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Aberrant Expression of Fas Ligand in Mice Deficient for the MHC Class II Transactivator

Tania S. Gourley,* Dipak R. Patel,* Kevin Nickerson,* Soon-Cheol Hong, † and Cheong-Hee Chang2✉✉

The MHC class II transactivator (CIITA) is a critical regulator of MHC class II genes and other genes involved in the Ag presentation pathway. CIITA-deficient mice lack MHC class II expression on almost all APCs. In this study, we show that these mice also have aberrant Fas ligand expression on both CD4 T cells and B cells. We found that Fas ligand expression was greatly increased on CIITA-deficient CD4 T cells during the Th1 differentiation process. However, both CIITA-deficient and control Th1 effector cells up-regulated Fas ligand to similar levels if cells were reactivated. The introduction of CIITA into primary CD4 T cells via retroviral infection resulted in a reduction in the level of Fas ligand and delay in apoptosis after activation. Interestingly, activated B cells from the CIITA-deficient mice also showed increased levels of Fas ligand that could be to some degree inhibited by the introduction of IL-4. The Journal of Immunology, 2002, 168: 4414–4419.

The major histocompatibility complex class II transactivator (CIITA) is the master regulator of MHC class II gene expression. CIITA was originally identified in a group of patients with bare lymphocyte syndrome. These patients lack MHC class II expression on their APCs due to a point mutation in the CIITA gene (1). CIITA is critical for both the constitutive as well as the IFN-γ-induced MHC class II expression (2, 3). These findings were further confirmed in CIITA-deficient (CIITA−/−) mice (4). These mice lack MHC class II expression on APCs, although residual MHC class II expression has been reported on a subset of dendritic cells (5). However, CIITA−/− mice have a severe reduction in the number of CD4 T cells due to the lack of positive selection in the thymus (4).

Other genes have also been shown to be affected by CIITA, including the invariant chain (6, 7), HLA-DM (7), MHC class I (8), and HIV virus long terminal repeat expression (9). We have reported that CIITA inhibits IL-4 gene transcription (10). In CIITA−/− mice, IL-4 was aberrantly expressed in Th1 cells and constitutive expression of CIITA in a Th2 clone-repressed IL-4 gene transcription (10). The inhibition of IL-4 was due to the competition between CIITA and NF-AT for binding to the coactivator p300/CBP (11). Taken together, these data suggest that CIITA plays an important role in immune responses for both B and T cells.

Naive CD4 T cells require activation through the TCR to become effector Th1 or Th2 cells that participate in cellular or humoral immunity, respectively. However, the same TCR activation signals may result in preactivated T cells undergoing activation-induced cell death (AICD) (12). The Fas-Fas ligand cell death pathway has been shown to be critical for AICD, which is the mechanism to maintain homeostasis by eliminating unwanted T cells after an immune response (12). Mice that lack Fas or Fas ligand have a severe combined immune disorder in which lymphocytes proliferate out of control, resulting in autoimmune diseases (13, 14).

Fas ligand (CD95L) is a member of the TNF family and is expressed principally on activated T cells (15), NK cells (16), and cells at immune privileged sites (17). Interaction between Fas ligand and its receptor, Fas, results in the Fas-bearing cell undergoing apoptosis. We have demonstrated that the constitutive expression of CIITA in CD4 T cell lines resulted in the inhibition of Fas ligand expression (18). CD4 T cell lines expressing CIITA were not able to up-regulate Fas ligand after TCR activation and, as a consequence, were resistant to AICD and could not kill Fas-bearing target cells (18). The inhibition of Fas ligand by CIITA was also mediated via NF-AT (18). In this study, we show that mice deficient in CIITA have enhanced Fas ligand expression on developing Th1 cells and on LPS-stimulated B cells.

Materials and Methods
Abs and reagents
Abs specific for CD4 (H129.1.9), Fas ligand (MFL3), B220 (RA3-6B2), MHC class II I-Aβ (AF6-120.1), CD44 (IM7), CD45RB (23G2), and recombination IL-4 and IFN-γ were obtained from BD Pharmingen (San Diego, CA). The 2C11 (anti-mouse TCR), 2.4-G2 (anti-FcγR), 30H12 (anti-Thy-1), 1D3 (anti-CD19), TIB 105 (anti-CD8), and TIB 210 (anti-CD8) were purified from hybridoma supernatant. LPS was purchased from Sigma-Aldrich (St. Louis, MO). Guinea pig complement was obtained from Invitrogen (Carlsbad, CA). Anti-mouse Ig- and anti-rat Ig-coated magnetic beads were purchased from Polysciences (Warrington, PA). Flow cytometry was performed using BD Biosciences (Mountain View, CA) FACScan.

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3 Abbreviations used in this paper: CIITA, class II transactivator; AICD, activation-induced cell death; GFP, green fluorescence protein; MFI, mean fluorescence intensity; PI, propidium iodide.

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Mice and cell lines

CIITA+/-, Ab+/-, CIITA+/- 1-E, and Ab+/- 1-E mice were described elsewhere (4, 10, 19). The AND TCR transgenic mice were kindly provided by R. Miller (University of Michigan). C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were maintained under specific pathogen-free conditions at the University of Michigan Medical School animal facility. The Phoenix-Eco cell line was obtained from American Type Culture Collection (Manassas, VA) with permission from G. Nolan (Stanford University). The Jurkat T cell line, Phoenix-Eco cell line, and the primary CD4 T and B cells isolated from all mice were cultured in Click’s medium supplemented with 10% FBS, 2 mM glutamine, 100 µg/ml penicillin and streptomycin, and 10-5 M 2-ME.

RNA extraction and PCR

Total cytoplasmic RNA preparation, cDNA synthesis, and PCR were performed, as previously described (18). The following primers were used in the PCR reactions: mouse CIITA (forward, 5’-CTCACGCTTACGACTGG-3’; reverse, 5’-AGCCGGTGGCTTCTCTGGTCTGGAGG-3’); and γ-actin (forward, 5’-ACACCAATGTCGTCACCCAGGGC-3’; reverse, 5’-CCACACAGATCTGCTGAGC-3’).

Real-time PCR

Real-time PCR was used in analysis of RNA samples. Reverse-transcription reactions were done as described previously (18). Real-time PCR was performed on an ABI Prism 7000 Sequence Detection System using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and following manufacturer’s protocols. Primers and concentrations used were: FasL sense (300 nM), TGGGTAGACAGCAGTGCCAC; Fast antisense (300 nM), GGCACAAAGTATGAGCAGGG; GAPDH sense (50 nM), CCAOCCGTCCTCTCCTGGAC; GAPDH antisense (50 nM), ATAC CAGGAAAATGAGCTTGACAAAGT. Each of these primer sets gave a unique product. PCR assays were triplicated, and the data were pooled. Values obtained for levels of mRNAs were normalized to the levels of GAPDH mRNA.

Preparation of total and naïve CD4 T cells

CD4 T cells were enriched using negative selection, as described (10). To obtain naïve CD4 T cells, enriched total CD4 T cells were further sorted for the CD4+ , CD45RB+bright , and CD44high population. Memory CD4 T cells were prepared from enriched total CD4 T cells by sorting the CD4+ , CD45RBlow , and CD44high population. The sorted naïve and memory CD4 T cells were greater than 97% pure. Naïve and enriched CD4 T cells were driven to Th1 and Th2 by incubating cells on plate-bound anti-CD3 (5 µg/ml) in the presence of IL-2 (20 U/ml), IL-12 (3.5 ng/ml), and anti-IL-4 (11B11, 10 µg/ml) for Th1, and IL-2 (20 U/ml), IL-4 (10 ng/ml), and anti-IFN-γ (3000 U/ml) for Th2, for indicated time periods. For restimulation, CD4 T cells were reactivated with plate-bound anti-CD3 (5 µg/ml) and IL-2 (50 U/ml) for indicated time periods.

Preparation of retroviruses

Retroviral construct expressing GFP (RV-GFP) was generated by modifying the MSCV2.2 vector. An internal ribosome entry site and green fluorescence protein (GFP) were cloned into the EcoRI and SalI sites, respectively. To generate CIITA-expressing retroviruses (RV-CIITA/GFP), the B cell form of CIITA (10) was cloned into the Xhol site of the modified MSCV2.2 vector containing the internal ribosome entry site and GFP. CIITA and GFP were translated as separate proteins. The Phoenix-Eco packaging cells (3.5 x 107 cells) were transduced with either RV-CIITA/GFP (4 µg) or RV-GFP (4 µg) using CaP04 as described (11). The transduced Phoenix-Eco cells were cultured at 37°C for 24 h, then 32°C for an additional 24 h to allow efficient viral production. The supernatants containing either the GFP or CIITA/GFP viruses were filtered through a 0.45-µM filter and used immediately to infect primary CD4 T cells.

Retroviral infection of primary CD4 T cells

Total splenocytes from AND transgenic mice were stimulated overnight with pigeon cytochrome c (100 µg/ml) and IL-2 (30 U/ml). The activated T cells were infected with 1 part media to 2 parts of viral supernatant and polybrene (8 µg/ml), centrifuged at 1800 rpm for 45 min, then incubated at 37°C for 24 h before the addition of fresh medium containing IL-2 (30 U/ml). Cells were analyzed 6 days after infection.

Preparation of B cells

To prepare B cells, total splenocytes were isolated and RBCs were lysed in hemolysis buffer (NH4Cl, 0.155 M; Tris-HCl, 0.1 M, pH 7.4). Splenocytes were then incubated on ice with anti-Thy-1 (30H12) Ab for 30 min, washed, incubated at 37°C for 45 min in complement media (RPMI medium containing 20 nM HEPES, 3% BSA, and guinea pig complement), and isolated using a Ficoll gradient.

Thymidine release assay

The thymidine release assay was conducted, as previously described (18), with the following modifications. T-depleted splenocytes were stimulated with LPS (10 µg/ml), LPS + IL-4 (10 ng/ml), or LPS + IFN-γ (300 U/ml), or left untreated for 3 days before culturing with the [3H]thymidine-labeled target cells overnight.

Results

Expression patterns of the CIITA gene during Th1 cell differentiation

We have previously demonstrated that the CIITA gene is expressed in Th1, but not Th2 cells during the differentiation process (10). To determine the kinetics of CIITA expression, we examined endogenous CIITA gene expression during the Th1 and Th2 differentiation process. Naïve CD4 T cells from C57BL/6 mice were sorted, then CIITA expression was analyzed from unstimulated naïve CD4 T cells, or cells driven to Th1 or Th2. As shown in Fig. 1A, the CIITA gene was expressed in naïve CD4 T cells if cells were not stimulated. CIITA expression was then down-regulated, and the transcripts were barely detectable during day 1–2 after differentiation. However, the CIITA gene was reexpressed at day 4 in Th1, but not Th2 cells. We have shown previously that the re-expression of the CIITA gene in Th1 cells depends on IFN-γ signaling (10).

We next examined CIITA expression in memory CD4 T cells that were CD45RBbright and CD44mbright. RNA was prepared from freshly sorted memory cells or from cells stimulated with plate-bound anti-CD3 overnight. Similar to naïve CD4 T cells, CIITA transcripts were detected in fresh memory cells, but decreased after stimulation (Fig. 1B). To exclude the possibility that the CIITA
transcripts were coming from contaminating B cells, we analyzed the same RNA samples for the presence of CD19. CD19 transcripts were not detected (Fig. 1C). These data indicate that the CIITA gene is expressed in naive, differentiated Th1, and memory CD4 T cells. In addition, the initial TCR signaling down-regulates CIITA gene expression in both naive and memory CD4 T cells.

**CD4 T cells lacking CIITA express enhanced levels of Fas ligand during Th1 differentiation**

Our previous study showed that the overexpression of CIITA in CD4 T cell lines resulted in an inhibition of Fas ligand expression (18). We demonstrated in this study that the CIITA gene is expressed in naive CD4 T cells and during Th1 differentiation (Fig. 1A) (10). Based on these observations, we explored the possibility that CIITA expression during Th1 cell differentiation may prevent Fas ligand expression necessary for the survival of developing effector cells. This hypothesis then predicts that the lack of CIITA would increase the level of Fas ligand in CD4 T cells. We tested this by two different approaches.

First, we measured the level of Fas ligand mRNA during Th1 cell differentiation using RT-PCR and real-time PCR. As expected, Fas ligand mRNA levels were much lower when CIITA was expressed (Fig. 2A, days 0 and 4). Second, we compared Fas ligand expression of Th1 cells from the wild-type and CIITA−/− mice. CIITA−/− mice do not have the CD4 T cell population in the periphery due to the lack of MHC class II expression (4). Therefore, to study CIITA−/− CD4 T cells, we used CIITA−/− mice expressing the I-E transgene (CIITA−/− I-E) (10). As a control, mice that lack the MHC class II structural gene Aβ (Aβ−/−) carrying the same I-E transgene were analyzed (Aβ−/− I-E) (10). Using these mice, we examined Fas ligand expression during Th1 differentiation as well as after reactivation of Th1 cells.

CD4 T cells from CIITA−/− I-E or Aβ−/− I-E mice were enriched, then driven to Th1 for 5 days, and Fas ligand expression was monitored during this period by flow cytometry. Fresh CD4 T cells from CIITA−/− I-E mice expressed a higher level of Fas ligand compared with that of Aβ−/− I-E (Fig. 2B). Furthermore, the levels of Fas ligand on CD4 T cells from CIITA−/− I-E mice were higher than Aβ−/− I-E mice during the Th1 differentiation (Fig. 2B). We also observed variable kinetics of Fas ligand expression during the differentiation, as shown by different mice (Fig. 2B, compare Expts. 1 and 2). The low level of Fas ligand on CD4 T cells from Aβ−/− I-E mice during the Th1 differentiation process was similar to that of wild-type C57BL/6 mice (data not shown). We also compared the mRNA level of Fas ligand between naive and memory CD4 T cells, we used CIITA−/− mice from CIITA−/− Th1 cells express a higher level of the Fas ligand gene (Fig. 2C).

**Constitutive expression of CIITA in primary CD4 T cells inhibits activation-induced apoptosis**

When CIITA was overexpressed in CD4 T cell lines, Fas ligand expression was greatly reduced after TCR activation (18). To determine whether this was reproducible in primary CD4 T cells and to provide further evidence in support of CIITA as a negative regulator of Fas ligand, we employed a retroviral system to introduce CIITA into primary CD4 T cells. Enriched CD4 T cells from AND TCR transgenic mice were activated overnight with cytochrome c Ags, followed by infection with supernatant containing retroviruses expressing GFP alone, or GFP and CIITA. The CD4 T cells were analyzed at the end of primary stimulation or after reactivation. As shown in Fig. 3A, the efficiency of infection was comparable between the CIITA and the control viruses. However, cells infected with virus expressing GFP with CIITA were MHC class II positive, indicating that CIITA was functional (Fig. 3B).

We next tested Fas ligand levels after 6 h of secondary stimulation, since longer activation reduces cell survival significantly. When Fas ligand expression was compared in GFP-negative cells, the two populations showed similar levels (Fig. 3C, compare lanes 3 and 4). However, the GFP-positive cells from CIITA virus-infected cells showed a reduction in the mean fluorescence intensity (MFI) of Fas ligand compared with the control GFP-positive cells (Fig. 3C, compare lanes 1 and 2). The MFI on CIITA-expressing
cells was consistently lower over three independent experiments, despite the difference being small. To determine whether the small reduction in the Fas ligand level is functionally significant, we assessed the number of cells undergoing apoptosis after TCR activation. For this purpose, the same population of cells that were used for Fas ligand staining was subjected to annexin V that binds to phosphatidylserine that presents on the outer membrane in cells undergoing apoptosis. There was little difference in the number of annexin V-positive cells at all time points between the uninfected cells represented by GFP-negative populations (Fig. 3D). In contrast, the number of annexin V- and GFP-positive CD4 T cells were significantly reduced if cells were infected with the virus expressing CIITA (Fig. 3D). To further support the hypothesis that CIITA regulates cell death processes, we assessed the number of cells that survived after cells were activated overnight by anti-CD3. When the GFP-positive cell populations were compared before and after activation, CIITA virus-infected cells were more resistant to cell death measured by both propidium iodide (PI) staining and trypan blue exclusion assays (Fig. 3E). These data suggest that the small reduction in Fas ligand levels seen by CIITA resulted in the delayed onset of CD4 T cell apoptosis.

B cells from CIITA⁻/⁻ mice also have enhanced Fas ligand expression

It is well documented that Fas ligand plays an important role for T cells. However, the role of Fas ligand on B cells is less clear (20, 21). CIITA is expressed and is critical for the Ag presentation function of B cells. If CIITA regulates Fas ligand expression in CD4 T cells, it may have a similar role in B cells. We examined B cells to determine whether CIITA deficiency affects Fas ligand expression in B cells. To do this, we enriched B cells from CIITA⁻/⁻ or Aβ⁻/⁻ mice without the I-E transgene. B cells were then cultured for 3 days in the presence of LPS, LPS + IL-4, LPS + IFN-γ, or left untreated. As previously demonstrated by other groups (20, 21), Fas ligand was detectable on B cells upon stimulation with LPS (Fig. 4A). Fas ligand was also detectable after LPS + IFN-γ stimulation (Fig. 4A). However, Fas ligand levels were very low on B cells stimulated with LPS in the presence of IL-4 (Fig. 4A). Under all conditions, the percentages of B

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**FIGURE 3.** Constitutive expression of CIITA in CD4 T cells reduces Fas ligand expression and apoptosis. A. The CIITA and control retroviruses infected CD4 T cells at comparable levels. Total splenocytes from AND TCR transgenic mice were activated with pigeon cytochrome c (100 μg/ml) overnight, followed by infection with supernatant containing retroviruses expressing GFP alone (control virus) or CIITA and GFP (CIITA virus). The cells were then cultured 6 more days before the analysis for GFP expression using flow cytometry. B. CD4 T cells infected with CIITA viruses expressed MHC class II. Infected cells were analyzed for MHC class II expression (I-Aβ) by flow cytometry at day 6 postinfection. The solid and the dotted lines represent the GFP-positive and GFP-negative cells, respectively. The plots shown were gated on CD4⁺ T cells. C. CIITA virus-infected CD4 T cells expressed a lower level of Fas ligand. CD4 T cells infected with either CIITA or the control viruses were cultured for 6 days after infection, and activated with plate-bound anti-CD3 (5 μg/ml) in the presence of IL-2 (50 U/ml) for 6 h. The data are represented as MFI of Fas ligand of two independent experiments. The error bar for CIITA⁺ GFP⁻ is too small to be shown. D. CIITA virus-infected CD4 T cells have a reduced number of annexin V-positive cells. Annexin V binding was determined at 6 days postinfection (0 h), 3 and 6 h after secondary stimulation with anti-CD3 (5 μg/ml) and IL-2 (50 U/ml). Numbers represent the percentage of cells in corresponding populations. Plots were gated on CD4⁺ T cells. E. CIITA virus-infected cells are more resistant to cell death. PI staining and trypan blue exclusion assays were performed using cells that were prepared the same way as in C, except that cells were reactivated overnight. Percentage of cell survival was calculated using total CD4 T cells (number of live cells after stimulation divided by the number of input cells). PI plots were gated on GFP-positive cells. Data shown are representative of two independent experiments.
cells expressing Fas ligand were greater than those in CIITA (Fig. 4A). Furthermore, as shown in Fig. 4B, the level of Fas ligand was also higher on CIITA−/− B cells regardless of the treatment. Fas ligand expression on Aβ−/− B cells was comparable with that of wild-type C57BL/6 mice (data not shown).

We next asked whether increased Fas ligand expression on CIITA−/− B cells affects their ability to kill Fas-bearing target cells. To test this, B cells stimulated under different conditions were cultured with [3H]thymidine-labeled Jurkat T cells. We then compared the level of thymidine release that is indicative of DNA fragmentation of target cells upon cell death. Fig. 4C shows that the B cells stimulated with LPS or LPS + IFN-γ had higher levels of killing activity. As expected, very little killing was seen by B cells that were unstimulated or stimulated with LPS + IL-4. CIITA−/− B cells that express higher levels of Fas ligand exhibited significantly increased killing activity compared with Aβ−/− B cells under all conditions. Similar data were obtained using A20 B cells as a target (data not shown). Our data suggest that the lack of CIITA expression is at least partly responsible for the enhanced level of killing activity.

Discussion

Our current study revealed that CIITA plays a different role in Fas ligand expression between naive and effector CD4 T cells. CIITA seems to be an important regulator of the Fas ligand gene expression during Th1 cell differentiation since CIITA−/− Th1 cells expressed an enhanced level of Fas ligand (Fig. 2, B and C). Therefore, the role of CIITA may be to protect differentiating Th1 cells from cell death by inhibiting Fas ligand expression. It should be noted that the level of CIITA transcripts in effector and memory cells decreased after activation (Fig. 1B), which may contribute to the up-regulation of Fas ligand upon activation. It is possible, therefore, that CIITA could protect effector CD4 T cells under a certain environment in vivo. Interestingly, CIITA−/− I-E mice have fewer mature CD4 T cells in the periphery than Aβ−/− I-E mice (C.-H. Chang, unpublished data). It is tempting to speculate that the elevated level of Fas ligand in CIITA-deficient CD4 T cells is responsible for the decreased survival of CD4 T cells in vivo. However, we cannot exclude the possibility that restoring MHC class II expression in the absence of other CIITA-mediated functions is not sufficient to reconstitute the entire CD4 T cell compartment in CIITA−/− mice, whereas the phenotype of Aβ−/− mice can be completely rescued by I-E transgene expression.

Fas ligand expression seems to be regulated at multiple levels consistent with the previous reports (23, 24). As we have shown in this work, the regulation of Fas ligand expression is mediated at the level of transcription. However, developing Th1 cells from the wild-type mice do not express an appreciable amount of Fas ligand on the cell surface, despite the presence of its mRNA. It is possible that Fas ligand mRNA present during Th1 cell differentiation is not sufficient to cause significant cell surface expression. Alternately, Fas ligand protein may not be exported to the cell surface until cells become the effector. CIITA−/− Th1 cells exhibited an enhanced level of mRNA and earlier expression of Fas ligand proteins on the cell surface. It is likely that elevated mRNA is responsible for enhanced cell surface expression in CIITA−/− Th1 cells, although we cannot rule out the possibility that CIITA regulates both transcription and export processes.

Previously, we showed that CD4 T cell lines stably transfected with CIITA expressed reduced levels of Fas ligand on the cell surface (18). These cells were also protected from AICD (18). Consistent with these observations, introduction of CIITA to primary CD4 T cells using retroviruses resulted in reduction in Fas ligand expression and the onset of apoptosis (Fig. 3, C–E). However, the degree of inhibition was much smaller. At least two possibilities explain the difference. First, the timing of CIITA expression by retroviral infection may not be ideal to repress Fas ligand expression. As CIITA gene transcription is a dynamic process during Th1 cell differentiation (Fig. 1), the kinetics of CIITA expression could be an important factor to modulate Fas ligand expression. Since retroviruses cannot infect resting cells, primary lymphocytes have to be prestimulated to allow infection. It is conceivable, therefore, that CIITA expression in cells that are already stimulated is not as effective as in naive cells. The second possibility is that the level of CIITA protein expressed by the retroviral system is not sufficient to inhibit the expression of the endogenous Fas ligand gene. It is well documented that the activation of MHC class II gene transcription can be initiated by a trace amount of CIITA protein (22). However, the efficient inhibitory function of CIITA may require a higher level of CIITA protein. Since the
amount of endogenous CIITA protein is too low to be detected by Western blot, it is technically challenging to compare CIITA levels between different systems.

CIITA is transcribed from at least three different promoters, resulting in three different functional isoforms (25). Each isoform is preferentially expressed in each type of APC; forms I, III, and IV are responsible in activating MHC class II genes in dendritic cells, B cells, and IFN-γ-inducible cells, respectively (25). When we examined CIITA gene transcription during Th cell differentiation, we used a primer set that is specific for the exons common to all isoforms (Fig. 1, A and B). However, we could not detect the CIITA transcripts using the primer set specific for each isoform (T. S. Gourley, unpublished data). This suggests that CD4 T cells express a unique isoform of CIITA. Furthermore, it raises another possibility that the introduction of the B cell-specific isoform of CIITA to CD4 T cells may not be relevant in vivo. Unfortunately, this issue cannot be resolved until the T cell-specific isoform of CIITA is identified and cloned.

A number of studies reported that B cells express membrane-bound Fas ligand (20, 21). In agreement with our data, Tian et al. (21) recently showed that B cells activated by LPS expressed Fas ligand, which was able to kill both B and T cells. In their system, adoptive transfer of activated B cells into prediabetic nonobese diabetic mice prevented spontaneous autoimmunity mediated by Th1 cells. Our data suggest that the modulation of Fas ligand expression on B cells is at least partly controlled by CIITA (Fig. 4, A and B). Interestingly, a stimulus that sends a proliferation signal such as LPS in conjunction with IL-4 did not induce Fas ligand expression. In contrast, the level of Fas ligand was enhanced upon treatment with LPS, or LPS in the presence of IFN-γ, which are not proliferative signals for B cells. Consistent with these observations, the level of endogenous CIITA gene transcripts in B cells increases upon treatment with LPS and IL-4 (C.-H. Chang, unpublished data).

B cells lacking CIITA expressed a higher level of Fas ligand and killed the Fas-bearing target cells more efficiently (Fig. 4C). However, the overall B cell numbers in CIITA−/− mice are comparable with that of the wild-type mice (C.-H. Chang, unpublished data). B cells may be protected from cell death by an unknown mechanism that differs from T cells. It has been reported that human naive B cells are protected from apoptosis by the sole addition of IL-4 in culture in the absence of activation (26). Resting naive B cells also express a high level of the IL-4R α-chain (26). It seems that, in addition to CIITA, a signaling molecule(s) that is induced by IL-4 prevents Fas ligand expression and cell death.

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