

The Role of the MHC Class II Transactivator in Class II Expression and Antigen Presentation by Astrocytes and in Susceptibility to Central Nervous System Autoimmune Disease¹

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The role of the MHC class II transactivator (CIITA) in Ag presentation by astrocytes and susceptibility to experimental autoimmune encephalomyelitis (EAE) was examined using CIITA-deficient mice and newly created transgenic mice that used the glial fibrillary acidic protein promoter to target CIITA expression in astrocytes. CIITA was required for class II expression on astrocytes. Like class II-deficient mice, CIITA-deficient mice were resistant to EAE by immunization with CNS autoantigen, although T cells from immunized CIITA-deficient, but not class II-deficient, mice proliferated and secreted Th1 cytokines. CIITA-deficient splenic APC presented encephalitogenic peptide to purified wild-type encephalitogenic CD4⁺ T cells, indicating that CIITA-independent mechanisms can be used for class II-restricted Ag presentation in lymphoid tissue. CIITA-deficient mice were also resistant to EAE by adoptive transfer of encephalitogenic class II-restricted CD4⁺ Th1 cells, indicating that CIITA-dependent class II expression was required for CNS Ag presentation. Despite constitutive CIITA-driven class II expression on astrocytes in vivo, glial fibrillary acidic protein-CIITA transgenic mice were no more susceptible to EAE than controls. CIITA-transfected astrocytes presented peptide Ag, but in contrast to IFN- γ -activated astrocytes, they could not process and present native Ag. CIITA-transfected astrocytes did not express cathepsin S without IFN- γ activation, indicating that CIITA does not regulate other elements that may be required for Ag processing by astrocytes. Although our results demonstrate that CIITA-directed class II expression is required for EAE induction, CIITA-directed class II expression by astrocytes does not appear to increase EAE susceptibility. These results do not support the role of astrocytes as APC for class II-restricted Ag presentation during the induction phase of EAE. *The Journal of Immunology*, 2002, 169: 6720–6732.

Experimental autoimmune encephalomyelitis (EAE)³ is a CNS inflammatory demyelinating disease that serves as a model for multiple sclerosis (MS) and other organ-specific autoimmune diseases (1, 2). EAE is mediated primarily by CD4⁺ Th1 cells that recognize CNS self-Ags in association with MHC class II molecules expressed on the surface of APC (1, 2). Ag presentation may be required at different stages of EAE patho-

genesis. Peripheral (outside the CNS) activation facilitates T cell entry into the CNS (3, 4). Ag processing and presentation by non-professional resident CNS APC may be required for recognition of CNS autoantigen and T cell activation during initial inflammation, and during progression to chronic and relapsing stages of CNS demyelinating disease (3, 5–7).

Astrocytes are the most abundant CNS glial cell population (8). In contrast with microglia, a more professional resident CNS APC that participates in CNS inflammatory disease (4), the role of astrocytes in Ag presentation and T cell activation in CNS inflammatory disease is controversial (9–12). When activated by IFN- γ in vitro, astrocytes up-regulate MHC class II and class I molecules and can present Ag to CD4⁺ Th1 cells or CD8⁺ T cells, respectively (8, 12–15). Although MHC class II molecules have been detected on astrocytes within inflammatory lesions of MS (16, 17) and EAE (18, 19) in certain studies, class II expression on astrocytes has not been consistently observed (16), raising questions regarding their contribution to class II-restricted Ag presentation and CD4⁺ T cell activation in vivo.

The MHC class II transactivator (CIITA) (20–24), a transcriptional coactivator, is the key intermediate that directs constitutive and IFN- γ -inducible expression of MHC class II genes in professional and nonprofessional APC, respectively (25). CIITA is differentially regulated by nonhomologous promoters (26). Murine astrocytes use primarily CIITA pIV for IFN- γ -inducible CIITA expression (20, 22–24, 27), while perivascular microglia, hemopoietically derived APC, use both CIITA pI and pIV for IFN- γ -inducible CIITA expression (27, 28). As CIITA also regulates expression of the invariant chain (Ii) and H-2M (25, 29), two

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³ Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; CIITA, class II transactivator; Ii, invariant chain; GFAP, glial fibrillary acidic protein; Tg, transgenic; Cat, cathepsin; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; h, human; m, murine; BTD, biotinylated.

molecules involved in MHC class II maturation and endocytic processing (30), CIITA has been described as a global regulator for genes involved in Ag presentation (29). CIITA can also promote IFN- γ -inducible MHC class I expression on certain types of human cells (31, 32). Although it is known that IFN- γ -activated astrocytes are capable of processing native CNS autoantigen for presentation to CD4⁺ T cells (20, 21), the role of CIITA in Ag processing and presentation by astrocytes has not been directly addressed.

In this investigation, we tested the hypothesis that CIITA-directed class II expression was necessary for T cell activation in CNS inflammation. Using the glial fibrillary acidic protein (GFAP) promoter we created GFAP-CIITA transgenic (Tg) mice to examine whether constitutive CIITA-directed class II expression by astrocytes could promote EAE induction (33–36). CIITA was required for class II expression by astrocytes. Like class II-deficient mice, CIITA-deficient mice were resistant to EAE by active immunization with CNS autoantigen. In contrast with class II-deficient mice, T cells from immunized CIITA-deficient mice proliferated and secreted IL-2 and IFN- γ , although to a lesser extent than wild-type mice, indicating that a CIITA-independent mechanism(s) can contribute to priming of peripheral T cells in CIITA-deficient mice. However, upon adoptive transfer of wild-type encephalitogenic CD4⁺ T cells, CIITA-deficient recipient mice did not develop clinical or histologic signs of EAE, which indicated that CIITA-directed class II expression was required for CNS Ag presentation. Despite constitutive CIITA-driven cell surface class II expression on astrocytes *in vivo*, GFAP-CIITA Tg mice were no more susceptible to EAE than control mice. IFN- γ -activated astrocytes could present encephalitogenic peptide or process native CNS autoantigen for presentation to CD4⁺ T cells, although unactivated CIITA-transfected astrocytes could present peptide only. IFN- γ -activated, but not unactivated CIITA-transfected, astrocytes up-regulated cathepsin (Cat) S, a cysteine protease involved in myelin basic protein (MBP) degradation (37). Although CIITA is required for MHC class II expression and presentation of peptide Ag by astrocytes, it does not direct expression of other elements in the endocytic pathway that may be required for processing and presentation of native CNS autoantigen.

Materials and Methods

Peptides and Abs

Mouse myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 (MEVGWYRSPFSRVVHLYRNGK) and MBP peptide Ac1–11 (ASQKRPSQRHG) were synthesized by solid-phase Fmoc chemistry by Quality Control Biochemicals (Hopkinton, MA). After cleavage from the solid support and deprotection of the amino acid side chains, peptides were purified by reversed-phase HPLC (C18 column, YMC). Major peaks, analyzed by matrix-assisted laser desorption ionization-time of flight mass spectrometry and HPLC, contained >95% of the desired product. Native guinea pig MBP was purified as described (38). Rat IgG2b anti-mouse I-A^b (M5/114), mouse IgG2a anti-mouse MHC class II mAb 10–2.16 (anti-I-A^{k/u}), mouse anti-mouse H-2K^b/D^b (28-8-6), the mouse IgG1 pan anti-H-2D mAb (8F12; recognizes haplotypes b, d, k, p, q, and s; B10.PL mice express H-2D^d), and isotype control Abs (rat IgG2b A95-1 and mouse IgG2a G155-178) were purchased from BD Pharmingen (San Diego, CA). Rabbit anti-bovine anti-GFAP Ab was purchased from DAKO (Santa Barbara, CA).

Astrocytes and transfections

Primary astrocyte cultures were obtained from wild-type C57BL/6 mice and CIITA-deficient mice (39) using techniques described previously (20). The immortalized astrocyte lines used in this study, described previously (20), were derived from primary B10.PL (H-2^u) astrocyte cultures. To transfect astrocyte lines and to generate GFAP-CIITA Tg mice, human CIITA cDNA (provided by L. Glimcher, Boston, MA) was subcloned into the unique *Bam*HI site of *Gfa2* (provided by M. Brenner, Birmingham, AL), a construct that contains a 2.2-kb 5'-flanking sequence (–2163 to +47) derived from the human (h) GFAP promoter and directs astrocyte-

specific gene expression *in vitro* and *in vivo* in Tg mice (33). Correct orientation was established by PCR and confirmed by DNA sequencing the regions encompassing both 5'- and 3'-CIITA integration sites. Astrocytes (1×10^5 ; lines 2.1 and 3.2) were cotransfected with 10 μ g of the *BgIII*-excised fragment containing hGFAP-hCIITA or hGFAP-0, the *Gfa2* construct without a cDNA insert, which was used as a control, and 1 μ g of the linearized (*Hind*III-digested) plasmid containing hygromycin resistance using 20 μ l of lipofectin (Life Technologies, Grand Island, NY). Hygromycin (200 μ g/ml) was added 48 h after transfection. After 14 days, 45–50% of the surviving cells constitutively expressed MHC class II molecules on their cell surface by FACS analysis. Transfected astrocytes with the highest 1% class II expression were sorted and used for experimentation. None of cells transfected with GFAP-0 constitutively expressed class II molecules.

T cells

MOG p35–55-specific T cells were isolated 10–14 days after immunization of C57BL/6 female mice with MOG p35–55 as described previously (5). PJR-25 is a T cell clone derived from PL/J mice that is specific for MBP Ac1–11 in association with I-A^u (40) and proliferates in response to intact mouse, bovine, guinea pig, and human MBP (2). Purified MOG p35–55-specific CD4⁺ T cells, used to obviate concern for APC carryover in the experiment to assess Ag presentation by CIITA-deficient splenic APC, were isolated from a MOG p35–55-specific T cell line using a density separation medium (StemCell Technologies, Vancouver, British Columbia, Canada) containing mAbs against CD11b (Mac-1), CD45R (B220), CD8, erythroid cells (TER119), and myeloid differentiation Ag (Gr-1) per manufacturer's recommendations. The purity of CD4⁺ T cells derived from a MOG p35–55-specific T cell line was 95–96% as determined by flow cytometry.

Proliferation assays

For primary proliferative responses, 5×10^5 spleen or lymph node cells removed from MOG p35–55-immunized mice were cultured in 0.2-ml serum-free medium, X-Vivo 20 (BioWhittaker, Walkersville, MD) supplemented with 5×10^{-5} M 2-ME, 2 mM glutamine, 100 μ g/ml penicillin, and 100 μ g/ml streptomycin. After 72 h, culture with appropriate concentrations of MOG p35–55, cultures were pulsed with 1 μ Ci [³H]thymidine and harvested 16 h later. In the experiment using CD4-enriched MOG p35–55-specific T cells, 1×10^4 CD4⁺ T cells were cultured with 5×10^5 gamma-irradiated syngenic splenic APC, pulsed at 48 h, and harvested 16 h later. For Ag presentation by astrocytes, these APC were treated with mitomycin C (60 μ g/ml per 10^6 APC) for 1 h at 37°C, washed three times, and plated at 4×10^4 cells/well with 1×10^4 PJR-25 T cells and appropriate concentrations of MBP Ac1–11 or native MBP. These cultures were also pulsed at 48 h and harvested 16 h later. The mean cpm of [³H]thymidine incorporation was calculated for triplicate cultures. SDs of triplicate cultures are shown.

Mice

Wild-type C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). MHC class II-deficient mice were purchased from Taconic Farms (Rockville, MD). CIITA-deficient mice, bred onto the C57BL/6 background, have been described (39). GFAP-CIITA Tg mice were generated using the same hGFAP promoter construct, *Gfa2* (33), used for transfection of astrocytes (see *Astrocytes and transfections* in *Materials and Methods*). The *Gfa2* promoter construct has been shown to direct transgene expression in the cerebellum, brainstem, spinal cord, and cerebral hemispheres of GFAP-*lacZ* mice (34). hCIITA cDNA was subcloned into this construct and correct orientation was determined as described for transfection studies above. After digestion of the GFAP-CIITA construct with *BgIII* to remove excess plasmid DNA, the fragment containing GFAP-CIITA was microinjected into C57BL/6 oocytes. From the initial microinjections, three founders (1, 19, and 22) were identified by PCR and confirmed by Southern blot analysis using genomic tail DNA (7.5 μ g) that was digested with *EcoRI* and hybridized with a 550-bp PCR-amplified fragment from the GFAP-hCIITA construct containing the overlapping sequence from the hGFAP promoter and hCIITA cDNA. Specifically, after electrophoresis of *EcoRI*-digested genomic DNA in 0.75% agarose, the gel was transferred onto nitrocellulose membrane. After a 1 h prehybridization at 65°C, ³²P-labeled GFAP-CIITA probe was added and hybridized overnight at 65°C. The membrane was washed two times with $2 \times$ SSC 0.1% SDS at room temperature (rt) for 15 min, then once in $0.1 \times$ SSC 0.1% SDS at 65°C for 30 min.

EAE induction and clinical evaluation

Eight- to 10-wk-old C57BL/6 female mice received a s.c. injection in the flank of 100 μ g of mouse MOG p35–55 in 0.1 ml of PBS emulsified in an equal volume of CFA supplemented with 2 mg/ml *Mycobacterium tuberculosis* H37RA (MT; Difco, Detroit, MI). Immediately thereafter and again 48 h later, mice received an i.v. injection of 400 ng of pertussis toxin (PT) in 0.2 ml of PBS. For adoptive transfer, spleen cells from MOG p35–55-immunized donor mice were cultured with 25 μ g/ml MOG p35–55 for 72 h. After Ficoll, cells were washed three times. T cell blasts, which were differentiated from other splenocytes by size under microscopic observation, were counted. Recipient mice were injected i.v. with 2.0×10^7 T cell blasts in 0.5 ml of PBS. Immediately thereafter and again 48 h later, mice received an i.p. injection of 400 ng of PT in 0.2 ml of PBS. Individual animals were observed daily, and clinical scores were assessed in a blinded fashion on a 0–6 scale as follows: 0 = no clinical disease, 1 = loss of tail tone only, 2 = mild monoparesis or paraparesis, 3 = moderately severe paraparesis, 4 = paraplegia, 5 = quadruparesis, and 6 = moribund or death. At least two independent experiments were conducted with a minimum of four mice per group.

Histology

C57BL/6 wild-type mice and GFAP-CIITA Tg mice were anesthetized by isoflurane inhalation and perfused with PBS containing 4% (v/v) paraformaldehyde. Fixed CNS tissues were embedded in paraffin wax, sectioned, and stained with H&E. Tissue used for MHC class II immunohistochemistry were deparaffinized and dried. Sodium citrate was used for Ag retrieval at a concentration of 10 mM, using the microwave method. Tissue sections were blocked with 0.3% H₂O₂, and then incubated with rat anti-mouse I-A^b (M5/114) in Tween PBS containing 3% normal goat serum, 1% BSA, and 0.3% Triton X (TX)-100 overnight at 4°C. Secondary biotinylated (BTD) Ab (rabbit anti-rat, mouse adsorbed; Vector Laboratories, Burlingame, CA) was applied for 1 h at rt, followed by incubation in AB-Complex mixture (Vector Laboratories). A DAB substrate kit (Vector Laboratories) was used as a chromagen and was counterstained with hematoxylin. Double immunofluorescence staining, used to identify MHC class II expression in Tg astrocytes, was performed using M5/114, and rabbit anti-GFAP Ab (DAKO). Tissue sections were incubated with anti-I-A^b for 48 h at 4°C. Staining with secondary Ab BTD rabbit anti-rat IgG (mouse adsorbed; Vector Laboratories) was applied for 2 h at rt, before adding Texas Red-conjugated streptavidin (Vector Laboratories) for 2 h at rt. Sections were mounted onto slides in fluorescent mounting medium (DAKO), then stained with rabbit anti-GFAP Ab (DAKO) for 48 h at 4°C, before adding BTD goat anti-rabbit IgG. FITC-conjugated streptavidin was added, sections were left in ddH₂O for 5 min, mounted in fluorescent mounting medium (DAKO), and permeabilized in 10% TX-100 in PBS. 0.3% TX-100 (Sigma-Aldrich) with 0.8% BSA in PBS was used as washing buffer. All tissue sections were examined in blinded fashion.

mRNA analysis

RNA from immortalized astrocyte clones or primary astrocytes was prepared from $\sim 10^8$ cells using the RNeasy Mini kit (Qiagen, Valencia, CA). RT-PCR and PCR were performed using the Access RT-PCR system (Promega, Madison, WI). The following PCR primers (designed from published sequences; Refs. 41–43) were purchased from Operon Technologies (Alameda, CA) while the β -actin primers were purchased from Stratagene (La Jolla, CA): CIITA, 5'-CCCTGCGTGTGATGGATGTC-3', 5'-GTTGCCCTAGCGTCTTCAG-3'; Ii, 5'-GAGGCTAGAGCCATGGATGAC-3', 5'-AGATGCTTCAGATTCTCGGG-3'; H-2Ma, 5'-CTACGAGATGTTGATGCGGGAAGT-3', 5'-GTGTAGCGGTCAATCTCGTGTGTC-3'; H-2Mb, 5'-GGACCCACAGGACTTCACATACT-3', 5'-GCCGTCTTCCTCTGTTGCTGTGG-3'; β -actin, 5'-TGTGATGGTGGGAATGGGTCAG-3', 5'-TTTGATGTCACGCACGATTTCC-3'; cathepsinS, 5'-TGGGCTTTCAGTCTGTGG-3', 5'-AGCAACCACAAGAACC-3'.

For PCR detection of β -actin, CIITA, Ii, H-2Ma, and H-2Mb, cDNA was amplified 35 cycles: 95°C, 15 s; 58°C, 30 s; and 72°C, 30 s. The mRNA for endosomal cysteine proteases was analyzed by quantitative PCR as recently described (44). In brief, primers for cathepsin S, B, H, and L were designed for multiplex RT-PCR and Taqman using Primer Express software (PerkinElmer, Foster City, CA) and purchased from Bioscience Technologies (Novato, CA). Cycle threshold (Ct) values for each gene product were converted to relative copy number based on normalization to GAPDH (44).

Flow cytometry

IFN- γ -activated (100 U/ml IFN- γ for 48 h) and -unactivated astrocytes were removed from flasks using PBS-EDTA and counted. Cells/sample (1×10^6) were washed in FACS buffer (PBS containing 0.5% BSA and 10

mM sodium azide) and stained. Anti-MHC class II mAb 10-2.16 (anti-I-A^{k,u}) (BD Pharmingen) (45) was used for MHC class II staining of B10.PL astrocyte lines and mAb M5/114 (anti-I-A^b) was used for class II staining of C57BL/6 astrocytes. The pan anti-H-2D mAb 8F12 (BD Pharmingen) was used for MHC class I staining of both B10.PL astrocyte lines and C57BL/6 primary astrocytes. Samples were stained with the specific mAb or isotype-matched control Ab for 30 min on ice, washed, and then incubated with a FITC-labeled rat anti-mouse secondary Ab for 15 min on ice. Following three washes, samples were analyzed on a FACSsort (BD Biosciences, San Jose, CA) using 10,000 events per sample.

Cytokine analysis

Cell culture supernatants were collected at 24-h (IL-2), 72-h (IFN- γ), and 120-h (IL-4 and IL-10) incubations for cytokine analysis. Quantitative ELISA was performed using paired mAbs specific for corresponding cytokines per manufacturer's recommendations (BD Pharmingen). The results or ELISA are expressed as an average of triplicate wells \pm SD. A SOFTmax ELISA plate reader and software was used for data analysis (Molecular Devices, Sunnyvale, CA).

Results

CIITA is required for IFN- γ -inducible MHC class II, but not MHC class I, expression on astrocytes

Astrocytes isolated from CIITA-deficient mice were used to examine whether CIITA was required for IFN- γ -inducible class II expression on astrocytes. There was essentially no detectable class II expression on unstimulated wild-type or CIITA-deficient astrocytes (Fig. 1). In contrast with wild-type astrocytes, IFN- γ -activated CIITA-deficient astrocytes did not up-regulate cell surface MHC class II expression. As it was previously demonstrated that CIITA could also participate in IFN- γ -inducible class I expression, we examined class I expression on these astrocytes. Cell surface class I cell surface expression on CIITA-deficient astrocytes was inducible by IFN- γ , indicating that a CIITA-independent pathway is used for IFN- γ -inducible MHC class I expression in these cells.

Mice deficient in CIITA are resistant to EAE

Having established that CIITA was required for class II expression on astrocytes, we used CIITA-deficient mice to examine the influence of CIITA deficiency on in vivo susceptibility to EAE. Following immunization with MOG p35–55, CIITA-deficient, and class II-deficient mice failed to develop clinical (Fig. 2A; Table I) or histological signs of disease (data not shown). However, as a result of deficient thymic-positive selection, CIITA-deficient and class II-deficient mice have a reduction of peripheral CD4⁺ T cells (39, 46, 47). Thus, lack of EAE susceptibility could reflect defects in either APC or T cell compartments. To further investigate these possibilities, we examined T cell responses from MOG-p35–55-immunized CIITA-deficient and class II-deficient mice. Lymph node cells from MOG p35–55-immunized CIITA-deficient mice produced the Th1 cytokines IFN- γ (Fig. 3A) and IL-2 (Fig. 3B), and proliferated when stimulated by MOG p35–55 in vitro (Fig. 3C). However, both cytokine production and proliferative responses were significantly lower in comparison to those in C57BL/6 wild-type mice (Fig. 3, A–C). The same observations were made in splenocytes from CIITA-deficient mice (data not shown). In contrast, lymph node cells from MOG p35–55-immunized MHC class II-deficient mice showed no Th1 cytokine production (Fig. 4, A and B) or proliferative responses (Fig. 4C) to MOG p35–55 stimulation. IL-4 and IL-10, two Th2 cytokines, were not detected in cultures from any mice tested (data not shown). Proliferative responses in wild-type (Fig. 3D, inset) and CIITA-deficient mice (Fig. 3D) immunized with MOG p35–55 were inhibited by anti-MHC class II mAb and, to a much lesser extent, by anti-MHC class I mAb, indicating that the T cell response to MOG p35–55 was primarily restricted by class II molecules. No significant inhibition of proliferation was observed in cultures using appropriate isotype control Abs (data not shown).

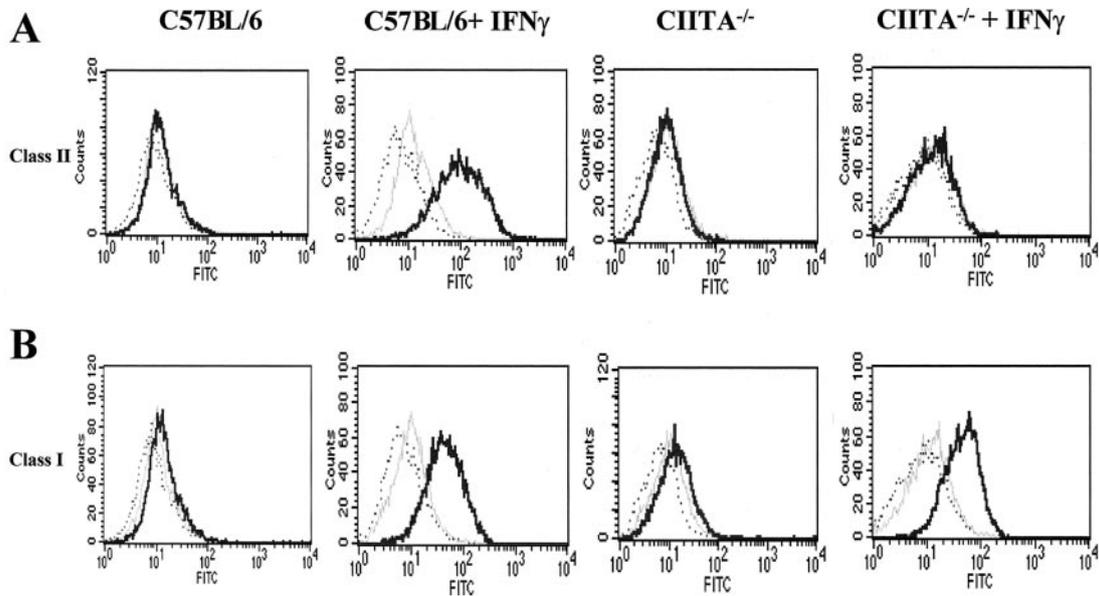


FIGURE 1. IFN- γ induces class I (*B*), but not class II (*A*), expression on CIITA-deficient astrocytes. *A*, Bold lines represent staining with anti-H-2K^b/D^b mAb (M5/114), thin gray lines represent staining with rat mAb (A-95-1) isotype control, and the stippled lines represent unstained control. *B*, Bold lines represent staining with anti-I-A^b mAb (28-8-6), thin gray lines represent staining with mouse isotype control, and the stippled lines represent unstained control. Primary astrocyte cultures were generated from C57BL/6 and CIITA-deficient mice.

As our results indicated that CIITA-deficient mice could prime p35–55-specific T cells, we examined whether splenic APC from naive CIITA-deficient mice could present MOG p35–55 to wild-

type encephalitogenic MOG p35–55-specific CD4⁺ T cells. As shown in Fig. 3*E*, CIITA-deficient APC could present MOG p35–55 to these cells, although not as efficiently as wild-type splenic APC. It was possible that these MOG p35–55-specific T cells may have contained irradiated APC from prior Ag stimulations. Thus, to eliminate this possibility of “APC carryover”, MOG p35–55-specific CD4⁺ T cells were purified. Similarly, CIITA-deficient APC presented MOG p35–55 to these purified CD4 T cells (Fig. 3*F*).

To further distinguish the influence of defects in Ag presentation by APC from alterations in selection of an encephalitogenic T cell repertoire, we investigated whether activated wild-type encephalitogenic T cells could induce CNS autoimmune disease when adoptively transferred into CIITA-deficient mice. In contrast to wild-type recipient mice, CIITA-deficient mice did not develop clinical EAE (Fig. 2*B*; Table II). Furthermore, histological signs of EAE or class II expression were not observed in the CNS of CIITA-deficient mice (data not shown). Thus, while CIITA-independent Ag presentation may occur in peripheral lymphoid tissue, CNS class II-restricted Ag presentation during EAE induction is CIITA-dependent.

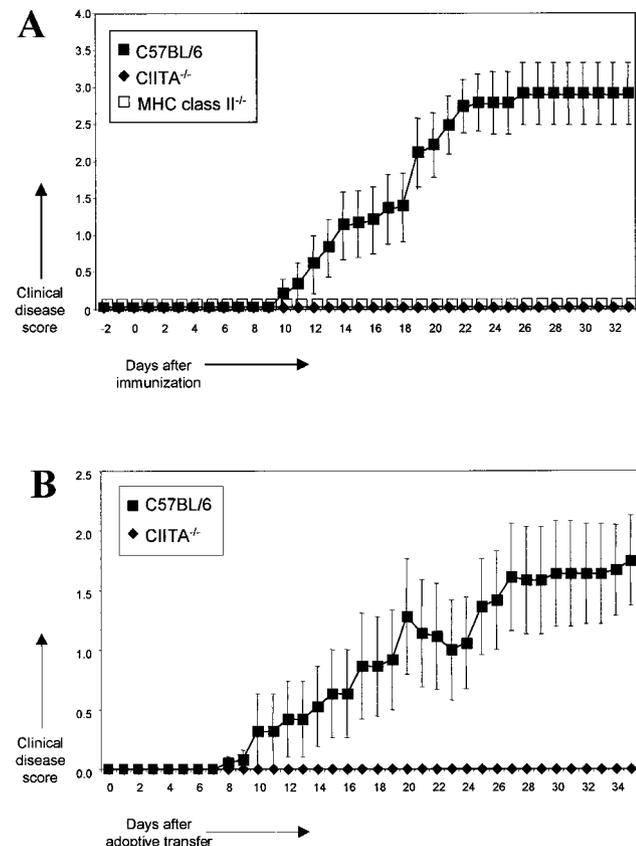


FIGURE 2. CIITA-deficient mice are resistant to EAE. Mean clinical score of mice (*A*) immunized with 100 μ g of pMOG 35–55, or (*B*) after adoptive i.v. transfer with 2.0×10^7 MOG p35–55-specific T cells. Mice were scored daily for clinical EAE as described in *Materials and Methods*.

Table I. Immunization with MOG p35–55 causes EAE in C57BL/6 wild-type, but not CIITA-deficient, mice

Group	Incidence	Mean Maximum Disease Score ^a	Mean Day of Disease Onset ^b
C57BL/6	20/20 ^c	3.1 \pm 0.29	17 \pm 0.9
CIITA ^{-/-}	0/12 ^c	0	0
MHC class II ^{-/-}	0/8 ^d	0	0

^a Mean of the maximum clinical disease score of individual animals in a group, shown with SEM. Individual animals were observed daily, and clinical scores were assessed in a blinded fashion on a 0–6 scale as follows: 0 = no clinical disease, 1 = loss of tail tone only, 2 = mild paraparesis, 3 = moderately severe paraparesis, 4 = paraplegia, 5 = moribund, or 6 = death.

^b Mean day of disease onset of individual animals in a group, shown with SEM.

^c Number represents the sum of animals used in three experiments.

^d Number represents the sum of animals used in two experiments.

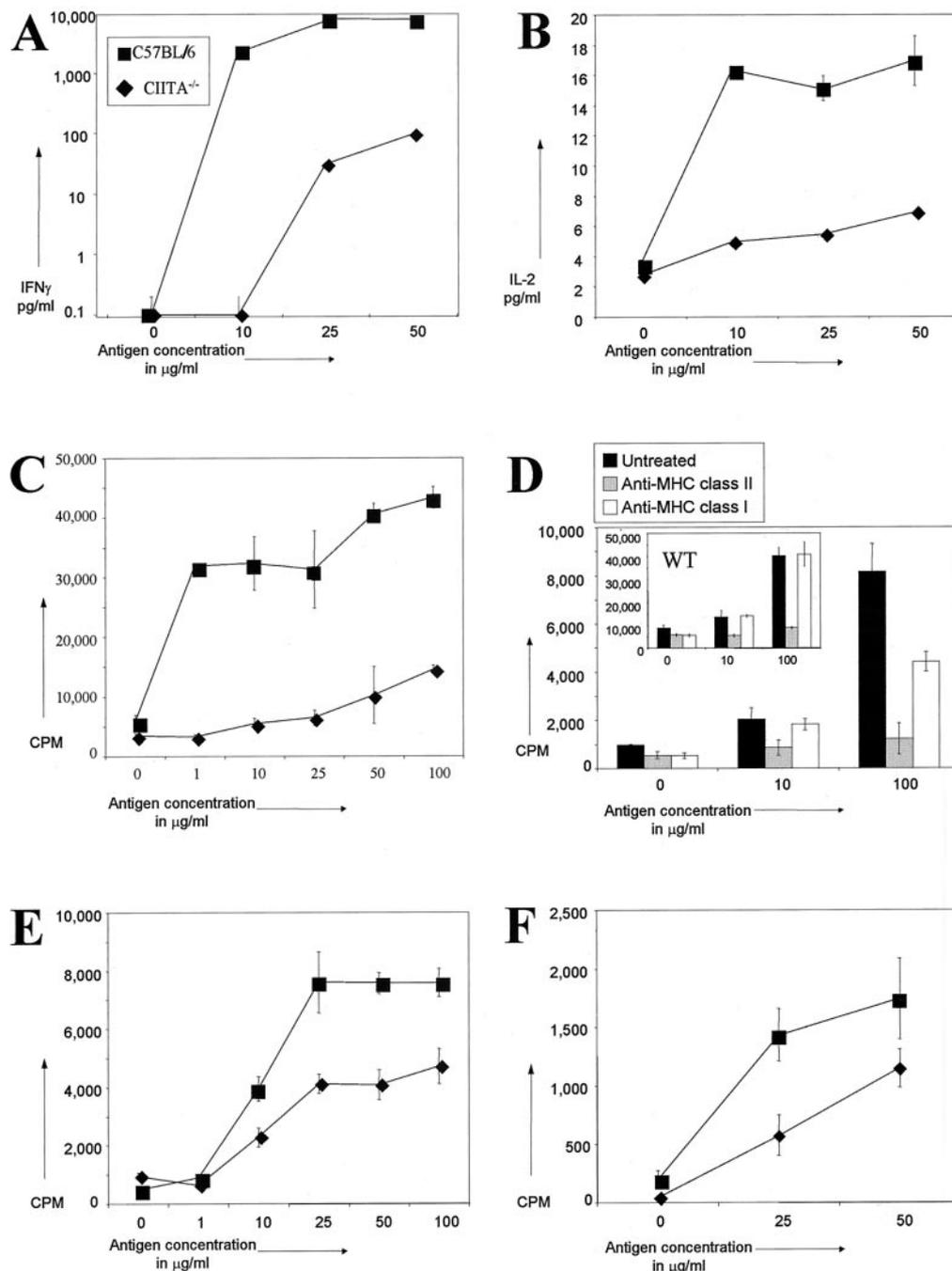


FIGURE 3. MOG p35–55 immunization induces an MHC class II-restricted T cell response in CIITA-deficient mice and CIITA-deficient splenic APC present MOG p35–55 to encephalitogenic T cells. Lymph node cells from CIITA-deficient mice immunized with MOG p35–55 produced the Th1 cytokines (A) IFN- γ and (B) IL-2, and (C) proliferated to MOG p35–55, although these responses were diminished in comparison with p35–55-immunized C57BL/6 wild-type mice. D, Proliferation of lymph node cells from CIITA-deficient mice and C57BL/6 wild-type mice (*inset*) was inhibited by anti-MHC class II mAb, and to a lesser extent by anti-MHC class I mAb. Lymph node cells (5×10^5) from CIITA-deficient mice were incubated in the presence or absence of the indicated Abs with various Ag concentrations. Irradiated splenic APC from CIITA-deficient mice were capable of presenting MOG p35–55 to p35–55-specific T cells (E) before and (F) after CD4⁺ purification.

CIITA directs expression of MHC class II, Ii, H-2M, and MHC class I on astrocytes

Having examined the role of CIITA deficiency in expression of class II molecules by astrocytes and EAE susceptibility, we proceeded to investigate how constitutive overexpression of CIITA in astrocytes influenced their expression of class II molecules and endocytic processing elements. Astrocyte lines 2.1 and 3.2 were derived from B10.PL (H-2^u) mice (20). Previously, we have shown that these astrocytes up-regulate CIITA, Ii, H-2M, and MHC class

II molecules after activation with IFN- γ (20). In Fig. 5, it can be seen that astrocytes transfected with GFAP-human (h) CIITA expressed hCIITA, but not murine (m) CIITA, without stimulation with IFN- γ . GFAP-CIITA-transfected 2.1 and 3.2 cells expressed Ii and H-2Mb mRNA without IFN- γ stimulation. In contrast, astrocytes transfected with GFAP vector only did not up-regulate CIITA, Ii, or H-2Mb. We observed previously that H-2Ma was expressed constitutively by these cells (20). As shown in Fig. 6, GFAP-CIITA-transfected astrocytes also up-regulated cell surface

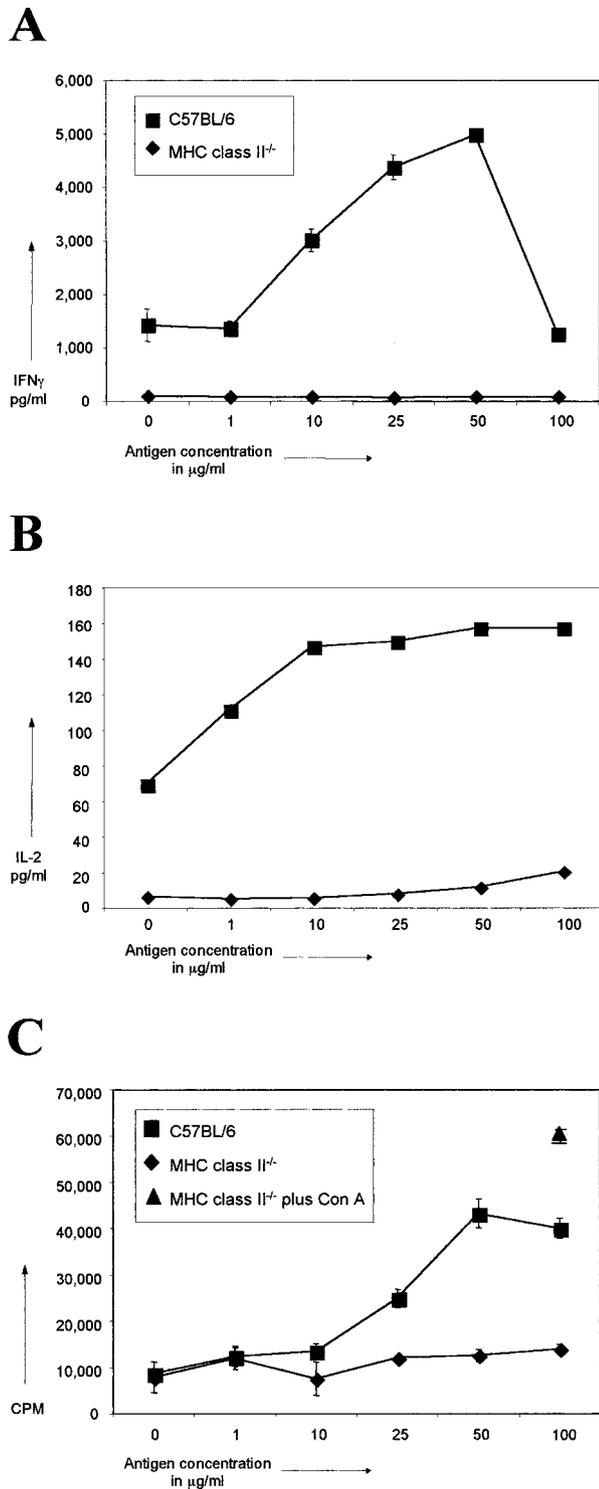


FIGURE 4. MOG p35–55 immunization of MHC class II-deficient mice does not induce Th1 cytokine secretion or significant proliferation. Lymph node cells from MOG p35–55-immunized MHC class II-deficient mice did not produce significant quantities of the Th1 cytokines (A) IFN- γ and (B) IL-2 in comparison with lymph node cells from C57BL/6 wild-type mice. C, Lymph node cells from MHC class II-deficient mice did not proliferate when cultured with MOG p35–55. Proliferative response to Con A (5 μ g/ml) by CIITA-deficient mice is shown as a control.

MHC class II molecules, whereas GFAP vector only-transfected cells did not. As it has been observed that CIITA participates in IFN- γ -inducible MHC class I regulation in certain human cell types (31, 32, 48) and studies suggest that astrocytes may activate

CD8⁺ T cells (12, 15, 49, 50), we examined expression of MHC class I molecules on untransfected, GFAP-CIITA-transfected, and IFN- γ -activated astrocytes. As shown in Fig. 6, CIITA induced MHC class I expression on 2.1 astrocytes. Similarly, CIITA transfection of astrocyte line 3.2 caused up-regulation of either class II or class I molecules, although 3.2 expressed a constitutive level of MHC class I molecules (data not shown). Results from these transfection studies clearly demonstrated that CIITA promotes MHC class I expression in astrocytes, while analysis of CIITA-deficient astrocytes showed that CIITA was not required for IFN- γ -inducible class I expression (Fig. 1). Thus, IFN- γ -inducible class I expression involves both CIITA-dependent and CIITA-independent pathways.

Constitutive CIITA expression in astrocytes is not sufficient to promote CNS autoimmune disease

To examine the role of CIITA in class II expression and Ag presentation by astrocytes in vivo, we generated GFAP-CIITA Tg mice, using the human GFAP promoter construct, *Gfa2* (Fig. 7A), which has been used to target transgene expression in astrocytes (33, 34). Three GFAP-CIITA Tg lines were identified by Southern blot analysis (Fig. 7B). Although these founder lines exhibited germline transmission, line 1 did not show substantial CIITA mRNA or class II protein expression on astrocytes. Founder lines 19 and 22, which up-regulated CIITA and class II expression, were used for further study. Similar clinical and histologic results were obtained for both lines 19 and 22. Initially, MHC class II expression was examined in CNS tissue. Naive GFAP-CIITA Tg mice expressed MHC class II molecules on astrocytes in the brainstem (Fig. 8, B and D), corpus callosum, spinal cord, and cerebral hemispheres (data not shown). MHC class II surface expression was not detectable in the CNS of nonimmunized wild-type mice by immunohistochemistry (Fig. 8A) or two-color immunofluorescence (Fig. 8C). Cell surface staining of MHC class II was also detected on some astrocytes from GFAP-CIITA Tg mice by FACS analysis, but not on astrocytes from wild-type mice (data not shown). We did not detect differences in class II expression in spleen, thymus, lymph node, kidney, or heart in GFAP-CIITA Tg mice (data not shown).

GFAP-CIITA Tg mice did not develop spontaneous EAE. When evaluated for EAE susceptibility after immunization with MOG p35–55, GFAP-CIITA mice developed EAE with similar onset, incidence, and clinical severity as wild-type mice (Fig. 9; Table III). In several experiments, we did not detect significant differences in the number of CNS inflammatory lesions in wild-type (Fig. 10A) and GFAP-CIITA Tg mice (Fig. 10B). Essentially no cell surface MHC class II expression was detected on astrocytes in EAE lesions in wild-type mice when examined by two-color immunofluorescence for GFAP and MHC class II molecules (Fig. 10C). MHC class II was detected on some astrocytes in some CNS inflammatory lesions in GFAP-CIITA mice (Fig. 10D), although these lesions were similar in size to the EAE lesions observed in wild-type mice. Thus, constitutive GFAP-CIITA-directed class II expression on astrocytes did not promote clinical or histologic EAE.

Astrocytes transfected with CIITA can present CNS autoantigenic peptide, but not native protein, to encephalitogenic T cells

Certain possible reasons why GFAP-CIITA-directed class II expression did not influence EAE susceptibility were examined. Using our in vitro astrocyte model (20, 51), we observed that GFAP-CIITA-transfected astrocytes, derived from H-2^d mice, could present MBP Ac1–11 to encephalitogenic MBP Ac1–11-specific T

Table II. Adoptive transfer of encephalitogenic MOG p35–55-specific T cells causes EAE in C57BL/6 wild-type, but not CIITA-deficient, mice

Donor Cells	Recipient Mice	Incidence ^a	Mean Maximum Disease Score ^b	Mean Day of Disease Onset ^c
Wild type	Wild type	12/19	2.5 ± 0.48	22 ± 2.5
Wild type	CIITA ^{-/-}	0/13	0	0

^a Number represents the sum of animals used in three experiments.

^b Mean of the maximum clinical disease score of individual animals in a group, shown with SEM. Individual animals were observed daily, and clinical scores were assessed in a blinded fashion on a 0–6 scale as follows: 0 = no clinical disease, 1 = loss of tail tone only, 2 = mild paraparesis, 3 = moderately severe paraparesis, 4 = paraplegia, 5 = moribund, or 6 = death.

^c Mean day of disease onset of individual animals in a group, shown with SEM.

cells. In contrast, astrocytes that were transfected with GFAP vector only did not (Fig. 11B). Previously, by comparing the capability to present native MBP and MBP Ac1–11 by live and fixed IFN- γ -activated astrocytes, we formally demonstrated that processing was required for presentation of native MBP to encephalitogenic MBP Ac1–11-specific T cells and that astrocytes were capable of processing native MBP (20). As shown in Fig. 11, A and B, GFAP-CIITA-transfected astrocytes did not present native MBP without IFN- γ -activation. Thus, although CIITA directs expression of MHC class II molecules and is responsible for up-regulation of Ii and H-2M, additional IFN- γ -inducible, but CIITA-independent, gene products may also participate in the endocytic processing of MBP. Furthermore, CIITA transfection did not cause up-regulation of certain costimulatory molecules (data not shown) that may also facilitate *in vivo* Ag presentation.

CIITA transfection of astrocytes does not induce all elements required for endocytic processing

Cat S, an IFN- γ -inducible lysosomal cysteine protease expressed by macrophages and microglia (52), participates in endocytic processing (53, 54) and MBP degradation (37). We examined Cat S expression in unstimulated astrocytes, IFN- γ -activated astrocytes, and GFAP-CIITA-transfected astrocytes. Cats B and L, which are also cysteine proteases, were examined for comparison. mRNA transcripts for Cats B and L were detected in a constitutive manner and were several orders of magnitude higher than Cat S (Table IV).

Cats B and L did not increase after IFN- γ activation of astrocyte line 3.2, whereas a 2.2-fold increase of Cat L was observed in 2.1 cells (Table IV). However, Cat S expression was up-regulated ~10-fold (8.31 for astrocyte line, 2.1 and 12.7 for astrocyte line 3.2) in both of these astrocyte lines. In contrast with IFN- γ -activated astrocytes, CIITA-transfected astrocytes did not show substantial increase in expression of Cat S, B, or L. Thus, in astrocytes derived from B10.PL mice, Cat S is up-regulated in an IFN- γ -dependent, CIITA-independent manner.

Discussion

CIITA has been described as the master regulator for IFN- γ -inducible and constitutive class II expression (25). Class II expression is difficult to detect in the CIITA-deficient mice and thymic-positive selection of CD4⁺ T cells is impaired (39). Nevertheless, evidence indicates that CIITA-independent class II expression does exist (39, 46, 47). In this report, we have shown that immunization of CIITA-deficient mice with the encephalitogenic MOG p35–55 caused a limited priming of class II-restricted T cells that secreted Th1 cytokines. These results indicate that a small component of CIITA-independent class II-restricted Ag presentation can occur *in vivo* in the lymphoid tissue of these mice. In this regard, we detected a minimal level of class II molecules on CD11c⁺ dendritic cells that were activated with IL-4 and LPS (data not shown). CIITA-deficient splenic APC were also capable of presenting MOG peptide to purified encephalitogenic CD4⁺ T

FIGURE 5. Astrocytes transfected with GFAP-CIITA up-regulate Ii and H-2M mRNA. Astrocyte line 2.1 was transfected with either GFAP-hCIITA or GFAP-0 (vector only). Unactivated and IFN- γ -activated (100 U/ml for 24 h) untransfected astrocyte lines were examined in parallel for expression of mouse CIITA, Ii, and H-2M mRNA. RT-PCR was performed using oligonucleotide primers described in *Materials and Methods*. Analysis of β -actin mRNA is shown as a positive control.

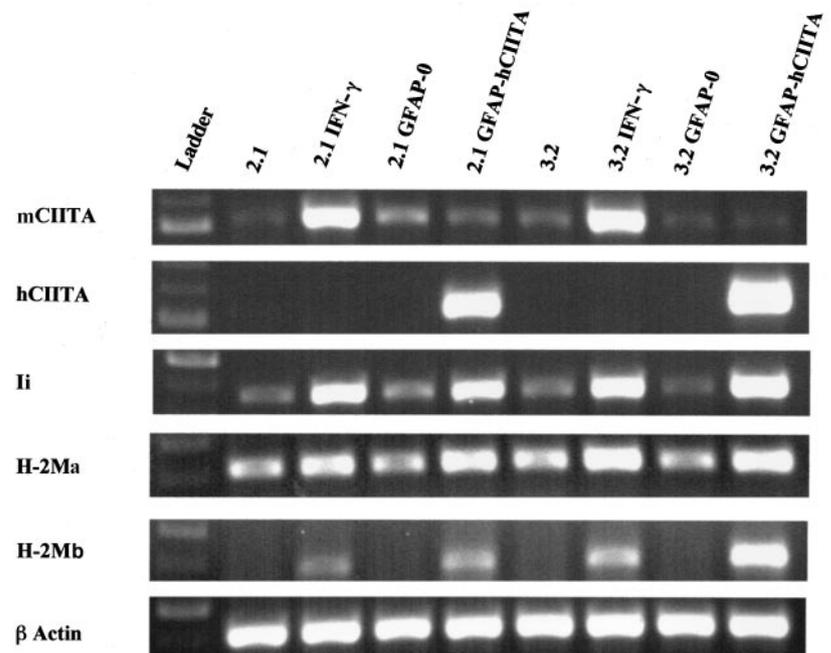
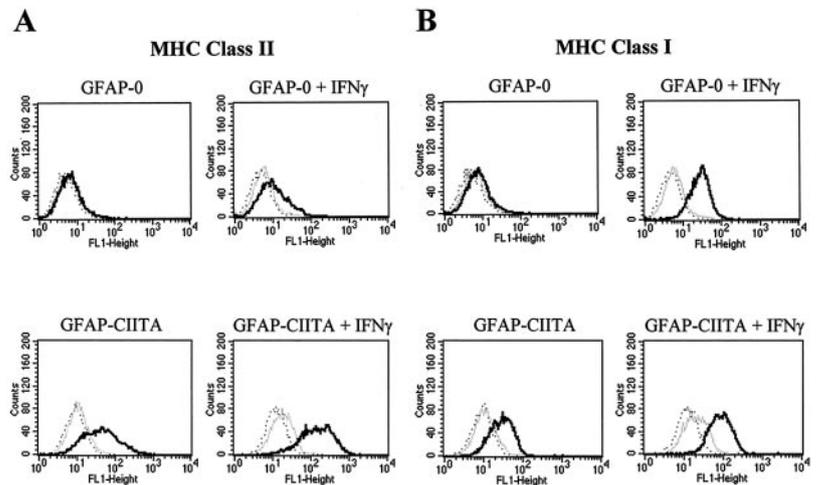


FIGURE 6. Astrocytes transfected with CIITA up-regulate MHC class II and class I molecules. Astrocyte line 2.1 transfected with GFAP-CIITA or GFAP-0 (vector only) were evaluated for constitutive and IFN- γ -inducible expression of (A) MHC class II molecules and (B) MHC class I molecules. Medium-treated or IFN- γ -activated (100 U/ml IFN- γ for 48 h) astrocytes were evaluated for expression of MHC class II molecules by staining with anti I-A^{k.u} mAb (10-2.16), or for MHC class I molecules by staining with pan anti-H-2^d mAb (8F12).



cells in vitro. However, CIITA-deficient mice were resistant to clinical or histologic EAE induced by active immunization or by adoptive transfer of wild-type encephalitogenic class II-restricted CD4⁺ T cells, and CNS class II expression was not detected in

CIITA-deficient mice. Class II-restricted CNS Ag presentation is clearly CIITA-dependent.

Whether astrocytes serve as APC for class II-restricted Ag presentation in CNS inflammatory disease is controversial (8, 10, 20). Using the GFAP promoter to direct CIITA expression in astrocytes, we examined how constitutive class II expression by astrocytes influenced EAE induction. Astrocytes transfected with CIITA in vitro up-regulated class II, Ii, and H-2M. Astrocytes in GFAP-CIITA Tg mice expressed cell surface class II molecules. These Tg mice did not develop spontaneous CNS inflammation and, in comparison to control mice, there was no significant difference in EAE onset or clinical severity. We also did not observe a significant difference in histologic EAE, although some astrocytes within EAE lesions did express class II molecules. Thus, our results indicate that CIITA-driven class II expression by astrocytes did not promote induction of EAE.

Results from a previous study indicated that Ag processing by CNS APC was required for initial class II-restricted Ag presentation and T cell activation in CNS autoimmunity (5). It was also demonstrated that IFN- γ -activated astrocytes up-regulated CIITA, Ii, and H-2M and were capable of processing and presenting native Ag to class II-restricted CD4⁺ T cells in vitro (20). However, the influence of CIITA on presentation of native Ags that require processing appears cell type-dependent (55–60). Introduction of CIITA into murine sarcoma cells (57), human intestinal epithelial cells (58), human hepatocarcinoma cells (59), and human fibroblasts (60) conferred the capability of these nonprofessional APC to process and present certain native proteins to CD4⁺ T cells without IFN- γ activation. In contrast, human melanoma cells transfected with CIITA up-regulated Ii, HLA-DM, and MHC class II molecules and could present peptide Ag, but could not process intact Ag for presentation to CD4⁺ T cells without IFN- γ activation (55). When transfected with CIITA, unactivated murine CD4⁺ T cells, which do not normally express CIITA or MHC class II genes (56, 61), up-regulated Ii, H-2M, and MHC class II molecules and could present peptide, but could not process intact protein (56). In this report, we observed that CIITA-transfected astrocytes up-regulated cell surface class II molecules, Ii, and H-2M and could present encephalitogenic peptide, but could not process native MBP without activation by IFN- γ . Furthermore, whereas IFN- γ -activated astrocytes up-regulated Cat S, a cysteine protease involved in endocytic processing and MBP degradation (62), CIITA-transfected astrocytes did not. Thus, one possible reason why the GFAP-CIITA Tg mice were no more susceptible to EAE induction than control mice is that CIITA did not confer the

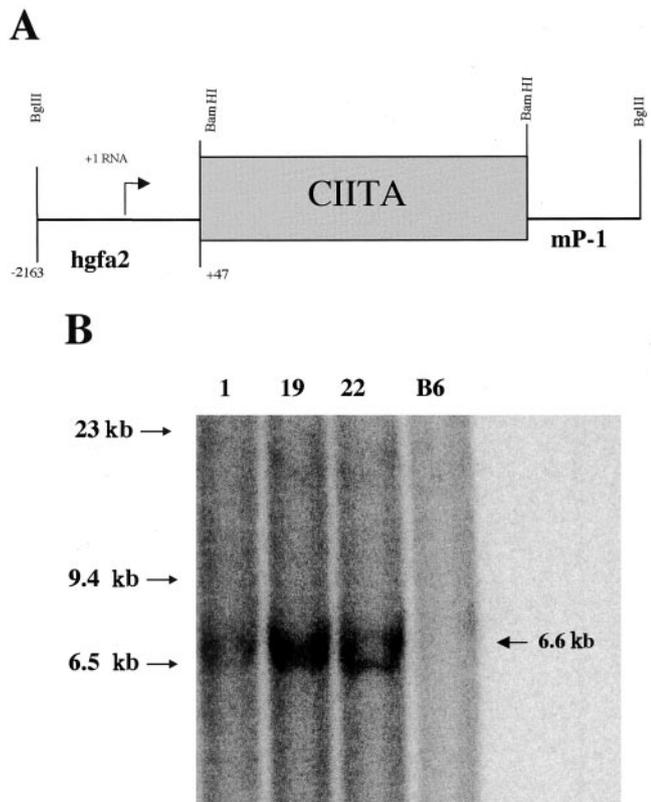


FIGURE 7. Generation of Gfa2-CIITA Tg mice. *A*, The Gfa2-CIITA transgene is shown. The hGFAP fragment spans bp -2163 to +47 relative to the transcriptional start site and has the natural initiating ATG at bp +15 converted to TTG by site-directed mutagenesis, so that the protein synthesis initiates within the CIITA. Also present are the coding region for CIITA and a segment of the mouse protamine-1 gene (mP-1) that provides an intron and a polyadenylation site. *B*, Southern blot confirmation for Gfa2-CIITA Tg founder lines is shown. Samples of the genomic tail DNA (7.5 μ g) were digested with *Eco*RI and hybridized with a 550-bp PCR-amplified fragment from the GFAP-hCIITA construct containing sequence from the human GFAP promoter and hCIITA cDNA. Hybridization to C57BL/6 genomic DNA is shown as a negative control.

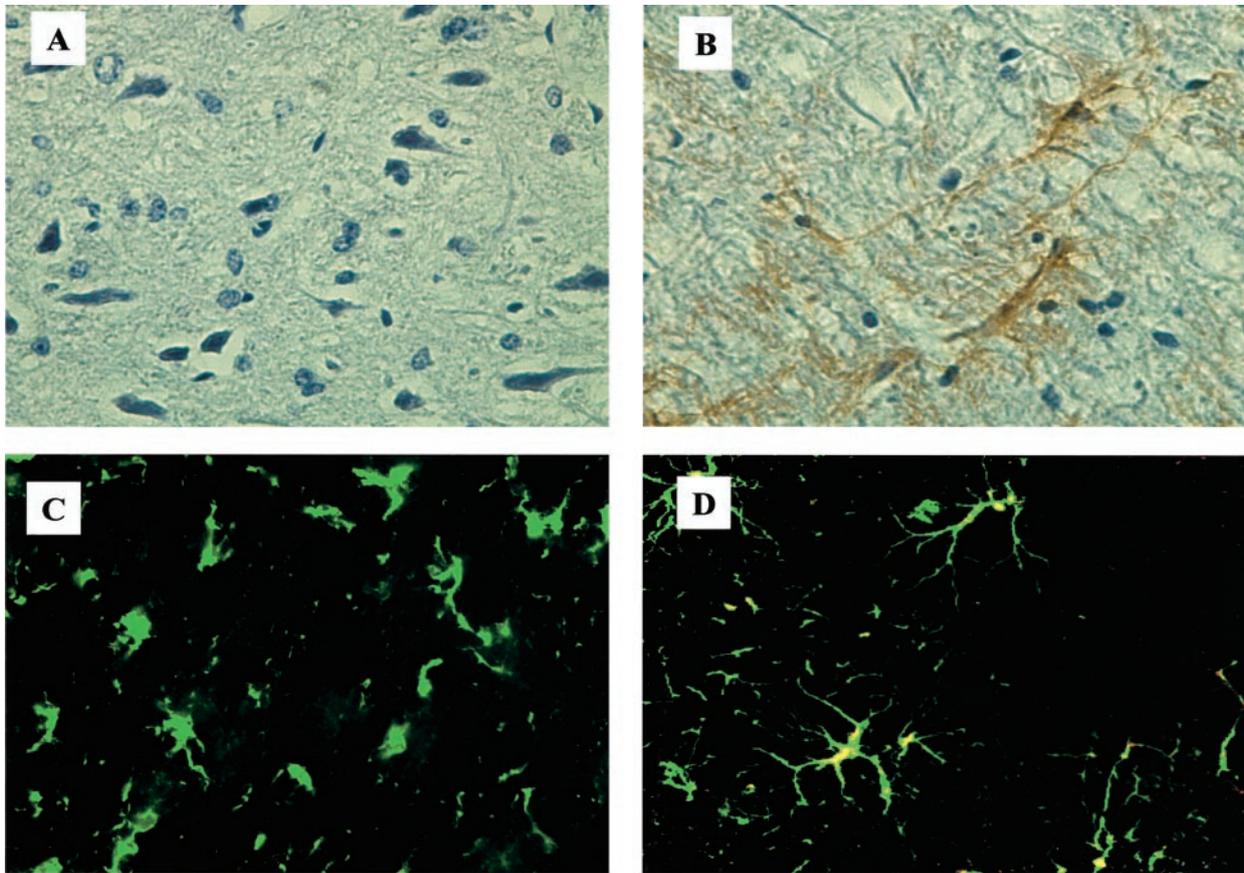


FIGURE 8. MHC class II surface molecules are expressed on astrocytes in unimmunized GFAP-CIITA Tg, but not wild-type, mice. *A*, Immunostaining does not reveal MHC class II expression in brain stem tissue of C57BL/6 wild-type mice, while *(B)* MHC class II staining is positive in brain stem tissue of GFAP-CIITA mice (magnification, $\times 20$). No staining is observed when GFAP-CIITA Tg brain stem tissue was stained with isotype-matched control Ab (data not shown). *C*, Two color immunofluorescence of brain stem sections stained by double immunofluorescence for MHC class II (PE (red)-labeled M5/114) and GFAP (FITC (green)-labeled) does not show MHC staining on astrocytes from C57BL/6 mice, *(D)* whereas MHC class II expression in brain stem tissue sections from GFAP-hCIITA mice colocalizes to astrocytes when analyzed by confocal microscopy (magnification, $\times 60$). Yellow represents costaining with PE and FITC.

capability to process native CNS autoantigen by astrocytes *in vivo*. It is possible that transgenic targeting of CIITA to microglia, more potent resident CNS APCs that express certain cysteine proteases involved in Ag processing (52), might promote CNS Ag presentation and EAE susceptibility.

There are other possible explanations of why the GFAP-CIITA transgenic mice did not have increased susceptibility to clinical or histologic MOG p35–55-induced EAE. It is known that IFN- γ -activated astrocytes up-regulate B7-1 (CD80) and B7-2 (CD86) costimulatory molecules (51, 63). Although transfection of

FIGURE 9. Onset and severity of EAE is similar in MOG p35–55 immunized wild-type and GFAP-CIITA Tg mice. Mice were immunized with 100 μg of MOG p35–55. Mice were scored daily for clinical EAE as described in *Materials and Methods*.

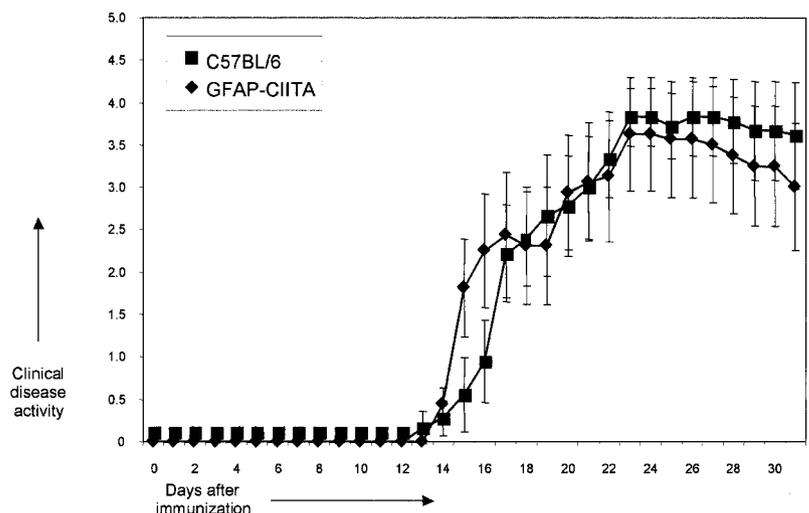


Table III. Comparison of clinical and histologic EAE scores in GFAP-CIITA Tg mice and C57BL/6 wild-type mice induced by immunization with MOG p35–55

Group	Incidence	Mean Maximum Disease Score ^a	Mean Day of Disease Onset ^b	Mean Inflammatory Infiltrates in Brain/Spinal Cord ^c
C57BL/6	17/19	4.0 ± 0.46	16 ± 0.9	8 (±3)/5 (±2)
GFAP-CIITA	13/17	4.2 ± 0.54	15 ± 1.1	8 (±3)/5 (±2)

^a Mean of the maximum clinical disease score of individual animals in a group, shown with SEM.

^b Mean day of disease onset of individual animals in a group, shown with SEM.

^c Inflammatory infiltrates were counted on one midline sagittal brain slice and two axial spinal cord levels.

astrocytes with CIITA caused up-regulation of class II molecules, it did not alter expression of B7-1 or B7-2 costimulatory molecules (data not shown). The GFAP promoter construct directs constitutive transgene expression in certain CNS anatomic locations (33, 34). Thus, it is possible that paralysis was not observed because class II expression was not induced on astrocytes within pyramidal tracts. This is unlikely as the GFAP promoter construct used induced transgene expression in the brainstem and spinal cord. EAE lesions commonly occur in the spinal cord and brainstem and we did not observe differences in histologic EAE.

Originally named for its pivotal function in MHC class II regulation, CIITA also has a role in MHC class I expression (31, 48). Specifically, it was demonstrated that CIITA is responsible for IFN- γ -inducible MHC class I expression on certain types of human cells, an effect that requires the cAMP response element (CRE) site α (31, 48), a *cis*-acting 5'-regulatory element of the MHC class I promoter involved in IFN- γ -inducible gene expression (64). CIITA induces MHC class I expression on some, but not all, types of human cells examined, suggesting that CIITA may

up-regulate MHC class I expression when the basal levels of other elements involved in MHC class I expression, such as β_2 microglobulin, TAP, and low molecular weight protein (LMP), were not limiting (31, 48). It was also observed that macrophages, APC of hemopoietic origin, isolated from CIITA-deficient mice up-regulated MHC class I molecules in response to IFN- γ , which suggested to those investigators that CIITA was not critically involved in IFN- γ -induced up-regulation of MHC class I genes (46). In this investigation, we examined the role of CIITA in MHC class I expression using CIITA-transfected and CIITA-deficient astrocytes. CIITA transfection of murine astrocytes caused MHC class I up-regulation. IFN- γ activation of CIITA-deficient murine astrocytes also induced class I expression. Thus, it is clear that both CIITA-dependent and CIITA-independent pathways can be used for IFN- γ -inducible MHC class I expression in murine astrocytes.

Two recent studies have shown that CD8⁺ T cells can participate as effector cells in EAE induction (49, 50). In the study by Sun et al. (50), it was observed that the MOG p35–55-specific proliferative response of p35–55-immunized wild-type C57BL/6 mice

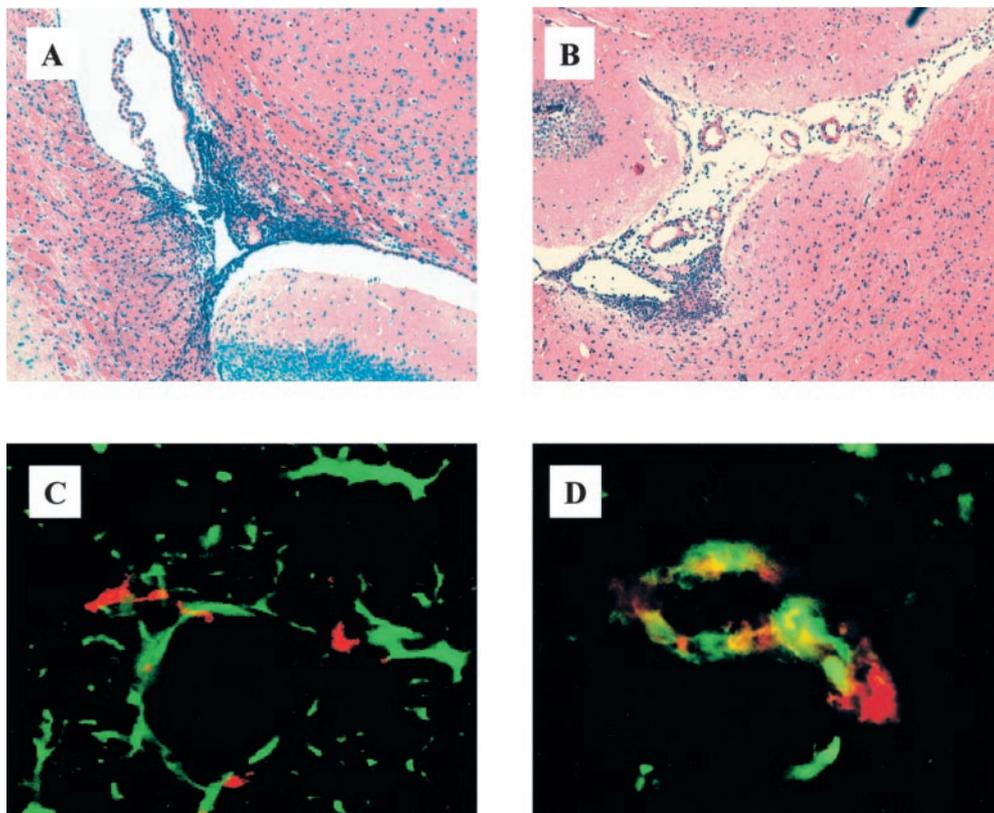


FIGURE 10. Histological evidence of EAE is similar in wild-type and GFAP-CIITA Tg mice. Brain stem sections from (A) C57BL/6 wild-type mice and (B) GFAP-CIITA Tg mice were stained with H&E as described in *Materials and Methods* (magnification, $\times 20$). C, Two color immunofluorescence for MHC class II (PE-labeled) and GFAP (FITC-labeled) shows MHC staining in inflammatory cuffs that does not colocalize to astrocytes in C57BL/6 wild-type mice, (D) while some astrocytes in EAE lesions of GFAP-CIITA Tg mice expressed MHC class II molecules (magnification, $\times 60$).

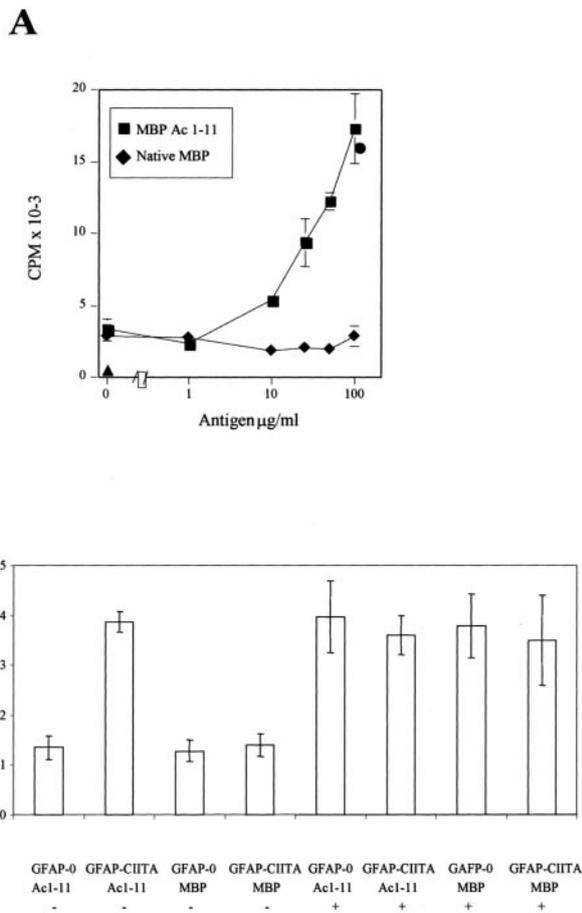


FIGURE 11. CIITA-transfected astrocytes that express constitutive class II molecules present MBP peptide Ac1-11 to encephalitogenic MBP Ac1-11-specific CD4⁺ T cells, but cannot present native MBP without IFN- γ activation. *A*, Unstimulated GFAP-CIITA-transfected astrocytes were capable of presenting MBP Ac1-11 but not native guinea pig MBP, to MBP Ac1-11-specific CD4⁺ T cells. Presentation of guinea pig MBP (●) or no Ag (▲) by B10.PL splenocytes is shown for comparison. *B*, Astrocytes transfected with vector only (GFAP-0) could not present either MBP Ac1-11 or native MBP. Activation with IFN- γ restored the capability to process and present native MBP by both GFAP-CIITA and GFAP-0 cells. Before coculture with T cells and Ag, astrocytes were treated with medium alone or IFN- γ (100 U/ml) for 48 h. In all panels, 4×10^4 mitomycin C-treated astrocytes were cultured with 1×10^4 MBP Ac1-11-specific T cells. After 48 h culture with Ag, cultures (triplicate) were pulsed with 1 mg/well [³H]thymidine for 16 h.

was inhibited by anti-class I, but not anti-class II, mAb. In contrast with their results, using the same anti-class I and anti-class II mAbs, as well as using isotype control mAbs, we consistently observed that the primary T cell proliferative response in p35-55-immunized wild-type C57BL/6 mice was restricted mostly by class II molecules. Similarly, the T cell proliferative response in p35-55-immunized CIITA-deficient mice was primarily inhibited by anti-class II mAb. Furthermore, in three separate experiments, we did not detect significant p35-55-specific proliferative responses in lymph node cells from p35-55-immunized class II-deficient mice. These mice, like CIITA-deficient, Ii-deficient, and H-2M-deficient mice (5), which have defects in the class II pathway, were also resistant to EAE induction. Our results do not conflict with the study by Huseby et al. (49) who generated MBP-specific CD8⁺ T cells using a determinant presented through the MHC class I pathway. Although it is known that CD8⁺ T cells are found along with CD4⁺ T cells within CNS demyelinating lesions

Table IV. Relative mRNA copy number (fold stimulation)^a

Cathepsin	Untreated	IFN- γ	GFAP-hCIITA
Line 2.1			
B	1.61E + 09	2.39E + 09 (1.49)	2.57E + 09 (1.60)
L	5.17E + 08	1.14E + 09 (2.20)	1.14E + 09 (2.20)
S	3.10E + 04	2.58E + 05 (8.31)	5.80E + 04 (1.90)
Line 3.2			
B	2.34E + 09	2.20E + 09 (0.94)	1.53E + 09 (0.65)
L	9.84E + 08	7.99E + 08 (0.84)	2.82E + 08 (0.29)
S	7.03E + 04	8.93E + 05 (12.7)	<E + 03

^a mRNA copy number of GAPDH in each sample was calculated and used to normalize Cat S mRNA copy numbers among the samples.

of MS (65) and EAE (66), and it is recognized that CD8⁺ T cells may have an important role as effector cells in CNS inflammation, our results indicate that “CD4⁺ T cell help” is required for CD8⁺ priming to MOG p35-55.

The results in this report do not eliminate the possibility that astrocytes participate in class II-restricted Ag presentation in vivo in CNS inflammatory disease or that they do not promote CNS inflammation. Although we did not detect class II expression on astrocytes in the acute CNS inflammatory lesions in wild-type mice with EAE and CIITA-directed constitutive class II expression on astrocytes did not appear to promote EAE induction, class II molecules have been detected on astrocytes in CNS lesions of relapsing EAE in SJL/J mice (18). Astrocytes can secrete TNF- α , a proinflammatory Th1 cytokine (67), and astrocyte-targeted secretion of proinflammatory cytokines does promote CNS inflammation (68, 69). Astrocytes may also serve as APC for presentation of Ag to class I-restricted CD8⁺ T cells (12). However, substantial evidence indicates that astrocytes serve to down-regulate proinflammatory CNS responses (70). Astrocytes produce TGF- β , an immunosuppressive cytokine (9), and some astrocytes can promote naive Th0 cells to differentiate into Th2 cells in vitro (51). In summary, our results do not support the in vivo role of astrocytes as APC for class II-restricted Ag presentation to proinflammatory T cells during the induction phase of this EAE model.

Note added in proof. Very shortly after submission of this manuscript, Tompkins et al. (71) reported that the CIITA-deficient mice were resistant to EAE by immunization with MOG p35-55 or by adoptive transfer of encephalitogenic T cells. They also determined that the proliferative response to MOG p35-55 in wild-type C57BL/6 mice immunized with p35-55 was restricted primarily by class II, and not class I, molecules. We are quite pleased that their results and our observations published here and previously (5) support each other.

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