Suppression of Lymphocyte Spontaneous Proliferative Response by Proteolipid Protein Peptide in Patients with HAM/TSP*

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To understand the immune mechanism suggested in HTLV-I-associated myelopathy (HAM/TSP), we investigated T cell response to proteolipid protein (PLP). Because of high autologous proliferative response (APR) of peripheral blood mononuclear cells (PBMC) in culture, the lymphocyte proliferation assay was not useful in this disease. Unexpectedly, however, APR was profoundly (70-98%) suppressed in 6 of 9 cases when PLP peptide 105-124 was added in the culture. PLP peptide 85-104 or 145-159 also suppressed APR in a few cases. Time course study showed that the peptide-mediated suppression became apparent after day 4 in culture. The results can be interpreted as that suppressor cells recognizing the PLP peptides were present in the PBMC of HAM/TSP patients and suppressed the APR as the consequence of antigen specific response. This may indicate that a T cell response to certain PLP determinants is involved in the pathomechanism of HAM/TSP at least in part. Molecular mimicry between PLP and HTLV-I may account for the T cell sensitization to PLP in HAM/TSP.

KEY WORDS: Proteolipid protein; HAM/TSP; T cell; myelin; HTLV-I.

INTRODUCTION

Human T-lymphotropic virus type I (HTLV-I)-associated myelopathy (1)/HTLV-I-associated tropical spastic paraparesis (2) (HAM/TSP) is caused by HTLV-I infection. It is a form of chronic progressive myelitis characterized by infiltration of CD4+ as well as CD8+ T cells and macrophages (3). Neuropathologically, axonal degeneration is predominant but certain demyelinating plaques do occur (4). Since the viral antigen is hardly shown in the cells within central nervous system (CNS), including infiltrating T cells, immune mechanisms are suggested for the CNS pathology (5). This is supported by the fact that there exist immunological abnormalities in HAM/TSP patients, which include the presence of activated T cells in the peripheral blood and cerebrospinal fluid (6), polyclonal B cell activation, and autologous proliferative response (APR) of T cells in vitro (7, 8). In addition, there are female preponderance, HLA association (9, 10), and frequent coexistence with autoimmune diseases such as rheumatoid arthritis, uveitis, Sjögren syndrome, bronchopneumonitis and others. HTLV-I tax transgenic mice developed a condition resembling Sjögren syndrome (11). However, exact immune mechanisms have not been elucidated yet. Therefore, we conducted here a proliferation assay of peripheral blood mononuclear cells (PBMC) against a myelin antigen proteolipid protein (PLP) using synthetic peptides.

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peptides. Results show specific suppression of APR with certain PLP peptides, and suggest that the sensitization of T cells to PLP may be present and play a significant role in HAM/TSP patients.

**EXPERIMENTAL PROCEDURE**

Thirteen patients with HAM/TSP were studied; they were 7 women and 6 men, and their age ranged 35-72 (mean, 60) years. For control, 20 healthy subjects, 10 women and 10 men (mean 31.9 years) were included. From heparinized blood, PBMC were separated by Ficoll density gradient sedimentation, suspended to a final concentration of $2 \times 10^6$ cells/ml in RPMI 1640 with 10% heat-inactivated autologous serum, the cell suspension seeded onto 96-well flat bottom plates at 100 µl/well was cultured at 37°C in a humidified 5% CO$_2$ atmosphere for 6 days, and then, the culture was pulsed with 18.5 KBq of [3H]TdR for 16 hrs. At the start of culture, 40 µg/ml of synthetic PLP peptide 85-104, 95-116, 105-124, 118-139, 130-148, 139-155, or 145-159 (Table I) was added, and thymidine incorporation was measured in a β-counter (Beta-plate 105, Pharmacia). For control, $2 \times 10^6$ cells/ml from healthy subjects were seeded and thymidine incorporation was studied similarly. For the time course study, thymidine incorporation during the last 16 hrs was measured on day 3, 4, and 6. For blocking studies, anti-HLA-DR (clone L234), -DQ (clone SK10), and -DP (clone B7/21) monoclonal antibodies (mAb) were obtained from Becton-Dickinson and an appropriate dose of each mAb (1.5 µg/ml) was added to the culture from the start. To identify the sequence homology between PLP peptide and HTLV-I, a software for computer homology search (GENETYX, Software Development, Tokyo) was used.

**RESULTS**

Nine of the 13 HAM/TSP patients showed significantly high APR (cmp > 20,000). The remaining cases who did not show significant APR had been under treatment with corticosteroids, α-interferon and others. Interestingly, in 6 of the 9 patients, APR was suppressed by one or two of the PLP peptides. APR in the 6 cases was profoundly suppressed in response to PLP peptide 105-124. In addition to PLP 105-124, PLP 85-104 in 2 cases, and PLP 145-159 in another case were inhibitory for APR (Fig. 1). Time course study showed that the peptide-induced inhibition of APR occurred at a later stage between day 4 and day 6 (Fig. 2). When mAbs were added to the culture, anti-HLA-DR significantly suppressed APR, but anti-HLA-DQ and -DP showed only marginal effects. In the presence of anti-HLA-DR mAb, the inhibitory effect of the PLP peptides was no more detectable (Fig. 3).

Among the 4 patients who did not show APR, 2 patients responded to one of the PLP peptides (S.I. > 2.0); one is to PLP 105-124 and the other to PLP 145-159. None of 20 healthy subjects showed APR. A few healthy subjects showed mild proliferative response to the PLP peptide 85-104, 95-116, 105-124, 118-139, 130-148, 139-155, or 145-159.

When a homology search was done between PLP peptides and HTLV-I and II, we found 87.5% homology between PLP105-122 and HTLV-I ENV321-336, 67.7% homology between PLP105-119 and HTLV-I TAT244-258, and 100% homology between PLP105-119 and HTLV-II ENV323-333, if both identical and conserved amino acids were considered as homologous (Table II).

**DISCUSSION**

One of the most characteristic immunological features in HAM/TSP is the spontaneous proliferative re-

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**Table I. Amino Acid Sequence of PLP Peptides Used for Study**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>PLP 85-104</td>
<td>LLAEGFYTTGAVRQIFGDYK</td>
</tr>
<tr>
<td>PLP 95-116</td>
<td>AVRQIFGDYKTTCIGKGLSATV</td>
</tr>
<tr>
<td>PLP 105-124</td>
<td>TTCIGKGLSATVTGGQKGRG</td>
</tr>
<tr>
<td>PLP 118-139</td>
<td>GGGKGRSGRSGHOAHSLSERVCH</td>
</tr>
<tr>
<td>PLP 130-148</td>
<td>QAHSLERV5HSLGKWGLHP</td>
</tr>
<tr>
<td>PLP 139-155</td>
<td>HCLGKWGLHPDKFVVGIT</td>
</tr>
<tr>
<td>PLP 145-159</td>
<td>LGHDPDFKFVGGITYALT</td>
</tr>
</tbody>
</table>

*Serine substituted for cysteine is underlined.