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*J Immunol* 2001; 166:6907-6913; doi: 10.4049/jimmunol.166.11.6907

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Thiopalmitoylation of Myelin Proteolipid Protein Epitopes Enhances Immunogenicity and Encephalitogenicity

Judith M. Greer, Bé rangère Denis, Raymond A. Sobel, and Elisabeth Trifilieff

Proteolipid protein (PLP) is the most abundant protein of CNS myelin, and is posttranslationally acylated by covalent attachment of long chain fatty acids to cysteine residues in the polypeptide backbone via thioester linkages. PLP acylation is highly conserved throughout evolution and during brain development, and is thought to play an important role in the normal functioning of PLP and in myelin stability. Cys108 is the major acylation site (7), but a total of six acylation sites have been reported (8). The acylation sites Cys108 and Cys140 are within the encephalitogenic PLP epitopes PLP104-117 and PLP139-151, respectively (1, 4, 9, 10). Reactivity to these epitopes is also found in some patients with multiple sclerosis (MS) (11-15). However, the contribution that the thioacyl side chains make to the immunogenicity and encephalitogenicity of PLP has not been studied.

It has been shown that lipopeptides formed by the attachment of acyl side chains to peptides either via stable amide bonds (16-19) or via the more labile thioester linkage (20), as is found in PLP, can act as natural adjuvants for the induction of Ab and CTL responses. We postulated that if thioacylated PLP lipopeptides show similar immune-enhancing properties, their release from the native protein during demyelination in MS or experimental autoimmune encephalomyelitis (EAE) might lead to enhanced autoimmune activation directed against PLP.

The aim of the present study was to determine whether thioacylated PLP lipopeptides affect the development of autoreactivity differently from their nonacylated counterparts. PLP peptides PLP104-117 and PLP139-151 were synthesized with a palmitic acid side chain attached via a thioester linkage (thiopalmitoylated; designated S-palm-PLP104-117 and S-palm-PLP139-151), as occurs in the native protein. Mice immunized with these peptides showed significant increases in T cell proliferative responses, and the incidence and duration of clinical EAE were enhanced. In contrast, peptides synthesized with a palmitic acid side chain attached to the N terminus via the amide group (N-palmitoylated (N-palm)-PLP104-117 and N-palm-PLP139-151) were only weakly immunogenic and not encephalitogenic, suggesting that the type of linkage between the peptide and the fatty acid may be important for the induction of CD4+ T cells. These results imply that immune responses induced by physiologically thioacylated PLP lipopeptides that are released with myelin breakdown may play a role in prolongation of autoimmune inflammatory demyelinating diseases in vivo.

Materials and Methods

Mice

Female SJL/J mice were purchased from the Animal Resources Center (Murdoch, Western Australia). Mice were age matched for each experiment, and were immunized at 7-10 wk of age.

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0022-1767/01/502.00
Antigens

Peptides PLP<sub>104-117</sub> and PLP<sub>139-151</sub> were synthesized by solid-phase synthesis using a Fmoc/tBu strategy. The thiopalmitylation of residues Cys<sub>108</sub> and Cys<sub>140</sub> was performed on the resin-bound peptide after selective deprotection of the Cys side chain (21). The N-palm peptides were obtained by coupling activated palmitic acid on the N terminus residue. After cleavage from the resin, the crude peptides were lyophilized and purified by C18 RP-HPLC. The purity of the peptides was ≥95%, and their identities were confirmed by electrospray mass spectrometry. The sequences of the peptides are shown in Table I. In some experiments, peptides PLP<sub>104-99</sub> and PLP<sub>178-191</sub> or guinea pig myelin basic protein (MBP), prepared as previously described (22), were used as control Ags.

Active induction of EAE

Mice were injected s.c. in the flank with 50 μg of peptide and 400 μg of Mycobacterium tuberculosis H37Ra (Difco, Detroit, MI) in an emulsion consisting of equal volumes of water and CFA (Difco). Peptides were dissolved at a concentration of 5 mg/ml in 0.2 M acetic acid, and diluted to the appropriate concentration with distilled water. Each mouse was also injected i.v. on days 0 and 3 with 300 ng Bordetella pertussis toxin (List Biological Laboratories, Campbell, CA).

Clinical and histological evaluation

Mice were assessed clinically, as previously described (4, 23), according to the following criteria: 0, no disease; 1, decreased tail tone or slightly clumsy gait; 2, tail atony and/or moderately clumsy gait and/or poor righting ability; 3, limb weakness; 4, limb paralysis; 5, moribund state. Animals were sacrificed within 7 days of the initial appearance of clinical signs of disease or within 7 days of a relapse. Some mice that showed no clinical signs were also sacrificed for histological analysis. Brains and spinal cords were removed and fixed in 10% phosphate-buffered Formalin, and paraffin-embedded sections were stained with luxol fast blue hematoxylin and eosin for light microscopy. Histological disease was quantitated by counting the inflammatory foci in meninges and parenchyma, as previously described (23).

Proliferation assays

Pooled lymph node cells (LNC) were prepared from inguinal and axillary lymph nodes from two to five mice injected s.c. 9–10 days earlier with 50 μg of peptide in CFA. The in vitro responses of LNC were assessed in triplicate in 96-well flat-bottom microtiter plates. Three hundred thousand LNC were added to each well, together with tissue culture medium as a control or various Ags. Cells were incubated for 72 h at 37°C in 5% CO<sub>2</sub>-Percoll in RPMI 1640 medium supplemented with 10% FCS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. After washing, cells were resuspended in wash buffer and analyzed using a FACStar flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

Results

Effect of lipopeptides on induction of proliferative responses

Mice were immunized with nonacylated, S-palm, or N-palm PLP<sub>104-117</sub> or PLP<sub>139-151</sub> in CFA. After 9 days, lymph nodes were removed, and proliferation of the LNC in response to the immunizing peptide and other Ags was tested. LNC from mice immunized with nonacylated PLP<sub>104-117</sub>, a subdominant epitope of PLP<sub>139-151</sub> (10), responded with a mean SI of ≥3 to concentrations ranging from 4.5 to 36 nmol/ml of the immunizing peptide and to concentrations of S-palm-PLP<sub>104-117</sub> greater than 18 nmol/ml, but made no significant response to the other concentrations of S-palm-PLP<sub>104-117</sub> or to the N-palm-PLP<sub>104-117</sub> peptides or unrelated Ags (guinea pig MBP or PLP<sub>178-191</sub>; data not shown) (Fig. 1A). The mean SI of LNC from mice immunized with S-palm-PLP<sub>104-117</sub> in response to PLP<sub>104-117</sub> was at least 4-fold greater than the response of LNC from mice immunized with PLP<sub>104-117</sub> (Fig. 1B). In addition, these LNC also responded with SI ≥3 to the S-palm-PLP<sub>104-117</sub> peptide, but not to N-palm-PLP<sub>104-117</sub>. They did not cross-react nonspecifically with palmitoylated PLP<sub>139-151</sub> peptides (data not shown), indicating that the T cell response is not directed against the palmitic acid side chain. The response of LNC from mice immunized with N-palm-PLP<sub>104-117</sub> to all Ags was minimal (Fig. 1C). Thus, maximal proliferative responses were observed in LNC from S-palm-PLP<sub>104-117</sub>-immunized mice.

PLP<sub>139-151</sub> is an immunodominant epitope of PLP (1, 4, 23), and LNC responses of PLP<sub>139-151</sub>-immunized mice to the nonacylated peptide were substantially greater than those seen in response to PLP<sub>104-117</sub> with mean SI ranging from 12 to 40 over the 1–35 nmol/ml peptide concentrations tested (Fig. 1D). The response to S-palm-PLP<sub>139-151</sub> was comparable with the response to PBS-T, and 100 μl of 1/1000 dilution of anti-mouse polyvalent Igs (Sigma, St. Louis, MO) was added to each well and incubated 2 h at 37°C. After extensive washing with PBS-T, 100 μl p-nitrophenylphosphate substrate (Sigma) was added to each well, and the plates were incubated 1 h in the dark at room temperature. The absorbance was read at 405 nm in a BioLumin 960 plate reader (Molecular Dynamics, Sunnyvale, CA). Data are expressed as the mean specific absorbance, which is the mean absorbance of test peptide-coated wells minus the mean absorbance of wells coated with the control peptide. ±SEM.

Flow cytometry

LNC or T cell lines were centrifuged through a Ficoll gradient and washed with PBS containing 1% FCS and 0.01% sodium azide (wash buffer). Aliquots of 1 million cells were incubated with Abs specific for CD4 (clone RM4-5; rat IgG2a) or CD8a (clone 53-6.7; rat IgG2a) together with Ab specific for the TCR β-chain (H57-597; hamster IgG) for 30 min at 4°C in the dark, followed by FITC-conjugate anti-rat κ-chain or PE-conjugated anti-hamster IgG for 30 min at 4°C in the dark. Isotype-matched primary Abs were used as controls. All Abs were purchased from Pharmingen (San Diego, CA) and were used at 1 μg/ml dilution in wash buffer. After washing, cells were resuspended in wash buffer and analyzed using a FACS-Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

Table I. Sequences of the peptides used in this study

<table>
<thead>
<tr>
<th>Peptide Designation</th>
<th>Sequence</th>
<th>Molecular Mass</th>
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<tbody>
<tr>
<td>PLP&lt;sub&gt;104-117&lt;/sub&gt;</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;N-KTICGKGLSATVT-COOH</td>
<td>1379.6</td>
</tr>
<tr>
<td>S-Palm-PLP&lt;sub&gt;104-117&lt;/sub&gt;</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;N-KTIC(Palm)GKGLSATVT-COOH</td>
<td>1618.1</td>
</tr>
<tr>
<td>N-Palm-PLP&lt;sub&gt;104-117&lt;/sub&gt;</td>
<td>Palm-KTTICGKGLSATVT-COOH</td>
<td>1618.1</td>
</tr>
<tr>
<td>PLP&lt;sub&gt;139-151&lt;/sub&gt;</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;N-HCLGKWLGHPDKF-COOH</td>
<td>1537.8</td>
</tr>
<tr>
<td>S-Palm-PLP&lt;sub&gt;139-151&lt;/sub&gt;</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;N-HC(Palm)GKLGKWLGHPDKF-COOH</td>
<td>1776.2</td>
</tr>
<tr>
<td>N-Palm-PLP&lt;sub&gt;139-151&lt;/sub&gt;</td>
<td>Palm-HCLGKWLGHPDKF-COOH</td>
<td>1776.2</td>
</tr>
<tr>
<td>PLP&lt;sub&gt;104-99&lt;/sub&gt;</td>
<td>TGTKKLIETYFSKNYQDYEY</td>
<td>2492.6</td>
</tr>
<tr>
<td>PLP&lt;sub&gt;178-191&lt;/sub&gt;</td>
<td>NWTITCQSIAPFSK</td>
<td>1583.7</td>
</tr>
</tbody>
</table>
the nonacylated peptide at the highest peptide concentrations, although at lower peptide concentrations, the response to S-palm-PLP<sub>139–151</sub> was greater than the response to the nonacylated peptide ($p = 0.03$ for peptide concentrations of 4 and 8 nmol/ml and $p = 0.06$ for lower concentrations). The response to the N-palm-PLP<sub>139–151</sub> peptide was significantly less than to either of the other two peptides. The same pattern of reactivity was seen in LNC from mice immunized with S-palm-PLP<sub>139–151</sub> (Fig. 1E), except that the responses to all three peptides were stronger than those in mice immunized with nonacylated PLP<sub>139–151</sub>. The responses to S-palm-PLP<sub>139–151</sub> were significantly greater than the response to nonacylated peptide for concentrations $< 8$ nmol/ml. For LNC from mice immunized with N-palm-PLP<sub>139–151</sub>, the magnitude of the responses was much less than responses of LNC from mice immunized with nonacylated PLP<sub>139–151</sub> or S-palm-PLP<sub>139–151</sub> (Fig. 1F).

**Phenotype of T cells from lipopeptide-immunized mice**

Lipopeptides have been widely used for induction of CD8<sup>+</sup> CTL responses; however, encephalitogenic T cells have invariably been found to be CD4<sup>+</sup> Th cells. Therefore, we investigated whether the T cells from lipopeptide-immunized mice were predominantly of the CD4<sup>+</sup> or CD8<sup>+</sup> phenotype. The CD4/CD8 ratio was measured for T cells taken from mice injected with nonacylated, S-palm, or N-palm peptide 9 days previously. As expected from the proliferative data, mice injected with S-palm peptides showed an increased CD4/CD8 ratio compared with cells from mice injected with nonacylated peptide, suggesting a stronger CD4<sup>+</sup> T cell response. In contrast, T cells taken from mice immunized with either N-palm-PLP<sub>104–117</sub> or N-palm-PLP<sub>139–151</sub> showed a reduction in the CD4/CD8 ratio compared with mice immunized with the corresponding nonacylated peptide or S-palm peptide, suggesting that the N-palm peptides either do not induce as strong a CD4<sup>+</sup> response or skew the response in favor of a CD8<sup>+</sup> T cell response (Table II). After one in vitro stimulation, the percentages of activated CD4<sup>+</sup> T cells specific for nonacylated PLP<sub>139–151</sub> and S-palm-PLP<sub>139–151</sub> were 92% and 95%, respectively. In addition, the percentage of activated T cells of the CD8 phenotype responding to N-palm-PLP<sub>139–151</sub> was substantially higher than the percentage of CD8<sup>+</sup> T cells responding to PLP<sub>139–151</sub> (16.1% vs 5.5%); however, <10% of $\alpha\beta$TCR<sup>+</sup> cells responded to the N-palm peptide, compared with nearly 50% of $\alpha\beta$TCR<sup>+</sup> cells stimulated with the nonacylated peptide. In contrast, after four in vitro stimulations, almost all of the responding cells were CD4<sup>+</sup> for T cells specific for both the nonacylated and N-palm-PLP<sub>139–151</sub> peptides. T cell lines specific for N-palm-PLP<sub>104–117</sub> could not be generated.

**Effect of lipopeptides on induction of Ab responses**

The ability of the thioacylated PLP lipopeptides to affect the production of Ab was investigated. Sera from mice immunized with nonacylated PLP<sub>104–117</sub> make a moderate response to that peptide, but recognize the immobilized S-palm-PLP<sub>104–117</sub> or N-palm-PLP<sub>104–117</sub> peptides very poorly (Fig. 2A). In contrast, sera from mice immunized with S-palm-PLP<sub>104–117</sub> showed a much stronger Ab response to the nonacylated peptide and to the S-palm-PLP<sub>104–117</sub>, but these Abs did not interact with the N-palm peptide (Fig. 2B). Sera from mice immunized with N-palm-PLP<sub>104–117</sub> did not contain Abs specific for any of the peptides (Fig. 2C). The lack of cross-reactivity between any of the sera and N-palm-PLP<sub>104–117</sub> suggests that the Abs against PLP<sub>104–117</sub> may be directed against an N-terminal region of the peptide, and that the attachment of the acyl side chain to this part of the peptide may interfere spatially with Ab/Ag interaction. None of the sera reacted with PLP<sub>40–59</sub>, which was used as a control peptide in each assay (data not shown).

Nonacylated PLP<sub>39–151</sub> induces a strong Ab response to both the nonacylated and S-palm peptides (Fig. 2D). In comparison,
S-palm-PLP139–151 induced Abs with higher titers for the nonacylated and S-palm peptides (Fig. 2E). Abs induced by both the nonacylated and S-palm peptides bound very well to immobilized N-palm-PLP139–151, suggesting either that the palmitic acid attached at the N terminus enhances binding of this peptide to the plate, or that it may act as a spacer between the plate and the peptide, and allow improved binding of the Ab to the peptide. N-palm-PLP139–151 induced a moderate Ab response against itself, but these Abs did not cross-react with nonacylated or S-palm peptide (Fig. 2F). None of the Abs bound nonspecifically to the unrelated control peptide PLP40–59 (data not shown).

**EAE induction with lipopeptides**

The nonacylated and acylated peptides were tested for their ability to induce EAE in vivo (Table III). PLP104–117 is only weakly encephalitogenic in SJL/J mice, and during the 95 days that mice immunized with this peptide were followed, none developed EAE. Histologically, two of these mice had small numbers of inflammatory cells in the meninges, but none in the parenchyma. In contrast, four of four SJL/J mice immunized with S-palm-PLP104–117 developed EAE with an average day of onset of the first attack of EAE of 40.5 days. All of the S-palm-PLP104–117-immunized mice subsequently recovered and developed one or more relapses before they were perfused for histology. Histologically, these mice showed evidence of demyelinated plaque-like lesions in the spinal cord, with some Wallerian degeneration.

PLP139–151 is highly encephalitogenic in SJL/J mice, and four of four mice immunized with PLP139–151 developed EAE (Table III). Mice immunized with the S-palm-PLP139–151 peptide induced EAE with the same incidence and mean day of onset of EAE. The mean clinical score of the mice immunized with S-palm-PLP139–151 was slightly higher than that of mice immunized with the nonacylated peptide, but this difference was not statistically significant (p = 0.06). The most striking feature of EAE induced by S-palm-PLP139–151 peptide was that the duration of the first attack of EAE was significantly longer in the mice immunized with S-palm-PLP139–151 (p = 0.007). In addition, mice immunized with S-palm-PLP139–151 developed a more chronic disease (i.e., more severe disease with more frequent relapses) than did the mice immunized with the nonacylated peptide (Fig. 3). The mean number of CNS-inflammatory lesions was slightly higher in the mice immunized with S-palm-PLP139–151 compared with mice immunized with PLP139–151 (140 ± 48 vs 116 ± 78), reflecting their higher clinical scores. Histologically, no differences in the composition or distribution of the lesions induced by the S-palm-PLP139–151 peptide compared with those induced by the nonacylated PLP139–151 peptide could be detected. A representative lesion from the spinal cord of a mouse immunized with S-palm-PLP139–151 is shown in Fig. 4. As expected from the in vitro proliferation assays, none of the mice immunized with N-palm peptides developed EAE (Table III).

**Discussion**

Posttranslational modifications of myelin proteins have been largely overlooked in studies of the development of autoreactivity to those proteins in demyelinating diseases such as MS; however, recent evidence suggests that they may be important. For example, it has been shown that MS patients accumulate over time a posttranslationally modified form of MBP, citrullinated MBP (24, 25), which appears to induce a stronger immune response than non-citrullinated MBP (26). We now show that another posttranslational modification, namely palmitoylation of peptides of PLP via a thioester linkage, as occurs in the native protein in myelin, can enhance autoimmune responses to PLP in an animal model of MS.

PLP palmitoylation is a dynamic and reversible event, and, although the dynamics of PLP palmitoylation change during development (5), the total percentage of acyl side chains covalently linked to PLP remains remarkably constant at ~3 mol of fatty acids per mol of PLP.

**Table III. Comparison of features of the first episode of EAE induced by nonacylated or palmitoylated PLP peptides**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Incidence</th>
<th>Day of Onset</th>
<th>Score</th>
<th>Duration (days)</th>
</tr>
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<tbody>
<tr>
<td>PLP104–117</td>
<td>0/4</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S-palm-PLP104–117</td>
<td>4/4</td>
<td>40.5 ± 29.9</td>
<td>3.0 ± 0</td>
<td>7.7 ± 0.5</td>
</tr>
<tr>
<td>N-palm-PLP104–117</td>
<td>0/4</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PLP139–151</td>
<td>4/4</td>
<td>11.0 ± 1.4</td>
<td>3.0 ± 0.7</td>
<td>5.8 ± 1.8</td>
</tr>
<tr>
<td>S-palm-PLP139–151</td>
<td>4/4</td>
<td>11.0 ± 0</td>
<td>4.3 ± 0.5</td>
<td>12.8 ± 2.8b</td>
</tr>
<tr>
<td>N-palm-PLP139–151</td>
<td>0/4</td>
<td></td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*The incidence of disease and clinical score for mice immunized with S-palm-PLP104–117 are significantly different from those for mice immunized with PLP104–117 (p < 0.001).*

*The duration of the first episode of disease in mice immunized with S-palm-PLP139–151 is significantly different than that of mice immunized with PLP139–151 (p = 0.007).*

**FIGURE 2.** Ab responses to palmitoylated and nonacylated peptides. Sera were collected from mice immunized with the peptide shown above each graph, and then tested for their ability to interact with nonacylated peptide (●), S-palm peptide (■), or N-palm peptide (▲) immobilized on ELISA plates. Each point is the mean specific absorbance ± SEM of sera (n = 8 for A–C and F; n = 10 for D; n = 6 for E).
acids/mol of protein throughout the life of the animal (6). The physiological role(s) of the acyl groups bound to PLP has not been fully elucidated. Several lines of evidence support the concept that the number of acyl side chains, the conformation of PLP in the myelin membrane, and the stability of the myelin sheath are interrelated, and that changes in one may lead to changes in the others. For example, in adrenoleukodystrophy, another human disease in which there is an inflammatory infiltrate with demyelination in the CNS, there is an increase in the proportion of very long chain fatty acids in PLP that may contribute to the myelin instability characteristic of this disorder (27). In addition, it has been shown that during spontaneous demyelination in transgenic mice carrying multiple copies of cDNA for DM20, the alternatively spliced isoform of PLP, the amount of palmitic acid linked to PLP increases 3-fold (28).

Regardless of whether the demyelination in these cases was a causal factor or consequence of altered palmitoylation of PLP, the fact that PLP is normally thioacylated has important implications for EAE and MS. Although palmitic acid attached to peptide via a stable amide bond can enhance CTL and Ab responses to the peptide (16–19), a recent study using a canine parvovirus model showed that peptides palmitoylated via the much more labile thioester bond enhanced Ab production even more than did N-palm peptides (20). Thus, thioacylation of PLP may have immunological consequences due to the release of S-palm PLP peptides during demyelination and their subsequent enhanced uptake and presentation to cells of the immune system.

The results of the present study confirm that S-palm peptides can induce greater Ab responses than nonthioacylated peptides. They also show that immunization with S-palm PLP lipopeptides can enhance autoimmune CD4+ Th cell reactivity. For S-palm-PLP139–151, this enhancement occurs in both the induction and effector phases. Mice immunized with the S-palm-PLP139–151 show increased responses to all three peptides compared with mice immunized with nonacylated peptide, indicating effects on the induction phase. Furthermore, the proliferative response against lower concentrations of S-palm-PLP139–151 of LNC from mice immunized with either nonacylated or S-palm-PLP139–151 is consistently greater than the response to the nonacylated peptide, indicating that acylation also influences the effector phase.

By contrast, the enhancement in the effector phase is not as clear for S-palm-PLP104–117. If the S-palm peptides enter the cell by endocytic mechanisms involving passage through lysosomes, then it would be expected that the acyl groups would be removed from the peptides by palmitoyl protein thioesterases, which are a major component of the lysosome (29). However, it has recently been shown that small hydrophobic or lipid-containing molecules can enter macrophages via several other pathways (reviewed in Ref. 30), although it is not yet known how organelles involved in these pathways interact with MHC class II-containing compartments. Therefore, the possibility exists that the peptide might bind to MHC class II molecules with the lipid side chain still attached. This could potentially affect recognition of the peptide by T cells. It is known that the threonine residue at position 117 of peptide PLP104–117 is critical for encephalitogenicity in SJL/J mice (10), and thus it would not be expected that palmitoylation of residue Cys108 would directly influence the formation of the trimolecular complex and the recognition of this peptide. However, the bulky fatty acid might induce an altered conformation of the peptide, which could result in S-palm-PLP104–117 acting as a partial agonist. Several previous studies (31, 32) have found that expansion of T cells on a partial agonist can lead to stronger responses against the agonist peptide, similar to the situation we have described in this study, in which LNC from mice immunized with S-palm-PLP104–117 showed an increased response to the nonacylated peptide, but not to S-palm-PLP104–117. Furthermore, there may also be differences in the ability of various types of APC to take up, process, and present this S-palm peptide. In particular, APC in the LNC preparations used in the proliferation assays may not process and present S-palm peptides via the same pathways as APC that process and present the encephalitogenic palmitoylated peptides in vivo.

The importance of the type of linkage between the peptide and the palmitic acid for enhancement of Th cell responses was investigated by comparing N-palm peptides with a stable amide linkage to S-palm peptides, in which there is a labile linkage between peptide and fatty acid. Rather than enhancing the immune responses, the N-palm peptides appeared to have some suppressive effects. A similar suppressive effect has been reported in EAE studies using N-palm peptides of MBP (33–35). N-palm peptides with acid chain formulations containing from one to three palmitic acid.

FIGURE 3. Pattern of disease in mice immunized with PLP139–151 (A) or S-palm-PLP139–151 (B). Each panel represents the clinical course of disease for an individual animal. The duration of the first episode of EAE was significantly increased in mice immunized with S-palm-PLP139–151 compared with those immunized with PLP139–151 (p = 0.007). Mice were followed for up to 95 days, or perfused for histology at an earlier time point (indicated by †) if their clinical signs were severe.

FIGURE 4. Leptomeningeal mononuclear cell infiltrate (arrow) and a large demyelinated plaque-like lesion in the spinal cord of an SJL/J mouse immunized with S-palm-PLP139–151. Intact white matter (white asterisk) is on the right side of the field. Luxol fast blue hematoxylin and eosin. Magnification, ×428.
residues have been used to induce CD8\(^+\) CTL responses in several other systems (16–19, 36–42). Although these N-palmitoyl peptides show similar CTL-enhancing properties, irrespective of the number of palmitic acid residues attached, they appear to exert their effects by different mechanisms (18, 39–42). For peptides palmitoylated by attachment of a single palmitic acid moiety via an amide bond at the N terminus, the lipid tail may facilitate passive translocation of the peptide through the plasma membrane into the cytosol, where it could enter the MHC class I pathway (39, 40). Therefore, it seems likely that the poor immunogenicity of the N-palmitoylated MBP and MBP peptides, and their immunosuppressive effects, may be due to the peptide entering a MHC class I presentation pathway. Because the epitopes used in the studies are MHC class II epitopes that may not bind to MHC class I with high affinity, this might produce no effective response. Alternatively, they may stimulate a weak MHC class I–restricted response in vivo, as suggested by the data in Table II. Such a response might down-regulate the CD4\(^+\) response.

The observation that S-palmitoylated peptides, in contrast to N-palmitoylated peptides, preferentially induce Th cell responses may be useful in the design of peptide-based vaccines. For such usage, the mechanisms by which S-palmitoylated peptides are taken up by APC must be determined. As noted above, it is possible that different types of APC may take up S-palmitoylated peptides by different pathways. Furthermore, it is not yet known whether the acyl side chain needs to be located within the immunogenic epitope or merely in the vicinity of the epitope of interest to induce the immune-enhancing effects. In addition, it has recently been demonstrated that some N-palmitoylated lipopeptides, but not the corresponding nonacylated peptides, can activate macrophages via CD14 (41) or Toll-like receptor 2 (42) pathways, suggesting that the lipid moiety itself can promote interaction with these receptors. It remains to be determined whether S-palmitoylated peptides can also activate APC via pathways of the innate immune system.

Thioacylation might help to explain the dominance of PLP\(_{139–151}\) in demyelinating diseases in SJL/J mice. When SJL/J mice are immunized with an equimolar mixture of nonacylated encephalitogenic peptides PLP\(_{139–151}\) and PLP\(_{178–191}\), responses to both peptides are equally strong (10, 23). By contrast, if EAE is induced by immunization of SJL/J mice with whole spinal cord homogenate, which contains many potential autoantigens (4, 10, 43–45), including PLP with covalently attached fatty acids, the dominant immune response is to PLP\(_{139–151}\) (46). Additionally, if EAE is induced in SJL/J mice with another autoantigen such as MBP, or if these mice are infected with Thielier’s murine encephalomyelitis virus and allowed to recover, PLP\(_{139–151}\) is the first new epitope against which autoreactivity subsequently develops (47, 48). The myelin breakdown products generated as a consequence of tissue injury in EAE and in Thielier’s virus encephalitis would most likely contain PLP with the acyl chains still covalently attached. We postulate that the presence of the thioacyl side chain in these conditions may skew the response toward naturally thioacylated peptides such as PLP\(_{139–151}\). It has also been shown that SJL/J mice naturally have a high precursor frequency of cells potentially responsive to PLP\(_{139–151}\), which appears to be due, in part at least, to the presence of DM-20 (which does not contain the PLP\(_{139–151}\) epitope), but not PLP, in the thymus (49, 50). However, whether a relationship also exists between thioacylation and the development of the repertoire is presently unknown.

PLP is not the only well-characterized autoantigen that is known to be thioacylated. For example, GAD-65, P0, erythrocyte band 3, and rhodopsin, putative autoantigens for insulin-dependent diabetes mellitus, autoimmune neuritis, autoimmune hemolytic anemia, and autoimmune uveoretinitis, respectively, are all thioacylated (51–54). An increased uptake of thioacylated peptide Ags and/or increased activation of APC due to the presence of the thioacyl side chain might result in a greater tendency for autoreactivity to spread to thioacylated Ags. If thioacylated lipopeptides act as natural adjuvants by stimulating APC through receptors of the innate immune system, then it may be that polymorphisms in some of these receptors, e.g., the Toll-like receptors, which are thought to be highly polymorphic in humans (55), could correlate with development of autoreactivity to particular Ags, and may increase the susceptibility of individuals to the development of chronic autoimmune disease. The relevance of the present findings to human disease, particularly MS, remains to be determined.

Acknowledgments

We thank Diane Muller for technical assistance, and Dr. Marjorie Lees for critical reading of the manuscript.

References


