the cell death and the NF-κB activation pathway. Here, we demonstrated for the first time that the CARD participates in transcriptional regulation in the context of DC-CIITA. DC-CIITA is a unique transcription factor possessing a CARD. CIITA CARD did not interact with several CARD-containing proteins tested so far, including caspase-1, -2, -4, -9, -IAP-1, RICK, Nod1, Nod2, ARC, Bel-10, CARD-12, and ICEBERG. In addition, neither cell death nor NF-κB activation seemed to be affected by DC-CIITA (Fig. 2). Rather, the CARD in the context of CIITA confers a higher activity for CIITA as a transcription factor to activate the MHC class II genes but not the molecules involved in cell death.

The mechanism by which the CARD serves as a transcriptional regulator of CIITA is not clear. We ruled out the possibility that DC-CIITA performs other functions specific for DCs. The CARD of CIITA with the CARD of a partner. To test this, we generated a construct expressing the wild-type CARD itself or the point mutant of the CARD with the L27Q amino acid substitution. We co-expressed wild-type or mutant CARD in 293T cells with DC-CIITA or B-CIITA in the presence of the Exo-luciferase reporter. The wild-type, but not CARD(L27Q), reduced the transactivation potential of DC-CIITA (Fig. 5C, lanes 1–3). The inhibition was specific for DC-CIITA because wild-type CARD did not affect the ability of B-CIITA to transactivate the MHC class II promoter (Fig. 5C, lanes 4–6).

The CARD is a critical domain mediating protein-protein interactions and is known to be present in proteins in the cell death and the NF-κB activation pathway. Here, we demonstrated for the first time that the CARD participates in transcriptional regulation in the context of DC-CIITA. DC-CIITA is a unique transcription factor possessing a CARD. CIITA CARD did not interact with several CARD-containing proteins tested so far, including caspase-1, -2, -4, -9, -IAP-1, RICK, Nod1, Nod2, ARC, Bel-10, CARD-12, and ICEBERG. In addition, neither cell death nor NF-κB activation seemed to be affected by DC-CIITA (Fig. 2). Rather, the CARD in the context of CIITA confers a higher activity for CIITA as a transcription factor to activate the MHC class II genes but not the molecules involved in cell death.

The mechanism by which the CARD serves as a transcriptional regulator of CIITA is not clear. We ruled out the possibility that the higher transactivation activity of DC-CIITA can be attributed to more efficient nuclear translocation and/or increased accumulation in the nucleus (Fig. 3D). Because one amino acid substitution at a conserved residue known to be critical for CARD-CARD interaction abrogates the CARD activity, the CARD of CIITA may act by recruiting a protein. Interactions between CARDS have been shown to be selective (19, 23). For example, the CARD of Apaf-1 interacts with caspase-9 whereas the CARD of Nod1 binds preferentially to RICK CARD (19, 23, 29). Therefore, it is likely that CIITA CARD recognizes a CARD of an unidentified protein(s), possibly a transcription factor(s), or a protein that cooperates with the DC-CIITA complex to enhance transactivation of the MHC class II gene. It would be of great interest to identify the partner molecule for DC-CIITA.

The conservation of the domain organization and the sequence homology between DC-CIITA and Nod1 suggest that these proteins may have originated from a common ancestral protein. Nod1 has been shown to confer responsiveness to bacterial lipopolysaccharides (30), indicating that Nod1 may have been evolved to become a sensor of pathogens. DC-CIITA, on the other hand, might be adapted as a transcription factor to induce MHC class II expression, which initiates the cascade of T cell-mediated immune responses resulting in elimination of pathogens. Thus, both DC-CIITA and Nod1 may have been evolved from an ancestral protein that might have been involved in host defenses against pathogens.

DCs are considered the most efficient antigen-presenting cells. They take up pathogens and antigens efficiently, express high levels of MHC class II, migrate from the sites of antigen acquisition to secondary lymphoid organs, and stimulate T cells (31). Our data suggest that the presence of the CARD is at least partly responsible for the enhanced level of MHC class II expression in DCs. However, we cannot rule out the possibility that DC-CIITA performs other functions specific for DCs. The assessment of the role of CARD in vivo would clarify the role of DC-CIITA and provide an insight toward a better understanding of differences between CIITA isoforms.

Acknowledgments—We thank Dr. Laurie Glimcher for providing the anti-CIITA antibody. We are also very grateful to Dr. Wes Dunnicliff for thoughtful discussions and critical reading of the manuscript.
