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Mice Lacking the MHC Class II Transactivator (CIITA) Show Tissue-Specific Impairment of MHC Class II Expression

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Summary

CIITA activates the expression of multiple genes involved in antigen presentation and it is believed to be required for both constitutive and IFNγ-inducible expression of these genes. To understand the role of CIITA in vivo, we have used gene targeting to generate mice that lack CIITA. CIITA-deficient (−/−) mice do not express conventional MHC class II molecules on the surface of splenic B cells and dendritic cells. In addition, macrophages resident in the peritoneal cavity do not express MHC class II molecules upon IFNγ stimulation nor do somatic tissues of mice injected with IFNγ, in contrast with wild-type mice. The levels of Ii and H-2M gene transcripts are substantially decreased but not absent in CIITA (−/−) mice. The transcription of nonconventional MHC class II genes is, however, not affected by CIITA deficiency. A subset of thymic epithelial cells express MHC class II molecules. Nonetheless, very few mature CD4 T cells are present in the periphery of CIITA (−/−) mice despite MHC class II expression in the thymus. Consequently, CIITA (−/−) mice are impaired in T-dependent antigen responses and MHC class II–mediated allogeneic reponses.

Introduction

Major histocompatibility complex (MHC) class II molecules are heterodimeric cell surface glycoproteins whose expression is critical for the development of CD4 T cells and the ability of the vertebrate to mount an immune response. These molecules are expressed on antigen-presenting cells such as B cells, macrophages, and dendritic cells, which take up, process, and present antigens to CD4 T cells. The expression of MHC class II on B cells is required for the collaboration between B and T cells that is necessary for an efficient antibody response. These molecules are also expressed on epithelial cells of the thymus, where CD4 T cells go through positive and negative selection to generate the mature T cell repertoire. The proper expression of MHC class II molecules is therefore crucial to regulate immune responses. Specifically, the lack of MHC class II expression can cause immunodeficiency (reviewed by Mach et al., 1994), whereas aberrant expression might result in autoimmune responses.

The molecular mechanisms responsible for the regulation of MHC class II gene expression in B cells have been extensively studied (reviewed by Glimcher and Kara, 1992). Many *cis* regulatory elements have been identified and DNA binding proteins have been characterized that could potentially be involved in the regulation of MHC class II gene expression. The conserved promoter elements, X, Y, and W boxes are present in all class II genes including human and mouse. The X box, subdivided into X1 and X2 boxes, has shown to be sufficient for B cell–specific expression (Sloan and Boss, 1988; Tsang et al., 1988; Sloan et al., 1992) and is required for interferon-γ (IFNγ) induction (Boss and Strominger, 1986; Basta et al., 1988; Tsang et al., 1988, 1990; Sloan et al., 1992). The X box is recognized by multiple proteins, RFX (Reith et al., 1988), X2BP (Hasegawa and Boss, 1991), and hXBP-1 (Liou et al., 1990; Ono et al., 1991). The Y box element, an inverted CCAAT sequence, binds the factor NF-Y (Hooft van Huijsduijnen et al., 1990) and functions to augment X box–directed expression (Hasegawa et al., 1993; Riley et al., 1995). W box elements and factors are the least conserved and characterized although several studies showed the significance of W box in both B cell–specific and IFNγ-inducible expression (Basta et al., 1988; Tsang et al., 1988, 1990; Cogswell et al., 1991; Sloan et al., 1992; Hasegawa et al., 1993).

Bare lymphocyte syndrome (BLS) is a hereditary severe combined immunodeficiency disease. It is characterized by the lack of HLA class II gene expression and a reduced number of mature CD4 T cells in the periphery of BLS patients (Mach et al., 1994). There is genetic heterogeneity among BLS patients and studies demonstrated two distinct biochemical phenotypes that have been subsequently grouped as A, B, and C. Cells derived from patients in group A and the in vitro-generated mutant Raji cell line RJ2.2.5 show intact MHC class II structural genes and the normal profile of DNA binding proteins to the MHC class II promoter elements (Kara and Glimcher, 1991). These results suggested that the requirement of other factor(s) necessary to activate class II genes. Recently it has been shown that a cDNA encoding a factor called CIITA can complement MHC class II expression in these cells and that the defect in BLS-2 cells and RJ2.2.5 is caused by a mutation in a splice junction of an exon of the CIITA gene and deletion of a part of the CIITA gene, respectively (Steimle et al., 1993).

CIITA is required for both constitutive and IFNγ-inducible expression of MHC class II genes (Steimle et al., 1993, 1994; Chang et al., 1994). In addition, introduction of the CIITA gene driven by a constitutive promoter is sufficient to activate MHC class II genes in plasmacytoma cells or mouse T cells where MHC class II genes are not normally expressed (Silacci et al., 1994; Chang et al., 1995). Furthermore, in cell lines CIITA regulates

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the expression of HLA-DM and invariant chain (Ii) genes that are involved in antigen presentation (Chang and Flavell, 1995; Chin et al., 1995; Kern et al., 1995). To ascertain the role of CIITA in vivo, we have used gene targeting to generate CIITA-deficient (−/−) mice. In this study, we show that CIITA is a critical transcriptional factor for the expression of conventional MHC class II genes as well as Ii and H-2M genes in vivo and that CIITA (−/−) mice can be utilized as a model system for human BLS type II. However, nonconventional MHC class II gene transcription does not require CIITA.

Results

Generation of CIITA (−/−) Mice

Since the structure of the mouse CIITA genomic DNA is not yet characterized, we screened a mouse genomic DNA λ phage library derived from the 129/Sv strain. The probe used for the screening was prepared by reverse transcriptase-polymerase chain reaction (RT–PCR) using mouse B cells with a set of primers from 2881–2901 and 3331–3351 nt. (The numbers correspond to human cDNA sequence published by Steimle et al., 1993). A defect in this region in human CIITA results in MHC class II deficiency. Preliminary studies showed that the sequence of this region is conserved between human and mouse (data not shown). Positive phage clones were partially mapped and a targeting vector was constructed by replacing a fragment containing exons shown to be critical for CIITA function and together with downstream sequences with a neomycin-resistance gene (Figure 1A). The herpes simplex virus–thymidine kinase gene was placed 10 kb downstream of the CIITA gene for negative selection against nonhomologous integration. The targeting vector was transfected into embryonic stem cells by electroporation, and transfectants were selected with G418 and gancyclovir. Resistant clones were screened by PCR and homologous recombinants were confirmed by Southern blot analyses. Four clones were identified and injected into C57BL/6J blastocysts to generate chimeric mice. We obtained two lines of germline-transmitted mice carrying the mutation at the CIITA locus and homozygous mice were generated by intercrosses of heterozygous mice (Figure 1B). Homozygous mice do not express detectable levels of CIITA mRNA, whereas wild-type mice do express CIITA message, confirming that the substitution introduced in the CIITA gene results in a null mutation (Figure 1C). CIITA (−/−) mice appeared healthy and exhibited no pathologic changes by gross examination when housed under specific pathogen-free conditions.

MHC Class II Gene Expression on B Cells and Dendritic Cells of CIITA (−/−) Mice

We first tested the constitutive expression of MHC class II molecules on B cells by staining total splenocytes with antibodies recognizing B cells (B220) and MHC class II (I-A^b). As shown in Figure 2A, I-A expression is not detectable in B cells derived from CIITA (−/−) mice. Likewise, I-A expression is not inducible upon stimulation of B cells with either lipopolysaccharide or interleukin-4 (IL-4). In contrast, B cells from CIITA (−/−) mice up-regulated the costimulatory molecule B7-2 when stimulated in addition with these same agents and proliferated normally compared with wild type (data not shown). These data suggest that the lack of MHC class II induction by these stimuli is not due to a global defect in the signaling pathway of B cell activation in CIITA (−/−) mice.

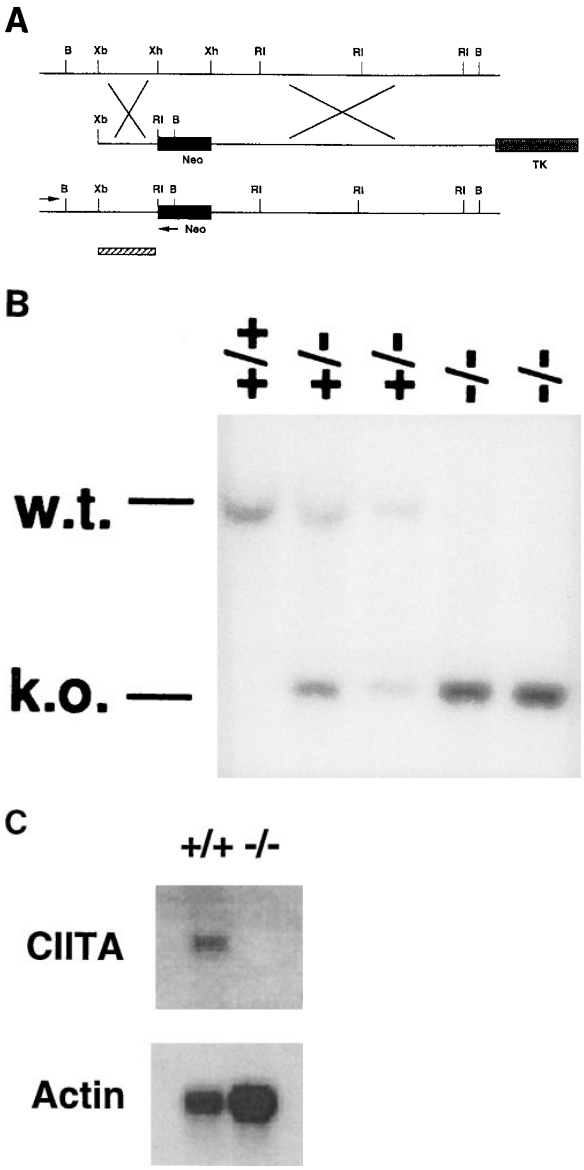

Figure 1. Construction of Targeting Vector and Southern Blot Analysis

(A) The line represents the genomic sequences of CIITA locus. Closed and shaded boxes represent *neo* and *tk* cassette, respectively. Arrows indicate primers used for PCR to screen homologous recombinants. The probe used for Southern blot analysis is shown at the bottom. Abbreviations for restriction sites are as follows: B, BamHI; Xb, XbaI; Xh, XhoI; RI, EcoRI.

(B) Analysis of offspring from CIITA heterozygote intercrosses. Tail DNA was digested with BamHI and EcoRI and analyzed by Southern blotting with the probe indicated in (A). Wild-type and the recombined alleles are shown.

(C) Northern blot analysis of poly(A)$^+$ mRNA from CIITA (+/+) and CIITA (−/−) mice. The membrane was hybridized with a probe derived from the human CIITA cDNA (DraIII–NotI).

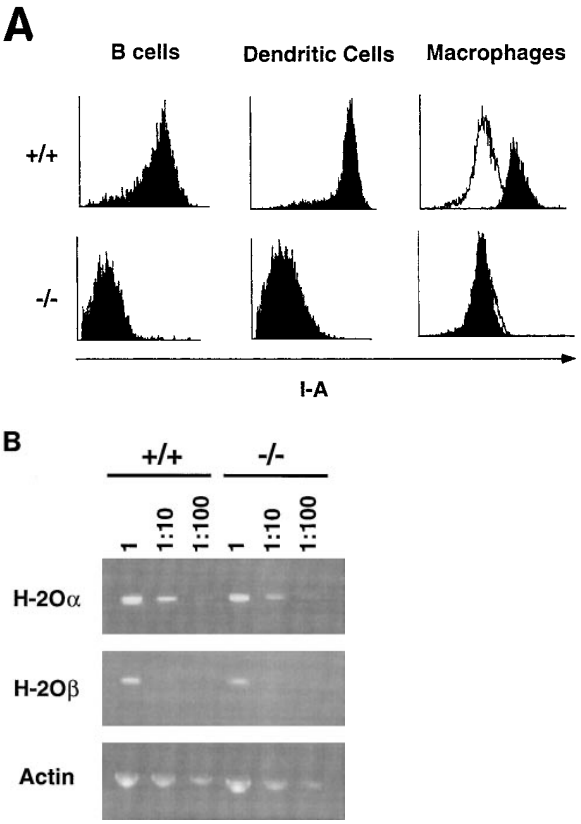

Figure 2. Expression of MHC Class II Molecules

(A) Cell surface expression of conventional MHC class II molecules. Single-cell suspensions were prepared from spleen of wild-type (+/+) and CIITA-deficient mice (−/−). Cells (1×10^6) were analyzed by two-color staining using biotinylated I-A^b (AF6-120, Pharmingen) followed by phycoerythrin-conjugated avidin and fluorescein isothiocyanate (FITC)-conjugated B220 (RA3–6B2, Pharmingen) for B cells, FITC-conjugated N418 (gift of K. Bottomly, Yale University) for dendritic cells, or FITC-conjugated MacI for macrophages (gift of K. Bottomly, Yale University).

(B) The expression of nonconventional MHC class II molecules. Total RNA was prepared from spleen and first-strand cDNA was synthesized using 2 μg of DNAse I-digested RNA and reverse transcriptase (GIBCO BRL). PCR was performed as described previously (Chang et al., 1994).

Previous studies showed that CIITA regulates all MHC class II genes (Steimle et al., 1993). To test whether the expression of I-E is affected by CIITA deficiency, RNA from total splenocytes were analyzed. Since CIITA (−/−) mice do not express I-E protein due to a mutation in the Eα gene (Mathis et al., 1983), we analyzed the level of Eβ gene transcripts by RT–PCR. As shown in Figure 4, Eβ transcripts are barely detectable in CIITA (−/−) splenocytes. It should be noted that the very low level of Aα transcripts were detectable in CIITA (−/−) mice. This shows that CIITA regulates the transcription of both MHC class II, I-A and I-E.

We also examined the expression of I-A on dendritic cells that normally express high levels of MHC class II molecules on the cell surface. Dendritic cells were first enriched from total splenocytes and stained with the dendritic cell marker (N418) and I-A. Dendritic cells from CIITA (−/−) mice did not express I-A, whereas wild-type dendritic cells expressed high levels of I-A (Figure 2A).

To determine whether all MHC class II genes are affected by CIITA deficiency, the expression of the nonconventional MHC class II genes, H-2Oα and H-2Oβ was tested (Wake and Flavell, 1985; Karlsson et al., 1991; Karlsson and Peterson, 1992). To do this, RNA prepared from total splenocytes was analyzed by RT–PCR. As shown in Figure 2B, comparable levels of both H-2Oα and H-2Oβ transcripts are found between CIITA (+/+) and CIITA (−/−) mice.

IFNγ-Inducible Expression of MHC Class II Genes Is Impaired in CIITA (−/−) Mice

The expression of MHC class II genes can be induced in macrophages upon activation with IFNγ. We therefore examined whether I-A expression is inducible in macrophages upon IFNγ stimulation in the absence of CIITA. Resident peritoneal macrophages were isolated from both wild-type and CIITA (−/−) mice, treated with IFNγ for 2 days, and analyzed by flow cytometry. I-A expression was induced after wild-type macrophages were treated with IFNγ (Figure 2). In contrast, macrophages derived from CIITA (−/−) mice did not express MHC class II molecules upon IFNγ stimulation (Figure 2). MHC class I molecules were, however, induced normally in CIITA (−/−) macrophages (data not shown), showing that the IFNγ signaling pathway is unaffected in these mice.

We also tested whether cells from CIITA (−/−) mice other than macrophages express MHC class II molecules upon IFNγ treatment. To do this, mice were injected intravenously with IFNγ for 3 consecutive days and the expression of MHC class II was analyzed by either flow cytometry or immunohistochemistry. In agreement with previous studies (Momburg et al., 1986a, 1986b), the constitutive expression of MHC class I molecules was detected in many organs tested, such as spleen, thymus, liver, kidney, heart, and lung of both CIITA (+/+) and CIITA (−/−) mice and levels were increased upon IFNγ injection (data not shown). Although CIITA (+/+) mice showed inducible expression of MHC class II in spleen, thymus, liver, and lung, MHC class II molecules were not detected from organs of CIITA (−/−) mice with the exception of thymus, which showed sparse positive staining for MHC class II (Figure 3). However, MHC class II level in the thymus of CIITA (−/−) was not inducible upon IFNγ injection (data not shown).

To characterize the nature of the cells expressing MHC class II molecules in the thymus of CIITA (−/−) mice, thymic sections were stained with various antibodies. As shown previously (Cosgrove et al., 1991; Grusby et al., 1991), in the control thymus MHC class II is confined to all cortical epithelial cells, medullary epithelial cells, and interdigitating cells (Figure 3A). In contrast, MHC class II molecules in CIITA (−/−) mice are expressed only in subsets of stromal cells (Figure 3B). In these mice, cortical MHC class II expression is confined to epithelial reticular cells (compare Figures 3B and 3C), but not all epithelial cells express MHC class II molecules. Even for those cells that stained positive, the staining intensity varies. Small groups of epithelial cells deeper in the cortex express MHC class II at higher levels than other MHC class II–expressing cells. Bright MHC class II–positive cells are found in the medulla, characterized by strong cytoplasmic staining.

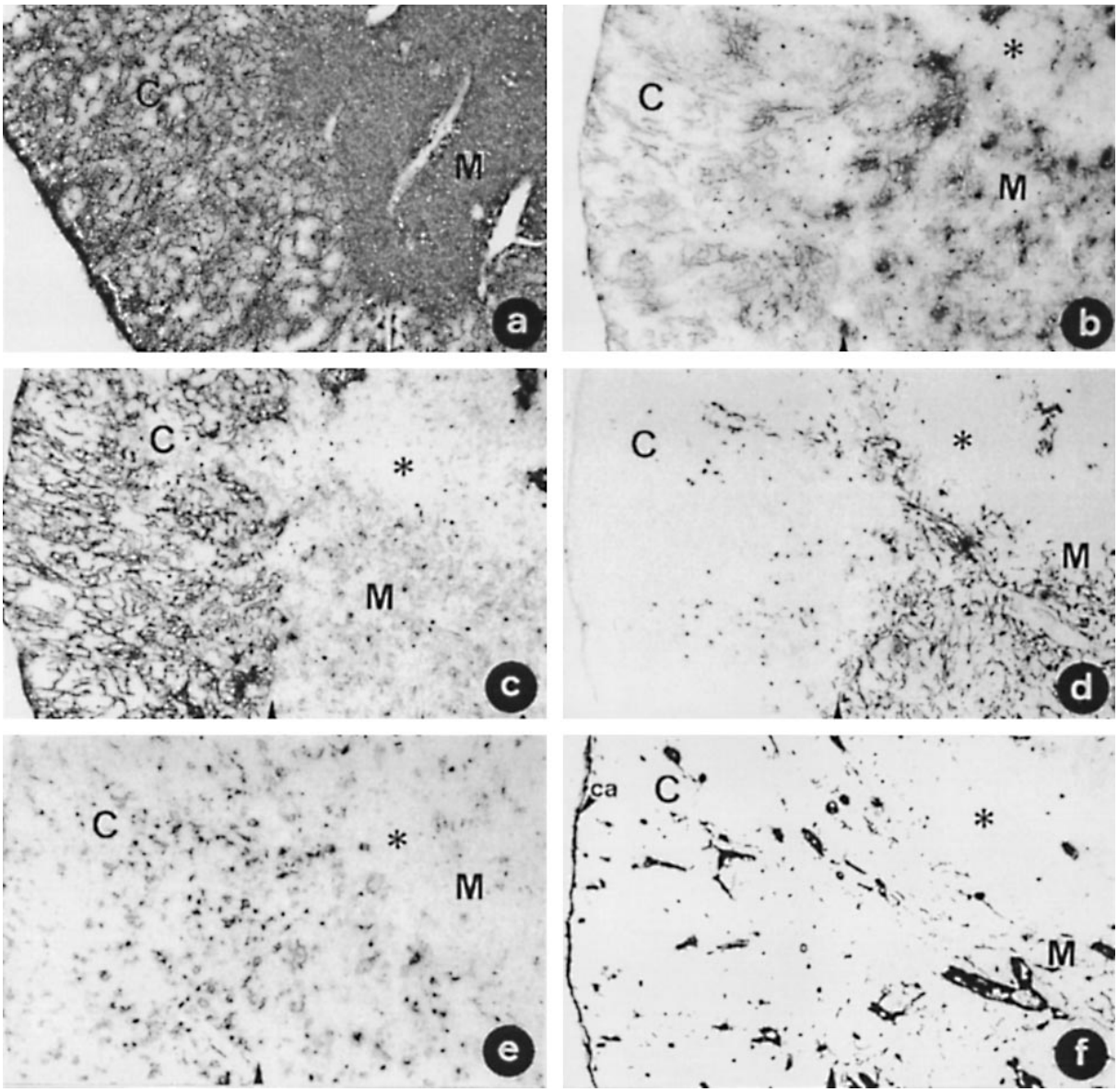

Figure 3. Immunohistochemistry of Thymic Sections of CIITA (−/−) Mice

Thymic section of the wild-type (A) and CIITA (−/−) (B) mice were stained with M5/114 for MHC class II molecules. Adjacent sections of CIITA (−/−) mice were stained with NLDC145 (C), ER-TR5 (D), a mix of three anti-macrophage monoclonal antibodies (F4/80, ER-MP3, ER-TR9) (E), and ER-TR7 (F). C, cortex; M, medulla; ca, thymic capsule. The arrows in B–F indicate the cortico–medullary junction. The asterisk indicates reticular-free domains.

Such cells are most probably interdigitating reticular cells (IDCs), since this staining pattern does not correspond to staining patterns observed in adjacent sections stained with antibodies to medullary epithelial cells (Figure 3D), macrophages (Figure 3E), or fibroblasts (Figure 3F). Furthermore, the cell size, together with the bright cytoplasmic MHC class II expression is indicative of IDCs (Rouse and van Ewijk, 1979; van Ewijk et al., 1980). The general stromal architecture of the thymus of CIITA (−/−) mice corresponds to the thymus of control mice except for the presence of reticular-free areas (Figures 3B–3F, asterisks). Such areas not only occur in the medulla but also are found in the cortex. The role of such areas is, at present, uncertain.

The MHC class II molecules detected in the thymus of CIITA (−/−) mice is bona fide I-A, not I-E, because neither thymic section of CIITA (−/−) mice stained with an isotype-matched control antibody to I-E (a class II molecules not expressed in H-2^b mice due to a mutation in the Eα gene; Mathis et al., 1983) nor thymic sections of Aβ (−/−) mouse stained with the same I-A antibody showed positive staining (data not shown).

Reduced Expression of Ii and H-2M in CIITA (−/−) Mice

We and others have previously shown that expression of CIITA in cell lines activates not only the expression of HLA-DR but also the Ii and HLA-DM genes involved

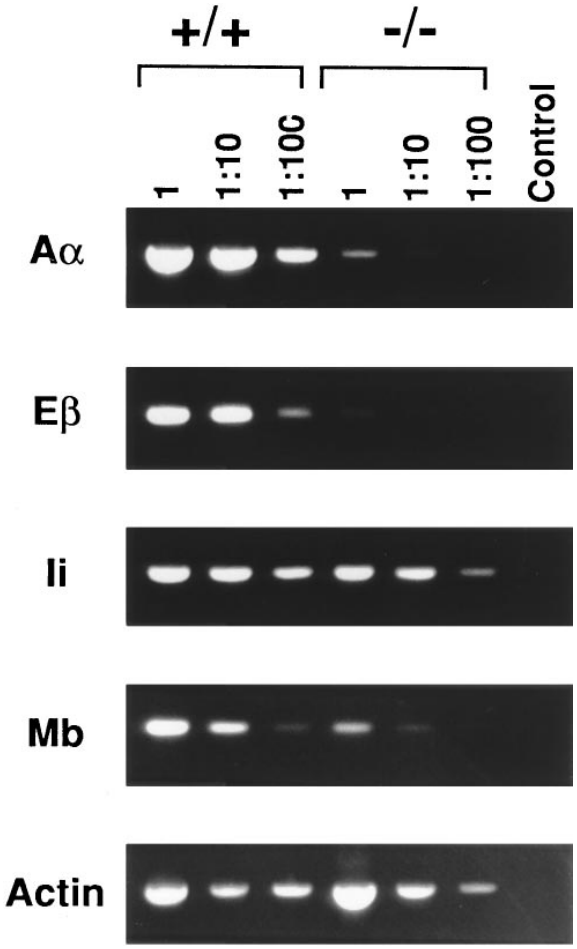

Figure 4. Expression of Ii and H-2M Genes in CIITA (+/+) and (−/−) Mice

Total RNA was prepared from spleen and first-strand cDNA was synthesized using 2 μg of DNAse I-digested RNA and reverse transcriptase (GIBCO BRL). PCR was performed as described (Chang et al., 1994). The control lane has the same components except first-strand product.

in antigen presentation (Chang and Flavell, 1995; Chin et al., 1995; Kern et al., 1995). To examine whether CIITA is required for the expression of these genes in vivo, RNA prepared from total splenocytes was analyzed by RT–PCR. As shown in Figure 4, the levels of Ii and H-2M gene transcripts are substantially decreased in CIITA (−/−) splenocytes, suggesting that CIITA is required for the optimal expression of these genes.

Lack of Positive Selection of CD4 T Cells in CIITA (−/−) Mice

It is believed that normal development of mature $CD4^+$ T cells requires MHC class II molecules to be present on thymic epithelial cells (Denning et al., 1988; Hugo et al., 1992; Jenkinson et al., 1992; van Ewijk et al., 1994; Hollander et al., 1995). Since CIITA (−/−) mice exhibit an unusual pattern of MHC class II expression in the thymus, the profile of T cells in the periphery was examined to determine whether the residual MHC class II molecules in the CIITA (−/−) thymus were sufficient to direct CD4 T cell development. T cells from lymph nodes of both CIITA (+/+) and (−/−) mice were prepared and analyzed (Figure 5A). Although CIITA (+/+) mice showed normal representation of CD4 T cells in the periphery, there was a drastic reduction of CD4 T cells in CIITA (−/−) mice (Figure 5A). The remaining $CD4^+$ cells express high levels of CD44, increased levels of CD69 and IL-2R, but lower levels of αβ T cell receptor (TCR) on the cell surface (Figure 5A). These CD4 T cells show a similar phenotype to the CD4 T cells in the periphery of Aβ (−/−) mice (Chan et al., 1993).

T cells in the thymus were also examined in the CIITA (−/−) mice. As expected from the peripheral T cell profile, CD4 single-positive T cells are not present in CIITA (−/−) mice (Figure 5B). There is, however, a residual population of cells that has been described previously to be at an intermediate stage of development, which are $CD4^{hi}CD8^{int}$. This cell population does not show much difference in the expression of the cell surface markers, CD44, HSA, and IL-2R, compared with the same population of CIITA (+/+) thymocytes.

Given that there is a massive reduction of mature $CD4^+$ T cells in the periphery of CIITA (−/−) mice, we have tested the T cell repertoire with a panel of Vα and Vβ antibodies in CIITA (−/−) mice. There was no major difference in the TCR usage of CD4 T cells with the exception of Vα2- and Vβ14-expressing T cells that were reduced in CIITA (−/−) as compared with CIITA (+/+) mice (Table 1). CD8 T cells, however, do not show any alteration of the TCR repertoire. These results are consistent with data generated previously using Aβ (−/−) mice (Chan et al., 1993).

T Cell Function

To assess whether T cells have any intrinsic defects due to CIITA deficiency, T cells were tested for their proliferation against various stimuli. First, we stimulated total lymph node T cells with either concanavalin A (ConA) or anti-CD3 antibody and examined them for the expression of different activation markers. The CD44, CD69, and IL-2R expression pattern of CD4 and CD8 T cells from wild-type and CIITA (−/−) mice was indistinguishable (data not shown). In addition, T cells from CIITA (−/−) mice proliferated normally upon activation with ConA or anti-CD3 antibody, suggesting that the TCR-mediated response is not impaired in CIITA (−/−) mice (Figures 6A and 6B). T cells were also tested for allogeneic responses. We first analyzed the response of total T cells from CIITA (−/−) mice to allogeneic stimulator B10.BR splenocytes (class I and class II difference). As shown in Figure 6, T cells from CIITA (−/−) mice showed comparable proliferation to CIITA (+/+) mice when B10.BR splenocytes were used as stimulators (Figure 6C). However, T cells from CIITA (+/+) but not from CIITA (−/−) mice proliferated against splenocytes from bm12 mice, whose MHC differs only at the MHC class II locus, indicating that CD4 T cell responses were greatly diminished, most likely owing to the reduction of peripheral CD4 T cells (Figure 6D).

We have tested whether $CD4^+$ cells from CIITA (−/−) mice can respond to antigen stimulation. To do this, mice were immunized with the T-dependent antigen

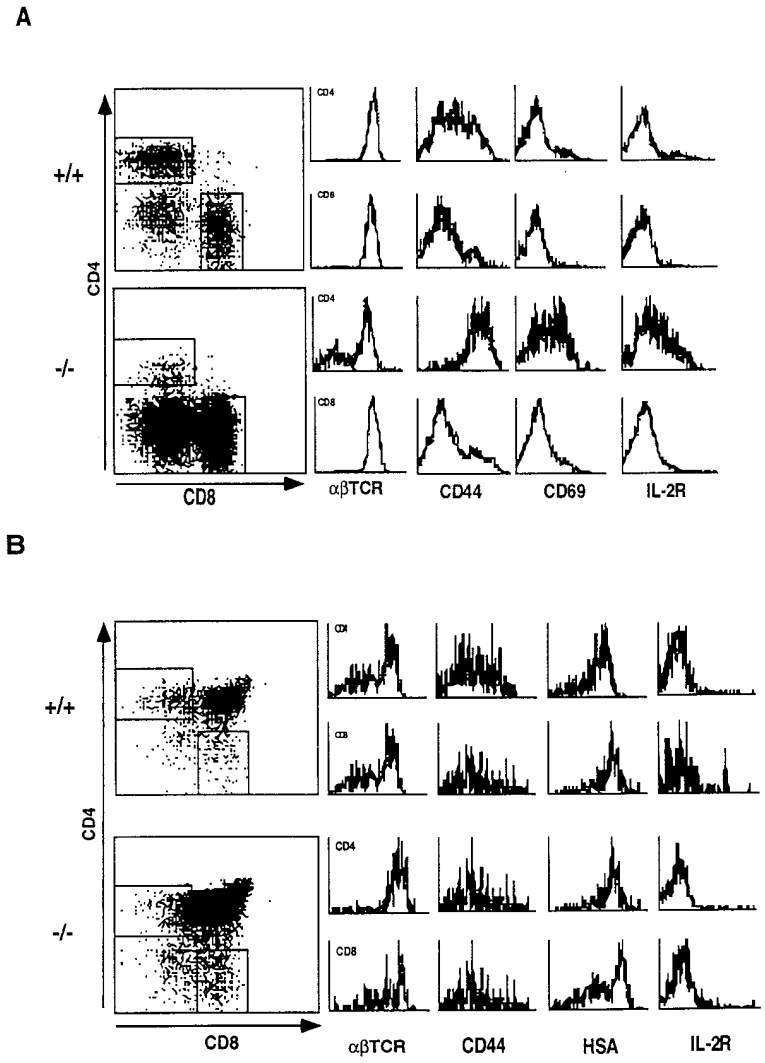

Figure 5. Analysis of T Cells from CIITA-Deficient Mice

Single-cell suspension was prepared from lymph nodes (A) and thymus (B) of control (+/+) and CIITA (−/−) mice and analyzed by three-color flow cytometry as described in Experimental Procedures. The leftmost panels are dot-plots of cells stained for CD4 and CD8. To the right are profiles of each staining indicated for gated $CD4^+CD8^-$ and $CD4^-CD8^+$ populations.

keyhole limpet hemocyanin (KLH) and analyzed. T cells were purified from draining lymph nodes 9 days after immunization and their proliferative responses against KLH were measured. As predicted from the absence of MHC class II, T cells from CIITA (−/−) mice proliferate very poorly, indicating that these mice do not respond to KLH immunization (Figure 6E).

CD4 T Cells Derived from Mice Lacking CIITA Can Develop Normally in a Wild-Type Thymic Environment

Despite MHC class II expression in the thymus of CIITA (−/−) mice, CD4 T cells are not selected and matured. There are two possible explanations for this phenotype. The first is that CIITA may be a critical factor for controlling CD4 T cell development. If this were true, CD4 T cells would not develop without CIITA regardless of MHC class II expression in the thymus. The other, more likely, possibility, however, is that CIITA deficiency results in a defect in thymic epithelial cells, not in CD4 T cells. This could be due either to a subpopulation of thymic epithelial cells expressing class II molecules that is unable to provide all the necessary signals required for appropriate maturation of CD4 T cells, such as medullary epithelium (van Ewijk et al., 1988; Burkly et al., 1990), or that the functional property of these thymic epithelial cells would depend on appropriate MHC class II–peptide complexes, which may be deficient in the CIITA (−/−) thymus even in the presence of MHC class II expression. In this case, CD4 T cells would not develop in the CIITA (−/−) thymus, but they would mature if they are present in an appropriate selecting environment such as the CIITA (+/+) thymus. We, therefore, tested these hypotheses by performing cell transfer experiments. Day 16 fetal liver cells from either CIITA (+/+) or CIITA (−/−) embryos were isolated and transferred into irradiated CIITA (+/+) or CIITA (−/−) recipients. In addition, we used irradiated C57BL/6 Thy-1.1 mice, which differ at the Thy-1 locus, as recipients to distinguish donor C57BL/6 cells (Thy-1.2) from the recipient (Thy-1.1). Cells from spleen, thymus, and lymph nodes from both chimeric animals were analyzed 2 months after transfer for their T cell profiles. In chimeric mice where the recipient and the donor were CIITA (+/+), peripheral $CD4^+$ and $CD8^+$ T cells were reconstituted at normal levels (Figure 7A). In contrast, the irradiated CIITA (−/−) mice

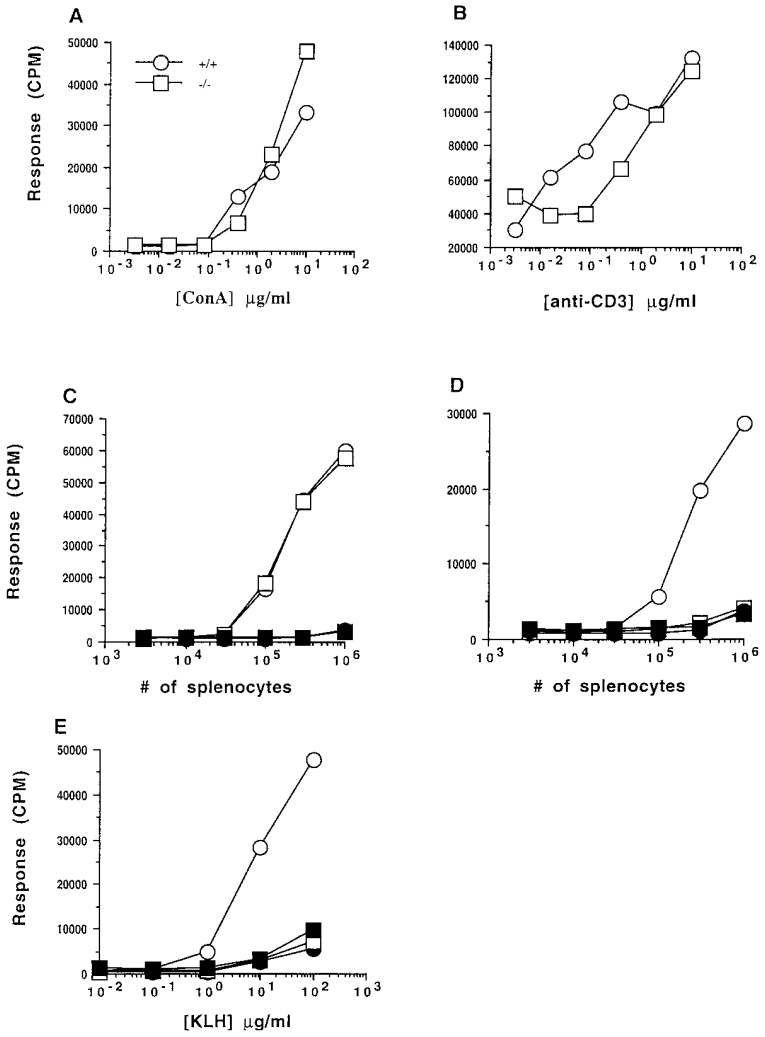

Figure 6. Proliferative Response of T Cells

(A–B) Response of lymph node T cells to ConA (A) or anti-CD3 antibody stimulation (B). T cells (2×10^5) from CIITA (+/+) (open circles) and CIITA (−/−) (open squares) mice were stimulated with different concentrations of stimuli for 72 hr. Proliferative responses were measured by [^{3}H]thymidine incorporation.

(C–D) Allogeneic response of T cells against B10.BR splenocytes (C) or bm12 splenocytes (D). Lymph node cells (2×10^5) from CIITA (+/+) (circles) and CIITA (−/−) (squares) mice were stimulated with different numbers of allogeneic splenocytes (open symbols) or syngeneic C57BL/6 (B6) splenocytes (closed symbols) for 72 hr.

(E) KLH recall response of T cells. Lymph node T cells were isolated from CIITA (+/+) (open circles) and CIITA (−/−) (open squares) mice immunized in vivo with 50 μg of KLH and stimulated in vitro in the presence of increasing amount of KLH. For comparison, unimmunized CIITA (+/+) (closed circles) and CIITA (−/−) (closed squares) are shown.

reconstituted with either CIITA (+/+) or CIITA (−/−) donor cells did not show significant reconstitution of CD4 T cells but did generate levels of CD8 T cells comparable to wild type (Figures 7B and 7D). CIITA (−/−) donor cells, however, repopulated the CD4 T cell compartment in irradiated wild-type mice to a comparable level as the reconstitution with CIITA (+/+) donor cells (Figure 7C). We also tested whether these cells are derived from donor cells rather than from residual cells that survived in recipients after irradiation. As shown in Figures 7E and 7F, B cells in CIITA (−/−) mice reconstituted with CIITA (+/+) cells or CIITA (+/+) mice reconstituted with CIITA (−/−) cells were I-A positive and negative, respectively, confirming that they are derived from donor cells. Furthermore, C57BL/6 Thy-1.1 mice reconstituted with CIITA (−/−) donor cells (Thy-1.2) showed mature CD4 T cells in the periphery, the majority of which were Thy-1.2 positive cells (Figures 7G and 7H). These data suggest that the lack of positive selection of CD4 T cells in CIITA (−/−) mice is not due to an intrinsic defect of CD4 T cells. The most reasonable explanation is that the subset of epithelial cells expressing MHC class II molecules in the thymus of CIITA (−/−) mice is unable to support the maturation of CD4 T cells.

Discussion

CIITA is believed to be required for the constitutive and IFNγ-inducible expression of MHC class II genes (Steimle et al., 1993, 1994; Chang et al., 1994). Here, we have shown that CIITA is a critical transactivator for both constitutive and IFNγ-inducible expression of MHC class II genes in vivo. CIITA (−/−) mice, therefore, recapitulate the phenotype of human BLS-2 patients who show lack of MHC class II molecules on all antigen-presenting cells and reduced number of CD4 T cells in the periphery. It should be noted that both Aα and Eβ transcripts are eliminated in CIITA (−/−) mice, suggesting that CIITA regulates both loci and possibly all MHC class II genes. This is consistent with previous studies, which show that human BLS-2 patients lack HLA-DR, DP, and DQ transcripts and the transfection of CIITA into cells defective in CIITA expression restores the expression of all these MHC class II genes (Steimle et al., 1993). This observation is not surprising since MHC class II genes share common promoter elements, in particular the X box, which has been shown to be the most crucial element to interact with CIITA (Riley et al., 1995; Zhou and Glimcher, 1995). However, MHC class II

Table 1. TCR Variable Region Usage in Peripheral T Cells

T cells	Header	Vα2	Vα3.2	Vα8	Vα11	Vβ2	Vβ5	Vβ6	Vβ8.2	Vβ10	Vβ11	Vβ14
CD4	control	12.72	0.49	2.85	4.21	6.37	3.15	8.55	17.11	4.12	5.56	10.89
		13.50	0.46	2.59	8.46	4.87	5.14	8.63	22.70	4.08	3.72	9.90
	CIITA	4.55	2.11	3.02	2.99	4.63	4.83	8.96	20.11	4.06	5.21	2.70
	−/−	5.62	1.10	3.99	7.40	2.83	9.18	10.42	16.79	1.68	6.32	2.46
		4.64	4.37	2.80	2.17	3.73	6.38	11.38	22.96	2.24	3.53	5.34
CD8	control	8.40	1.33	5.30	4.37	5.15	11.44	7.95	18.84	4.16	9.94	5.25
		10.06	1.49	4.25	6.51	3.12	14.25	7.12	21.19	4.15	5.47	4.14
	CIITA	7.03	2.58	4.02	1.84	5.22	10.36	7.59	16.98	5.48	5.76	2.57
	−/−	8.77	1.18	4.02	4.32	4.48	17.97	8.72	15.25	3.51	3.81	3.34
		6.00	2.03	3.79	1.50	3.91	15.18	8.60	15.05	3.18	5.44	3.49

Numbers represent the percent of T cells in the $CD4^+$ or $CD8^+$ population displaying a particular Vα or Vβ. Each value comes from triple staining of spleen cells or lymph node cells from an individual mouse. Controls are always negative littermates.

transcription is not abolished completely in CIITA (−/−) mice. It is possible that MHC class II genes can be transcribed at a very low basal level without CIITA, although this level may not have physiological significance.

It has been reported that the regulation of nonconventional MHC class II gene is different from that of conventional class II genes. The tissue distribution is limited such that H-2O molecules are detectable in B cells and thymic medullary epithelial cells (Wake and Flavell, 1985; Karlsson et al., 1991; Karlsson and Peterson, 1992). In addition, the transcription of H-2O is not inducible by IFNγ treatment, a mechanism that is believed to involve CIITA (Wake and Flavell, 1985; Steimle et al., 1993, 1994; Chang et al., 1994). Our data shows that the transcription of H-2O gene does not depend on CIITA and therefore that the mechanism of nonconventional MHC class II transcription is distinct from that of conventional MHC class II genes.

We and others have shown previously that CIITA is required not only for the expression of HLA-DR but also the Ii and HLA-DM genes, which are necessary for antigen presentation (Chang and Flavell, 1995; Chin et al., 1995; Kern et al., 1995). CIITA (−/−) mice, however, still express Ii and H-2M genes, albeit at substantially reduced levels. This may due to subtle differences in the organization of promoter elements among these genes. The phenotype observed, however, is consistent with the data generated using cells derived from BLS patients where there is leaky expression of HLA-DR, HLA-DM, and Ii (Chang and Flavell, 1995). CIITA, therefore, is a critical transactivator for the expression of MHC class II genes but to a lesser degree for Ii and H-2M gene expression.

CIITA (−/−) mice are viable and B cells from CIITA (−/−) mice do not appear to show any abnormalities other than the defect in the expression of MHC class II, Ii, and H-2M genes. Specifically, these B cells can mature, proliferate normally, and up-regulate costimulatory molecules upon stimulation by lipopolysaccharide or IL-4. Our data suggests that there is an alternative way to up-regulate costimulatory molecules in the absence of MHC class II, although it has shown that the up-regulation of costimulatory molecules can be regulated by the signaling through the MHC class II cytoplasmic domain (Nabavi et al., 1992). In addition, T cells of CIITA (−/−) mice respond normally to TCR-mediated stimuli. All these data suggest that CIITA probably does not regulate other essential genes except for genes involved in antigen presentation.

CIITA (−/−) and Aβ (−/−) mice share many similarities. Both mice show substantially reduced numbers of mature CD4 T cells in the periphery, the same pattern of surface markers on CD4 T cells, and a similar TCR repertoire (Cosgrove et al., 1991; Grusby et al., 1991; Chan et al., 1993). In addition, both mice show defective T cell responses to T-dependent antigens. CIITA (−/−) mice, however, exhibit a unique phenotype. First, both MHC class II mRNAs and, hence, cell surface I-A and I-E molecules are absent in CIITA (−/−) mice. Thus, CIITA (−/−) mice carrying MHC haplotypes other than $H\text{-}2^b$ do not express I-A as well as I-E (unpublished data). CIITA (−/−) mice should be, therefore, very useful to generate mice deficient in any MHC class II haplotype while retaining endogenous MHC class I gene expression.

Second, it is interesting that a subset of thymic epithelial cells of CIITA (−/−) mice but not Aβ (−/−) express MHC class II even without induction by IFNγ in CIITA (−/−) mice. MHC class II is expressed in subsets of cortical epithelial cells as well as in interdigitating reticular cells in the medulla. There are two possible explanations for this observation. First, a subset of thymic epithelial cells might utilize different transcriptional machinery for MHC class II genes. It is not yet clear what mechanism regulates MHC class II gene transcription in these cells but it is at least clear that MHC class II genes can be transcribed without CIITA, possibly at a reduced level since MHC class II molecules are detectable in the thymus of CIITA (−/−) mice. The other possibility is that cytokine production in the thymus may activate MHC class II transcription through a CIITA-independent pathway. Although MHC class II molecules were only detectable in the thymus of CIITA (−/−) mice whether or not they were treated with IFNγ, the most potent known cytokine that stimulates MHC class II gene transcription, we cannot rule out the possibility that other cytokines can up-regulate MHC class II gene transcription.

Although CIITA (−/−) mice express normal levels of nonconventional MHC class II mRNAs, it appears that the MHC class II molecules detected in the thymus are

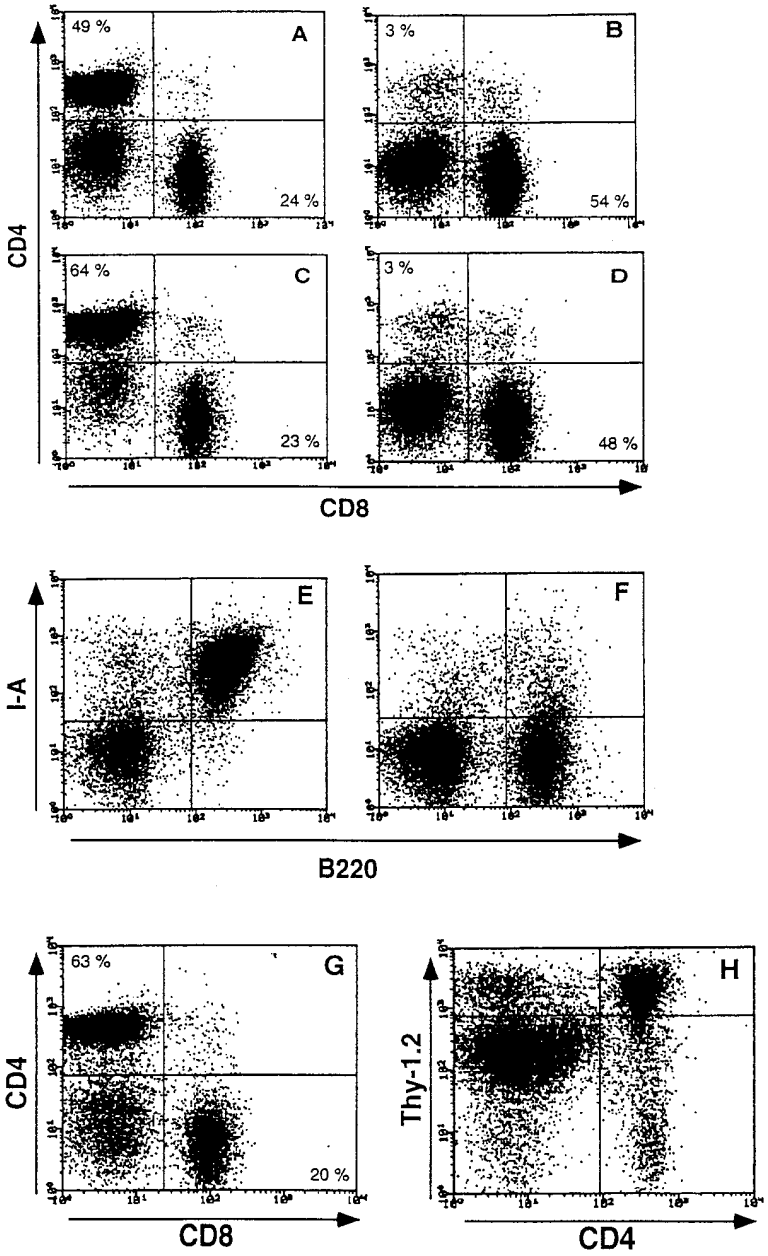

Figure 7. T Cell Profiles from Chimeric Mice after Fetal Liver Cell Transfer

Lymph node T cells were prepared and stained with CD4 and CD8 antibodies as described.

(A) Lymph node cells from an irradiated CIITA (+/+) animal reconstituted with CIITA (+/+) fetal liver cells.

(B) Lymph node cells from an irradiated CIITA (−/−) animal reconstituted with CIITA (+/+) fetal liver cells.

(C) Lymph node cells from an irradiated CIITA (+/+) animal reconstituted with CIITA (−/−) fetal liver cells.

(D) Lymph node cells from an irradiated CIITA (−/−) animal reconstituted with CIITA (−/−) fetal liver cells.

(E) Spleen cells from an irradiated CIITA (−/−) animal reconstituted with CIITA (+/+) fetal liver cells.

(F) Spleen cells from an irradiated CIITA (+/+) animal reconstituted with CIITA (−/−) fetal liver cells.

(G and H) Lymph node cells from an irradiated C57BL/6 Thy1.1 animal reconstituted with CIITA (−/−) fetal liver cells. We scored 25,000 events.

conventional I-A/I-E. This conclusion is drawn from the following observations: MHC class II molecules can be detected in both the cortex and medulla of CIITA (−/−) mice, whereas H-2O expresses only in the medulla (Karlsson et al., 1991; Karlsson and Peterson, 1992). Furthermore, we have shown, using the same reagents, that MHC class II molecules are not present in thymic sections of Aβ (−/−) mice where H-2O expression is not disturbed. It also has been suggested that the regulation of MHC class II expression is different in the thymus because BLS patients show significant number of CD4 T cells that appear to have a normal repertoire (Griscelli et al., 1989; Lambert et al., 1991, 1992). Nonetheless, thymic expression of MHC class II in CIITA (−/−) mice is not sufficient to direct positive selection of CD4 T cells. This difference can be explained by the fact that CIITA (−/−) mice have a null mutation, which was generated by deleting more than one exon of the CIITA gene, whereas at least some human BLS patients have a point mutation in the splicing junction (Steimle et al., 1993). It is possible, therefore, human BLS patient may express mutated CIITA molecules that can function partially. The other possibility is that the selection process of CD4 T cells or even the requirement of CIITA for MHC class II expression in humans is different from that of mice, although there is no direct evidence to support this notion.

Many studies demonstrate that the interaction between thymic stromal cells and hematopoietic cells is important for T cell development (Denning et al., 1988; Hugo et al., 1992; Jenkinson et al., 1992; van Ewijk et al., 1994; Hollander et al., 1995). We have shown that

the CIITA (−/−) thymus cannot select CD4 T cells even when the T cell precursors derive from normal CIITA (+/+) mice. Although it seems that T cells of CIITA (−/−) mice do not show any intrinsic defect, it is still possible that CIITA regulates genes other than MHC class II, Ii, and H-2M genes in cell types that are essential for CD4 T cell maturation in the thymus. We believe that CIITA (−/−) mice can serve as an excellent model system to study human BLS-2 phenotype and genotype.

Experimental Procedures

Construction of Targeting Vector

Phage clones of the mouse CIITA gene were isolated from a 129/Sv mouse DNA library (Stratagene, California). The probe used to screen the library was generated from mouse B cell (A20) RNA by RT–PCR using primers corresponding 2881–2092 and 3331–3352 of human CIITA cDNA (Steimle et al., 1993). The XhoI fragment containing exons was replaced with pGK–*neo* cassette in the opposite transcriptional orientation.

Generation of Mutant Mice

The linearized targeting vector was electroporated into D3 embryonic stem cells and G418-resistant colonies were selected as described (Elliott et al., 1994). Homologous recombinant clones were screened first by PCR using the following primers: 5′-CAGCTTGACTAGGACCGGTCT-3′, which is located in outside of the targeting vector, and 5′-AGTGGAGGAATGAGCTGGCCC-3′ in 3′ end of pGK–*neo* gene (Figure 1A). PCR conditions were 30 cycles of 1 min of denaturation at 94°C followed by 1 min of annealing at 60°C and 1 min of elongation at 72°C. Positive clones identified by PCR were confirmed by Southern blot analysis after digestion with EcoRI and BamHI. These clones were expanded and injected into C57BL/6J blastocysts. Resulting chimeric animals were backcrossed to C57BL/6J mice, and germline transmission was scored by coat color. Heterozygous mice were identified by Southern blot analysis and were intercrossed to produce homozygotes.

Cytofluorometric Analyses

The following antibodies used in experiments were purchased from Pharmingen: AF6-120.1 for I-A; 14.4.4S for I-E; H129.1.9 for CD4; 53-6.7 for CD8; H57-597 for TCRβ; M1/69 for heat-stable antigen; IM7 for CD44; H1.2F3 for CD69; 7D4 for IL-2R α chain; 1G10 for B7-1; GL1 for B7-2; S7 for CD43. RA3-6B2 and 53-6.7 were used for CD45RA (B220) and CD4, respectively, and they were from GIBCO BRL. Y-3JP for I-A and MacI for macrophages were a gift from C. Janeway (Yale University) and K. Bottomly (Yale University), respectively.

Thymus, lymph nodes, and spleen cell suspensions were prepared in Bruff's medium and the spleen erythrocytes were lysed by hypotonic shock. Cells were resuspended in cold phosphate-buffered saline (PBS) supplemented with 1% fetal bovine serum. Staining was performed in the same buffer. To enrich dendritic cell population, total splenocytes were cultured for 2 hr, washed vigorously to remove nonadherent cells, and incubated at 37°C overnight. The next day, cells in suspension were used in further experiments. Peritoneal macrophages were harvested by washing the peritoneal cavity with 5 ml of PBS. Cells were cultured for 2 hr and nonadherent cells were removed and the remaining macrophages were cultured overnight either with or without 100 U/ml of murine IFNγ. Flow cytometry was performed using FACStar (Becton-Dickinson).

RNA Analyses

RNA from tissues was prepared by acid guanidium–thiocyanate method (Chomczynski and Sacchi 1987). The preparation of poly(A)$^+$ RNA and Northern blot analysis were as described (Sambrook et al., 1989). First-strand cDNA was synthesized using reverse transcriptase (GIBCO BRL, Maryland) and PCR was performed as described previously (Chang et al., 1994). The following primers were used for PCR reactions: Aα, 5′-GAAGACGACATTGAGGCCGACCACG-3′, 5′-TAAAGGCCCTGGGTGTCTGGAGGTG-3′; Eβ, 5′-GAGAACCTGCGCTTCGACAGC-3′, 5′-CACCTGGCAGGTGTAAACCTC-3′; Ii, 5′-GAGGCTAGAGCCATGGATGAC-3′, 5′-AGATGCTTCAGATTCTCTGGG-3′; Mb, 5′-TGAATTTGGGGTGCTGTATCC-3′, 5′-TGCTGAACCACGCAGGTGTAG-3′; H-2Oα, 5′-CCTTCTACCAATCTTACGACG-3′, 5′-GTGTGCCTGTGATCATGAGCAC-3′; H-2Oβ, 5′-CTGCAGCAGCACAACCTGCTG-3′, 5′-CTCCACAGATGCTTTCTGAGC-3′. Actin primers were as described (Chang et al., 1994).

IFNγ Injection

Mice 6–8 weeks of age were injected intravenously on three consecutive days with different doses of murine IFNγ (Calbiochem, California), 1,000 U, 10,000 U, and 100,000 U, or with PBS as a control. On day 4, mice were sacrificed and organs were taken out and frozen.

Immunohistology

Details of this method have been published before (van Ewijk et al., 1988). In brief, thymic tissue was snapfrozen in Tissue Tek and 6 μm frozen sections were cut on a Leitz cryostat. Sections were acetone-dipped for 5 s and stored no longer than 1 week in a dessicator at room temperature. Sections were incubated with monoclonal antibodies in moist chambers for 30 min, followed by rinsing of the sections in PBS supplemented with 0.01% Tween-20. Next, sections were incubated with a rabbit anti-immunoglobulin–horseradish peroxidase conjugate (DAKO) for 30 min. After extensive rinsing, the sections were developed using di-amino-benzidene. Sections were photographed with a Zeiss photomicroscope equipped with Plan Apo objectives using a Schott IL4 interference filter. Antibodies used for immunohistology were M5/114, and Y-3JP for MHC class II, F4/80 and MacI for macrophages, and NLDC-145 for dendritic cells.

Proliferation Assays

Lymph node T cells were isolated by depletion of B cells using goat anti-mouse immunoglobulin magnetic beads (Collaborative Research, Massachusetts), followed by two cycles of exposure to a magnetic field. Proliferation assays were performed by plating out cells in 96-well plates containing 200 μl of media. The amount of each stimulus and the number of cells plated are indicated in the figure legends. After 2 days, 1 μCi of [^{3}H]thymidine was added to each well for 12 hr and [^{3}H]thymidine and the incorporation into DNA was measured.

T-Dependent Immune Response

Mice 6–8 weeks of age were injected with 25 μg of KLH in complete Freund's adjuvant into each hind footpad and with 50 μg of KLH into the base of tail. T cells were prepared from draining lymph nodes 9 days after immunization and cultured with KLH for 2 days. Proliferation assays were performed as described.

Fetal Liver Cell Transfer

Recipient animals were irradiated with 920 rads (1 rad = 0.01 Gy) delivered by a cesium source and then rested for 1 day. Fetal liver cells from 16 days of gestation were prepared and 5×10^6 cells were injected intravenously.

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