

# PEPTIDE-SPECIFIC PREVENTION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

## Neonatal Tolerance Induced to the Dominant T Cell Determinant of Myelin Basic Protein

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Experimental allergic encephalomyelitis (EAE)<sup>1</sup> is an animal model of antigen-specific, T cell-mediated autoimmune disease. The characteristic clinical and histological features of chronic relapsing paralysis and demyelination with perivascular mononuclear cell infiltration of the central nervous system (CNS) resemble human multiple sclerosis (1, 2). EAE is mediated by T cells of the helper phenotype (CD4<sup>+</sup>, CD8<sup>-</sup>, IL-2R<sup>+</sup>) as detected in the CNS lesions of EAE animals (3). The involvement of CD4<sup>+</sup> T cells is readily demonstrated by the transfer of MBP-specific T cell lines and clones to rats (3, 4) and mice (5, 6) and by the fact that EAE can be treated with antibody to CD4 but not to other T cell subsets or B cells (7). EAE can be actively induced by immunization with synthetic peptides corresponding to different regions of myelin basic protein (MBP), as well as with the whole molecule (5, 8). The  $\alpha$ -acetylated, NH<sub>2</sub>-terminal, nonapeptide of MBP (1-9NAc) (identical in rat, mouse, and human MBP) is the dominant encephalitogenic T cell epitope in the PL/J and B10.PL (H-2<sup>u</sup>) mouse strains, eliciting rapid onset of disease (5). The encephalitogenic peptide determinants vary in different MHC haplotypes (8, 9). For example, the disease-inducing T cell determinants for the SJL/J (H-2<sup>s</sup>) strain have recently been localized within the mouse MBP COOH-terminal region 81-104 (10).

Activation of T cells involves the recognition of both MHC and peptide (11). Thus, EAE has been treated with mAbs to TCR V $\beta$ 8 determinants (found on most T cells directed to the dominant 1-9NAc determinant) (12, 13) and to MHC Ia molecules (7). However, we wished to investigate the alternative of using antigen to tolerize autoreactive T cells. Previous studies tolerizing animals against whole MBP have been successful in preventing EAE (14, 15), suggesting that tolerance against T cell determinants might provide a specific approach toward therapy. Neonatal administration of minimal immunogenic cytochrome and lysozyme peptides in IFA was previously shown to greatly reduce the adult T cell proliferative response, with exquisite peptide specificity (16, 17).

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<sup>1</sup> *Abbreviations used in this paper:* CNS, central nervous system; EAE, experimental allergic encephalomyelitis; HEL, hen egg-white lysozyme; LNC, lymph node cells; MBP, myelin basic protein.

Here, experiments are presented on mice tolerized during the neonatal period to the 1-9NAc peptide. We investigated whether reduction in both the *in vitro* T cell proliferation and disease assays would occur, after challenge of the tolerant mice with 1-9NAc or MBP. This approach could show whether peptides might be used for specific prophylaxis with the advantages of greater purity and potentially fewer side effects than whole MBP (14, 15, 18, 19). It also enabled us to investigate tolerance to a dominant determinant on a self antigen that is normally sequestered from the peripheral circulation, and does not induce tolerance by clonal elimination during development (20, 21).

### Materials and Methods

*Mice.* Male and female B10.PL mice (5-6 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME) and were maintained and bred in our animal facilities at UCLA.

*Antigens.* The synthetic peptide,  $\alpha$ -amino acetylated MBP, residues 1-9 (1-9NAc), was kindly provided by the Cetus Corp. (Emeryville, CA) or synthesized as previously described (10). The sequence of 1-9NAc is as follows: Acetyl-Ala-Ser-Gln-Lys-Arg-Pro-Ser-Gln-Arg.

MBP was isolated from mouse or rat brains (Pel-Freez Biologicals, Rogers, AR); delipidation was followed by acid extraction and analysis by SDS-PAGE (22). Hen eggwhite lysozyme (HEL), 5 $\times$  crystallized, was obtained from Societa Prodotti Antibiotici (Milan, Italy). This was additionally purified by elution as a single peak from a BioRex-70 column (Bio-Rad Laboratories, Richmond, CA) in 0.2 M sodium phosphate buffer at pH 7.18.

*Bordetella Pertussis.* *B. pertussis* organisms were provided as a generous gift by Dr. Robert Fritz (Emory University, Atlanta, GA) and also purchased from the Michigan Department of Public Health (Lansing, MI). Purified *pertussigen* was purchased from Porton Products Ltd. (Porton Down, UK).

*Tolerance Induction.* Neonatal mice were injected intraperitoneally, according to a previous protocol (16), at 24 and 72 h after birth with 50  $\mu$ l containing either 14 nmol (15  $\mu$ g) of 1-9NAc, or 7 nmol (100  $\mu$ g) of HEL, emulsified 1:1 with IFA (Gibco Laboratories, Grand Island, NY).

*EAE Induction and Grading.* Adult B10.PL mice (7-11 wk old) were immunized subcutaneously in the tail base with a 100- $\mu$ l mixture containing 100  $\mu$ g of 1-9NAc or 200  $\mu$ g of rat MBP, emulsified 1:1 in CFA. Subsequently, either 10<sup>10</sup> heat-killed *B. pertussis* (extensively washed in saline) or 0.025  $\mu$ g of purified *pertussigen* (in 100  $\mu$ l saline) were given intravenously 24 and 72 h later. (Both preparations were used during the course of these experiments and were found to be equally effective.) Animals were observed daily and graded as follows: 1, loss of tail tone; 2, hind limb weakness; 3, difficulty turning over, severe limb weakness or mild paralysis; 4, severe to total paralysis; 5, dead.

*T Cell Proliferation Assays.* Adult B10.PL mice (7-11 wk old) were immunized subcutaneously (tail base) with 100  $\mu$ l containing: 20  $\mu$ g of 1-9NAc or 100  $\mu$ g of mouse MBP in saline, emulsified 1:1 in CFA. 10 d later, draining lymph node cells (LNC) were removed and tested for proliferation according to a previous protocol (16): cells were cultured for 5 d with antigens at concentrations ranging from 0.03 to 30  $\mu$ M, in HL-1 serum-free medium (Ventrex Laboratories, Portland, ME) supplemented with 2 mM glutamine, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin. 1  $\mu$ Ci [<sup>3</sup>H]thymidine was added for the last 16 h of culture and the incorporation was measured by scintillation counting.

### Results

*MBP Peptide 1-9NAc Is a Major T Cell Epitope in B10.PL Mice.* To establish peptide-induced EAE in B10.PL mice, we first demonstrated that in this strain 1-9NAc is a dominant T cell determinant on MBP (Table I). After immunization of normal adult mice with mouse MBP, an *in vitro* proliferative response is obtained to itself and to the 1-9NAc determinant: the *in vitro* response to 1-9NAc is stronger than

TABLE I  
*1-9NAc Is the Dominant Proliferative Epitope on MBP  
 in the B10.PL (H-2<sup>u</sup>) Mouse Strain*

Antigen priming	In vitro antigen				
	MBP		1-9NAc		PPD
	3 $\mu$ M	0.3 $\mu$ M	30 $\mu$ M	3 $\mu$ M	
MBP	19.2 $\pm$ 12.8*	10.3 $\pm$ 8.1	69.4 $\pm$ 20.8	45.5 $\pm$ 14.1	91.8 $\pm$ 6.6
1-9NAc	2.7 $\pm$ 1.5	7.2 $\pm$ 0.3	39.2 $\pm$ 2.1	7.1 $\pm$ 2.3	104.6 $\pm$ 4.2

\* Values represent the mean [<sup>3</sup>H]TdR uptake of triplicate cultures ( $\times 10^{-3} \pm$  SD). [<sup>3</sup>H]TdR incorporation is expressed as counts per minute, with background (media alone) values between 1 and 3  $\times 10^3$  subtracted ( $\Delta$ cpm). Adult mice were immunized with either mouse MBP/CFA (100  $\mu$ g/100  $\mu$ l) or 1-9NAc/CFA (20  $\mu$ g/100  $\mu$ l) subcutaneously in the base of the tail. LNC were harvested 10 d later for proliferation assay. The antigen concentration range used was 3-30  $\mu$ M for 1-9NAc and 0.3-3  $\mu$ M for mouse MBP.

to whole MBP at the 3- $\mu$ M dose. (For MBP a greater dose than this appears to be toxic.) Likewise, good in vitro proliferation to MBP and 1-9NAc can be induced by immunization with the 1-9NAc determinant itself. These results are consistent with those of Zamvil et al. (5) showing that 1-9NAc is a major proliferative T cell determinant on MBP. It was apparent that individual mice varied in their response to MBP and 1-9NAc. For example, in vitro proliferation to MBP was undetectable in one-third of mice immunized with MBP, although a response to 1-9NAc was seen in the same animals (data not shown).

*B10.PL Mice Can Be Neonatally Tolerized to the Self Peptide 1-9NAc.* To determine if tolerance could be induced to the self MBP peptide 1-9NAc, we used the protocol previously described for induction of neonatal tolerance to cytochrome and lysozyme peptides (16, 17). The proliferative T cell response to 1-9NAc was considerably lower within 1-9NAc-primed LNC in peptide-tolerized mice compared with the proliferation in LNC from HEL-tolerized mice (Fig. 1 A). Likewise, the 1-9NAc response of whole MBP-primed LNC was reduced in 1-9NAc-tolerized mice, in contrast to HEL-tolerized control animals (Fig. 1 B).

*The Immunodominant 1-9NAc Epitope Tolerizes the In Vitro Response to Whole MBP.* Similarly, in mice tolerized to 1-9NAc and immunized with mouse MBP, the in vitro LNC proliferation to mouse MBP was virtually eliminated (Fig. 1 C). Mouse MBP was used in preference to rat MBP in these in vitro studies, assuming crossreactivity of amino acid sequences of the encephalitogenic determinants. The 1-9NAc determinant for example is identical between these two species and although heterologous MBP may be used to activate autoreactive T cells, the pathogenic activity is specifically against self tissue. In particular, we wished to avoid proliferation induced by epitopes specific to rat MBP (5). Again, variation between individual animals was apparent, reflecting that found in normal mice. For example, two of six HEL-tolerized mice did not respond to MBP (Fig. 1 C).

*Induction of EAE in B10.PL Mice.* Chronic relapsing EAE was established in B10.PL mice (H-2<sup>u</sup>) by immunization with the synthetic peptide 1-9NAc. The incidence of EAE in normal untreated B10.PL mice is shown in Table II. 100  $\mu$ g of the peptide 1-9NAc is more efficient in causing disease than 200  $\mu$ g of native rat MBP. The peptide results in much higher average severity and earlier mean day of onset of

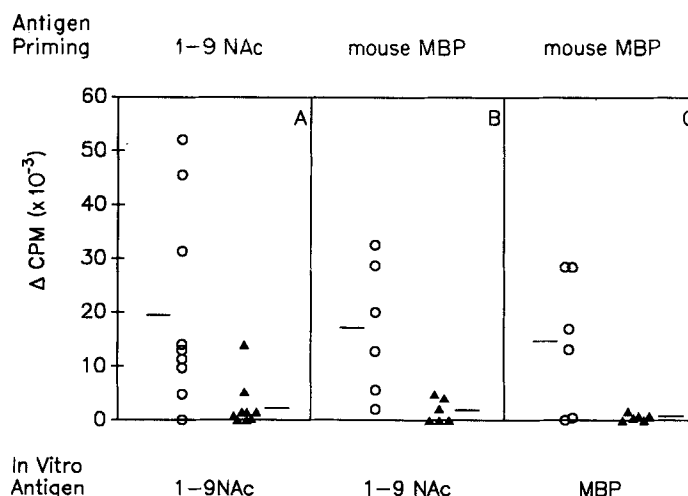


FIGURE 1. In vitro tolerance induced by 1-9NAc. Mice were tolerized by injection at 24 and 72 h after birth with 50  $\mu$ l containing 14 nmol of 1-9NAc/IFA. Adults were then immunized with either 1-9NAc/CFA (20  $\mu$ g/100  $\mu$ l) or mouse MBP/CFA (100  $\mu$ g/100 $\mu$ l) subcutaneously in the base of the tail. LNC were harvested 10 days later for proliferation assay as described in the legend to Table I. In vitro proliferation was tested to 1-9NAc at an optimal concentration of 30  $\mu$ M (A and B) or MBP at 3  $\mu$ M (C). The arithmetic mean of the antigen-specific response for each group is represented by a horizontal bar. The symbols are as follows: (O) control, HEL-tolerized mice, immunized with 1-9NAc (A) or mouse MBP (B and C); filled triangles (▲) represent 1-9NAc tolerized mice immunized with 1-9NAc (A) or mouse MBP (B and C).

disease, 3.2 and 12.6 d, respectively, compared with 1.3 and 17.5 d, for the whole molecule. The characteristic disease course of three mice is shown in Fig. 2. This pattern is similar to that described for PL/J, SJL, and (PL/J  $\times$  SJL) $F_1$  mice (5). The pattern of disease severity varied for individual animals, although interestingly, most relapses tended to occur simultaneously.

TABLE II  
EAE Is Induced in Normal B10.PL Mice after Immunization  
with 1-9NAc and Rat MBP

Immunogen	Dose	Disease incidence*	Disease score†	Total group score‡	Mean day of onset
	$\mu$ g				
1-9NAc	100	14/16 (86.5%)	3.2 $\pm$ 1.1	2.8 $\pm$ 1.3	12.6 $\pm$ 3.6
Rat MBP	200	4/5 (80%)	1.3 $\pm$ 0.4	1.0 $\pm$ 0.3	17.5 $\pm$ 9.3

\* Disease incidence is expressed as the number of animals showing clinical signs of EAE/the total number per group. Animals were immunized for EAE by injection of the above dose of antigen in CFA, given  $10^{10}$  *B. pertussis* in saline intravenously at 24 and 72 h, then monitored daily.

† The disease score indicates the average of the maximum severity scores for each diseased animal per group. Mice were graded as follows: 1, loss of tail tone; 2, hind limb weakness; 3, difficulty turning over, severe limb weakness or mild paralysis; 4, severe to total paralysis; 5, dead.

‡ The total group score indicates the sum of maximum disease scores/total number of animals per group.

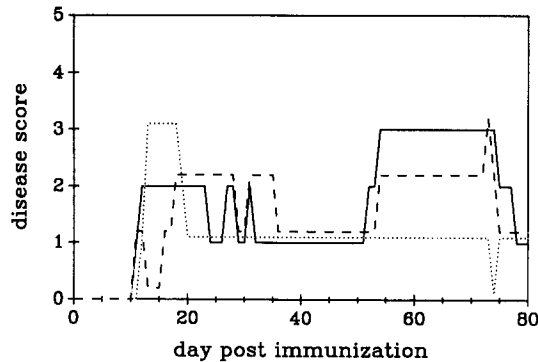


FIGURE 2. Chronic relapsing paralysis induced in 3 B10.PL mice. Mice were immunized with 1-9NAc/CFA, given *B. pertussis* in saline intravenously at 24 and 72 hours, then monitored daily. Disease severity was graded from 1 to 5 as described in Table II.

*Neonatal Tolerance to 1-9NAc Successfully Decreases the Incidence of 1-9NAc-induced EAE.* A considerable reduction in disease incidence was found in mice tolerized to 1-9NAc, and subsequently immunized with the same peptide in adulthood (Table III). This is approximately half of that seen in control, HEL-tolerized mice and is statistically significant (according to  $\chi^2$  analysis with Yates correction factor ( $p$  is 0.05)). In those 1-9NAc-tolerized mice that did get EAE, the disease score (the average maximum score of diseased animals) was one full grade lower. The total group score, including all animals (with and without EAE), clearly expresses this difference in disease activity with a score of 0.8 for all 1-9NAc-tolerized mice and 2.7 for the HEL-tolerized controls. Also there appeared to be a correlation between reduced incidence and mean day of onset for clinical disease, which was later (day 14) for 1-9NAc-tolerized mice than for HEL-tolerized controls (day 11.9). Fig. 3 illustrates the absence of EAE in the majority of 1-9NAc-tolerized mice following immunization with 1-9NAc. It also shows the tendency of diseased animals in the tolerized group to have lower grade symptoms than controls.

To improve the degree of tolerance, we are investigating several parameters such as dose of tolerogen or age of challenge, but most importantly, using 1-11NAc as the tolerogen. This MBP 1-11 peptide appears to be more immunogenic than 1-9NAc (5); tolerization with 1-11 may more effectively eliminate T cells of all affinities that bind 1-9NAc.

*Failure to Suppress MBP-induced EAE in Mice Tolerized to 1-9NAc.* Immunization of 1-9NAc-tolerized mice with rat MBP produces EAE in the same proportion of animals (50%) as in the control, HEL-tolerized groups (Table IV). The difference

TABLE III  
Tolerance to EAE Induction Is Achieved in B10.PL Mice  
Treated Neonatally with 1-9NAc

Tolerogen	Antigen	Disease incidence*	Disease score <sup>†</sup>	Total group score <sup>§</sup>	Mean day of onset
1-9NAc	1-9NAc	9/23 (39%)	2.1 ± 1.1	0.8 ± 0.9	14.0 ± 3.8
HEL	1-9NAc	17/20 (85%)	3.1 ± 1.2	2.7 ± 1.0	11.9 ± 2.4

See footnotes for Table II.

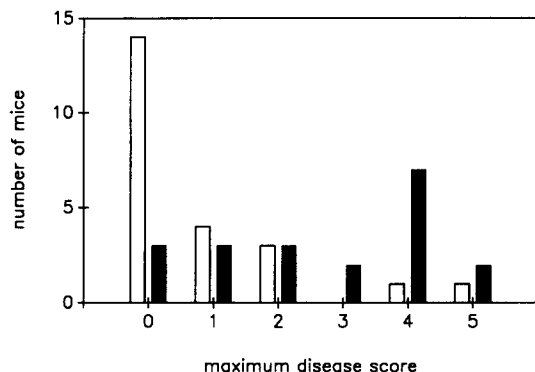


FIGURE 3. Maximal disease severity in neonatally tolerized mice. Mice were tolerized with 1-9NAc/IFA as described in Fig. 1, then immunized for disease and graded for signs of EAE as outlined in the legend to Table II. Open bars represent 1-9NAc-tolerized animals; closed bars represent control, HEL-tolerized animals.

in disease incidence between rat MBP-primed mice in Table II and HEL-tolerized animals in Table IV may be due to variation between the different MBP preparations used. The less efficient disease induction in rat MBP-immunized mice (compared with mice given 1-9NAc) can presumably be remedied by a higher dose. It is clear, however, that even at this limiting dose, tolerance to 1-9NAc did not alter the disease incidence. This shows that there is at least one additional disease-inducing determinant on rat MBP, other than 1-9NAc, which crossreacts with the autologous protein at the T cell level.

*Peptide 31-50 Contains a Second Disease Determinant on MBP.* The possible existence of T cell determinants overlapping peptic cleavage sites of MBP (for example, at residue 37 [23]) was tested using peptides spanning the whole MBP molecule. We found that the peptide 31-50 (crossreactive between mouse and rat MBP) can normally induce strong LNC proliferation in the B10.PL strain (Kono, D., unpublished results). However, unlike the 1-9NAc determinant, there is no detectable *in vitro* response to peptide 31-50 after priming with the whole MBP molecule.

In experiments with 1-9NAc- and control, HEL-tolerized mice, T cell proliferation to peptide 31-50 is equivalent in LNC from both groups (accounting for individual response variation) (Fig. 4). This is an indication of specificity of the peptide-induced tolerance. We also have data showing that MBP peptide 31-50 causes EAE in B10.PL mice (Kono, D., unpublished results). This is consistent with the results of others describing a T cell proliferative and encephalitogenic determinant between residues 35-47 on MBP for PL/J and (PL/J × SJL/J)<sub>F1</sub> mice (24).

These data suggest that in comparison to 1-9NAc, peptide 31-50 represents a sub-

TABLE IV  
Tolerance to 1-9NAc Has No Effect on EAE Induction in B10.PL Mice  
after Immunization with Rat MBP

Tolerogen	Antigen	Disease incidence*	Disease score <sup>†</sup>	Total group score <sup>§</sup>	Mean day of onset
1-9NAc	rat MBP	8/16 (50%)	2.4 ± 1.1	1.2 ± 1.3	12.9 ± 4.4
HEL	rat MBP	5/10 (50%)	2.6 ± 0.9	1.3 ± 1.4	13.6 ± 3.3

See footnotes for Table II.

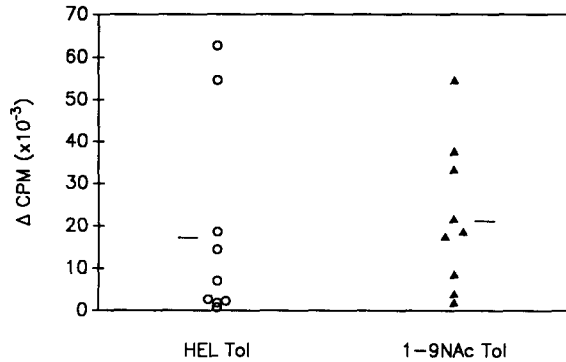


FIGURE 4. In vitro proliferation to MBP peptide 31-50 in 1-9NAc- and HEL-tolerized mice. Mice were immunized with peptide 31-50/CFA (20  $\mu\text{g}/100 \mu\text{l}$ ) subcutaneously in the base of the tail. LNC were harvested 10 d later for proliferation assay as described in the legend to Table I. In vitro proliferation was tested to peptide 31-50 at an optimal concentration of 30  $\mu\text{M}$ . (○) Control, HEL-tolerized mice; (▲) 1-9NAc-tolerized mice.

dominant T cell determinant on MBP, but may be relevant to in vivo disease activity. We are therefore examining whether peptide 31-50 includes the determinant causing EAE in those 1-9NAc-tolerized mice which were subsequently challenged with rat MBP.

#### Discussion

Normal adult B10.PL mice mount a good proliferative response in vitro to MBP, and this response appears to be predominantly directed against a determinant on the  $\text{NH}_2$ -terminal peptide, 1-9NAc (Table I). Tolerance induced in neonatal mice to this peptide, greatly reduces not only the response to the peptide itself but also the response to the whole protein (Fig. 1). This confirms the immunodominant status of the 1-9NAc determinant in the proliferative response. Indeed, 1-9NAc is also the predominant EAE-inducing peptide in B10.PL mice (Table II), and neonatal tolerance induced to 1-9NAc leads to a specific reduction in incidence and severity of peptide-induced EAE (Table III). However, in contrast to the lack of a proliferative response to MBP in 1-9NAc tolerant mice (Fig. 1), MBP induces disease in these animals at the same frequency and to an equal severity, as in control mice (Table IV). This suggests that one or more additional disease-inducing determinants exist for H-2<sup>u</sup> mice. EAE-inducing activity appears to be a more sensitive assay than in vitro proliferation for detecting T cell responses specific for minor determinants.

Experiments in a variety of systems indicate that a hierarchy of responsiveness exists to a multideterminant antigen at the T cell level, in which the proliferative response appears to be limited to a few dominant determinants. Other potential (subdominant) determinants exist that are only revealed by peptide immunization (10, 25, 26). It is possible that the hierarchy of determinants defined by disease induction could be different from that defined by the in vitro proliferation assay. However, this would seem unlikely and instead we would assume that the apparent difference is one of sensitivity. There are several possible reasons why a subdominant determinant may be capable of inducing disease but not a proliferative response. For example, these autoreactive clones may accumulate within the CNS leaving low numbers remaining in the periphery, precluding their detection by the proliferation assay.

One possible candidate for an additional disease-inducing determinant on MBP exists on peptide 31-50. This determinant is subdominant in the proliferative re-

sponse in that no response to it is observed after MBP immunization, but a good response is obtained after priming with peptide 31-50 itself. Peptide 31-50 can also induce disease. A similar result, using the MBP peptide 35-47, has been found by others (24). 35-47 induces a good proliferative response to itself as well as disease in susceptible PL/J mice. This peptide determinant is only a minor component in the anti-MBP T cell proliferative response, judging by the low frequency of 35-47-specific T cell clones compared with 1-9-specific ones (27).

A panel of synthetic peptides, overlapping in sequence and spanning the whole molecule, is being analyzed both *in vivo* and *in vitro* for the purpose of identifying all encephalitogenic determinants in the H-2<sup>u</sup> haplotype. In the previous work of others, no additional disease determinants were detected in synthetic peptides spanning amino acids 1-37 of rat MBP (5), although B10.PL mice gave a T proliferative response to all fragments of guinea pig MBP (1-37, 43-88, and 89-169 [28]). Peptic digestion of the MBP molecule at these cleavage sites (residues 37 and 88) may have destroyed other dominant or subdominant encephalitogenic determinants (22). Since *in vitro* proliferation studies alone clearly do not indicate all disease-inducing T cell determinants on a self antigen, the neonatal tolerance system in EAE is ideal for addressing these questions.

Our data suggest that B10.PL mice do not spontaneously develop autoimmune encephalomyelitis, owing to the sequestration of MBP from the immune system. Clearly autoreactive T cells are not deleted in these animals, but normally they are not activated. These T cells can be tolerized by administration of peptide to neonatal mice. Presumably neonatal exposure to exogenous peptide causes inactivation or deletion as has been proposed in neonatal tolerance to cytochrome and lysozyme peptides (16, 17). The ability to inactivate 1-9Nac-reactive cells shows that lack of tolerance is not primarily due to an insensitivity of the T cells; rather it suggests absence of MBP determinants from the developing thymus (20, 21). This may result from insufficient quantities of MBP in neonatal mice to reach the thymus and induce tolerance (29).

In the normal adult, the blood-brain barrier usually will prevent trafficking of significant numbers of autoreactive T cells through the CNS; this is in contrast with more extensive trafficking during the autoimmune state (30-33). Additionally, the presentation of MBP in an immunogenic form within the CNS may only occur when class II expression is abnormally induced (33-35). As a consequence, competent, but normally unstimulated MBP-specific T cells could be always present in the adult animal. This last view is supported by the finding that EAE can be induced in mice with autologous MBP (28). In addition, in previous studies with the SJL strain, the *in vitro* proliferative T cell response to autologous MBP was as strong as to the heterologous protein (Kono, D., unpublished results).

Recently, EAE induction by MBP peptide 1-11 and whole MBP was partially prevented in PL/J and B10.PL mice by treatment with an antibody specific for the TCR V $\beta$ 8 gene segment. This V $\beta$ 8 gene segment was found to be used in ~70-80% of T cells recognizing the 1-9Nac determinant (12, 13, 36). T cell tolerization with peptide epitopes, as shown here, has the advantage of greater specificity than V $\beta$ 8-targeted elimination and furthermore addresses all cells with specificity for 1-9Nac, including those using other TCR genes. Our data, emphasizing the role of additional determinants other than 1-9Nac, suggest that those mice immunized with whole MBP that developed symptoms after anti-V $\beta$ 8 therapy were probably reacting



to non-1-9NAc epitopes. The response to peptide 35-47, which is I-E<sup>u</sup> restricted, is largely composed of T cells not expressing V $\beta$ 8 (24).

For the purpose of eventual human treatment, it would be of crucial importance to attempt to turn off disease in the preimmunized mouse. Through the tolerance mechanism, even selected T cells of high affinity should be able to be rendered tolerant. Peptide-induced tolerance has the advantage that it minimizes the potential side effects of therapy with whole MBP (14, 15, 18, 19). Administration of tolerogenic peptides might be attempted in various ways. For example, Higgins and Weiner (19) have induced oral tolerance to MBP and MBP peptides in rats and have successfully tolerized T cells throughout the animal despite the localized nature of the stimulus. A large dose of antigen may be needed to induce oral tolerance although this would not necessarily be a contra-indication if peptide-induced tolerance were long lasting. A second approach might be that used by Jenkins and Schwartz (37) for their *in vitro* induction of unresponsiveness in T cell clones (which actually can be regarded as representative of activated cell populations). Thus, ECDI-fixed spleen cells pulsed with peptide may be excellent inducers of tolerance.

The failure to induce tolerance to whole MBP after tolerization with the major encephalitogenic determinant 1-9NAc showed that subdominant disease-inducing determinants can play a significant role in autoimmune disease. The diversity of potential responses to MBP may prove a major obstacle to the use of either peptides (for tolerance induction) or anti-TCR antibodies for the elimination of self-reactive T cells. However, with attention to the caveats discussed above, it is realistic to predict that peptide-induced tolerance is potentially a successful approach to the treatment of autoimmune disease.

### Summary

Experimental allergic encephalomyelitis (EAE) is a model of antigen-specific T cell-mediated autoimmune disease. The  $\alpha$ -acetylated, NH<sub>2</sub>-terminal nine amino acids (1-9NAc) of myelin basic protein (MBP) represents the dominant T cell epitope for the induction of EAE in the B10.PL (H-2<sup>u</sup>) strain.

We tolerized neonatal B10.PL mice to 1-9NAc and studied the proliferative responses to this peptide and to whole MBP. Mice exposed to 1-9NAc in the neonatal period were tolerant to subsequent challenge at the proliferative T cell level. Similarly, in the 1-9NAc-tolerant group, both the incidence and severity of 1-9NAc induced EAE were greatly reduced. The fact that we were able to tolerize mice normally responsive to MBP suggests that this self antigen is sequestered (within the central nervous system) and hence tolerance to it is not normally induced.

No significant difference in disease incidence was seen in response to rat MBP between control animals and 1-9NAc-tolerized mice (50% in both groups), demonstrating the presence of at least one additional encephalitogenic determinant elsewhere on the molecule.

We have successfully prevented disease induction by peptide-induced tolerization. Tolerance induction by peptides provides a new and specific strategy in the prevention of autoimmunity. However, it will be clearly necessary to fully define all epitopes potentially capable of inducing pathogenic T cells to ensure complete and effective therapy of T cell-mediated autoimmune disease.

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