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The MHC class II (MHC-II) transactivator (CIITA) is the master transcriptional regulator of genes involved in MHC-II-restricted Ag presentation. Fine tuning of CIITA gene expression determines the cell type-specific expression of MHC-II genes. This regulation is achieved by the selective usage of multiple CIITA promoters. It has recently been suggested that CIITA also contributes to Th cell differentiation by suppressing IL-4 expression in Th1 cells. In this study, we show that endogenous CIITA is expressed at low levels in activated mouse T cells. Importantly CIITA is not regulated differentially in murine and human Th1 and Th2 cells. Ectopic expression of a CIITA transgene in multiple mouse cell types including T cells, does not interfere with normal development of CD4+ T cells. However, upon TCR activation the CIITA transgenic CD4+ T cells preferentially differentiate into IL-4-secreting Th2-type cells. These results imply that CIITA is not a direct Th1-specific repressor of the IL-4 gene and that tight control over the expression of CIITA and MHC-II is required to maintain the normal balance between Th1 and Th2 responses. The Journal of Immunology, 2003, 170: 1150–1157.

Major histocompatibility complex class II (MHC-II) molecules present peptides to the TCR of CD4+ T cells. The interaction between MHC-II and TCR is essential for the development, survival, and activation of CD4+ T cells. MHC-II molecules are cell surface αβ heterodimers. There are three isotypes in humans (HLA-DP, -DQ, and -DR) and two in mice (I-A and I-E) (1). In addition, nonclassical MHC-II molecules encoded by related genes are also located in the MHC (HLA-DM and -DO in humans and H-2M and H-2O in mice) (1). These nonclassical molecules and the invariant chain are implicated in peptide loading and intracellular transport of MHC-II molecules. They are essential for inducing the mature conformation of MHC-II molecules and for efficient peptide presentation to the TCR (2, 3).

In contrast to MHC class I (MHC-I) proteins, MHC-II molecules are largely restricted to professional bone marrow-derived APCs such as B lymphocytes, dendritic cells (DCs), and macrophages. MHC-II expression is modulated during the development of APCs. For example, IL-4-stimulated B cells increase MHC-II expression, while plasma cells shut down this expression (4). Mature DCs up-regulate cell surface MHC-II expression, but turn off de novo synthesis of MHC-II transactivator (CIITA) and MHC-II proteins (5). MHC-II expression is also found on APCs that are not bone marrow-derived, namely the cortical thymic epithelial cells. In addition, various epithelia and endothelia express MHC-II molecules at variable levels (6, 7). Finally, most cell types can be induced to synthesize MHC-II molecules in response to cytokines such as IFN-γ. The importance of IFN-γ-induced MHC-II expression, or the expression that is found on epithelia and endothelia, is not clear.

The expression of classical and nonclassical MHC-II genes is regulated primarily by CIITA, which is encoded by the AIR-I gene (1, 8, 9). Fine tuning of CIITA expression determines the cell type specificity and level of MHC-II expression. This regulation is achieved by the selective usage of three independent CIITA promoters (pl, pII, and pIV) exhibiting different activities (10). Promoters pl and pII are active in bone marrow-derived APCs such as DCs and B cells. Promoter pIV is activated by IFN-γ and other cell types. In addition, pIV is essential for CIITA and MHC-II expression in thymic epithelial cells and thus for positive selection of CD4+ thymocytes (11).

Tightly regulated expression of MHC-II molecules is crucial for the adaptive immune system. It is essential to ensure controlled responses to pathogens (1). For example, macrophages and DCs increase MHC-II expression during the course of infections. It is thus not surprising that CIITA, as the key regulator of MHC-II expression, is targeted by several pathogens (1, 9). Increased CIITA and MHC-II expression also occur in normal and abnormal immune responses. Abnormal MHC-II expression is for instance found in many autoimmune conditions such as rheumatoid arthritis and lupus nephritis (6). In contrast, deficient MHC-II expression leads to severe immunodeficiency (9). Mutations in genes coding for transcription factors required for MHC-II expression cause the
bare lymphocyte syndrome. Mutations in the CIITA gene in both humans and mice result in a CD4+ T cell deficiency and in reduced T-dependent immune responses (9, 12).

CIITA knockout mouse models have allowed an assessment of the extent of CIITA-independent transcription of MHC-II genes. In two CIITA knockout mice (12, 13), transcription of classical MHC-II genes is drastically reduced in all cell types except for a few cortical thymic cells (12). Expression of the nonclassical MHC-II genes DM and DO is likewise reduced (12, 13). Transcripts of the invariant chain gene are reduced to a lesser extent (12, 13). In the third CIITA knockout mouse, in which there is an infarame deletion that may allow synthesis of a partially functional protein, residual expression of classical MHC-II molecules is found on DCs in lymph nodes (LN) (14). As a functional consequence of the deficiency in CIITA, MHC-II-restricted peptide presentation in these knockout mice is severely affected for several reasons. There is defective expression of cell surface MHC-II molecules, reduced peptide loading due to the lack of DM, and an immature floppyl conformation of the residual cell surface MHC-II molecules because of reduced invariant chain expression (1–3). In summary, it is fair to state that CIITA is indeed the master regulator of MHC-II gene transcription and MHC-II-restricted Ag presentation.

In addition to its tight control over MHC-II genes, CIITA has recently also been postulated to participate in CD4+ Th cell differentiation by repressing IL-4 gene expression (15, 16). This hypothesis was based on three lines of evidence. First, CD4+ T cells derived from CIITA knockout mice produced elevated levels of IL-4 and other Th2-type cytokines upon activation (15). In these CIITA−/− mice, development of the CIITA-deficient Th cells was ensured by the introduction of an I-Eβ and β transgene driven by the ubiquitously expressed MHC-I promoter. Second, CIITA mRNA was reported to be found in wild-type (WT) activated mouse CD4+ T cell preparations under Th1, but not Th2, conditions (15, 17). Finally, transfection experiments have suggested that CIITA might suppress IL-4 transcription in Th1 cells by competing with NFAT for binding to the general coactivator CREB-binding protein (CBP) (16). These reports supported the notion that CIITA could be a Th1-specific factor that functions as a direct repressor of IL-4 expression. One prediction of this model is that enhanced CIITA expression in CD4+ T cells would lead to IL-4 repression and enhanced Th1 responses (15).

To determine whether CIITA could indeed play a role in Th cell differentiation, we have performed a rigorous quantitative analysis of endogenous CIITA expression in activated mouse and human CD4+ T cells undergoing differentiation into Th1 and Th2 cells. In contrast to expectations, we find that endogenous CIITA expression is not regulated differentially during Th1-Th2 differentiation, in mouse and human T cells. Furthermore, we have studied the consequences of deregulated CIITA expression on Th cell differentiation by exploiting a new transgenic mouse strain that expresses CIITA ectopically in many cell types, including CD4+ T cells. We demonstrate that perturbing the normal pattern of CIITA and MHC-II expression results in a severe Th2 bias during CD4+ T cell activation. Our findings are in contradiction with the view that CIITA is a direct repressor of IL-4 expression.

Materials and Methods

Mouse

The transgenic CIITA construct will be described in detail elsewhere (L. A. Otten, V. Steinle, H. Acha-Orbea, and W. Reith, manuscript in preparation). Briefly, the mouse CIITA cDNA was placed under the control of the Sr promoter (18, 19) and the two fragments (MT5 and MT3) of the locus control region of the metallothionine gene (20). The transgenic mice were generated in a C57BL/6 x CBA/J F2 background. The MC42 transgenic line was backcrossed into CBA/J for at least nine generations. BALB/cJ and C57BL/6J mice were purchased from Harlan Laboratories (Horst, Holland). All the animals were maintained under conventional conditions.

FACS analysis

Mouse cells were incubated with mAbs against Fc-R (2-4G2) to block Fc-mediated nonspecific binding and fluorescently labeled Abs directed against B220 (RA3-6B2; Caltag Laboratories, Zürich, Switzerland), I-E (14–4–4S), I-A and I-E (2G9), CD3 (17A2), CD4 (L3T4), CD5 (53.7.3), CD25 (PC61; Caltag Laboratories), CD62 ligand (CD62L) (Mel14; Caltag Laboratories), and CD69 (H1.2F3). Unless otherwise indicated, the Abs were from BD Biosciences (San Diego, CA). To analyze double-negative mouse thymocytes, all cells expressing mature T cell and non-T cell markers were excluded as described (21). Human cells were labeled with anti-CXCR3 (clone no. 49801; R&D Systems, Minneapolis, MN), CRTH2 (provided by Dr. K. Nagata (R&D Center, BML, Kawagoe, Japan), and HLA-DR (L243).

Mouse lymphocyte purification

CD4+ CD62L− cells were isolated from peripheral LN and spleens. For RNA analysis, the cells were purified using MACS CD4 beads (Miltenyi Biotech, Bergisch Gladbach, Germany) followed by FACS selection for B220+ CD62L+CD4+ cells. The last selection achieves >99.5% purity. Splenic B cells were purified using Thy1-based complement depletion (90% purity). For ELISA, the cells were sorted in two steps using MACS beads for positive selection of CD4+ and CD62L− cells.

Mouse Th cell differentiation and cytokine detection in vitro

Naive CD4+ CD62L− cells were plated in flat-bottom 96-well Costar plates (Miller, Plan see Ouate, Switzerland) with immobilized anti-CD3 (5 μg/ml) at 105 cells/200 μl. They were cultured in DMEM, 10% FCS, 50 μM 2-ME, 2 mM L-glutamine, 10 mM HEPES, and 100 U/ml penicillin and streptomycin. For Th1 conditions, rIL-12 (5 ng/ml; BD Biosciences) and anti IL-4 (11B11, 20 μg/ml) were included. For Th2 conditions, rIL-4 (50 ng/ml; BD Biosciences), anti IL-12 (C17.15, 10 μg/ml), and anti-IFN-γ (XMG1.2, 10 μg/ml) were included. After 4 days, the cells were transferred to a new plate and cultured with IL-2 (10 U/ml) for 2 days. On day 7, the cells were washed twice and restimulated overnight with anti-CD3 (5 μg/ml). IFN-γ and IL-4 proteins in supernatants were measured by ELISA (BD Biosciences) with a detection limit at 50 and 20 pg/ml, respectively.

Quantitative transcript analysis

For mouse samples, quantitative RT-PCR using SYBR green was performed with a Light Cycler (Roche, Rotkreuz, Switzerland). Total RNA was purified using TRIzol (Invitrogen, Basel, Switzerland) and reverse-transcribed using random nonamers and SuperScript II (Invitrogen). Intron-spanning primers were used to avoid amplification of genomic DNA. The mouse primers are as follows, with F indicating forward and R indicating reverse: TATA-binding protein (TBP) F, 5′-ACCTCGTGCAAGAATGCTGAA-3′; TBP R, 5′-GTTCCGTGCTCTTATCTTCTA-3′; IFN-γ F, 5′-GGATGTGATTAGTCTACGCTTCTCCTGAGG-3′; IL-4 F, 5′-CCCGAGATGTGACAGCCAAC-3′; IL-4 R, 5′-AGGCCCTACAGGCGCTACTC-3′; CIITA F, 5′-CTCTTCCATCAAGCTCTCAAATG-3′; CIITA R, 5′-TGCGCGTACCAGGCTCC-3′. Amplification plots were analyzed using the second derivative method with LC data analysis 3.5 software (Roche). Corrections for amplification efficiency were included. For human samples, TaqMan RT-PCR for CIITA and 18S RNA was performed as reported (5).

Human T lymphocyte differentiation in vitro

PBLs used in this study were obtained from two informed adult volunteers in accordance with the ethical standards of the regional committee on human experimentation. In vitro differentiation was performed as previously described (22, 23). Briefly, CD3+ CD4+ cells were first purified to 99% by negative magnetic separation against CD8, CD14, CD16, CD19, CD20, CD44, CD45R0, CD45RA, and B. To obtain a pure Th2-oriented cell line, CD3+ CD4+ CRT2+ cells were positively selected from the CD3+ CD4+ population by MACS system (Miltenyi Biotech) (23). CD3+ CD4+ and CD3+ CD4+ CRT2− cells were then stimulated, in RPMI 1640 medium containing 10% heat-inactivated FCS with plate-bound anti-CD3 (5 μg/ml) plus anti-CD28 (10 μg/ml) mAbs, IL-2 (25 IU/ml), and in the presence of IL-12 (2.5 ng/ml) or IL-4 (2 ng/ml), respectively. On day 12, all the T cell lines were monitored for surface expression of IFN-γ and IL-4 proteins by ELISA.
molecular expression and stimulated for detection of the intracellular synthesis of IL-4 and IFN-γ.

B cells were purified from PBLs using MACS CD19-beads (Miltenyi Biotec).

Results

CIITA expression is not regulated differentially in Th1 and Th2 cells

To address a potential role of CIITA in Th cell differentiation we used real-time RT-PCR to obtain reliable measurements of CIITA expression levels in activated mouse CD4⁺ T cells. Such rigorous quantitative measurements have not been reported previously. Activation of highly purified naive CD4⁺ T cells (CD4⁺CD62L⁺) was performed in vitro with plate-bound anti-CD3 mAb under unbiased, Th1 or Th2 conditions. Results obtained with CD4⁺ T cells from mice of C57BL/6, BALB/c, and CBA strains were similar. Interstrain differences were less important than the experimental variability and the results were therefore pooled (Fig. 1A). Before activation, the naive CD4⁺ T cells expressed CIITA mRNA at a 10-fold lower level than B cells. This level was not enhanced during activation. Instead, a further transient 10-fold reduction in CIITA mRNA was observed after 48 h, while CIITA levels were similar at 0 and 96 h. At the two time points analyzed (48 and 96 h), the unbiased, Th1 and Th2 conditions did not influence the expression of CIITA mRNA differentially. Based on previous quantitative RNase protection experiments, we have estimated that mouse B cells express only about five copies of CIITA mRNA per cell (24, 25). It follows that there is an average of only 0.5–0.05 copies of CIITA mRNA per cell in the naive and activated CD4⁺ T cell preparations. This implies that there is either an intermittent expression of CIITA transcripts in most purified cells or a continuous expression in a minor subpopulation of cells.

To search for an eventual heterogeneity of CIITA and MHC-II expression in the mouse CD4⁺ T cell population, we performed FACS analysis on purified T cells before and following anti-CD3 stimulation (Fig. 1B). No significant MHC-II expression was detected on CD4⁺ T cells before or after activation for 48 or 96 h. No subpopulation with high MHC-II expression was found. These results show that naive mouse CD4⁺ T cells and activated Th1 and Th2 cells express low amounts of CIITA mRNA, and that this level is not sufficient to induce cell surface MHC-II expression that is detectable by FACS. In addition, no evidence was found for the existence of a minority of CD4⁺ T cells that express high levels of CIITA and MHC-II.

To determine whether the low levels of CIITA transcripts present in mouse CD4⁺ T cells are functionally relevant, we examined MHC-II expression at the mRNA level by a more sensitive assay (Fig. 1C). I-Aα mRNA levels were measured by real-time RT-PCR and compared with CIITA mRNA levels in the same purified CD4⁺ T cells at 0, 48, and 96 h of stimulation under unbiased, Th1, and Th2 conditions. The CD4⁺ T cells expressed I-Aα mRNA at levels that are 30- to 2300-fold lower than in B cells. Under all conditions, CIITA and I-Aα levels were tightly correlated in the CD4⁺ T cells (r² = 0.91). These results suggest that CIITA protein is synthesized in the highly purified CD4⁺ T cells and that MHC-II transcription in these cells occurs at very low levels. However, these levels are too low to give rise to detectable cell surface MHC-II expression (Fig. 1B).

In contrast to mice (26), it is well-established that human T cells up-regulate expression of CIITA and MHC-II during activation in vitro and in vivo (27-29). Therefore, we extended our analysis to human Th1 and Th2 CD4⁺ T cells. Polyclonal Th1 and Th2 cell lines were generated from purified peripheral blood CD4⁺ T lymphocytes. Real-time PCR measurements of CIITA mRNA were conducted immediately after isolation or after a week of differentiation (Fig. 2A). In the ex vivo CD4⁺ T cell population, CIITA is expressed 20-fold less than in purified peripheral blood B cells. In vitro-activated T cells up-regulated CIITA mRNA expression under Th1 and Th2 conditions. The observed 2- and 3-fold increase is underestimated because the ex vivo CD4⁺ T cell population was composed of both naive and activated T cells, which are negative and positive for CIITA expression, respectively (28). The same cells were also analyzed by FACS for HLA-DR expression and the Th1-Th2 differentiation markers CXCR3 and CCR7 (2). In contrast to the situation observed during mouse T cell activation (Fig. 1B), the human Th1 and Th2 cells clearly induced cell surface MHC-II molecules (Fig. 2B). The percentage of HLA-DR-positive cells in Th1 or Th2 cells was 2.5- to 10-fold higher than in ex vivo CD4⁺ T cells. Importantly, HLA-DR expression was increased in both the Th1 and Th2 cells. It is to be noted that expression of CIITA and HLA-DR was more variable under Th1 conditions than under Th2 conditions.
Taken together, the combined mouse and human results demonstrate that the CIITA gene is not regulated differently during the development of Th1 and Th2 cells. Moreover, in mouse T cells the low amount of CIITA is not sufficient to significantly activate MHC-II expression. These results are difficult to reconcile with a key role of CIITA in direct repression of the IL-4 gene specifically in Th1 cells.

**Ectopic MHC-II expression in CIITA transgenic mice**

Activated CIITA-deficient mouse CD4+ T cells were reported to express increased levels of IL-4, suggesting that CIITA functions as a repressor of the IL-4 gene (15). It follows that increased CIITA expression in CD4+ T cells should result in an inhibition of IL-4 expression. Therefore, we turned to a new strain of transgenic mice expressing CIITA in T cells (L. A. Otten, V. Steimle, H. Acha-Orbea, W. Reith, manuscript in preparation). These mice were generated to study the importance of properly regulated CIITA and MHC-II expression for the regulation of the immune response.

In the transgenic construct, expression of mouse CIITA cDNA is controlled by the SRα promoter, which drives high transcription and translation levels (18), and the locus control region of the metallothioneine gene (20), which has been reported to provide copy number-dependent transgene expression to heterologous promoters (Fig. 3A). The transgenic lines showed standard Mendelian transmission of the transgene and the absence of overt developmental defects or lethality under conventional housing conditions. The transgenic line MC42, having high transgene expression, was chosen for further analysis. This transgenic line expresses the CIITA transgene in all organs tested, including the...
skin, liver, kidney, lung, and various lymphoid organs (data not shown). As a consequence, MHC-II mRNA expression is increased in many of these organs to levels that are comparable to those found in WT lymphoid tissues.

To examine the effect of the CIITA transgene on cell surface MHC-II expression in T cells, we first performed a FACS analysis of cells from the spleen and peripheral LNs of the transgenic mice and littermate controls. The proportion of I-E-positive cells in the non-B cell (B220-negative) fraction was greatly enhanced in both transgenic organs (Fig. 3B). The level of I-E expression in these transgenic T cells was very similar to that observed in WT B cells and was only marginally increased in the transgenic B cells (Fig. 3B). Because the level of MHC-II expression is dependent on the concentration of CIITA (24), these results indicate that the CIITA transgene is expressed at significant levels.

The expression of CIITA and I-Aα transcripts was next examined by real-time RT-PCR in highly purified naive CD4+ T cells, either directly after FACS sorting or after activation using anti-CD3 mAb under unbiased conditions (Fig. 3C). In nonactivated transgenic CD4+ T cells, CIITA transcripts were only four times less abundant than endogenous CIITA mRNA in nontransgenic littermate B cells. During activation, an increase in CIITA transgene expression was observed, leading to levels 2-fold higher than in control B cells. In nonactivated transgenic CD4+ T cells, I-Aα transcript abundance was 15% of that in control B cells. Upon activation, the expression of I-Aα was increased and reached 56% of that in control B cells. Importantly, the expression of CIITA and I-Aα mRNA was induced to a similar extent under unbiased, Th1, or Th2 culture conditions (data not shown). To further assess the functional impact of CIITA transgene expression in CD4+ T cells we analyzed MHC-II expression by FACS (Fig. 3D). Transgenic T cells subjected to activation with anti-CD3 mAb were examined. MHC-II expression was clearly induced by the CIITA transgene in both the resting and activated transgenic CD4+ T cells. The mean level of MHC-II expression was increased during activation. This was the consequence of an induction of MHC-II expression in previously MHC-II-negative/intermediate cells (Fig. 3D). These results confirm that the MC42 line expresses the CIITA transgene in CD4+ T cells at levels that are sufficient to induce high MHC-II expression. Moreover, the maintenance of CIITA transgene expression during T cell activation permits an analysis of whether CIITA expression can repress IL-4 expression as previously proposed (15).

**CD4+ T lymphocyte development in CIITA transgenic mice**

The development of bone marrow-derived cells in the CIITA transgenic mice was assessed by several approaches. First, we evaluated cell counts in the primary and secondary lymphoid organs. Cell numbers in the transgenic and littermate mice were comparable in the bone marrow, thymus, and LN. The only documented difference was a 20% increase in cell number often found in the transgenic spleens. Second, we analyzed the different developmental stages of the T lymphoid lineage by FACS. In the thymus, modest MHC-II expression was detected in 21% of transgenic double-negative (CD8-CD4-) thymocytes (Fig. 4A). In more mature thymocytes, we did not find differences in the proportion of double-positive and single-positive thymocytes (Fig. 4B). At these developmental stages, the majority of the transgenic cells express MHC-II molecules. In the periphery, the proportion of CD8+ and CD4+ T cells was not altered in the transgenic mice (Fig. 5A) In these mature T cells, transgene-mediated MHC-II expression was further increased with respect to thymocytes. Finally, we examined cell surface markers on peripheral CD4+ T cells in the spleen and LN of the transgenic mice and control littermates (Fig. 5B). Again, no major differences were observed. At best, a reproducible but very minor decrease in CD3 and CD4 and an extremely modest increase in CD5 were observed in the transgenic cells. We did not detect any change in the proportion of activated CD4+ T cells (CD25+, CD62Llow, CD69+inth). In summary, the ectopic CIITA and MHC-II expression in the CIITA transgenic mice does not perturb normal CD4+ T cell development. Therefore, these mice provide a valuable model to study the importance of correctly regulated CIITA and MHC-II expression for Th cell differentiation and function.

**Activated CD4+ T cells from the CIITA transgenic mice exhibit a strong Th2 bias**

Activation of CD4+ T cells leads to their proliferation and the induction of a cytokine secretion profile that characterizes their Th function. Primary stimulation of naive Th cells allows an examination of their intrinsic ability for cytokine expression and proliferation during Th1-Th2 differentiation (30). To determine the cytokine expression pattern of the CIITA transgenic Th cells, highly
Discussion

CIITA expression during Th development in human and mouse CD4+ T cells

The species-specific difference in MHC-II expression between human and mouse CD4+ T cells is well-established. In both humans and mice, naive CD4+ T cells are MHC-II-negative. In contrast, memory and activated human CD4+ T cells express MHC-II (27, 28), while activated mouse CD4+ T cells do not (17, 26). Our results are entirely consistent with these previous findings. We find that CIITA and MHC-II expression is indeed up-regulated during activation of human CD4+ T cells. In contrast, no induction of
CIITA or MHC-II is observed during activation of mouse CD4+ T cells. The difference in MHC-II induction between the two species can be accounted for by the differential induction of CIITA (Figs. 1 and 2) (26, 28, 29).

It has been suggested that activated Th1 CD4+ T cells express higher levels of CIITA than Th2 cells (15, 17). In this study, we therefore examined the impact of Th1-Th2 culture conditions on CIITA and MHC-II expression in both human and mouse CD4+ T cells. We demonstrated that MHC-II expression is not differentially regulated in activated Th1 and Th2 CD4+ T cells. In human T cells, HLA-DR expression can be activated to similar levels in the two cell populations. In mouse T cells, in contrast, MHC-II expression is not activated under either Th1 or Th2 conditions. Importantly, in both species no correlation was found between Th1-Th2 differentiation and levels of CIITA mRNA.

Our results are in sharp contrast to previous reports describing the presence of CIITA mRNA in activated mouse CD4+ T cell preparations under Th1, but not Th2, conditions (15, 17). The reasons for this discrepancy are not clear and may reside in the purity of the cell populations that were analyzed. In the two earlier reports, the presence of contaminants such as DCs in the CD4+ T cell population (97% purity) was not excluded (17). Moreover, the analysis of CIITA expression was performed by a conventional PCR approach that did not permit a quantitative evaluation of the expression levels (15, 17). In this respect it should also be mentioned that despite the signal for CIITA mRNA in these previous studies, no MHC-II expression could be detected at the surface of the T cells (15).

**CIITA is not a direct Th1-specific repressor of the IL-4 gene**

Previous transfection experiments have shown that CIITA is capable of repressing the IL-4 promoter in a T cell line (15). The mechanism proposed to be implicated in this repression involves the CIITA-mediated sequestration of the general coactivator CBP, which is required for IL-4 transcription (16). In addition, CIITA−/+ CD4+ T cells were found to express more IL-4 (15). On the basis of these results it has been proposed that CIITA contributes to Th differentiation by inhibiting IL-4 expression in Th1 cells. The results presented in this study render it unlikely that this mechanism is operative in vivo, at least under physiological conditions. First, CIITA expression in mouse T cells is not increased upon TCR activation. If anything, a transient decrease is observed after activation for 2 days. Second, mouse Th1 and Th2 cells do not express different levels of CIITA. Third, CIITA transgene expression in mouse CD4+ T cells, to levels sufficient to activate MHC-II expression in these cells, does not repress IL-4 expression. In fact, quite the opposite is observed: CIITA transgenic CD4+ T cells have a 4- to 10-fold greater propensity for expressing IL-4. Finally, human Th2 CD4+ T cells can express levels of CIITA as high as Th1 cells, indicating that there is no direct correlation between the level of CIITA expression and silencing of the IL-4 gene. Therefore, we believe that CBP sequestration and silencing of the IL-4 gene, which is readily observed when CIITA is overexpressed in transfection experiments (15, 17), probably does not occur in vivo under the low expression levels of CIITA found in Th cells.

**Increased Th2 response in CIITA transgenic mice**

CIITA−/+ CD4+ T cells were previously shown to express increased levels of IL-4 under Th1 conditions (15). Based on this finding it was suggested that CIITA represses the IL-4 gene in Th1 cells. This interpretation would predict that increased CIITA expression in CD4+ T cells should repress IL-4 expression. Our novel CIITA transgenic mice, which express CIITA in most transgenic hemopoietic cells, including CD4+ T cells, allowed us to test this prediction. In contrast to expectations, the CIITA transgenic CD4+ T cells exhibit a strong bias for differentiation into IL-4-secreting Th2-type cells. Surprisingly therefore, a bias toward Th2 differentiation is found in both the CIITA-deficient T cells (15) and the CIITA transgenic CD4+ T cells studied in this report.

To resolve the paradox that the same Th2 bias is found in the presence or absence of CIITA in T cells, we propose that the IL-4 gene is not under the direct control of CIITA. Instead we suggest that the Th2 bias might result from a feature shared by the two models, namely the ectopic expression of MHC-II molecules. In the CIITA transgenic mice, ectopic expression of CIITA, and hence MHC-II genes, is driven in multiple cell types by a ubiquitous viral promoter. In the CIITA−/+ system, positive selection of CD4+ T cells is rescued by an MHC-II transgene driven by an MHC-I promoter. As in our transgenic mice, the use of the MHC-I promoter leads to ectopic MHC-II expression in multiple cell types. The ectopic expression of cell surface MHC-II molecules is indeed quite similar in the two models: for instance, B220-negative splenocytes express MHC-II in both systems (Fig. 3A and Ref. 15). Therefore, we propose that the Th2 bias may be an indirect consequence of the engagement of the TCR on naive CD4+ T cells by MHC-II molecules on cells that ectopically express CIITA. In this respect, the naive transgenic CD4+ T cells would be precommitted toward Th2 development in either the thymus or the periphery. Consequently, expression of CIITA and MHC-II in the CD4+ T cells undergoing Th differentiation would not be relevant for the regulation of IL-4 expression and the Th1/Th2 balance. This interpretation is supported by the identical expression patterns of CIITA and MHC-II that we observed in Th1 and Th2 CD4+ T cells.

**CIITA transgenic mice: modulation of ectopic MHC-II expression**

In our transgenic mouse, the CIITA transgene induces MHC-II expression in most cell types of hemopoietic origin, including T cells. However, MHC-II expression levels induced by the transgene are variable. This is clearly exemplified by an examination of the T cell compartment. In double-negative thymocytes, low levels of MHC-II expression are found in a minority of the cells (20%). In double-positive and single-positive thymocytes, as well as in mature peripheral T cells, MHC-II expression is increased and evident in a majority of the cells (50–70%). Finally, T cell activation leads to a further increase in MHC-II expression (90–100% positive cells). This implies that the efficiency of transgene expression is modulated as a function of developmental stage and activation status. This is reminiscent of previous studies showing variable transgene expression in T cells. For example, transgenes under the control of CD2 or lck promoters showed reduced and variable levels of expression in thymocytes (31, 32). The transgenic lck promoter was active in peripheral T cells, in contrast to the endogenous lck gene (32). In addition, a retrovirus-based construct expressed GFP at higher levels in activated rat CD4+ T cells (33). The reasons for this variability in expression remain unclear. It may result from the fact that heterochromatin-mediated silencing of transgenes is reduced during differentiation (34, 35).

**Conclusion**

The current study highlights the importance of correctly regulated CIITA and MHC-II expression for the adaptive immune response. It was already well-established that deficient MHC-II expression leads to immunodeficiency because of defective MHC-II-restricted Ag presentation and a reduction in CD4+ T cells (8, 9). We now...
show that ectopic CIITA and MHC-II expression is also detrimental because it can induce a Th2 bias. This demonstrates that the tight regulation imposed in vivo on the cell type specificity and level of CIITA and MHC-II expression represents a key parameter in determining the balance between Th1 and Th2 responses. This finding may provide new insight into the importance of MHC-II expression in nonprofessional APCs, such as epithelia and endothelia, in normal and pathological conditions. Our CITAg transgenic mice will also be valuable to evaluate the impact of deregulated MHC-II expression on professional APCs that normally modulate CIITA expression during the final stages of their development. The analysis of the CIITA transgenic mice should permit an evaluation of the importance of these modulations in vivo.

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