intracellular components as a buffer against endogenous ROS by detoxifying H_2O_2. Relationship between oxidative modifications of signaling proteins and apoptosis has been also suggested [31]. Consequently, glutathione depletion by carnosol may increase oxidative damage to the proteins, triggering apoptotic signaling. A possibility is the oxidative damage to mitochondria [29]. Another possibility is the thioredoxin system, which functions as ROS scavenger like glutathione. Apoptosis signal-regulating kinase 1 (ASK1) forms the complex with thioredoxin under the normal condition [32, 33]. When thioredoxin is oxidized by ROS, ASK1 dissociates from the complex and then apoptotic signaling occurs. The increase of endogenous ROS by carnosol may be associated with the activation of apoptosis signal by ASK1. Because thioredoxin reductase 1 regulates redox status of thioredoxin, the increase of its expression in our experiments may reflect this hypothesis.

In several in vitro and in vivo experimental systems, carnosol has been reported to increase the level of glutathione and activity of glutathione-s-transferase (GST) that catalyzes the glutathione conjugation as one of the phase II enzymes [34–37]. Although the stimulation of glutathione metabolism by carnosol is conflicted with its depletion in our experimental system, several possibilities for the underlying mechanism of this confliction are considered. The first is the treatment conditions of carnosol. For example, when neuronal HT22 cells are treated with 5 μM carnosol for 24 h, the expression of phase II enzymes such as glutathione synthesis enzymes and several GST family enzymes is induced through Keap1/Nrf2 pathway [37]. In contrast, we used the eightfold higher concentration of carnosol (40 μM) and glutathione depletion was seen on the treatment for only 3 h. The second is the cell species. While carnosol has been reported to increase the glutathione level and GST activity as antioxidant in HT22 cells, hepatic cell, and rat liver [34–37], it also shows a large variety of action mechanisms against a number of different cell lines and cancer animal models [27]. Collectively, it is implied that concentrations and treatment times of carnosol and characters of cell lines contribute to the superiority of Keap1/Nrf2 and apoptosis pathways. To confirm this, examining the GST activity and expression of glutathione metabolism-related proteins in carnosol-treated ATL cells may be necessary. In addition, GST family enzymes have been also noticed as interesting target molecules for cancer therapy [38–40], supporting the importance of GST assay using not only ATL cell lines but also animal xenograft models of ATL.

Investigation into the action mechanism of carnosol in ATL cells may lead to the development of new therapeutic and preventive strategies for ATL. Studies using ATL cell lines and animal models are in progress, focusing on the target molecule of carnosol, the protein oxidization caused by glutathione depletion, and GST family enzymes.

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Conflict of interest None.

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Brief Report

Treatment with anti-tumor necrosis factor biologics in human T-lymphotropic virus type 1 positive patients with rheumatoid arthritis

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Abstract

Objectives: To investigate the response to and safety of anti-tumor necrosis factor (TNF) therapy in human T-lymphotropic virus type 1 (HTLV-1) positive patients with rheumatoid arthritis (RA).

Methods: Therapeutic response was evaluated in 10 HTLV-1 positive and 20 negative patients with RA (sex- and age- matched) at three months after the beginning of anti-TNF therapy using the European League Against Rheumatism improvement criteria. As secondary endpoints, discontinuation rate of anti-TNF therapy and safety, especially the development of adult T-cell leukemia (ATL), were evaluated over a 2-year period.

Results: Significantly higher baseline levels of C-reactive protein (CRP) were observed in HTLV-1 positive patients than in HTLV-1 negative patients (P= 0.0003). Response rate to anti-TNF therapy was lower in HTLV-1 positive patients than in HTLV-1 negative patients. The median levels of CRP, erythrocyte sedimentation rate, and DAS28 at 3 months after anti-TNF treatment in HTLV-1 positive patients were significantly higher than those in HTLV-1 negative patients (P= 0.003, P= 0.03 and P= 0.003, respectively). Discontinuation rate due to insufficient response was

significantly higher in HTLV-1 positive patients than in HTLV-1negative patients (P= 0.013). During the 2-year observation period, no patients developed ATL.

Conclusion: These data suggested that HTLV-1 positive patients with RA had higher inflammation and greater resistance to anti-TNF treatment than HTLV-1 negative patients. Further study is necessary to determine whether HTLV-1 infection should be measured when anti-TNF agents are administrated to patients with RA, especially in endemic areas.

Significance and Innovations

- It is important to know whether pre-existing infection may influence the effect of treatment with biologics in rheumatoid arthritis (RA). To date, however, few studies have been published on this point.
- We hypothesized that human T-lymphotropic virus type 1 (HTLV-1), which has been known to modify the function of T-cells, would influence the effect of anti-tumor necrosis factor (TNF) biologics.
- The present study showed that inflammatory markers were higher in HTLV-1 positive patients with RA than in HTLV-1 negative patients. Moreover, anti-TNF biologics revealed lower efficacy in HTLV-1 positive patients than in HTLV-1 negative patients.
- These results raised the important question of whether we should test HTLV-1 when we begin anti-TNF treatment in HTLV-1 endemic areas.

Introduction

Rheumatoid arthritis (RA) is characterized by systemic inflammation with proliferation of synovial cells and destruction of joint bone. The effectiveness of biologics, which target proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL) -1 and IL-6, has revolutionized the treatment of RA; however, reports have noted a less efficient response to biologics in approximately 30 percent of patients with RA (1). Patients with advanced and active RA tended to be resistant to biologics; however, the mechanism remains unclear.

Human T-lymphotropic virus type 1 (HTLV-1) is a causative agent of adult T-cell leukemia (ATL). The number of HTLV-1 carriers within the global population is estimated at 20 million. HTLV-1 is endemic in Japan, and a recent study reported the number of carriers to be one million (2). Chronic inflammatory diseases such as myelopathy, uveitis, Sjögren syndrome, arthritis, brocho-alveolitis, and polymyositis have been reported to be related to HTLV-1 infection (3,4). A study in Nagasaki, Japan showed the HTLV-1 positive rate in patients with RA to be higher than in blood donors (5). There have been reports of HTLV-1-associated arthropathy,

which had unique clinical characteristics such as the involvement of large joints, marked inflammation, and extra-articular symptoms (4,6). Recently, we reported two HTLV-1 positive patients with RA treated with anti-TNF agents showing lower effectiveness (7).

These data suggested the possibility that RA patients with HTLV-1 infection may have clinical features and responses to anti-rheumatic treatment that differ from HTLV-1 negative RA patients. Therefore, we performed a small retrospective study to evaluate the clinical response of 10 HTLV-1 positive and 20 negative RA patients treated with anti-TNF agents. Moreover, as secondary endpoints, discontinuation rate of anti-TNF therapy and its safety (development of HTLV-1 associated diseases, especially ATL) were also evaluated over a 2-year period.

Patients and methods

Patients

We retrospectively evaluated 124 Japanese patients with RA, who were treated with one of the following anti-TNF therapies as first biologic agents: infliximab (IFX), etanercept (ETN), or adalimumab (ADA). The initial diagnosis of RA was based on the 1987 diagnostic criteria of the American College of Rheumatology (ACR). Three of the patients were already known to be positive for HTLV-1 antibody before the beginning of anti-TNF treatment. Serum samples from the other 121 patients were tested for HTLV-1 antibody using Lumipulse HTLV-1 (FUJIREBIO INC. Tokyo, Japan) after obtaining informed consent, and seven of these tested positive. Therefore, a total of ten patients with RA were positive for HTLV-1 antibody. Then, two age (within 5 years) and anti-TNF agent matched HTLV-1 negative patients were selected for each HTLV-1 positive patient as controls in this cohort. Therefore, ten HTLV-1 positive and 20 HTLV-1 negative patients with RA were subjects of this study. The study protocol was approved by the institutional review board of University of Miyazaki.

The characteristics of these patients before anti-TNF therapy are

summarized in the Table. All patients were female. Only one HTLV-1 positive patient was negative for anti-citrullinated protein antibody (ACPA); however, she had poly-arthritis, and X-ray showed progressive bone erosion. Her clinical features fulfilled 1987 ACR criteria. In HTLV-1 positive patients, the anti-TNF agents, IFX, ETN, and ADA were administrated in three, six, and one patient, respectively.

Methods

Differences in background characteristics and clinical outcomes after anti-TNF treatment were evaluated between HTLV-1 positive and negative patients. The European League Against Rheumatism (EULAR) improvement criteria were used to evaluate clinical responses and disease activity. The patients were categorized into high, moderate, and low disease activity, and remission when the disease activity scores in 28 joints (DAS28) calculated by erythrocyte sedimentation rate (ESR) were >5.1, 3.2 to 5.1, 2.6 to 3.2, and <2.6, respectively. At three months after the beginning of anti-TNF therapy, DAS28 scores were evaluated and categorized into good, moderate or no-responders based on changes in DAS28 and the level of

DAS28 reached. Good responders were defined as patients who had a decrease in DAS28 from a baseline (ΔDAS28) of >1.2 and a DAS28 at 3 months of < 3.2; moderate responders had either a ΔDAS28 of >1.2 and a DAS28 at 3 months of ≧3.2 or a ΔDAS28 of 0.6 to 1.2 and a DAS28 at 3 months of < 5.1; and non-responders were those who had either a ΔDAS28 of < 0.6 or a DAS28 at 3 months of ≧5.1.

As secondary endpoints, discontinuation rate of anti-TNF therapy and safety (development of HTLV-1 associated diseases, especially ATL) were also evaluated during the 2-year period.

Statistical analysis

Results are expressed as median with interquartile range (IQR). A nonparametric test (Mann–Whitney U test) was used to compare disease activity markers, such as C-reactive protein (CRP), elevated ESR, tender joint counts in 28 joints (TJC28), swollen joint counts in 28 joints (SJC28), and DAS28 between HTLV-1 positive and negative patients with RA at baseline and after anti-TNF treatment. Fisher's exact test was used to compare the positive rates of rheumatoid factor (RF), those of ACPA, and

frequency of prednisolone (PSL) and methotrexate (MTX) use between HTLV-1 positive and negative patients. A nonparametric test (Wilcoxon sign rank test) was also used to compare change in disease activity markers before and after anti-TNF therapy. Log-rank test was used to compare the difference of the continuation periods of anti-TNF treatment between HTLV-1 positive and negative patients. P values of less than 0.05 were considered statistically significant. Data were analyzed by GraphPad Prism 5 for windows version 5.04 (GraphPad Software Inc., USA.).

Results

Background characteristics of patients prior to anti-TNF therapy (Table)

The level of serum CRP was higher in HTLV-1 positive patients than in HTLV-1 negative patients (median [IQR] 4.1 [4.2] vs. 0.7 [1.3] mg/dl, p= 0.0003). TJC28, SJC28 and DAS28 did not differ between HTLV-1 positive and negative patients. There were no differences in disease activity, including low disease activity/remission rate according to EULAR improvement criteria between HTLV-1 positive and negative patients.

The efficacy of anti-TNF treatment

The efficacy of anti-TNF treatment was assessed at 3 months after the beginning of treatment (Figure 1). The rate of good response in HTLV-1 positive patients was lower than that in HTLV-1 negative patients (10 % vs. 50 %) and the rate of no response in HTLV-1 positive patients was higher than that in HTLV-1 negative patients (30 % vs. 5 %) (Figure 1A). The rate of low disease activity/ remission in HTLV-1 negative patients was higher than that in HTLV-1 positive patients (50 % vs. 10 %) (Figure 1B).

The levels of CRP, ESR and DAS28 at 3 months after anti-TNF therapy were significantly decreased in HTLV-1 negative patients (median [IQR] 0.7

[1.3] vs. 0.1 [0.3] mg/dl, P= 0.0002, median [IQR] 65.0 [34.5] vs. 34.5 [25.7] mm/60min, P= 0.0004, and median [IQR] 5.2 [0.8] vs. 3.2 [0.8], P< 0.0001, respectively). On the other hand, in HTLV-1 positive patients, the levels of CRP and ESR at 3 months after anti-TNF therapy were lower than those before therapy (median [IQR] 4.1 [4.2] vs. 1.3 [3.4] mg/dl, P= 0.0645 and median [IQR] 74.5 [37.5] vs. 62.0 [44.5] mm/60min, P= 0.425, respectively); however, they did not reach statistical significance. DAS28 in HTLV-1 positive patients after the therapy was significantly lower than that before treatment (median [IQR] 5.8 [0.8] vs. 4.4 [1.1], P= 0.0137). The median levels of CRP, ESR, and DAS28 at 3 months after anti-TNF treatment in HTLV-1 positive patients were significantly higher than those in HTLV-1 negative patients (P= 0.003, P= 0.03 and P= 0.003, respectively) (Figure 1C).

During the 2-year observation period, anti -TNF therapy was discontinued in 6 HTLV-1 positive patients (2 cases due to adverse reaction and 4 cases due to lack of efficacy). On the other hand, anti -TNF therapy was discontinued only in 3 HTLV-1 negative patients (2 cases due to adverse reaction and one case due to lack of efficacy). Discontinuation rates due to any reason and due to insufficient effect were significantly higher in HTLV-1

positive patients (60% and 40%) than in HTLV-1 negative patients (15% and 5%) (P= 0.0053 and P= 0.013, respectively) (Figure 2).

Signs, symptoms and laboratory data showed no indication of the development of ATL in HTLV-1 positive patients during the 2-year observation period.

Discussion

The background levels of CRP in HTLV-1 positive patients with RA were higher than those in HTLV-1 negative patients with RA in the present study. HTLV-1 Tax protein has been reported to promote the production of IL-6 (8,9). Production of IL-6 from synovial cells was reported to be up-regulated in HTLV-1 positive patients with osteoarthritis (10). These data suggest that production of IL-6 could be up-regulated by HTLV-1 infection and may account for the high inflammation.

Moderate or better responses have been reported in 70-80% of Japanese patients with RA who received treatment with IFX or ETN (11-13). In the present study, the response rate (moderate or better) to anti-TNF treatment in HTLV-1 negative RA patients was 95%, which is consistent with rates reported in the previous studies. In contrast, in HTLV-1 positive patients, the decrease in CRP and ESR at 3 months after anti-TNF therapy did not reach statistical significance. The levels of CRP, ESR and DAS28 in HTLV-1 positive patients were significantly higher than those in HTLV-1 negative patients. According to EULAR improvement criteria, the rate of low disease activity and remission in HTLV-1 positive patients was much

lower than that in HTLV-1 negative patients (10 % vs. 50 %). Therefore, it is suggested that HTLV-1 positive patients with RA are resistant to anti-TNF therapy.

High serum levels of CRP have been reported as a factor in insufficient RA patient response to anti-TNF treatment (14). Therefore, it is still not clear whether the low response to anti-TNF in HTLV-1 positive patients is due to high inflammation or due to the HTLV-1 positivity itself. To clarify this question, a greater number of HTLV-1 patients with RA must be classified according to CRP level to examine the response to anti-TNF based on level of CRP. It is also not clear whether HTLV-1 positive patients with RA show insufficient response only to anti-TNF agents. Future study to clarify these questions is necessary.

During the 2-year observation period, there were no signs, symptoms, or laboratory data suggesting that HTLV-1 positive patients developed ATL. Viral markers such as HTLV-1 proviral loads or clonality of HTLV-1 infected cells were not measured in this study; however, a previous study of two HTLV-1 positive cases showed no change in these viral markers after receiving anti-TNF agents (7).

This retrospective study has a number of limitations. The number of HTLV-1 positive and negative patients was only 10 and 20, respectively, and, therefore, too small to reach a conclusion about the difference in response to anti-TNF therapy. Because the incidence of ATL among HTLV-1 carriers has been reported as only one out of 1000 person-years, a prospective study including a greater number of HTLV-1 positive patients and with longer observation periods is necessary to clarify the risk of ATL. At the same time, proviral loads and clonality of HTLV-1 infected cells should be measured.

The results of this study raise the question of whether HTLV-1 infection should be measured when anti-TNF agents are administrated in patients with RA, especially in endemic areas. Further study including a greater number of patients with longer periods of observation is necessary to reach a definite conclusion.

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