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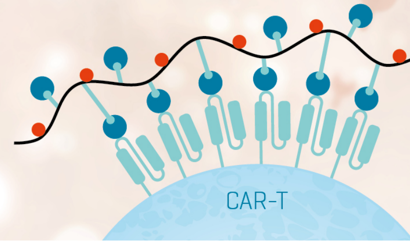

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PARENTAL MHC MOLECULE HAPLOTYPE EXPRESSION IN (SJL/J × SWR)F_1 MICE WITH ACUTE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS INDUCED WITH TWO DIFFERENT SYNTHETIC PEPTIDES OF MYELIN PROTEOLIPID PROTEIN[1]

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To determine if the Ag that induces an autoimmune disease influences parental MHC haplotype molecule expression in situ in MHC heterozygotes, acute experimental allergic encephalomyelitis (EAE) was induced with different encephalitogenic peptides in (SJL/J × SWR)F_1 mice. The mice were sensitized with either a synthetic peptide corresponding to mouse myelin proteolipid protein (PLP) residues 103-116 YKTTICGKGLSATV which induces EAE in SWR (H-2^q), but not SJL/J (H-2^s) mice or a synthetic peptide corresponding to PLP residues 139-151 HCLGKWLGHPDKF which is encephalitogenic in SJL/J but not SWR mice. Mice were killed when they were moribund or at 30 days after sensitization. Twelve of 18 F_1 mice given PLP peptide 103-116 and 12 of 17 mice given PLP peptide 139-151 developed EAE within 2 to 3 wk after sensitization. Cryostat sections of brain samples from F_1 and parental mice were immunostained with a panel of mAb identifying H-2^s and H-2^q class I and II MHC molecules. In brains of controls, class I MHC molecules were expressed on choroid plexus, endothelial cells, and microglia whereas class II MHC molecules were absent. In EAE lesions, class I and II MHC molecules were present on inflammatory and parenchymal cells, but the degree of parental haplotype molecule expression did not vary with the different peptide Ag tested. Thus, in (SJL/J × SWR)F_1 mice, myelin PLP peptides 103-116 and 139-151 are co-dominant Ag with respect to clinical and histologic disease and parental haplotype MHC molecule expression. We propose a unifying hypothesis consistent with these results and previous observations of differential Ia expression in (responder × non-responder)F_1 guinea pigs. We suggest that MHC molecules may bind locally derived peptide Ag in inflammatory sites and that these interactions influence levels of MHC haplotype molecules on APC.

EAE[3] is a useful autoimmune disease model for the study of mechanisms of cellular immune reactions in the CNS and shares features with human multiple sclerosis (1, 2). Acute EAE can be induced by sensitization of various species with whole CNS tissue, myelin, or its components. Immunopathologic studies have documented expression of MHC molecules on inflammatory and CNS resident cells in EAE (3–7). Thus, CNS resident cells may act as APC, but the specific cell populations and levels of expression in situ vary depending on the species studied and the nature of the disease model.

Previous studies of EAE (8) and delayed hypersensitivity reactions in (strain 2 × strain 13)F_1 guinea pigs (9) suggested that the degree of in situ expression of responder and non-responder parental haplotype Ia molecules in F_1 animals might vary in different cellular immune reactions and that expression was influenced by the Ag that promote the response. However, because only one inbred strain of this species is susceptible to EAE induced with whole CNS tissue, enhancement of both parental Ia molecules in immune responses to self CNS Ag could not be demonstrated in F_1 guinea pigs. Furthermore, because strain 2 and strain 13 guinea pigs are identical in class I MHC loci (10), possible differences in class I MHC haplotype expression could not be evaluated.

We have previously used synthetic peptides of PLP, the major CNS myelin protein, to identify encephalitogenic determinants in inbred mice. The peptide corresponding to mouse PLP residues 103-116 YKTTICGKGLSATV is encephalitogenic in the SWR (H-2^q) strain (11), but not in SJL/J mice whereas the peptide corresponding to residues 139-151 HCLGKWLGHPDKF is encephalitogenic in SJL/J (H-2^s) but not in SWR mice (12, 13). These findings now permit analysis of expression of MHC parental haplotype molecules in immune responses to different synthetic encephalitogenic peptides of mouse PLP in F_1 mice. Mice are particularly useful because molecular mechanisms of Ag presentation and regulation of both class I and II MHC molecule expression have been extensively characterized and a wide range of susceptibility to peptide Ag derived from CNS proteins has been documented.

In this study, we compared the encephalitogenic potencies of the SWR and the SJL/J synthetic encephalitogenic peptides in (SJL/J × SWR)F_1 mice and found that the two

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[3] Abbreviations used in this paper: EAE, acute experimental allergic encephalomyelitis; CNS, central nervous system; PLP, myelin proteolipid protein.

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peptides are co-dominant. We also tested the hypothesis that two different synthetic encephalitogenic peptides derived from PLP induce differential class I and class II MHC haplotype molecule expression depending on the peptide that induced the immune response. The findings indicate that there is not a differential expression of class I and II MHC molecules in these F_1 mice. Thus, patterns of cellular MHC molecule expression in situ in autoimmune responses to different peptide Ag may be indistinguishable.

MATERIALS AND METHODS

Mice. Female SJL/J and SWR mice, 5 to 8 wk of age, were purchased from The Jackson Laboratory, Bar Harbor, ME. (SJL/J × SWR)F_1 and (SWR × SJL/J)F_1 mice were bred at the E. K. Shriver Center. Mice were immunized between 10 and 14 wk of age. They were housed in groups of two to four mice/cage and provided food and water ad libitum. When severe clinical disease was present, affected mice were given daily nursing care consisting of hand feeding and hydration.

Peptide synthesis. Peptides were synthesized manually using the simultaneous multiple peptide synthesis ("tea bag") method (14) on a *p*-methylbenzhydrylamine resin. After cleavage from the resin, peptides were purified by reverse phase HPLC on a VYDAC-Flow C_{18} preparative column (The Sep/a/rations Group, Hesperia, CA). After purification, each peptide eluted as a single peak and the composition of each was confirmed by amino acid analysis.

Immunization. Mice were immunized s.c. in the abdominal flanks with 100 nmol mouse PLP residues 139-151 HCLGKWLGHPDKF (154 μg) or mouse PLP residues 103-116 YKTTICGKGLSATV (144 μg). Each immunization included 200 μg *Mycobacteria tuberculosis* H37RA (Difco Laboratories, Detroit, MI) in an emulsion of 200 μl of an equal volume of water and IFA (Difco). Each animal was also injected i.v. with 7.5×10^9 *Bordetella pertussis* bacilli (pertussis vaccine, lot no. WF262, Massachusetts Public Health Biologics Laboratories, Boston, MA) on days 0 and 3.

Clinical evaluation and death. Beginning on Day 7 after sensitization, the animals were weighed and monitored daily for clinical signs of EAE, as described (13). They were killed by cervical dislocation under ether anesthesia when they became moribund with neurologic disease or at 30 days. Half of the brains and the spinal cords were fixed in formalin and embedded in paraffin. Half of the brains and samples of spleen and heart were frozen in OCT Compound (Miles Laboratories, Naperville, IL) and stored at −70°C.

Histologic evaluation. Paraffin-embedded sections of CNS tissues were stained with Luxol fast blue-hematoxylin and eosin. To determine the severity of histologic disease, inflammatory foci, identified as perivascular clusters containing at least 20 mononuclear cells, were counted in leptomeninges and parenchyma.

Immunohistochemistry. Culture supernatants of mAb hybridomas (Table I) (16–24) were conjugated with biotin-N-hydroxysuccinimide ester (Bethesda Research Laboratories, Gaithersburg, MD) according to the manufacturer's instructions. Four to 6 μm thick tissue sections of brain, spleen, and heart samples were immunostained with biotinylated antibodies, as described (15). Sections were stained with rat mAb using an immunohistochemical staining kit for rat Ig (Vector Laboratories, Inc., Burlingame, CA). Spleen samples from parental and F_1 mice were used as positive controls with each incubation and for each mAb. Negative controls included the use of biotinylated mouse mAb to irrelevant MHC molecules.

To quantitate class I and II MHC molecule expression on inflammatory cells, proportions of stained cells/total nuclei in high power (400 ×) fields were determined in inflammatory foci in F_1 mice with EAE. Fields with perivascular inflammation were selected by placing the slides on the microscope stage so that vessel lumens were in the field centers. The numbers of fields counted in each sample were dependent on the numbers of foci present in the section. Student's *t*-test was used for statistical analysis.

RESULTS

Clinical and histologic disease. Beginning on day 10, 13 of 35 peptide-sensitized F_1 mice exhibited signs of EAE, regardless of which peptide was used for sensitization (Table II). The mean day of onset of clinical signs of peptide 103-116-sensitized F_1 mice was day 13 and that of peptide 139-151-sensitized F_1 mice was day 15. In both groups, the clinical signs progressed rapidly so that all but one of these mice had to be killed by day 18. One PLP peptide 103-116-sensitized mouse had disease onset on day 25 and was killed on day 26.

Paraffin sections of half brains and spinal cords in F_1 mice that showed clinical signs and 11 additional F_1 mice that had not shown neurologic signs had typical histologic EAE characterized by mixed mononuclear cell and neutrophil infiltrates in leptomeninges and CNS white matter (Fig. 1). Gray matter had fewer inflammatory foci and spinal nerve roots were free of inflammation. No inflammatory cell accumulations were identified in choroid plexuses. The greatest numbers of inflammatory foci were present in mice with severe clinical disease that had been killed by day 18. There were no differences in clinical or histologic disease between peptide 103-116- and peptide 139-151-sensitized F_1 mice.

SWR and SJL/J mice sensitized with peptides 103-116 and 139-151, respectively, also exhibited clinical and histologic or histologic EAE whereas SWR and SJL/J

TABLE I
mAb used for immunohistochemistry

mAb[a]	Isotype[b]	Specificity	Cross-Reactivity	Strain Identified	Reference
MHC class I					
16-1-2N	IgG2aκ	K^kD^k	$K^{q,p,r}$	SWR	(16)
28-11-5S	IgMκ	D^b	$D^{d,a}$ H-$2^{q,p}$	SWR	(17)
23-A-5-21S	IgMκ	D^b	$D^{d,a}$ H-$2^{q,p}$	SWR	(17)
15-1-5P	IgG2bκ	K^kD^k	$D^{d,s,q,r}$	SWR, SJL/J	(16)
34-1-2S	IgG2aκ	K^kD^k	$K^{b,s,r,q,p}$	SWR, SJL/J	(18)
M1/42[c]	IgG2a (Rat)	H-2 (monotypic)	NA[e]	SWR, SJL/J	(19)
MHC class II					
M5/114.15.2	IgG2b (Rat)	I-$A^{b,d,q}$ I-$E^{d,k}$	none	SWR	(20)
BP 107.2.2	IgG3	I-$A^{b,d}$	I-$A^{j,p,q,u,v}$	SWR	(21)
10-3.6.2	IgG2b	I-A^k	I-$A^{r,f,s}$	SJL/J	(22)
MK-S4	IgG2b	I-A^s	I-A^f	SJL/J	(23)
Controls					
MK-D6	IgG2aκ	I-A^d	none	none	(23)
FMC 16[d]	IgG2a	Human β-2 microglobulin	none	none	(24)

[a] Hybridomas from the American Type Culture Collection, Rockville, MD.
[b] Mouse Ig, unless otherwise indicated.
[c] Boehringer Mannheim Biochemicals, Indianapolis, IN.
[d] Accurate Chemical and Scientific Corporation, Westbury, NY
[e] Not applicable

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TABLE II

Clinical and histologic incidence of EAE in F1 and parental mice

Mice	Sensitization	Disease Incidence		Mean Inflammatory Foci[a] in Mice with Histologic Acute EAE ± SE (Range)
		Clinical[b]	Histologic[c]	
(SJL/J X SWR)F_1[d]	103-116/CFA/pertussis	6/18	12/18	30 ± 10 (2–85)
	139-151/CFA/pertussis	7/17	12/17	43 ± 9 (2–88)
	CFA/pertussis	0/8	0/8	
	Unsensitized	0/2	0/2	
SJL/J	103-116/CFA/pertussis	0/3	0/3	
	139-151/CFA/pertussis	1/3	3/3	62 ± 43 (2–168)
SWR	103-116/CFA/pertussis	1/2[e]	1/1	15
	139-151/CFA/pertussis	0/2	0/2	
	CFA/pertussis	0/2	0/2	

[a] Meningeal plus parenchymal foci counted in paraffin sections of half brains and spinal cords.
[b] Proportion of mice that showed clinical signs of EAE.
[c] Proportion of mice with inflammatory lesions in the CNS. All mice with clinical disease had CNS inflammation. Other mice did not exhibit clinical signs but had histologic EAE.
[d] Includes (SJL/J X SWR)F_1 and (SWR X SJL/J)F_1 mice.
[e] One SWR mouse died immediately after sensitization.

Figure 1. Acute EAE lesion in spinal cord of a PLP peptide 139-151-sensitized F_1 mouse. Clinical signs (weight loss) began on day 10 and the mouse was killed on day 13 after sensitization. Mononuclear cells and neutrophils comprise the infiltrates. Luxol fast blue-hematoxylin and eosin stain, 200×.

mice sensitized with peptides which in previous studies were not encephalitogenic in those strains did not show EAE. Mice injected with CFA and pertussis alone and uninjected mice also did not show clinical or histologic disease.

Class I MHC molecules. All anti-class I MHC mAb stained the majority of lymphoid cells in spleens of SWR and F_1 mice. In addition, mAb 15-1-5B, 34-1-2S, and M1/42 stained cells in the spleens of SJL/J mice. Minimal staining with anti-class I MHC molecule mAb was observed on vessels in hearts.

In the brains of control and peptide-sensitized mice without EAE, class I MHC molecules were found on scattered CNS vessels (Fig. 2*A*) and rare parenchymal dendritic cells. Staining was particularly prominent on choroid plexus (Fig. 2*B*). The most numerous stained cells and vessels were found in sections stained with rat mAb M1/42. There was a greater proportion of stained vessels with this antibody in F_1 than in SJL/J or SWR mouse brains, but there were no detectable differences in vascular expression between sensitized mice with and without EAE and controls. In EAE lesions, there was additional strong expression on ependymal cells, inflammatory cells, and on parenchymal dendritic cells which are morphologically consistent with microglia (Fig. 3). However, with each mAb, regardless of the peptide used for sensitization, the proportions of stained cells or vessels was the same. Results of quantitation of stained parenchymal cells in inflammatory foci are shown in Figure 4.

Class II MHC molecules. Class II molecules were absent in brains of controls and in peptide-sensitized mice without EAE. In inflammatory foci of mice with EAE, there were many mononuclear cells that were positively stained (Fig. 5). In the choroid plexus, small numbers of infiltrating mononuclear cells were class II^+, but the choroid plexus cells were not stained. Small numbers of capillary endothelial cells and parenchymal dendritic cells were also stained for class II molecules, but the degree of staining was not as prominent on these cells as it was for class I molecules. There were some variations in proportions of stained cells among the antibodies, and greater proportions of stained cells were identified with mAb M5/114.15.2 than with the other anti-I-A antibodies. However, there were no detectable differences in parental haplotype expression on any cells depending on which peptide induced the disease (Fig. 6).

There was some diffuse background parenchymal staining with the biotinylated mAb to irrelevant murine and human MHC molecules, but none with PBS. No class II MHC molecule-positive cells were observed in F_1 hearts, but lymphoid cells were class II^+ in spleens. Anti-I-A^q mAb only stained SWR and F_1 tissues and anti-I-A^s mAb only stained SJL/J and F_1 tissues.

No differences between (SWR × SJL/J)F_1 and (SJL/J × SWR)F_1 with respect to clinical disease, histologic severity, or MHC molecule expression were found. There were no differences in MHC molecule expression between unsensitized and CFA/Pertussis-sensitized control mice and peptide-sensitized mice without histologic disease.

DISCUSSION

The two different encephalitogenic PLP peptides tested induced identical clinical and histologic disease and are, therefore, co-dominant Ag in (SJL/J × SWR)F_1 mice. This result might not have been predicted on the basis of the known parental strain susceptibilities because F_1-unique MHC restricting elements have been found to alter the expected repertoire of immune responses in other MHC heterozygous mice (25, 26). In addition, there is a lack of co-dominance of two myelin basic protein peptide frag-

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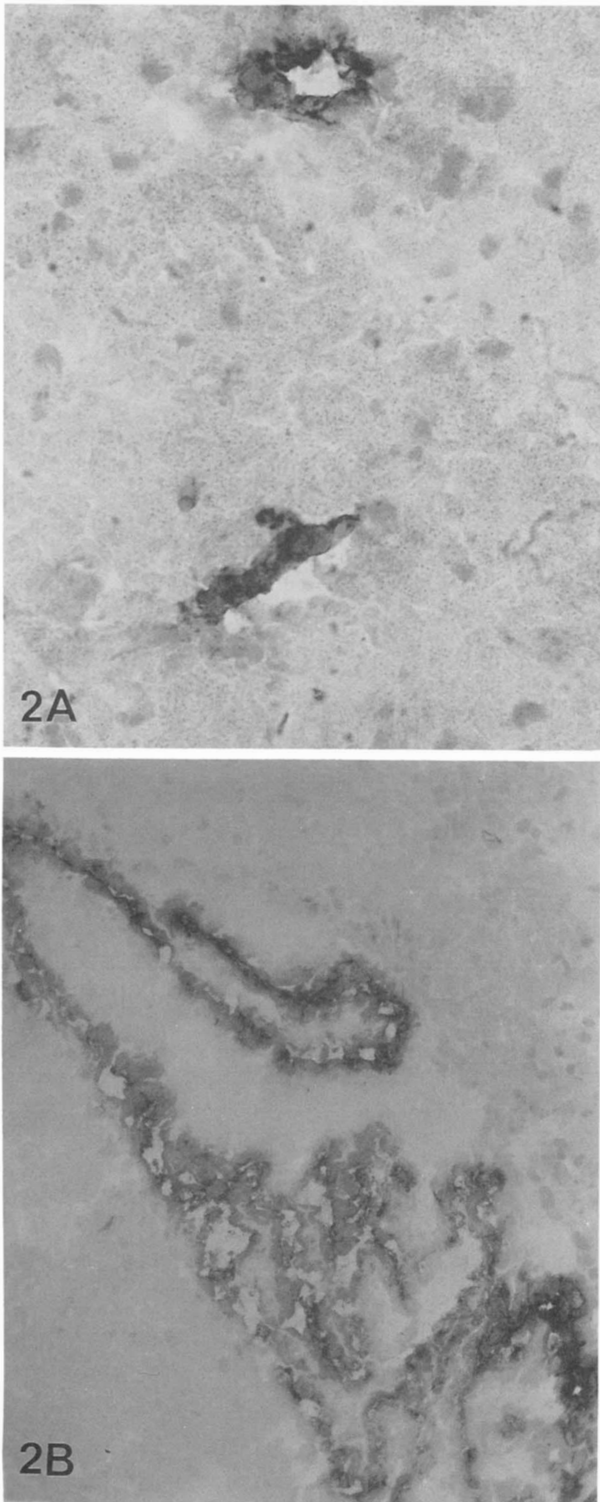

Figure 2. *A*, Class I MHC molecules on capillary endothelial cells in the brain of a PLP peptide 103-116-sensitized F_1 mouse which showed no clinical or histologic EAE. mAb M1/42 immunoperoxidase with hematoxylin, 500×. *B*, Class I MHC molecules on the choroid plexus in the brain of a control F_1 mouse. Staining is predominantly on basal surfaces of the choroid plexus cells. The adjacent parenchyma is unstained. mAb M1/42 with hematoxylin, 200×.

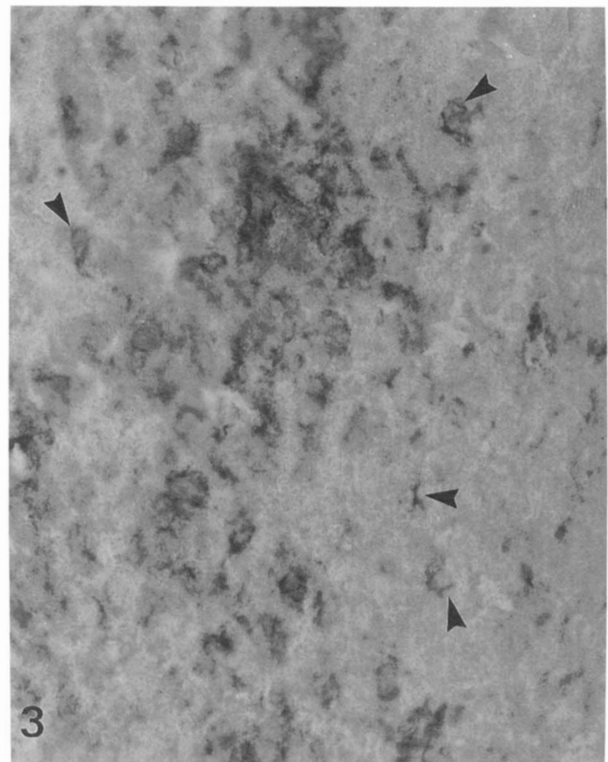

Figure 3. Class I MHC molecules in an inflammatory focus in the brain of an F_1 mouse sensitized with PLP peptide 139-151. Clinical signs began on day 14 and the mouse was killed on day 16 after sensitization. Mononuclear and dendritic cells (microglia, arrowheads) are stained. mAb M1/42 with hematoxylin, 500×.

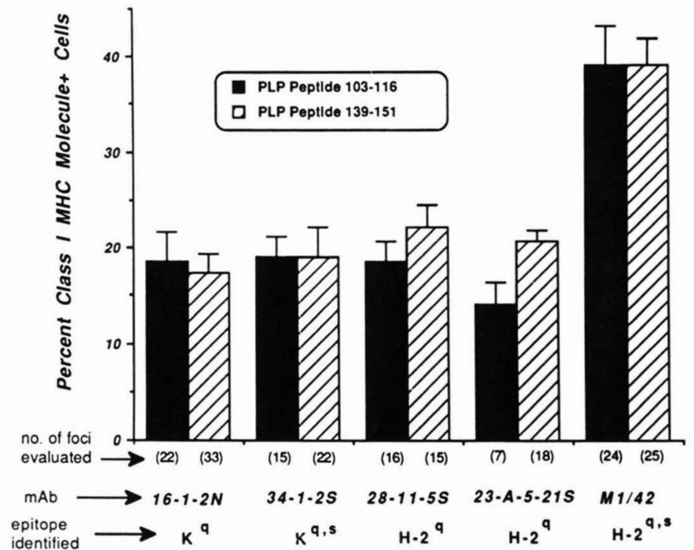

Figure 4. Quantitation of cellular class I MHC molecule expression in brain inflammatory foci of F_1 mice with acute EAE. Controls and sensitized mice without EAE showed staining only of CNS vessels and choroid plexus.

ments in actively induced EAE in (SJL/J × PL/J)F_1 mice (27). Thus, there are differences in patterns of immunodominance between PLP and myelin basic protein peptides that may indicate variations in the contributions of different peptide Ag to immune responses to whole CNS myelin. However, immune responses in (SJL/J × SWR)F_1 mice have not been analyzed at the molecular level and, for example, the extent of formation of heterodimeric MHC molecules (28–30) and their capacities for binding specific encephalitogenic peptides are not known in this strain combination.

In EAE, class I MHC molecule expression has received less attention than that of class II. Because class I MHC molecule haplotype preferences have been shown to occur in antiviral responses in F_1 mice (31), we analyzed class I MHC molecule expression in MHC heterozygous

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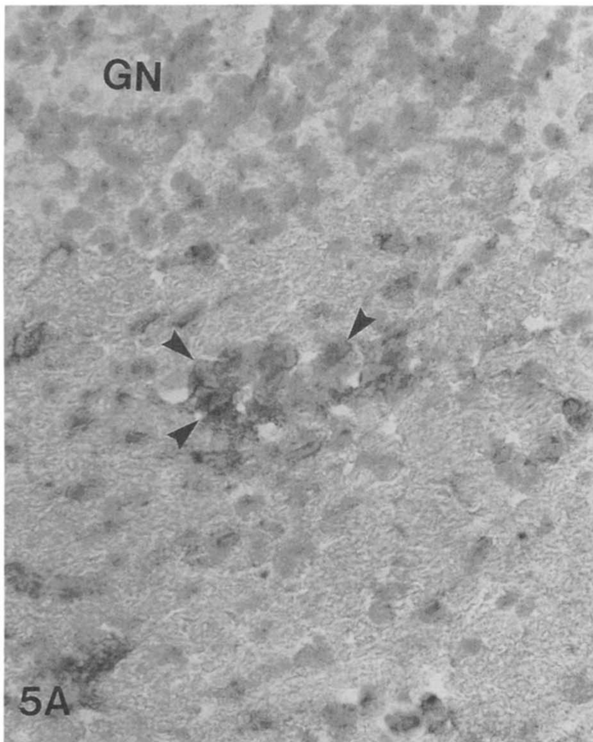

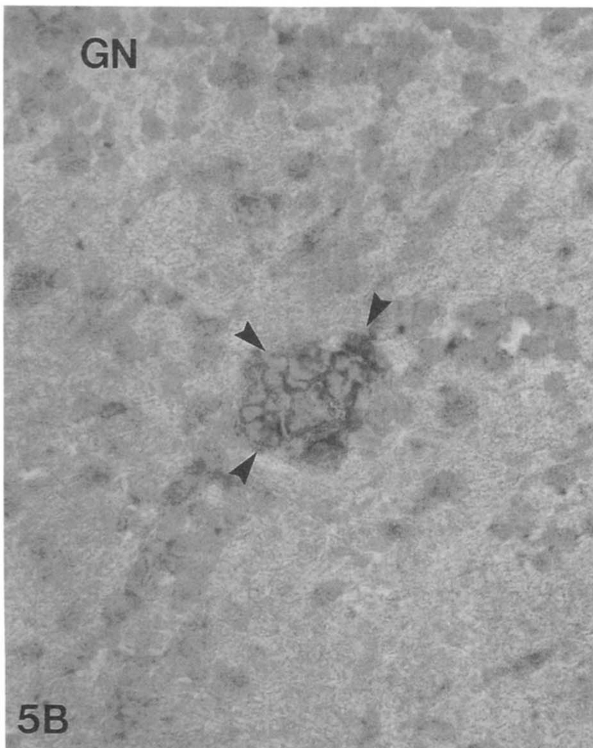

Figure 5. *A*, Inflammatory focus in the cerebellum of an F_1 mouse with EAE induced by sensitization with PLP peptide 103-116. Onset of weight loss occurred on day 15 and the animal was killed on day 18 after sensitization. I-A^q is predominantly expressed on perivascular inflammatory cells (*arrowheads*). Cerebellar granular layer neurons (*GN*) are not stained by immunoperoxidase. mAb M5/114.15.2 with hematoxylin, 500×. *B*, Adjacent serial section stained with a mAb to I-A^s. A similar staining pattern is present. mAb MKS4 with hematoxylin, 500×.

mice sensitized with different PLP peptides. No differences attributable to peptide sensitization were found. The strongest and most consistent staining of class I MHC molecules in the brains was on vessels and choroid plexus cells. Although choroid plexus cells present Ag in vitro (32), the choroid plexus is not the site of inflamma-

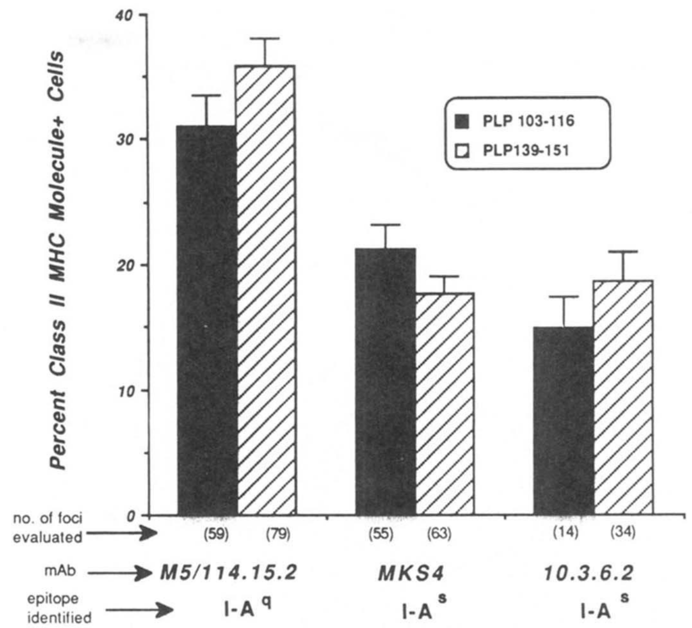

Figure 6. Quantitation of cellular class II MHC molecule expression in brain inflammatory foci of F_1 mice with acute EAE.

tory cell accumulation in EAE so that the immunopathologic significance of strong class I MHC expression on these cells in situ is uncertain.

Sensitization with the different peptides also did not result in detectable differences in parental haplotype class II MHC molecule expression in the brains of the F_1 mice. This observation might be related to distinct features of EAE in mice. Mouse T cells lack detectable surface Ia (33) so that B cells and macrophages may be the only Ia^+ mononuclear cells in inflammatory infiltrates. In addition, more neutrophils are present in EAE lesions in mice. Thus, for these two reasons there may be proportionately fewer Ia^+ inflammatory cells in EAE in mice compared to other species. Whereas parental haplotype Ia differences were previously observed on vessels in guinea pigs, less endothelial cell Ia is found in mice (6, 15). Therefore, even though they have been shown to present Ag in vitro (34), mouse CNS endothelial cells might not function as APC to the same extent in situ as they may in immune responses in other species. Furthermore, although the T cell response is necessary, *Bordetella pertussis* is generally required for active induction of EAE in mice (12, 35) and additional pathophysiologic mechanisms relating to effects of this adjuvant may be involved. Thus, possible differences in parental haplotype Ia expression on mononuclear and resident APC may have been relatively small and undetectable in this study.

Recent advances in understanding of molecular mechanisms of Ag presentation, and in particular, the formation of peptide/MHC molecule complexes (36, 37), suggest additional interpretations of our previous observations of differential parental haplotype Ia expression in (strain 2 × strain 13)F_1 guinea pigs (8, 9). Inasmuch as strain 2 guinea pigs are resistant to EAE induced with whole CNS tissue, the strain 2 haplotype Ia molecules expressed on APC in sensitized F_1 animals may not have bound neuroantigens in CNS inflammatory sites. In delayed hypersensitivity reactions, enhancement of both strain 2 and strain 13 parental haplotype expression corresponding

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to parental strain susceptibilities to synthetic (poly-GL, poly-GT) and xenogeneic (bovine and porcine insulin) Ag was demonstrated in different skin test sites. In that study, although each parental haplotype Ia molecule could bind the appropriate peptide, only foreign Ag were injected into the skin and, therefore, only formation of complexes of peptides derived from these Ag with susceptible parent Ia molecules could occur. In both guinea pig studies, the potential binding of peptides with the susceptible parent haplotype Ia molecule within the immune reaction site correlated with greater detection of that parental haplotype Ia molecule.

To explain our previous observations in guinea pigs and our current observations in mice, we hypothesize that multiple different peptides may be bound by MHC molecules within inflammatory sites and that the extent of the formation of peptide/MHC molecule complexes may influence levels of parental haplotype MHC molecule expression. In the present study and in contrast to the studies in guinea pigs, each parental mouse strain is susceptible to EAE and multiple encephalitogenic peptides, including both co-dominant PLP peptides, are normally present in the immune reaction site, i.e., the brain. Thus, only in the F_1 mice can it be assumed that each parental haplotype MHC molecule expressed on APC could bind one or more peptide Ag derived from self proteins in situ. The net result of multiple different peptides interacting with both parental haplotype MHC molecules in the mice may have been that detected levels of these molecules were indistinguishable. Although mechanisms by which peptide/MHC molecule binding may result in greater expression of one MHC molecule over another in a MHC molecule heterozygote are presently unclear, recent studies indicate that exogenous peptides can affect levels of cellular expression of MHC molecules (38–40). Differential turnover (41) or shedding (42) of peptide/MHC molecule complexes might also be involved in vivo. Our hypothesis implies that in an organ-specific autoimmune disease, such as EAE, the immune response involves MHC molecule binding of multiple Ag, even though the initial response may be provoked by sensitization with a single peptide.

The present findings have implications for strategies of therapy for autoimmune diseases that involve perturbation of peptide/MHC molecule interactions. Anti-Ia haplotype antibodies have been used to treat both EAE (43) and experimental autoimmune thyroiditis (44) in (responder × non-responder)F_1 mice. In both cases, mAb directed against the non-responder as well as the responder haplotype suppressed the disease in vivo. Our observation that there is equivalent in situ expression of parental MHC haplotype molecules in (SJL/J × SWR)F_1 mice suggests that in the anti-Ia haplotype experiments, antibody binding of the presumed non-responder epitope in the target organ might have been sufficient to alter peptide/Ia interactions overall and to down-regulate the autoimmune response. Exogenous peptides can also compete with encephalitogenic Ag and inhibit EAE (45, 46). However, chronic diseases may be further complicated by additional immune reactions to Ag generated as a consequence of tissue injury (47, 48) and T cell responses to CNS Ag may be polyclonal in vivo (49, 50). Our findings suggest that many peptide Ag may be involved in situ, and multiple competing peptides may be necessary for successful intervention in complex diseases, such as human multiple sclerosis.

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