Biochemical and Biophysical Research Communications **278,** 58–62 (2000)
doi:10.1006/bbrc.2000.3769, available online at http://www.idealibrary.com on IDEAL®

Class II Transactivator Suppresses Transcription of Thyroid-Specific Genes

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Received October 5, 2000

Class II transactivator (CIITA) is the master regulator of MHC class II genes, and mediates their induction by interferon gamma (IFNγ). To study the role of CIITA in modulating the expression of thyroid-specific genes, we cloned the full-length rat CIITA and use it to transfect a rat thyroid cell line. We found that only one type of CIITA, type IV, is induced in thyroid cells upon IFNγ stimulation, and that CIITA is capable not only of inducing the expression of MHC genes in the thyroid, but also of differentially suppressing the expression of thyroid-specific genes. These findings suggest new avenues for the development of thyroid autoimmune diseases.

Key Words: **MHC; thyroid; autoimmunity; transcription factors; antigen presentation; and gene regulation.**

Class II transactivator (CIITA) is a GTP-dependent transcription factor (1) that controls the expression of multiple genes involved in antigen presentation, such as the major histocompatibility complex (MHC) genes (2). CIITA is necessary and sufficient for the expression of MHC class II genes (2), and mediates their induction by interferon gamma (IFNγ) (3). Finally, CIITA can act not only as transcriptional activator, but also as repressor for other immune-related genes; for example, it suppress transcription of IL-4, IL-5, and IL-13 in T lymphocytes (4).

The discovery that MHC class II molecules are aberrantly expressed on the surface of thyroid epithelial cells in Graves' disease and Hashimoto's thyroiditis has provided an hypothesis to explain the initiation of these and other autoimmune diseases (5, 6). In fact, nonimmune cells can acquire the capacity to present their own autoantigens to T lymphocytes and initiate or perpetuate an autoimmune process (7). Additionally, aberrant class II expression does occur *in vivo* and can be returned to normal when patients are treated with agents that suppress their autoimmune thyroid disease (5, 8, 9). Also, an animal model of Graves' disease has been created by immunizing mice with fibroblasts containing overexpressed TSHR plus aberrant class II but not with either alone (10). For these reasons, the hypothesis deserves further study and the mechanisms by which class II and thyroid-specific genes might be coregulated deserves investigation.

MHC and thyroid-specific genes are coordinately regulated in the thyroid. It is known, for example, that thyrotropin (TSH) coordinately induces the expression of thyroglobulin (TG), thyroperoxidase (TPO), and the sodium iodide symporter (NIS) while decreasing the expression of the TSH receptor (TSHR), MHC class I, and MHC class II genes (11–17). The molecular basis for such coordinate regulation has been explained by the finding that transcription factors which regulate thyroid-specific gene expression in response to TSH also regulate MHC gene expression in the thyroid. Particular examples are thyroid transcription factor-1, a Y box factors, a single strand binding protein (SSBP-1), and the high mobility group protein, Sox-4 (12, 13, 15–17).

In contrast to TSH, interferon (IFNγ) induces the expression of the MHC genes but suppresses the expression of thyroid-specific genes (16–19). Such coordinate regulation of MHC and thyroid-specific genes may be important in maintaining self-tolerance. In fact, a reduced expression of thyroid-specific genes during ac-

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0006-291X/00 $35.00

tivation of MHC genes by IFNγ and, conversely, a reduced expression of MHC genes during activation of thyroid-specific genes by TSH can all function as a protective mechanism to suppress the expression of self antigens with MHC molecules. We recently overexpressed IFNγ gene in the thyroid and found that it did decrease expression of the NIS gene while increasing MHC class II (20). However, the action of IFNγ is mediated by the class II transactivator (CIITA); and CIITA might not have identical effects as does IFNγ (21).

The goals of the present study were, therefore, to investigate the role of CIITA on the expression of MHC and thyroid-specific genes, and the response of thyroidal CIITA to IFNγ. We cloned the rat CIITA and studied its transcriptional effects *in vitro*, using rat thyroid FRTL-5 cells. We show that CIITA not only induces the expression of MHC genes but also modulates the transcription of non-immune genes, such as TG, TPO, TSHR and NIS. We also show that only type IV CIITA is induced upon IFNγ stimulation, and that CIITA is the essential mediator of the effects of IFNγ action on activation and suppression of thyroidal gene transcription.

MATERIALS AND METHODS

Cloning of rat CIITA. A λgt11 library of rat spleen cDNA (Rat Spleen 5′-STRECH cDNA, Clontech, Palo Alto, CA) was screened by plaque hybridization using as probe a ^{32}P-labeled human type III CIITA cDNA fragment (16, 21). Nitrocellulose membranes were hybridized at 68°C for 12 h, then washed for 30 min each in 4× SSPE at 37°C, 2× SSPE at 37°C, and 1× SSPE at 65°C. Two clones were identified, purified (Lambda Kit from QIAGEN Inc., Valencia, CA), and sequenced. The 5′- and 3′-ends of the two cDNA clones were also extended using the Marathon-Ready Rat Spleen cDNA kit (Clontech), and sequenced. The genomic region flanking the 5′ end of rat CIITA was finally cloned using the Genome Walking kit (Clontech), and sequenced.

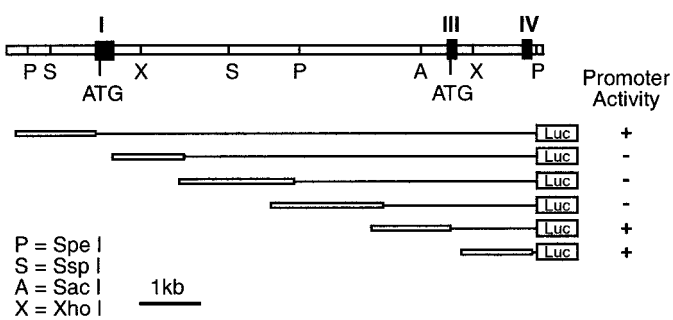

FIG. 1. Schematic representation of CITTA first exons and promoter activities. In the panel, three types of CIITA exon 1 are indicated by closed boxes and the introns by open boxes. Representative restriction enzyme and translation start sites are also shown. Intronic fragments were cloned in pGL3-Basic plasmid by PCR and transfected in FRTL-5 cells or LK35.2 mouse B cell hybridoma in the presence or absence of IFNγ. Luciferase promoter activity was measured and its presence indicated by (+) or (−).

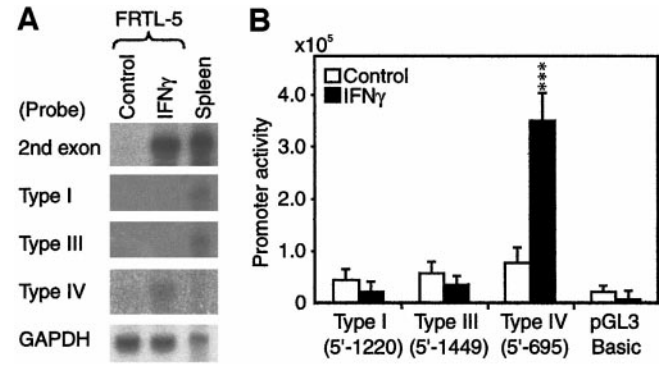

FIG. 2. Thyroidal expression of the three types of CIITA and their regulation by IFNγ. (A) Total RNA from untreated FRTL-5 cells, IFNγ-treated FRTL-5 cells, and splenocytes was probed with a 3′ common region of CIITA cDNA and with the three types of CIITA exon 1. (B) Promoter activity of the three types of CIITA exon 1, with or without IFNγ stimulation. Asterisks represent statistical significance with *P* values less than 0.001.

Reporter gene constructs, transfection, and measurement of promoter activity. For CIITA, the 5′-flanking region of type I, III, and IV exon 1 were subcloned in pGL3-Basic vector, which contains the luciferase reporter gene (Promega). For NIS promoter, reporter gene constructs were amplified by PCR using rat genomic DNA (Promega) as a template and specific primers (22), and then cloned into pGL3-Basic (Promega) and sequenced. For MHC class I, MHC class II, TG, TPO, and TSH-R promoters, the previously characterized chloramphenicol acetyl transferase reporter gene constructs (16, 17) were subcloned in pGL3-Basic (Promega) or pSEAP2-BASIC (Clontech) and sequenced. One μg of plasmid DNA was used to transfect FRTL-5 cells grown in 6-well plate using Lipofectamine Plus (GIBCO BRL, Gaithersburg, MD) as described (23–25). Promoter activity of luciferase and alkaline phosphatase was measured in a luminometer following the manufacturer's recommendations.

Others. Rat FRTL-5 thyroid cells (Interthyr Research Foundation, Baltimore, MD; ATCC CRL8305) were a fresh subclone (F1) with the properties described (11–13, 16, 17, 23–26). Extraction of total RNA and northern analysis were performed as described (23–25). All experiments were repeated at least three times and data shown mean ± SD, where P value greater than 0.05 was taken as significant.

RESULTS

Genomic features of rat CIITA. Sequencing of cDNA and genomic clones revealed that rat CIITA has three types of exon 1. They have been named type I, type II, and type IV following the nomenclature of the human CIITA gene (27) (Fig. 1 and Fig. 2A). Type I exon 1 (GenBank Accession No. AF251305) has its own translation initiation codon and is 83 and 72% identical to the mouse and human counterparts. Type III exon 1 (GenBank Accession No. AF251306) has also its own translation initiation codon and is the least similar to the mouse and human genes (62 and 46% identity, respectively). Type IV exon 1 (GenBank Accession No. AF251307), instead, consists only of untranslated sequences. For type IV CIITA the transcription starts in exon 2 and is in frame with that of type I and III

CIITAs. Each type of exon 1 is preceded by distinct promoter elements (GenBank Accession No. AF294912, AF294913, and AF294914 for type I, type III, and type IV promoter, respectively). Type II exon 1 is not present in the rat, similarly to the murine and differently from the human genome. This was confirmed by 5′ RACE cloning of cDNA and by sequencing of genomic clone, as well as by the lack of promoter activity between Type I and Type III exon 1, when reporter gene constructs were made in pGL3-Basic vector and transfected in FRTL-5 cells or LK35.2 mouse B cell hybridoma (Fig. 1).

Type IV CIITA encodes a protein of 1,059 amino acids that is identical to that encoded by type III and type I except for the N terminus; here, 21 and 101 additional amino acids are present in type III and type I CIITA, respectively. The rat type IV CIITA protein is 91% and 72% identical to the murine and human counterparts, respectively.

Type IV CIITA is the type expressed in the thyroid. The expression of the three types of exon 1 CIITA was studied by northern blotting using rat splenocytes and rat thyroid FRTL-5 cells stimulated with IFNγ. In thyrocytes, IFNγ induced expression of type IV exon 1, but not of type I and III (Fig. 2A). No CIITA mRNA was detected in the absence of IFNγ. In splenocytes, CIITA was constitutively expressed and showed the use of type I and III exon 1, but not of type IV exon 1 (Fig. 2A).

To confirm the specific induction of type IV CIITA in thyrocytes by IFNγ, we studied the promoter activity of each type of exon 1, using luciferase reporter gene constructs that included the 5′-flanking regions from each type exon 1. Upon transfection of FRTL-5 cells and IFNγ stimulation, we showed that only the activity of type IV exon 1 is increased, but not that of type I and III exon 1 (Fig. 2B). These results confirm that in thyroid cells the type of CIITA that is produced upon IFNγ stimulation is type IV.

CIITA induces the activity of MHC class I and class II promoters, but suppresses that of thyroid-specific gene promoters. After demonstrating in thyrocytes the induction of type IV CIITA upon IFNγ stimulation, we studied the effects of CIITA on the transcription of MHC class I and class II genes. Since other CIITA types might be induced in particular conditions, we examined the effects of all three types of CIITA. Full length type I, III or IV CIITA were all capable of similarly transactivating the 5′-1100, 5′-203, and 5′-127 regions of MHC class I promoter and the 5′-176 (Fig. 3A) and 5′-137 regions of MHC class II promoter in thyroid cells (Fig. 3B). This activation of MHC gene promoters was as strong as or better than that obtained after IFNγ stimulation (Fig. 3A vs 3C, and 3B vs 3D). These results indicate that although types I and III CIITA are not found in thyrocytes, they are capable

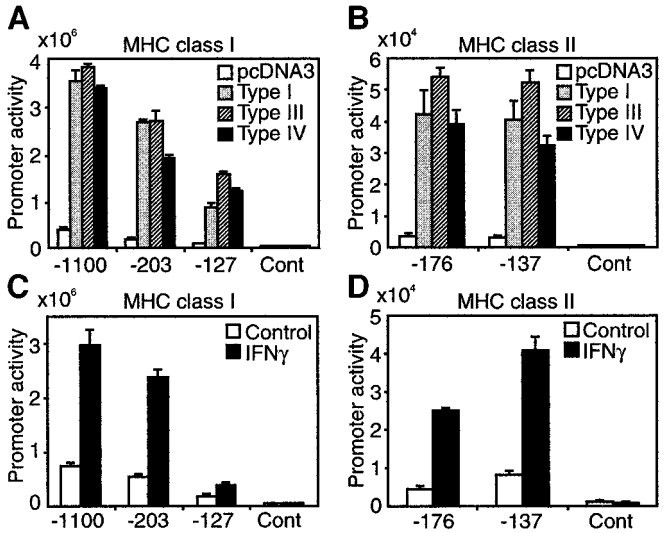

FIG. 3. All three rat CIITA types activate MHC class I and II promoters, as potently as IFNγ. (A, B) FRTL-5 cells cultured in 6-well plates were transfected with 1 μg of expression vector, each containing one of the three CIITA types, along with 1 μg of reporter gene constructs. (C, D) Rat IFNγ was added to the culture medium after transfection, at a final 100 U/ml concentration. Reporter gene activity was measured 48 h after transfection. *P* values were <0.01 in all cases where CIITA was transfected or IFNγ was added, except for IFNγ-treated 5′-127 MHC class I construct where *P* was <0.05.

of activating MHC gene transcription as well as type IV CIITA.

Finally, we tested the effect of the three CIITA types on transcription of thyroid-specific genes. FRTL-5 cells were transfected with reporter gene constructs containing minimal promoter regions of TG, TPO, TSHR and NIS. All three types of CIITA significantly suppressed the 5′-808, 5′-688 and 5′-207 regions of TG promoter (Fig. 4A) and the 5′-6300 and 5′-1372 regions of TPO promoter (Fig. 4B). In contrast, TSHR and, in particular, NIS promoter activity was only partially reduced by CIITA, and the results were not consistent among the three CIITA types (Figs. 4C and 4D). These results were consistent with those obtained upon IFNγ stimulation, where suppression of TG and TPO mRNA levels and promoter activities was more prominent than that of NIS and TSHR (data not shown).

DISCUSSION

This work was undertaken to study the effects of IFNγ-inducible genes on transcription of thyroid-specific genes. We focused on CIITA and showed that it regulates transcription not only of MHC genes, but also of thyroid-specific genes. We have demonstrated that CIITA strongly suppresses transcription of two thyroid-specific genes, namely TG and TPO.

In the thyroid, IFNγ was known to induce the aberrant expression of MHC class I and class II molecules

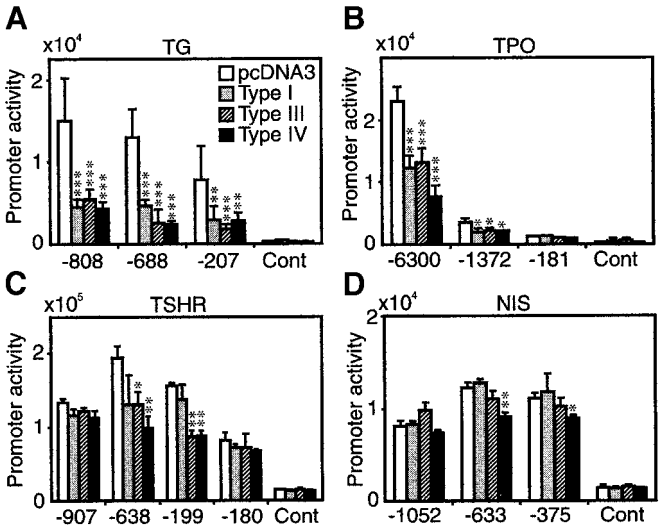

FIG. 4. Suppression of thyroid-specific gene transcription by CIITA. (A–D) One microgram μg of expression vector, each containing one of the three CIITA types, was transfected in FRTL-5 cells, along with 1 μg of reporter gene constructs. Reporter gene activity was measured 48 h after transfection. Promoter constructs of TG and TPO were in pGL3-Basic, TSHR, and NIS in pSEAP2-Basic. *, **, and *** denote statistical significance of $P < 0.5$, 0.01, and 0.001, respectively, compared to the basal promoter activity.

and to suppress the expression of thyroid-specific genes (16–19). The mechanism underlying this finding, however, was largely unknown. The present study indicates that type IV CIITA is induced by IFNγ and directly suppresses thyroid-specific gene transcription. CIITA has been considered as a co-activator of gene transcription of MHC-related genes, such as MHC class I, MHC class II, Invariant chain (Ii), HLA-DMA and DMB (28–31). However, its repressor activity was recently reported for IL-4, IL-5, and IL-13 genes in Th2 cells (4). Our results suggest the interesting possibility that other tissue-specific genes might be regulated by CIITA.

The present study clearly shows that there is a difference in response of thyroid-specific genes to IFNγ and CIITA. In fact, the response to IFNγ was more evident in the TG and TPO genes than is the NIS and TSHR genes. Among these, TG and TSHR are autoantigens associated with Hashimoto's thyroiditis and Graves' disease, respectively. The difference in the regulation of TG and TSHR genes by IFNγ/CIITA may suggest that a different mechanism determines which autoantigen is presented to the immune system. Thus, when MHC expression is induced by IFNγ or CIITA, TG expression is suppressed in order to prevent it from presentation to immune cells, whereas TSHR expression, which is not changed, might be more efficiently presented to immune cells by the thyrocyte MHC molecules. In such a case, anti-TSHR antibodies may be produced, resulting in autoimmune hyper- or hypothyroidism.

Presentation of self antigen in the context of MHC molecules to autoreactive T cells is considered the initial event in autoimmune diseases. This can be done by thyrocyte itself without professional antigen presenting cells (5–7, 25). Animal studies suggest the importance of self-antigen presentation by affected cells on development of autoimmune diseases. Thus, a number of different laboratories have attempted to induce Graves' disease in mice by immunizing various preparations of TSHR and resulted in only limited success. In contrast, immunizing MHC haplotype-matched cultured cells that express both TSHR and MHC class II provided successful results (10, 32). These studies, together with the present results, suggest the following possibility for the pathogenesis of Graves' disease. In a case when aberrant MHC expression is induced in the thyrocyte by CIITA, a TSHR specific peptide targeted by stimulatory antibodies is preferably presented. In such a situation, the expression of TG, which is a strong autoantigen, is suppressed. Such TSHR epitope may not be presented efficiently by antigen-presenting cells when soluble TSHR was used as immunogen. These hypotheses have to be proven by studies involving stimulation of T cell clones, as well as antigen processing and ubiquitination.

In conclusion, this study shows that type IV CIITA is the form induced in the thyroid upon IFNγ stimulation; that CIITA induces the thyroidal expression of MHC class I and II molecules as potently as IFNγ; and that CIITA suppresses the transcription of thyroid-specific genes in a pattern similar to that observed with IFNγ, suggesting that CIITA is the mediator of the suppression exerted by IFNγ on thyroid-specific genes.

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