Relationship between the Clinical Efficacy of Pentoxifylline Treatment and Elevation of Serum T Helper Type 2 Cytokine Levels in Patients with Human T-lymphotropic Virus Type I-Associated Myelopathy

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Object Previously, we reported the efficacy of pentoxifylline (PTX) treatment in human T-lymphotropic virus type I (HTLV-I)-associated myelopathy (HAM). Here, we clarify the relationship between the clinical efficacy of PTX treatment and elevation of T helper type 2 (Th2) cytokine levels in HAM patients. Patients and methods PTX (300mg) was administered daily by the oral route to 12 HAM patients for 4 weeks. We assessed the relationship between the changes in neurological status (motor disability scores, the degree of spasticity on neurological examination, and the time required to walk 10 m) and the changes in serum and cerebrospinal fluid (CSF) levels of interferon-γ (IFN-γ) as a Th1 cytokine and interleukin-4 and -10 (IL-4 and -10) as Th2 cytokines measured by an EASIA (enzyme-amplified sensitivity immunoassay) kit. Results PTX treatment induced incremental increases in the levels of IL-4 and IL-10 in both sera and CSF of 6 HAM patients. Clinical improvement was associated with this elevation in IL-4 and IL-10. PTX treatment also induced a decrease in IFN-γ levels in the sera of 6 HAM patients, but this was not correlated with clinical improvement. Conclusion These results suggest that the correction of the immunological imbalance in Th1 to Th2 cytokine responses, with upregulation of IL-4 and IL-10, may account for the clinical improvement in HAM patients treated with PTX.

Key words: human T-lymphotropic virus type I (HTLV-I)-associated myelopathy (HAM), pentoxifylline (PTX), interleukin (IL)-4, IL-10

Introduction

Human T-lymphotropic virus type I (HTLV-I)-associated myelopathy (HAM) is a chronic progressive neurological disorder characterized by bilateral pyramidal tract involvement and sphincteric disturbances (1). Various treatment modalities, including corticosteroids (2), interferon-α (3) and plasmapheresis (4), have proven to be limited or dubious benefit. Recently, we reported the efficacy of pentoxifylline (PTX) treatment in patients with HAM (5). Although PTX downregulates inflammatory cytokine production, such as tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and granulocyte-macrophage colony stimulating factor (GM-CSF), in peripheral blood mononuclear cells of HAM patients (6), the precise mechanisms by which PTX serves to ameliorate the motor disability in HAM are far from clear.

Recent studies indicate that T helper lymphocytes can be divided into two distinct populations based on the patterns of specific cytokines synthesized: T helper type 1 (Th1), which induces cell-mediated immunity, produces interleukin-2 (IL-2) and IFN-γ, whereas T helper type 2 (Th2), which induces humoral immunity, produces interleukin-4 and -10 (IL-4 and IL-10) (7, 8). For differentiation to Th1, IFN-γ and interleukin-12 (IL-12), which is mainly derived from antigen-presenting cells, are vitally important cytokines (9, 10). On the other hand, for differentiation to Th2, IL-4 is required (11). Imbalances between Th1 and Th2 cytokine responses appear to play a significant role in the pathogenesis of autoimmune or inflammatory diseases (12–14). In previous studies, we demonstrated that spontaneous production of inflammatory cytokines, such...
as IFN-γ, TNF-α and GM-CSF, but not IL-4, was simultaneously increased in cultured CD4+ T cells of HAM patients (15). Very recently, we demonstrated that both the serum and cerebrospinal fluid (CSF) level of IFN-γ are elevated in HAM patients with elevated levels of serum IL-12, compared to patients with other neurological diseases including anti-HTLV-I-seropositive carriers (16). These data suggested that the Th1 rather than Th2 cell population was dominant in peripheral blood CD4+ T lymphocytes of HAM patients.

In the present study, we employed a sensitive enzyme immunoassay to measure IFN-γ as a Th1 cytokine, and IL-4 and IL-10 as Th2 cytokines in sera and CSF of HAM patients before and after PTX treatment to ascertain whether changes in Th1 and Th2 cytokine levels could account for the clinical improvement following PTX therapy.

Results

Changes in Th1 and Th2 cytokine levels in sera and CSF after PTX treatment

As shown in Fig. 1A, PTX treatment induced a decremental reduction in serum IFN-γ levels in 6 of 12 HAM patients, whereas IL-4 and IL-10 levels increased in the sera of 6 and 5 HAM patients, respectively, after PTX treatment. Among these patients, IL-4 and IL-10 increased from undetectable to detectable levels in 3 and 4 HAM patients, respectively. On the other hand, as shown in Fig. 1B, IFN-γ levels in CSF decreased in 3 of 7 HAM patients after PTX treatment. Both IL-4 and IL-10 levels in CSF increased in 4 of 7 HAM patients.

Discussion

The present data indicate that the daily oral administration of 300 mg PTX for 4 weeks in HAM patients induced an incremental rise in Th2 (IL-4 and IL-10) cytokine levels in both oligoclonal system in which wells are coated with several monoclonal antibodies directed against distinct epitopes of each cytokine. Sera and CSF and appropriate standards were tested in duplicate. Detection was achieved by 2-hour incubation with horseradish peroxidase-conjugated immunoglobulin G (IgG) against the respective cytokine. After washing, the substrate solution (tetrathymethylbenzidine) was dispensed into each well and incubated for 15 minutes (IFN-γ) or 30 minutes (IL-4 and IL-10). The reaction was stopped with H₂SO₄ and the absorbances were measured using an optical densitometer. Determination of the individual cytokine was performed on all specimens in parallel. Each titer of these cytokines measured in duplicate was averaged. The minimum measurable level of each cytokine was 0.1 IU/ml for IFN-γ, 6.0 pg/ml for IL-4 and 2.8 pg/ml for IL-10.
Figure 1. Changes in (A) serum and (B) CSF levels of Th1 (IFN-γ) and Th2 (IL-4 and IL-10) cytokines before and after PTX treatment in HAM patients. (A) PTX treatment induced a decremental decrease in IFN-γ levels in sera of 6 of 12 patients, whereas IL-4 and IL-10 levels were increased in sera of 6 and 5 HAM patients, respectively, after PTX treatment. (B) IFN-γ levels in CSF were decreased in 3 of 7 HAM patients, while both IL-4 and IL-10 levels in CSF were increased in 4 of 7 HAM patients after PTX treatment. Before: before PTX treatment, After: after PTX treatment. The minimum level of each cytokine was 0.1 IU/ml for IFN-γ, 6.0 pg/ml for IL-4 and 2.8 pg/ml for IL-10, as measured by the enzyme-amplified sensitivity immunoassay.
Table 1. Comparison of the Efficacy of PTX Treatment between Patients with (group A) and without (group B) Elevated Levels of Th2 Cytokines in Sera

<table>
<thead>
<tr>
<th>Group (A)</th>
<th>Age</th>
<th>Sex</th>
<th>Duration of illness (years)</th>
<th>Motor disability score before treatment</th>
<th>Motor disability score after treatment</th>
<th>Improvement of spasticity</th>
<th>Reduction in 10-m walk time (%)</th>
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<td>8</td>
<td>6</td>
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Group (B)

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<th>Sex</th>
<th>Duration of illness (years)</th>
<th>Motor disability score before treatment</th>
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<tr>
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<tr>
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<td>19</td>
<td>8</td>
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</table>

a: clinically graded from 0 to 10.
b: time to walk 10 m before PTX treatment – time to walk 10 m after PTX treatment × 100 (%)c: became ambulatory with assistance after PTX treatment, but was unable to walk before PTX treatment.  
d: This case originally had no spasticity.


