INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Blockade of IL-2 Receptor Suppresses HTLV-I and IFN-γ Expression in Patients with HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis

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Abstract

Objective Th1 activation based on a high HTLV-I proviral load is one of the characteristic immunological abnormalities in the peripheral blood lymphocytes of patients with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). To clarify the cause of this abnormality with the potential to be one of the therapeutic targets, we analyzed the involvement of interleukin-2 (IL-2)/IL-2 receptor (IL-2R) signaling in HTLV-I and interferon-γ (IFN-γ), which is a representative Th1 cytokine, expression in peripheral blood $CD4^+$ T cells from HAM/TSP patients.

Patients and Methods Twelve patients with HAM/TSP were included in the study. After the peripheral blood $CD4^+$ T cells were treated in cultures under the presence of each anti-IL-2Rα, β,and γ blocking antiboby for 48 hours, both HTLV-I p19 antigen and IFN-γ levels in the culture supernatants were measured using ELISA methods. To check the influence on cell proliferation under these culture conditions, the numbers of viable cells were simultaneously determined by MTS assay.

Results Treatment with anti-IL-2Rα blocking antibody, but not anti-IL-2Rβ or anti-IL-2Rγ blocking antibody, suppressed HTLV-I p19 antigen expression levels. In addition, treatment with all types of anti-IL-2R blocking antibodies also suppressed IFN-γ expression levels. All of the types of anti-IL-2R blocking antibodies did not inhibit the proliferation.

Conclusion These results indicate that IL-2/IL-2R signaling is involved in HTLV-I and IFN-γ expression on peripheral blood $CD4^+$ T cells from HAM/TSP patients, suggesting that the interruption of this signaling has therapeutic potential against HAM/TSP in patients with the focus on the down-regulation of Th1 activation based on a high HTLV-I proviral load in the peripheral blood.

Key words: HAM/TSP, IL-2, IL-2 receptor, HTLV-I, Th1, IFN-γ

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Introduction

Human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is chronic progressive myelopathy characterized by bilateral pyramidal tract involvement and sphincteric disturbances (1). The main pathological feature of HAM/TSP is chronic inflammation of the spinal cord (2); however, it remains unclear why such chronic inflammation is induced in only a small proportion of HTLV-I-infected individuals.

Numerous studies have revealed immunological abnormalities related to a high HTLV-I proviral load in peripheral blood lymphocytes from HAM/TSP patients (3). HTLV-I preferentially infects $CD4^+$ T cells (4) and interleukin-2 receptors (IL-2R) are expressed on the surfaces of HTLV-I-infected $CD4^+$ T cells through the transactivation by HTLV-I tax (5). Although increased spontaneous peripheral blood lymphocyte (PBL) proliferation seems to be one of the most characteristic immunological abnormalities in PBL of HAM/

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TSP patients (6), this phenomenon relies on the activation of the autocrine loop of IL-2/IL-2R (6, 7) and IL-15/IL-15R (8). We previously proposed the importance of Th1 immune activation based on a high HTLV-I proviral load in the development of HAM/TSP (9) and more recently demonstrated that activation of the p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway may involve both Th1 activation and a high HTLV-I proviral load in HAM/TSP patients (10). Although it remains unclear why p38 MAPK signaling is activated in HAM/TSP patients, p38 MAPK signaling is one of the downstream targets of IL-2 signaling (11, 12). Therefore, the activation of IL-2/IL-2R signaling might involve the activation of p38 MAPK signaling and then subsequently induce Th1 activation with the high HTLV-I proviral load observed in HAM/TSP patients. If so, interruption of IL-2/IL-2R signaling might be a therapeutic strategy for HAM/TSP. From these viewpoints, we focused our analysis on the involvement of IL-2/IL-2R signaling in HTLV-I and interferon-γ (IFN-γ) expression in peripheral blood $CD4^+$ T cells from HAM/TSP patients.

Patients and Methods

Patients

Twelve patients with HAM/TSP (4 men and 8 women; mean age, 57.2 years; age range, 33-76 years) were included in the study. HAM/TSP diagnosis was based on previously described criteria (13). None of the patients had been treated with immunomodulatory drugs, including corticosteroids and interferon-α. The study protocol was explained in detail and signed informed consent was obtained from each subject before study participation. This research was approved by the institutional review boards of the authors' institutions.

Separation of peripheral blood $CD4^+$ T cells

Peripheral blood $CD4^+$-enriched T cells were separated, in negative selection, by the depletion of $CD8^+$ T cells from macrophage/B cell-depleted mononuclear cells using magnetic beads coated with anti-CD8 monoclonal antibody (Dynal Biotech ASA, Oslo, Norway), from peripheral blood mononuclear cells as described previously (10). $CD4^+$-enriched T cells were used as $CD4^+$ T cells. Analysis of the $CD4^+$ T cells using anti-CD4, anti-CD20, and anti-CD14 monoclonal antibodies showed that they were more than 90% pure with the presence of less than 1% B cells, and less than 1% monocytes as assessed by flow cytometry (Epics XL system II, Beckman-Coulter Electronics, Fullerton, CA).

Treatment of peripheral blood $CD4^+$ T cells with anti-IL-2Rα, β, and γ blocking antibody

To determine the appropriate concentration of anti-IL-2R blocking antibodies (R & D Systems, Minneapolis, MN) for the suppression of HTLV-I p19 antigen and IFN-γ expression, an IL-2-dependent HTLV-I-producing T cell line derived from a HAM/TSP patient (HCT-1) was treated with different concentrations of anti-IL-2R blocking antibodies, and the levels of HTLV-I p19 antigen and IFN-γ in each culture supernatant were determined (data not shown). Thereafter, peripheral blood $CD4^+$ T cells were cultured (1×10^6/ml) in the presence of 10 μg/ml of each anti-IL-2Rα (clone 22722), β (clone 27302), and γ (clone 38024) blocking antibody in RPMI 1640 supplemented with 20% fetal bovine serum (FBS) in 24-well culture plates for 48 hours. The culture medium was centrifuged (2,500 rpm, 10 min) and the supernatant was stored at -40℃ until use.

Measurement of HTLV-I p19 antigen and IFN-γ levels in culture supernatants

The level of HTLV-I p19 antigen in the culture supernatants was measured using the RETROtek HTLV p19 antigen enzyme-linked immunosorbent assay (ELISA) kit using the manufacturer's instructions (Zeptometrix Corporation, Buffalo, NY). The amount of HTLV-I p19 antigen was determined in duplicate. The minimum measurable level of HTLV-I p19 antigen was 25.0 pg/ml. The IFN-γ level in the culture supernatants was measured using an enzyme-amplified sensitivity immunoassay (EASIA) kit according to the manufacturer's instructions (Medgenix, Fleurus, Belgium). The amount of IFN-γ was determined in duplicate. The minimum measurable level of IFN-γ was 0.03 IU/ml.

MTS assay

When peripheral blood $CD4^+$ T cells were cultured in the presence of each anti-IL-2Rα, β, and γ blocking antibody to collect the culture supernatants for the assay of HTLV-I p19 antigen and IFN-γ levels, the number of viable cells was simultaneously determined by a modified MTT assay as described previously (10). Briefly, after peripheral blood $CD4^+$ T cells (1×10^5 cells/well) were cultured in the presence of 10 μg/ml of each anti-IL-2R antibody in 96-well flat-bottom plates for 48 hours, assays were performed by a nonradioactive cell proliferation method (Promega, Madison, WI). Cultures were studied in triplicate and the results were expressed as the mean optical density (OD).

Statistical analysis

The Wilcoxon-signed rank test was used for statistical analysis. Differences were considered statistically significant at $p<0.05$.

Results

Effect of anti-IL-2R blocking antibody on HTLV-I expression on peripheral blood $CD4^+$ T cells from HAM/TSP patients

As shown in Fig. 1a, the treatment of peripheral blood $CD4^+$ T cells from HAM/TSP patients with anti-IL-2Rα, but not anti-IL-2Rβ or anti-IL-2Rγ blocking antibody, signifi-

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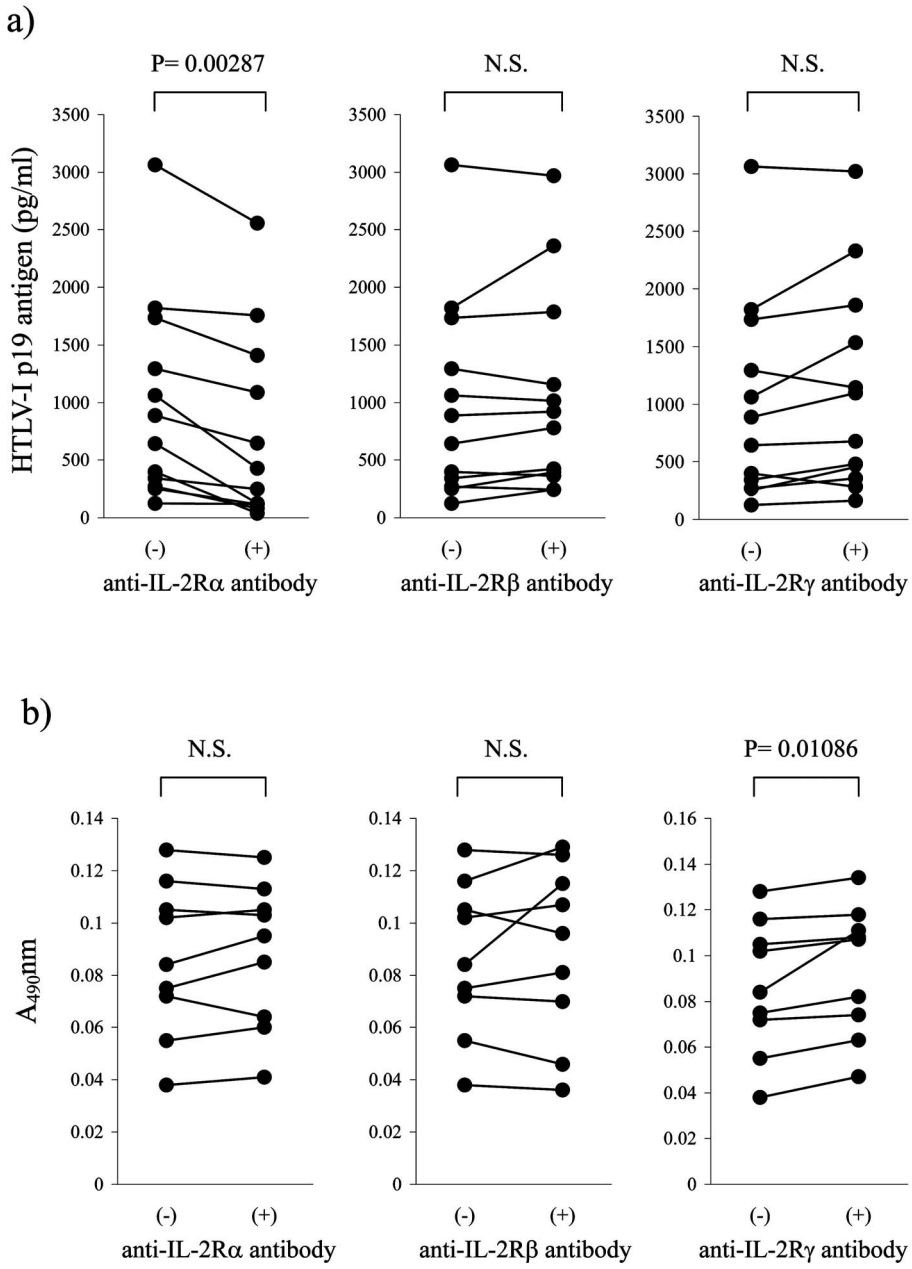

Figure 1. **Anti-IL-2Rα blocking antibody, but not anti-IL-2Rβ or γ blocking antibody, suppresses HTLV-I p19 antigen expression in peripheral blood CD4⁺ T cells from HAM/TSP patients. a) Peripheral blood CD4⁺ T cells were cultured (1 × 10⁶ /ml) in the absence or presence of 10 μg/ml of each anti-IL-2Rα, β, or γ blocking antibody for 48 hours, and culture supernatants were collected. Levels of HTLV-I p19 antigen in each culture supernatant were measured by ELISA (n = 12). N.S.: not significant. b) Cell viability assays (n = 9). Peripheral blood CD4⁺ T cells from HAM/TSP patients were cultured at 1 × 10⁵ /well, as in a) in 96-well plates. MTS assays were performed according to the manufacturer's instructions. Data were calculated as the mean OD titer in triplicate cultures at a wavelength of 490 nm. N.S.: not significant.**

cantly suppressed HTLV-I p19 antigen expression in peripheral blood CD4⁺ T cells of all HAM/TSP patients by about 2-90%. The IL-2/IL-2R autocrine loop is considered to be one of the factors responsible for spontaneous proliferation in PBL in HAM/TSP patients (6, 7). Thus, to confirm that the anti-IL-2Rα blocking antibody-induced suppression of HTLV-I p19 antigen expression was not dependent on the inhibition of cell proliferation or on cytotoxicity by each anti-IL-2R blocking antibody, we checked the changes in cell viability of peripheral blood CD4⁺ T cells treated with each anti-IL-2R blocking antibody in MTS assays as indicators of cell proliferation. Not all the anti-IL-2R blocking an-

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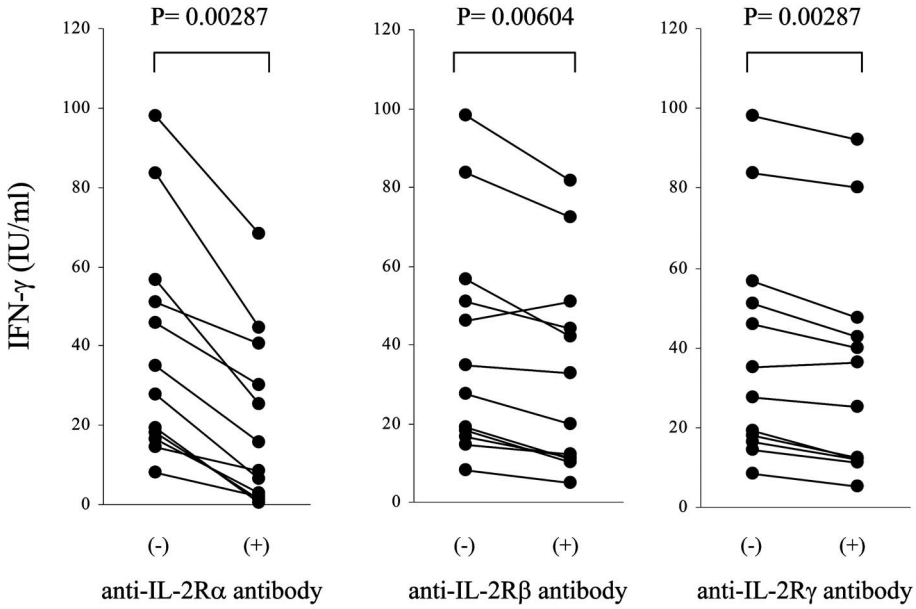

Figure 2. **All types of anti-IL-2R blocking antibody suppress IFN-γ expression in peripheral blood CD4⁺ T cells from HAM/TSP patients. Peripheral blood CD4⁺ T cells were cultured (1 × 10⁶ /ml) in the absence or presence of 10 μg/ml of each anti-IL-2Rα, β, or γ blocking antibody for 48 hours, and culture supernatants were collected. IFN-γ levels in each culture supernatant were measured by ELISA (n = 12).**

tibodies suppressed cell viability in our experiment system, such as 48-hour culture (Fig. 1b), indicating that treatment with these antibodies did not inhibit cell proliferation.

Effect of anti-IL-2R blocking antibody on IFN-γ expression on peripheral blood CD4⁺ T cells from HAM/TSP patients

In the case of IFN-γ expression, the treatment of peripheral blood CD4⁺ T cells from HAM/TSP patients with anti-IL-2Rα antibody significantly suppressed IFN-γ expression in peripheral blood CD4⁺ T cells of all HAM/TSP patients by about 21-99% in the same manner as the effect on HTLV-I p19 antigen expression (Fig. 2). In addition, the treatment of peripheral blood CD4⁺ T cells from HAM/TSP patients with anti-IL-2Rβ or anti-IL-2Rγ blocking antibody also significantly suppressed IFN-γ expression in peripheral blood CD4⁺ T cells of all HAM/TSP patients (Fig. 2). As shown in Fig. 1b, these suppressive effects were not dependent on the inhibition of cell proliferation or on cytotoxicity by each anti-IL-2R blocking antibody. There was no discernible relationship between the suppressive effect for HTLV-I p19 antigen and IFN-γ expression by the treatment with anti-IL-2Rα antibody and the duration or severity of illness, or the serum level of anti-HTLV-I antibody titer.

Discussion

In this report, we showed that the blockade of IL-2/IL-2R by anti-IL-2R blocking antibody, particularly anti-IL-2Rα blocking antibody, suppressed HTLV-I p19 antigen and IFN-γ expression in peripheral blood CD4⁺ T cells from HAM/TSP patients. IL-2/IL-2R and IL-15/IL-15R signaling, which is closely related to IL-2/IL-2R signaling, as an autocrine loop, was previously proposed as one of the most characteristic immunological abnormalities in HAM/TSP patients, such as spontaneous PBL proliferation (6). Indeed, although spontaneous PBL proliferation is an *in vitro* phenomenon, the disruption of IL-2/IL-2R or IL-15/IL-15R signaling might be an important target when considering therapeutic strategies for HAM/TSP patients (7, 8). Here, we have demonstrated that IL-2/IL-2R signaling is also an important signaling pathway for HTLV-I and IFN-γ expression in peripheral blood CD4⁺ T cells from HAM/TSP patients, suggesting that IL-2/IL-2R signaling involves Th1 activation based on high HTLV-I proviral load observed in PBL of HAM/TSP patients although a comparative study between HAM/TSP patients and other HTLV-I- infected individuals was not performed. In this study, although HTLV-I expression in peripheral blood CD4⁺ T cells of HAM/TSP patients was suppressed by treatment with anti-IL-2Rα blocking antibody, but not by anti-IL-2Rβ or IL-2Rγ blocking antibody, IFN-γ expression in peripheral blood CD4⁺ T cells of HAM/TSP patients was suppressed by treatment with not only anti-IL-2Rα blocking antibody but also anti-IL-2Rβ or IL-2Rγ blocking antibody. The reason for these discrepancies is unknown. However, the suppressive effect for IFN-γ expression seemed more significant in the treatment with anti-IL-2Rα blocking antibody than with anti-IL-2Rβ or IL-2Rγ blocking antibody. Therefore, these phenomena may be based on the fact that the IL-2Rα chain is a molecule involved in the initial stage of IL-2 binding in IL-2/IL-2R signal transduction

(12).

Lehky et al previously reported that treatment with humanized anti-Tac (IL-2R) for HAM/TSP patients induces the decrease of HTLV-I proviral load in PBL (14). Although the exact mechanism of how the reduction of HTLV-I proviral load is induced is unclear, this treatment might inhibit the new infection of non-HTLV-I-infected cells from HTLV-I-infected cells through the down-regulation of HTLV-I expression by the blockade of IL-2/IL-2R signaling *in vivo*, just as in our *in vitro* results, or alternatively induce apoptosis of HTLV-I-infected cells by the long-term inhibition of IL-2/IL-2R signaling *in vivo*.

In conclusion, we have demonstrated the involvement of IL-2/IL-2R signaling in HTLV-I and IFN-γ expression in peripheral blood $CD4^+$ T cells from HAM/TSP patients. The down-regulation of both HTLV-I expression and Th1 activation is an important condition when considering HAM/TSP treatment. Therefore, the IL-2/IL-2R signaling pathway may be a potential target in therapeutic strategies for HAM/TSP.

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