

HLA-DR4-IE Chimeric Class II Transgenic, Murine Class II-Deficient Mice Are Susceptible to Experimental Allergic Encephalomyelitis

By Kouichi Ito,* Hong-Jin Bian,* Margarita Molina,* Jihong Han,* Jeanne Magram,† Elizabeth Saar,† Charles Belunis,* David R. Bolin,* Reynaldo Arceo,§ Robert Campbell,* Fiorenza Falcioni,* Damir Vidović,* Juergen Hammer,|| and Zoltan A. Nagy*

From the *Department of Inflammation and Autoimmune Diseases, †Department of Biotechnology, and §Department of Toxicology & Pathology, Hoffmann-La Roche Inc., Nutley, New Jersey 07110-1199; and ||Roche Milano Ricerche, I-20132 Milan, Italy

Summary

To investigate the development of HLA-DR-associated autoimmune diseases, we generated transgenic (Tg) mice with HLA-DRA-IE α and HLA-DRB1*0401-IE β chimeric genes. The transgene-encoded proteins consisted of antigen-binding domains from HLA-DRA and HLA-DRB1*0401 molecules and the remaining domains from the IE^d- α and IE^d- β chains. The chimeric molecules showed the same antigen-binding specificity as HLA-DRB1*0401 molecules, and were functional in presenting antigens to T cells. The Tg mice were backcrossed to MHC class II-deficient (IA β ⁻, IE α ⁻) mice to eliminate any effect of endogenous MHC class II genes on the development of autoimmune diseases. As expected, IA $\alpha\beta$ or IE $\alpha\beta$ molecules were not expressed in Tg mice. Moreover, cell-surface expression of endogenous IE β associated with HLA-DRA-IE α was not detectable in several Tg mouse lines by flow cytometric analysis. The HLA-DRA-IE α /HLA-DRB1*0401-IE β molecules rescued the development of CD4⁺ T cells in MHC class II-deficient mice, but T cells expressing V β 5, V β 11, and V β 12 were specifically deleted.

Tg mice were immunized with peptides, myelin basic protein (MBP) 87-106 and proteolipid protein (PLP) 175-192, that are considered to be immunodominant epitopes in HLA-DR4 individuals. PLP175-192 provoked a strong proliferative response of lymph node T cells from Tg mice, and caused inflammatory lesions in white matter of the CNS and symptoms of experimental allergic encephalomyelitis (EAE). Immunization with MBP87-106 elicited a very weak proliferative T cell response and caused mild EAE. Non-Tg mice immunized with either PLP175-192 or MBP87-106 did not develop EAE. These results demonstrated that a human MHC class II binding site alone can confer susceptibility to an experimentally induced murine autoimmune disease.

Since HLA-DR4 allele is associated with the development of autoimmune diseases such as rheumatoid arthritis (RA)¹ and multiple sclerosis (MS), HLA-DR4/human CD4 Tg mice and HLA-DR4-IE Tg mice have been generated to investigate these diseases. These Tg mice are functional in activation of HLA-DR4-restricted T cells (1, 2). However, since the Tg mice express both HLA-DR and endogenous mouse MHC class II molecules, both

HLA-DR- and mouse MHC class II-restricted T cells are activated in response to antigens. Thus, the effect of HLA-DR on immune response and susceptibility to autoimmune disease is difficult to separate from that of the wild-type murine MHC class II molecules. In the present study, we produced HLA-DRA-IE α /HLA-DRB1*0401-IE β Tg mice, and backcrossed them to MHC class II-deficient mice to eliminate any effect of endogenous MHC class II proteins. Recently, the same strategy has been applied to HLA-DQ8 Tg mice (3).

MBP and PLP are putative autoantigens involved in the pathogenesis of MS and can induce EAE in mice and rats (4-6). Disease in these animal models can also be induced

¹Abbreviations used in this paper: EAE, experimental allergic encephalomyelitis; HA, Flu virus hemagglutinin; MBP, myelin basic protein; MMTV, mouse mammary tumor virus; MS, multiple sclerosis; PLP, proteolipid protein; RA, rheumatoid arthritis; Tg, transgenic.

by adoptive transfer of MBP- or PLP-reactive T cells into syngeneic recipients (7). In humans, MBP- or PLP-reactive HLA-DR-restricted T cells have been isolated from both MS patients and healthy controls (8–13). MBP-specific T cells in MS patients were activated while those from controls were in a resting state (13). Activated MBP- and PLP-reactive T cells might therefore be involved in the pathogenesis of MS. We show here that immunization of HLA-DRA-IE α /HLA-DRB1*0401-IE β Tg mice with PLP175-192 or MBP87-106 peptides induces the development of EAE.

Materials and Methods

Gene Constructs of HLA-DRA-IE α and HLA-DRB1*0401-IE β . The DNA carrying exon 2 of HLA-DRA was amplified from pPR541 cosmid DNA (14) with primers, 5' ACAGAA-GGTGTCAGGTAGACTGAAA3' and 5' GAGCACAGTTACTCTGGAGGTAC3' by polymerase chain reaction (PCR) using Pfu DNA polymerase. The amplicon carrying the HLA-DRA exon 2 was treated with T4 DNA polymerase and subcloned into the SmaI site of pBluescript vector (Stratagene, San Diego, CA). An 8-kb BglI fragment of IE α gene derived from no. 32.1 IE α cosmid DNA (15) was also treated with T4 DNA polymerase and subcloned into the SmaI site of pBluescript vector. The IE α genomic DNA, from which exon 2 was removed by BstEII and BglII digestion, was treated with Klenow Fragment of DNA polymerase I. This DNA was then ligated to the 1.5-kb DNA fragment carrying HLA-DRA exon 2 that was digested from pBluescript vector with EcoRV and Bsp106 and then treated with Klenow Fragment of DNA polymerase I.

A 7-kb EcoRI/KpnI DNA fragment carrying IE β exon 1 was ligated to a 10-kb EcoRI/KpnI DNA fragment carrying IE β exon 3–6 (16). The ligated DNA was subcloned into the KpnI site of pBluescript vector. The DNA fragment containing Exon 2 of HLA-DRB1*0401 was amplified from genomic DNA of Priess cells by PCR using Taq polymerase and primers, 5' TGA-AAGCGGTGCGTGCTGTTAA3' and 5' CCCCATACATTTCCCTTCCTTG3'. It was then subcloned into a pCRII vector (Invitrogen, San Diego, CA). The 0.85-kb EcoRI DNA fragment carrying exon 2 of HLA-DRB1*0401 was ligated to the EcoRI site of the IE β genomic DNA which lacks exon 2.

Transgenic Mice. The gene constructs were removed from plasmid by digestion with Not I and Bsp106 for HLA-DRA-IE α

and KpnI for HLA-DRB1*0401-IE β . They were coinjected into C57BL/6 fertilized eggs. Tg mice were backcrossed to C2D (IA β ⁻, IE α ⁻) mice on the C57BL/6 genetic background. Tg founders were first identified by PCR with 5' GGGAAGCAGGGG-GACTATGAC3' and 5' TTAGGGCAATGACTTCGTAGG3' primers for HLA-DRA-IE α and 5' TGAAAGCGGTGCGTG-CTGTTAA3' and 5' CACCCGCTCCGTCCCGTTGAA3' primers for HLA-DRB1*0401-IE β , and were then confirmed by southern blot analysis using probes derived from exon 2 of HLA-DRA and HLA-DRB1*0401 genes and by staining of peripheral blood cells using anti-HLA-DR mAb, L243. C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME), and C2D mice were purchased from GenPharm International (Mountain View, CA).

Flow Cytometry Analysis. Spleen cells or thymocytes (5×10^5) were suspended in 100 μ l of RPMI medium containing 2% FCS and 0.1% sodium azide and incubated on ice in the presence of mAb for 30 min. The cells were washed twice with PBS containing 0.1% sodium azide and 0.1% BSA, and then once with PBS containing 0.1% sodium azide. The cells were analyzed on a FACSort[®] using LYSIS II software. The following mAbs were used in the experiments: Anti-HLA-DR mAb, L243; anti-B220 mAb, RA3-6B2; anti-CD11 β mAb, M1/70; anti-IE β mAb, 17.3.3; anti-IA^b mAb, AF6-120.1; anti-mouse CD4 mAb, RM4-5; anti-mouse CD8 mAb, 53-6.7; anti-V β Abs (V β 2, B20.6; V β 3, KJ25; V β 4, KJ4; V β 5, MR9-4; V β 6, RR4-7; V β 7, TR310; V β 8, MR5-2; V β 9, MR10-2; V β 10, B21.5; V β 11, RR3-15; V β 12, MR11-1; V β 13, MR12-3; V β 14, 14-2; V β 17, KJ-23). The anti-HLA-DR mAb was purchased from Becton Dickinson (San Jose, CA), and all other mAbs were purchased from PharMingen (San Diego, CA).

T Cell Proliferation Assay. Mice were primed in the base of the tail with 50–200 μ g of peptide in Complete Freund's Adjuvant (CFA). 9 d later, inguinal and paraortic lymph node cells were isolated and assayed for T cell proliferation using 4×10^5 cells/well. The cells were cultured with an antigen for 63 h in RPMI medium containing 10% FCS, 50 u/ml penicillin/streptomycin, 2 mM glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, and 0.25 μ g/ml fungizone. The cells were pulsed with 2 μ Ci[methyl-³H]thymidine for 6–9 h, then harvested and counted in a scintillation counter.

Peptide Binding Assay. Peptides were prepared using solid phase synthesis procedures as described (17). Their sequences are described in Table 1. MHC class II molecules were purified from

Table 1. Antigen Binding Specificity to DRB1*0401-IE

Peptides	Sequence	50% Inhibitory Concentration (IC ₅₀ , μ M)	
		DRB1*0401	DRB1*0401-IE
m1-7	YRAMATL	0.120	0.130
S6-m1-7	YRAMASL	0.043	0.053
A6-m1-7	YRAMAAL	0.410	0.530
R6-m1-7	YRAMARL	>10	3.300
HA307-319	PKYVKQNTLKLAT	0.245	0.220
HA-R6	PKYVKQNRLLKLAT	>10	>10
MBP87-106	VVHFFKNIVTPRTPPPSQGK	0.158	0.153
PLP175-192	YIYFNTWTTTCQSIAPFSK	0.187	0.169

Priess cells (DRB1*0401), and I α -negative mouse B lymphoma M12.C3 cells (18) transfected with HLA-DRA-IE α and HLA-DRB1*0401-IE β genes. Transfection was carried out with 10 μ g of NotI-digested Neo^r-HLA-DRA-IE α and 20 μ g of KpnI-digested HLA-DRB1*0401-IE β under 960 mFD and 0.25 kV by a Bio-Rad Gene PulserTM. The binding peptides used in the study were radioiodinated using IODO-BEADS (Pierce, Rockford, IL) and the labeled peptide was separated from free ¹²⁵I by a Seppak C-18 column. The binding reactions were performed in 96-well Optiplates (Packard Instrument, Downers Grove, IL), which were blocked with 1 μ g/ml BSA in PBS. The affinity-purified MHC class II molecules (0.1–1 μ g), 75 nM ¹²⁵I-radiolabeled peptide, and various concentrations of competitor peptides were mixed in binding buffer (50 mM PBS, pH 7.5, 1% NP-40, 6 mM N-ethylmaleimide, 1 mM PMSF, 1.6 mM EDTA and a cocktail of protease inhibitors; aprotinin (100 U/ml), leupeptin (10 μ g/ml), pepstatin A (10 μ g/ml). After an 18–24-h incubation at room temperature, 10 μ l of anti-HLA-DR mAb (LB3.1)-conjugated SPA beads (Amersham Corp., Arlington Heights, IL) were added at a dilution of 1:7. After a 1–2-h incubation at room temperature, with gentle shaking, the plates were heat sealed and peptide binding was quantitated in a Packard Top Count Scintillation counter.

EAE Induction. EAE was induced by subcutaneous injection of 400 μ g of peptides emulsified in CFA containing 250 μ g of H37Ra *Mycobacterium tuberculosis*. 7 d later, mice were boosted by a second subcutaneous injection of the same amount of antigens used in the primary immunization. One hundred ng of pertussis toxin (List Biological Laboratories, Campbell, CA) was injected intravenously at the time of immunization and again 2 d later. Mice were assessed clinically according to the following criteria: 0, no disease; 1, tail atony; 2, hind-limb weakness; 3, hind-limb paralysis; 4, hind-limb paralysis and fore-limb paralysis or weakness; 5, moribund.

Histological Analysis. The paralyzed mice were sacrificed 30 d after primary challenge. The spinal cords and brains were removed and fixed in 10% phosphate-buffered formalin. The paraffin-embedded sections were stained with hematoxylin/eosin and examined under light microscopy.

Results

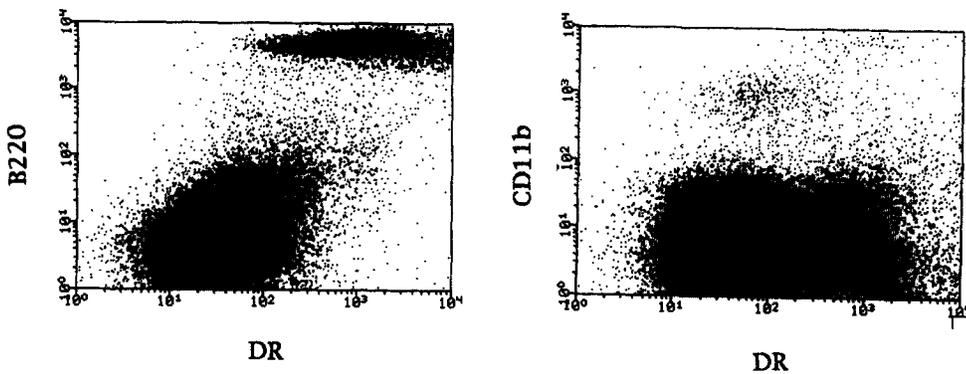
Generation of HLA-DRA-IE α /HLA-DRB1*0401-IE β Tg mice. We produced Tg mice with chimeric human-mouse MHC class II genes that encode proteins composed of the antigen binding domains from HLA-DRA and HLA-DRB1*0401, and the remaining domains from the IE α - α and β chains. We used IE genes, because HLA-DR molecules bear more homology to IE than to IA at the amino acid sequence level. The chimeric gene constructs were manipulated by replacing exon 2 of IE α - α or IE β - β genomic DNA with gene fragments containing exon 2 of HLA-DRA or HLA-DRB1*0401 respectively, as described in Materials and Methods. Tg mice produced by comicro-injection with the two gene constructs were backcrossed to MHC class II deficient (IA β ⁻,IE α ⁻) mice (19). As shown in Fig. 1 A, B220⁺ cells brightly expressed the chimeric MHC class II molecules, while CD11b⁺ cells expressed them dimly. This expression pattern was the same as that of murine MHC class II genes (data not shown), indicating that the transgenes were expressed in a regular, tissue-specific

manner. Since it has been reported that endogenous IE β molecules can associate with the HLA-DRA chain and the resulting mixed heterodimers are expressed on the cells in HLA-DRA Tg mice (20), we investigated cell surface expression of the HLA-DRA-IE α /IE β heterodimer. The IE β chain was not detected by FACS[®] analysis in 2 out of 5 Tg mouse lines. Representative Tg mouse lines are shown in Fig. 1 B. Transgenic mice not expressing endogenous IE β were used in the following experiments.

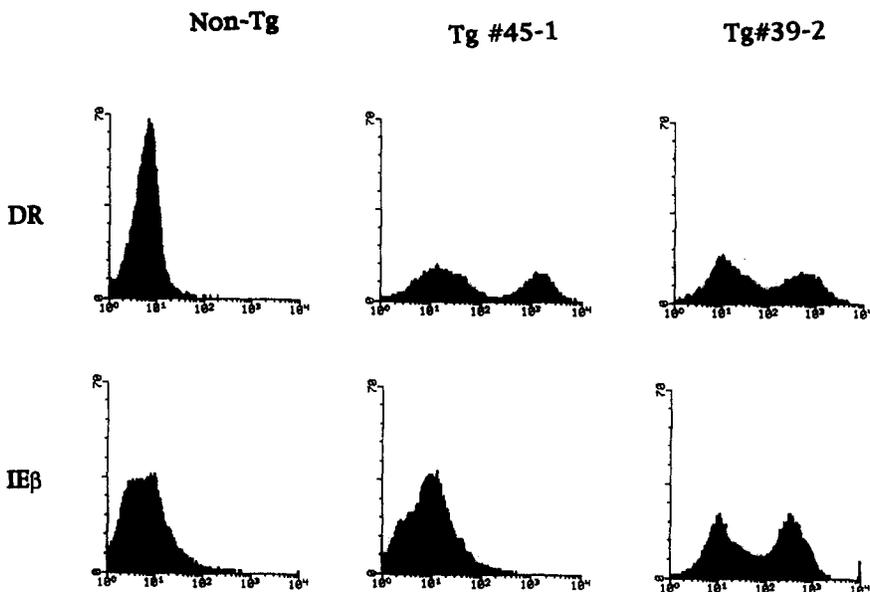
T Cell Development in Tg Mice. It has been shown that mature, MHC class II-restricted CD4⁺ T cells do not develop in MHC class II-deficient mice (19, 21). This evidence suggests that the expression of MHC class II molecules closely influences the development of CD4⁺ T cells in the thymus. Thus, we examined whether mouse T cells could develop properly in a human-mouse MHC class II environment. As shown in Fig. 2, CD4⁺ T cells developed normally in the thymus and spleen of both Tg (IA⁻,IE⁻) mice and their non-Tg (IA^{b+},IE⁻) littermates. We next examined the development of T cell repertoire in Tg (IA⁻,IE⁻) mice and found that T cells expressing V β 5-, V β 11-, or V β 12-TCR gene segments were specifically deleted. In contrast, V β 6⁺ and V β 10⁺ T cells were significantly increased in the spleen of Tg mice (Fig. 3).

Antigen-binding Specificity of HLA-DRB1*0401-IE Molecules. Since antigens bind to the first domain of MHC class II molecules, we expected similar antigen-binding specificity between HLA-DRB1*0401-IE and HLA-DRB1*0401 molecules. The most crucial residues in the peptide-binding motif of HLA-DRB1*0401 are an aromatic or aliphatic anchor residue at position 1, and a residue with a hydroxyl group at position 6 (22). Peptide m1-7 (YRAMATL) possesses the full binding motif for HLA-DRB1*0401, and thus, binds to HLA-DRB1*0401 with a high affinity (Table 1) (23). We used substitutions at position 6 of m1-7 to compare the binding specificity of the chimeric molecules with that of HLA-DRB1*0401. As shown in Table 1, amino acid substitutions at position 6 resulted in nearly identical changes in binding affinity for both HLA-DRB1*0401 and HLA-DRB1*0401-IE molecules. Although R6-m1-7 is an apparent exception, we should point out that the SPA peptide binding assay is less sensitive at peptide concentration higher than 10 μ M, and the IC₅₀ values, 3.3 and >10 μ M, both indicate that the peptides are very poor binders or non-binders for the respective molecules. Therefore, these results have indicated that the HLA-DRB1*0401-IE molecule mimics the HLA-DRB1*0401 molecule in terms of peptide binding specificity.

T Cell Response to Antigenic Peptides in Tg Mice. To study the HLA-DRB1*0401-restricted T cell response in Tg mice, Tg and non-Tg mice were immunized with HA307-319 or HA-R6. As shown in Table 1, HA307-319 is a good binder to both DRB1*0401 and DRB1*0401-IE, while HA-R6, a peptide with Arg substituted at position 314 of HA307-319 is nearly a non-binder. Immunization of Tg mice with HA307-319 elicited T cell proliferation, while HA-R6 did not (Fig. 4). The absence of proliferative response to HA-R6 is most likely due to a low binding affini-



(A)



(B)

Figure 1. HLA-DRA-IE α /HLA-DRB1*0401-IE β heterodimer is expressed in B220- or CD11b-positive population, while endogenous IE β is not expressed in a no. 45-1 Tg line. (A) Spleen cells from Tg mice were stained with anti-HLA-DR mAb-FITC and anti-B220 mAb-PE or anti-CD11b mAb-PE. (B) Spleen cells from non-Tg, no. 45-1 Tg, and no. 39-2 Tg mouse lines were stained with anti-HLA-DR mAb or anti-IE β mAb.

ity to HLA-DRB1*0401-IE molecules since an Arg residue at relative position 6 of the binding motif is not tolerated (see in Table 1). The T cell proliferation to HA307-319 was inhibited by the anti-HLA-DR mAb, LB3.1. Immunization of non-Tg mice with HA307-319 failed to elicit any T cell proliferation (Fig. 4). These results indicate that the T cell proliferation was HLA-DR-restricted, i.e., the HLA-DRA-IE α /HLA-DRB1*0401-IE β molecules present antigens effectively to T cells in the Tg mice.

Development of EAE in Tg Mice Immunized with PLP175-192 or MBP87-106. The major immunodominant epitopes of MBP are residues 87-106 and 143-168 for individuals with HLA-DR4 alleles. Based on the peptide-binding motif of HLA-DRB1*0401 (22) and peptide-binding results using overlapping 20-amino acid peptides of PLP (12), we assumed that PLP175-192 could be an immunodominant

epitope for HLA-DR4 individuals. As we expected, PLP175-192 binds HLA-DRB1*0401 as strongly as MBP87-106 (Table 1). The amino acid sequences of PLP175-192 and MBP87-106 are identical between human and mouse, and thus, they represent autoantigens for both species. We therefore examined the T cell proliferative responses with these peptides. As shown in Fig. 5 A, lymph node cells from SJL/J mice immunized with MBP87-106 proliferated in response to the antigen, while little or no proliferative response was detected in Tg mice immunized with MBP87-106. In contrast, immunization with PLP175-192 provoked a strong T cell response to this peptide in Tg mice (Fig. 5 B). T cell response to PLP175-192 was inhibited by both anti-DR α mAb, LB3.1, and anti-HLA-DR β mAb, 1-1C4 (24, 25), but not by anti-IE β mAb, 17.3.3 (Fig. 5 B). This result indicated that T cell proliferation to PLP175-

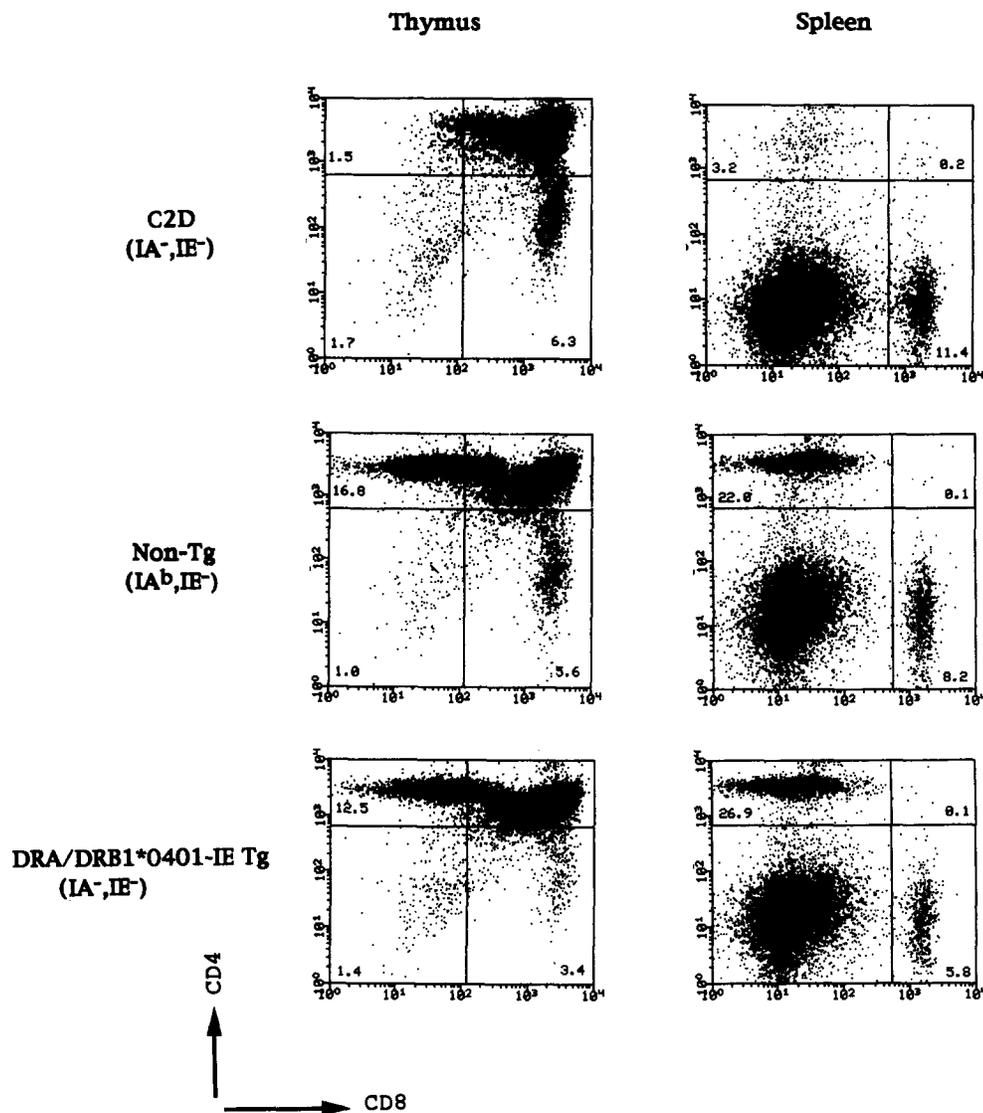


Figure 2. T cell development in Tg (IA⁻, IE⁻) mice. Single cell suspensions were made from thymus and spleen and stained with anti-CD8 mAb-FITC and anti-CD4 mAb-PE as described in Materials and Methods.

192 was restricted by the transgenic class II molecule. We then tested the development of EAE by immunizing with PLP175-192 or MBP87-106. As shown in Table 2 and Fig. 6, Tg mice immunized with PLP175-192 showed signs of EAE 14–17 d after primary immunization. Most of the mice immunized with PLP175-192 showed a progression of symptoms beginning with tail atony, followed by hind-limb paralysis, weakening of the forelimbs and eventually death (Fig. 6). In contrast, Tg mice immunized with MBP87-106 developed only a mild EAE. To examine that EAE was induced by DR4-restricted T cells, we used non-Tg/IA⁻, IE⁻ mice as a negative control. The mice did not develop the disease, thus the EAE development was mediated by DR4-IE chimeric molecules. Histological studies of the spinal cord and brain from the paralyzed Tg mice revealed numerous inflammatory foci in white matter, and demyelination and axonal degeneration of nerves were observed in the spinal cord (Fig. 7). The results have indicated that HLA-DRA-IE α /HLA-DRB1*0401-IE β Tg mice are susceptible to

EAE, and that PLP175-192 is a more potent encephalitogenic epitope than MBP87-106 in these mice.

Discussion

To study the development of HLA-DR4-linked autoimmune diseases, we generated transgenic mice possessing HLA-DR4-IE chimeric MHC class II genes, because it was unclear whether the murine CD4 molecule could be activated by HLA-DR4 molecules as effectively as it could be by mouse MHC class II molecules. Although it has been reported that murine CD4 interacts well with HLA-DR1 molecules (26), this cannot be extrapolated directly to HLA-DR4 molecules, since human CD4 does not interact equally well with all HLA-DR allotypes (27). Additionally, the cytoplasmic tail of MHC class II molecules plays an important role in T cell activation and in the recycling of MHC class II molecules (28). Signal transduction from the cytoplasmic tail regulates the development of Th1/Th2 T

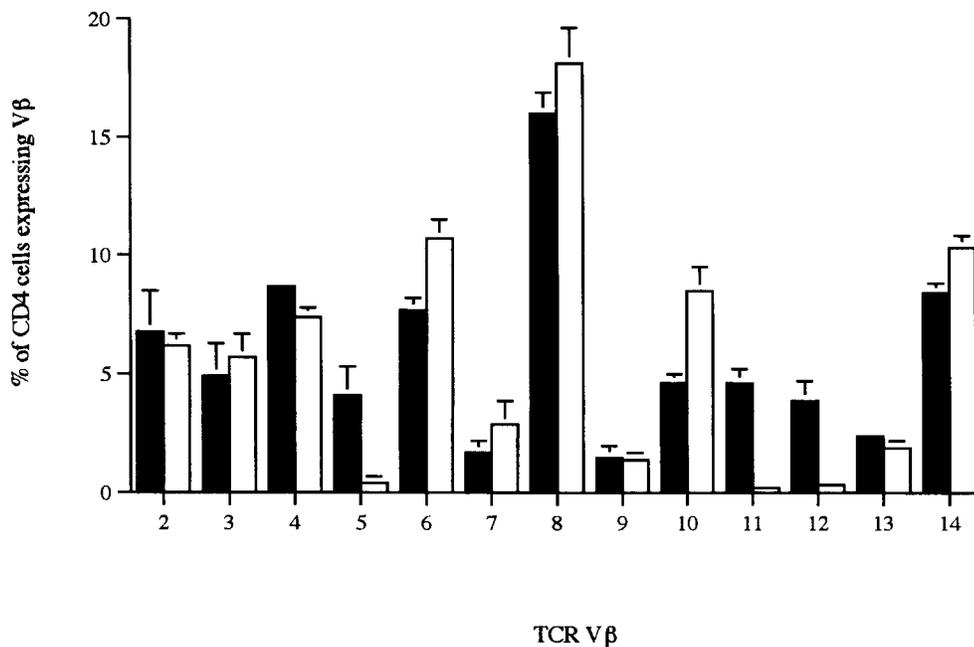


Figure 3. TCR V β expression on CD4⁺ T cells in spleen. Splenoocytes were stained with PE-conjugated anti-CD4 mAb and biotinylated anti-TCR V β mAbs, and FITC-streptavidin was used in the second step staining. Percentage of the cells which express both CD4 and TCR V β in a CD4⁺ T cell population was calculated. Error bars represent SD of three mice. ■, C57BL/6; □, Tg (IA⁻,IE⁻).

helper cells through the activation of B7 molecules (29), and this signal might be critical for the development of autoimmune diseases. Thus, human/mouse chimeric class II gene constructs appeared to be a better choice than full HLA-DR genes for production of HLA-DR Tg mice. However, we could not completely rule out the possibility that the joining of human first domains to murine second domains would produce an altered conformation of the human antigen binding site. Thus, we compared the antigen-binding specificity of HLA-DRB1*0401 and HLA-DRB1*0401-IE molecules, respectively. The specificity and affinity of antigen-binding by these molecules were very similar (Table 1). We also examined the peptide-binding specificity of another chimeric molecule we constructed, HLA-DRB1*0404-IE molecules. The peptide-binding specificity to HLA-DRB1*0404-IE molecules showed the same specificity to HLA-DRB1*0404 molecules (unpublished observation). These results indicated that HLA-DR4-IE molecules could substitute the HLA-DR4 molecules with respect to first domain-dependent functions in Tg mice.

As shown in Fig. 4 and previous reports (1, 2), HA307-319 specific DR4-restricted T cells were elicited to proliferate in Tg mice after immunization with the peptide. We next examined whether HA307-319-specific human T cell clones could be activated by the peptide presented by HLA-DR4-IE chimeric molecules. Although the clones were activated by HA307-319 presented by native DR4 molecules expressed on murine fibroblast cells, they were barely activated by the same peptide presented on splenoocytes from Tg mice (data not shown). This suggested that the association of human CD4 molecules with the chimeric class II was insufficient to activate these T cells.

To eliminate any influence of endogenous MHC class II on the development of autoimmune disease, the HLA-

DR4-IE Tg mice were backcrossed to MHC class II-deficient (IA β ⁻,IE α ⁻) mice. Endogenous IE β associated with HLA-DRA-IE α was not detected by FACS[®] analysis in certain Tg mouse lines (see in Fig. 1 B). Mice which express IE β have low copy number of transgene (1–10 copies), while IE β -non-expressing mice have high copy number (70–120 copies). Thus, the difference in expression might be due to either a dilution of mixed DRA-IE α /IE β

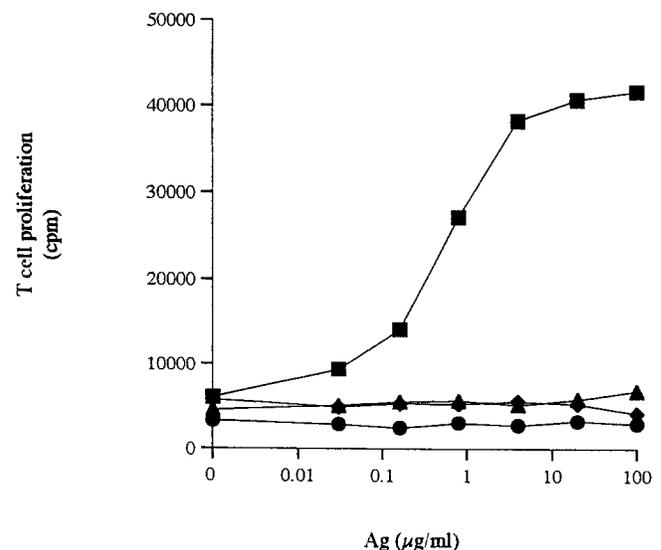


Figure 4. T cell proliferation in Tg and non-Tg mice immunized with antigenic peptides. Lymph node cells were isolated from mice immunized with antigen in CFA. Cells were cultured with antigen in the presence or absence of 20 μ g/ml of anti-DR mAb, LB3.1. Proliferative responses were measured as described in Material and Methods. ■, HA307-319; ●, HA-R6; ▲, HA307-319/anti-DR mAb; ◆, HA307-319/Non-Tg.

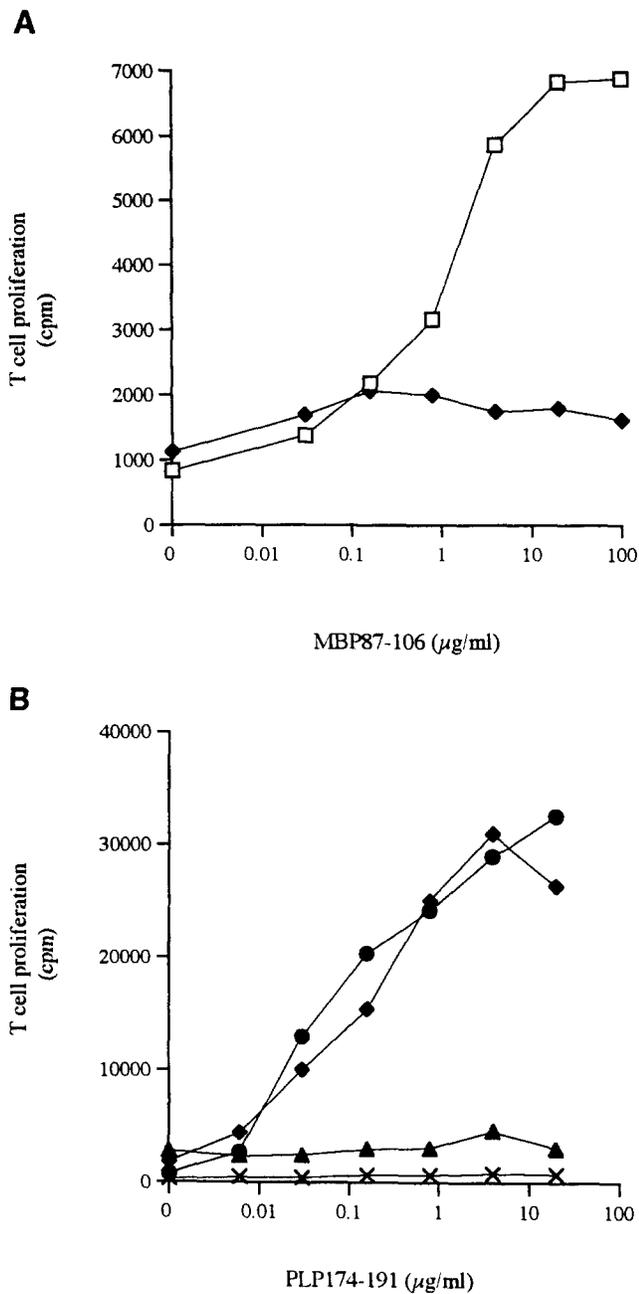


Figure 5. T cell response to MBP87-106 and PLP175-192 in Tg mice. (A) T cell proliferative response to MBP87-106. Tg mice and SJL/J mice were immunized with MBP87-106 and proliferation of lymph node cells in response to MBP87-106 was measured. (B) T cell proliferative response of Tg mice to PLP175-192 and its inhibition by anti-DR mAbs. Lymph node cells from Tg mice immunized with PLP175-192 were cultured with various concentrations of PLP175-192 in the presence or absence of 20 µg/ml of anti-DRα (LB3.1), anti-DRβ (1-1C4) or anti-IEβ (17.3.3) mAbs. (A) □, SJL/J/mice; ◆, Tg mice; (B) ◆, PLP174-191; ●, PLP174-19/anti-IEβ mAb; ▲, PLP174-191/anti-DRα mAb; ×, PLP174-191/anti-DRβ mAb.

molecules caused by the high expression of DRB1*0401-IEβ molecules, or decreased transcription of the IEβ gene due to limiting amounts of transcription factors. Experiments are currently in progress to clarify the mechanism of

Table 2. Development of EAE in DRB1*0401-IE Tg Mice

Antigen	Mice	Incidence	Onset Day (mean)	Severity					
				0	1	2	3	4	5
PLP 175-192 + CFA	Tg	5/5	15	0	0	0	0	1	4
	Non-Tg	0/5	—	5	0	0	0	0	0
MBP 87-106 + CFA	Tg	3/5	16	2	2	0	1	0	0
	Non-Tg	0/5	—	5	0	0	0	0	0
CFA	Tg	0/5	—	5	0	0	0	0	0

Clinical scoring is described in Materials and Methods.

differential gene expression. We also examined the association of endogenous IAα chain with HLA-DRB1*0401-IEβ protein and their cell-surface expression using the mice carrying a single HLA-DRB1*0401-IEβ transgene. The mixed heterodimer was not detected by 1-1C4 mAb specific for the HLA-DRβ chain (data not shown). These results have demonstrated that the Tg mice do not express any murine class II chains on the cell surface.

In the Tg mice, the thymus and spleen were of normal architecture. However, the Vβ5-, Vβ11-, Vβ12-, and Vβ17-expressing T cell subsets were strongly diminished or absent from Tg mice. A similar observation was reported in double Tg mice with DRB1*0401 and human CD4 genes (2). The lack of Vβ17-bearing T cells is due to a deficiency of the Vβ17 gene in C57BL/6 mice. C57BL/6 mice carry mouse mammary tumor viruses (MMTV) -8,-9, and -17

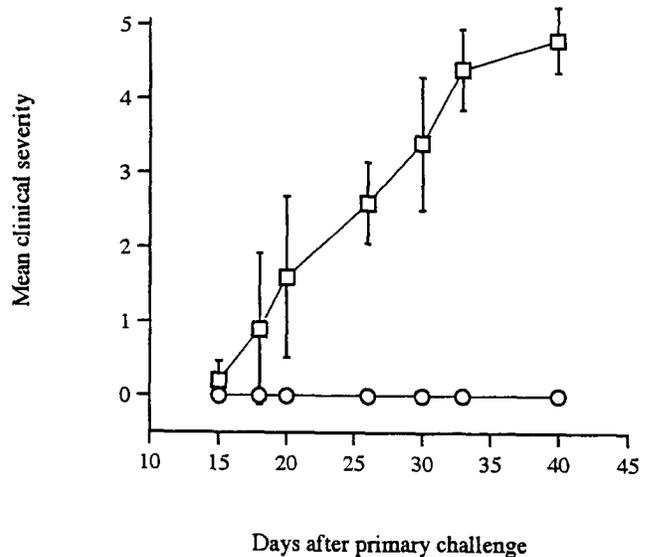


Figure 6. EAE development in Tg mice in response to PLP175-192. Clinical score is described in Materials and Methods. Error bars represent SD of five mice in Table 2. □, Tg mice; ○, Non-Tg mice.

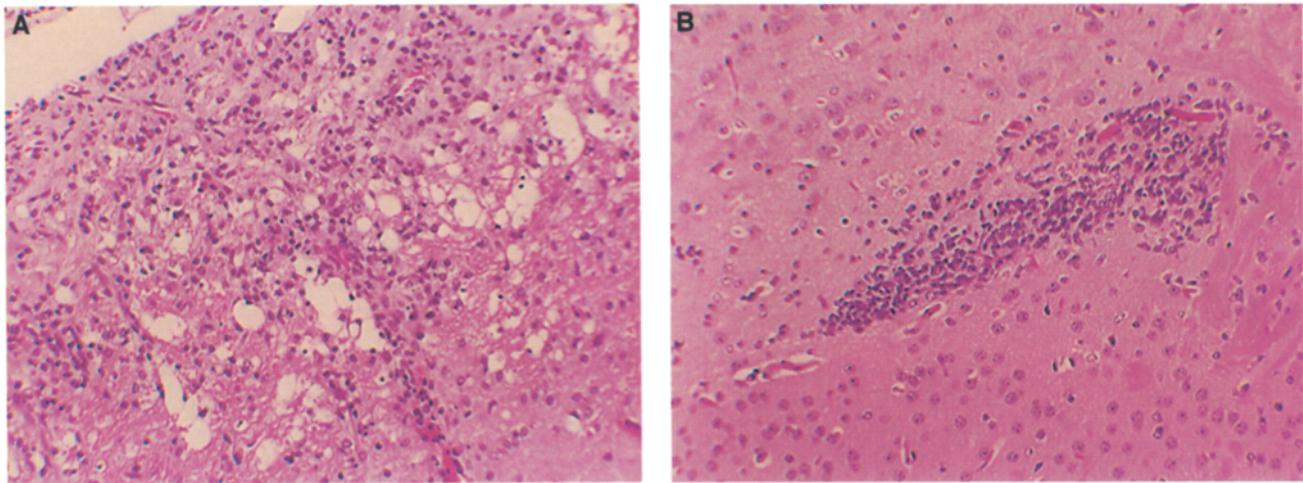


Figure 7. Histopathological analysis of spinal cord and brain from a paralyzed Tg mouse with PLP175-192. The magnification of pictures of (A) Spinal cord and (B) Brain is $\times 200$.

(30), which bind to IE and delete T cells expressing V β 5, V β 11, and V β 12 TCR segments (31). Thus, MMTV-encoded superantigens could bind to HLA-DR4-IE molecules and the MHC class II complexes with the superantigens deleted these T cell subsets.

As shown in Table 1 and Fig. 5 A, although SJL mice developed a proliferative T cell response to MBP87-106, a response was barely detectable in Tg mice in spite of the binding of MBP87-106 to DRB1*0401-IE molecules. A possible explanation for this discrepancy is that the thymic positive selection for MBP87-106-specific T cells is different between SJL and Tg mice because of their different MHC class II haplotypes. However, since MBP87-106 reactive T cells have been isolated from HLA-DRB1*0401 humans, it is more likely that the relevant specificity is missing from the TCR repertoire of Tg mice. This may be due to the limited homology of TCR genes between human and mouse (32), or the deletion of certain T cell subsets by endogenous MMTV integrants. Similar results were observed in SJL-TCR V β 8.2 Tg mice, which had a limited T cell repertoire because of allelic exclusion by the transgene (33). In these mice, immunization with a PLP peptide elicited a strong

T cell proliferation, while immunization with MBP87-106 barely stimulated T cells. The result suggests that the TCR diversity of MBP87-106-reactive T cells is more limited than that of PLP175-192-specific T cells. The same explanation may apply to the minimally-detectable T cell response to MBP87-106 in our DR4-IE Tg mice. The fact that these Tg mice developed mild EAE when immunized with MBP87-106 suggests that a small population of encephalitogenic MBP87-106 specific T cells does exist. However this T cell population may be too small to be detected in the primary T cell proliferation assay.

Herein, we have demonstrated that HLA-DRB1*0401-IE restricted T cells specific for PLP175-192 or MBP87-106 peptides cause encephalomyelitis in HLA-DR Tg mice. Thus, PLP175-192 and MBP87-106 could be encephalitogenic epitopes in individuals with HLA-DRB1*0401 allele. The results also demonstrate that a human MHC class II binding site alone can confer susceptibility to autoimmune disease. The Tg mice reported here will be useful to study autoantigens involved in the development of other autoimmune diseases associated with the HLA-DRB1*0401 allele.

We would like to thank Dr. Haruo Saito for providing us IE β cosmid DNA; Dr. David A. Hafler for the information of immunodominant epitopes of PLP in HLA-DR2 MS patients; Michelle Fanale, Raymond Makofske, Donald Cox, Diana Gaizband, and Himanshu Shah for excellent technical assistance; Doug Larrigan, Joseph Levine and John Duker for DNA sequencing; Rongshen Hsiao and Ludmilla Foppiani for oligonucleotides synthesis; Drs. Werner Haas and Kathy Myers for critically reading the manuscript; and Dr. Michael Steinmetz for encouragement.

Address correspondence to Kouichi Ito, Department of Inflammation and Autoimmune Diseases, Hoffman-La Roche, Inc., Bldg. 123, Rm 4331, 340 Kingsland St., Nutley, NJ 07110-1199.

Received for publication 27 December 1995 and in revised form 14 March 1996.

References

1. Woods, A., H.Y. Chen, M.E. Trumbauer, A. Sirotina, R. Cummings, and D.M. Zaller. 1994. Human major histocompatibility complex class II-restricted T cell responses in transgenic mice. *J. Exp. Med.* 180:173–181.
2. Fugger, L., S.A. Michie, I. Rulifson, C.B. Lock, and G.S. McDevitt. 1994. Expression of HLA-DR4 and human CD4 transgenes in mice determines the variable region β -chain T-cell repertoire and mediates an HLA-DR-restricted immune response. *Proc. Natl. Acad. Sci. USA.* 91:6151–6155.
3. Nabozny, G.H., J.M. Baisch, S. Cheng, D. Cosgrove, M.M. Griffiths, H.S. Luthra, and C.S. David. 1996. HLA-DQ8 transgenic mice are highly susceptible to collagen-induced arthritis: a novel model for human polyarthritis. *J. Exp. Med.* 183:27–37.
4. Pettinelli, C.B., R.B. Fritz, H.J. Chou C, and D.E.J. McFarlin. 1982. Encephalitogenic activity of guinea pig myelin basic protein in the SJL mouse. *Immunology.* 129:1209–1211.
5. Fritz, R.B., H.J. Chou C, and D.E. McFarlin. 1983. Relapsing murine experimental allergic encephalomyelitis induced by myelin basic protein. *J. Immunol.* 130:1024–1026.
6. Enddoh, M., T. Tabira, T. Kunishita, K. Sakai, T. Yamamura, and T. Taketomi. 1986. DM-20, a proteolipid apoprotein, is an encephalitogen of acute and relapsing autoimmune encephalomyelitis in mice. *J. Immunol.* 137:3832–3835.
7. Zamvil, S., P. Nelson, J. Trotter, D. Mitchell, R. Knobler, R. Fritz, and L. Steinman. 1985. T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination. *Nature (Lond.).* 317:355–358.
8. Ota, K., M. Matsui, E.L. Milford, G.A. Mackin, H.L. Weiner, and D.A. Hafler. 1990. T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. *Nature (Lond.).* 346:183–187.
9. Martin, R., D. Jaraquemada, M. Flerlage, J. Richert, J. Whitaker, E.O. Long, D.E. McFarlin, and H.F. McFarland. 1990. Fine specificity and HLA restriction of myelin basic protein-specific cytotoxic T cell lines from multiple sclerosis patients and healthy individuals. *J. Immunol.* 145:540–548.
10. Pette, M., K. Fujita, D. Wilkinson, D.M. Altmann, J. Trowsdale, G. Giegerich, A. Hinkkanen, J.T. Epplen, L. Kappos, and H. Wekerle. 1990. Myelin autoreactivity in multiple sclerosis: Recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple-sclerosis patients and healthy donors. *Proc. Natl. Acad. Sci. USA.* 87:7968–7972.
11. Martin, R., U. Utz, J.E. Coligan, J.R. Richert, M. Flerlage, E. Robinson, R. Stone, W.E. Biddison, D.E. McFarlin, and H.F. McFarland. 1992. Diversity in fine specificity and T cell receptor usage of the human CD4⁺ cytotoxic T cell response specific for the immunodominant myelin basic protein peptide 87–106. *J. Immunol.* 148:1359–1366.
12. Markovic-Plese, S., H. Fukaura, J. Zhang, A. Al-Sabbagh, S. Southwood, A. Sette, V.K. Kuchroo, and D.A. Hafler. 1995. T cell recognition of immunodominant and cryptic proteolipid protein epitopes in humans. *J. Immunol.* 155:982–992.
13. Zhang, J., S. Markovic-Plese, B. Lacet, J. Raus, H.L. Weiner, and D.A. Hafler. 1994. Increased frequency of Interleukin 2-responsive T cell specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. *J. Exp. Med.* 179:973–984.
14. Kawai, J., A. Ando, T. Sato, T. Nakatsuji, K. Tsuji, and H. Inoko. 1989. Analysis of gene structure and antigen determinants of DR2 antigens using DR gene transfer into mouse L cells. *J. Immunol.* 142:312–317.
15. Steinmetz, M., K. Minard, S. Horvath, J. McNicholas, J. Frelinger, C. Wake, E. Longe, B. Mach, and L. Hood. 1982. A molecular map of the immune response region from the major histocompatibility complex of the mouse. *Nature (Lond.).* 300:35–42.
16. Saito, H., R.A. Maki, L.K. Clayton, and S. Tonegawa. 1983. Complete primary structures of the E β chain and gene of the mouse major histocompatibility complex. *Proc. Natl. Acad. Sci. USA.* 80:5520–5524.
17. Barany, G., and R.B. Merrifield. 1980. The Peptides. Academic Press, New York. 2:1–287.
18. Glimcher, L.H., D.J. McKean, E. Choi, and G. Seidman. 1985. Complex regulation of class II gene expression: analysis with class II mutant cell lines. *J. Immunol.* 135:3542–3550.
19. Grusby, M.J., R.S. Johnson, V.E. Papaioannou, and L.H. Glimcher. 1991. Depletion of CD4⁺ T cells in major histocompatibility complex class II-deficient mice. *Science (Wash. DC).* 253:1417–1420.
20. Lawrence, S.K., L. Karisson, J. Price, V. Quaranta, Y. Ron, J. Sprent, and P.A. Peterson. 1989. Transgenic HLA-DR α faithfully reconstitutes IE-controlled immune functions and induces cross-tolerance to E α in E α ^o mutant mice. *Cell.* 58:583–594.
21. Gosgrove, D., D. Gray, A. Dierich, J. Kaufman, M. Lemeur, C. Benoist, and D. Mathis. 1991. Mice lacking MHC class II molecules. *Cell.* 66:1051–1066.
22. Hammer, J., P. Valsasini, K. Tolba, D. Bolin, J. Higelin, B. Takacs, and F. Sinigaglia. 1993. Promiscuous and allele-specific anchors in HLA-DR-binding peptides. *Cell.* 74:197–203.
23. Hammer, J., C. Belunis, D. Bolin, J. Papadopoulos, R. Walsky, J. Higelin, W. Danho, F. Sinigaglia, and Z.A. Nagy. 1994. High affinity binding of short peptides to MHC class II molecules by anchor combination. *Proc. Natl. Acad. Sci. USA.* 91:4456–4460.
24. Cammarota, G., A. Scheirle, B. Takacs, D.M. Doran, R. Knorr, W. Bannwarth, J. Guardiola, and F. Sinigaglia. 1992. Identification of a CD4 binding domain of HLA-DR molecule. *Nature (Lond.).* 356:799–801.
25. Vidović, D., F. Falcioni, B. Siklodi, C.J. Belunis, D.R. Bolin, K. Ito, and Z.A. Nagy. 1996. Downregulation of Class II major histocompatibility complex molecules on antigen-presenting cells by antibody fragments. *Eur. J. Immunol.* 25:3349–3355.
26. Altmann, D.M., D.C. Doue, A.J. Frater, C.M. Hetherington, H. Inoko, and J.I. Elliott. 1995. The T cell response of HLA-DR transgenic mice to human myelin basic protein and other antigens in the presence and absence of human CD4. *J. Exp. Med.* 181:867–875.
27. Fleury, S., J. Thibodeau, G. Croteau, N. Labrecque, H.E. Aronson, C. Cantin, E.O. Long, and R.P. Sekaly. 1995. HLA-DR polymorphism affect the interaction with CD4. *J. Exp. Med.* 182:733–741.
28. Pinet, V., M. Vergelli, R. Martin, O. Bakke, and E.O. Long. 1995. Antigen presentation mediated by recycling of surface HLA-DR molecule. *Nature (Lond.).* 375:603–606.
29. Kuchroo, V.K., M.P. Das, J.A. Brown, A.M. Ranger, S.S. Zamvil, R.A. Sobel, H.L. Weiner, N. Nabavi, and L.H. Glimcher. 1995. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: Application to autoimmune disease therapy. *Cell.* 80:707–718.
30. Lee, B.K., and E.M. Eicher. 1995. Segregation patterns of endogenous mouse mammary tumor viruses in five recombi-

- nant inbred strain sets. *J. Virol.* 64:4568–4572.
31. Scherer, M. T., L. Ignatowicz, A. Pullen, J. Kappler, and P. Marrack. 1995. The use of mammary tumor virus (Mtv)-negative and single-Mtv mice to evaluate the effects of endogenous viral superantigens on the T cell repertoire. *J. Exp. Med.* 182:1493–1504.
32. Lai, E., P. Concannon, and L. Hood. 1988. Conserved organization of the human and murine T-cell receptor β -gene families. *Nature (Lond.)*. 331:543–546.
33. Kuchroo, V.K., M. Collins, A. Al-Sabbagh, R.A. Sobel, M.J. Whitters, S.S. Zamvil, M.E. Dorf, D.A. Hafler, J.G. Seidman, H.L. Weiner, and I.J. Rimm. 1994. T cell receptor (TCR) usage determines disease susceptibility in experimental autoimmune encephalomyelitis: studies with TCR V β 8.2 transgenic mice. *J. Exp. Med.* 179:1659–1664.