Ⅳ. 研究成果の刊行物・別刷（抜粋）

Arthritis Care & Research
Vol. 66, No. 5, May 2014, pp 788–792
DOI 10.1002/acr.22205

BRIEF REPORT

Treatment With Anti–Tumor Necrosis Factor Biologic Agents in Human T Lymphotropic Virus Type I–Positive Patients With Rheumatoid Arthritis

KUNIHIKO UMEKITA,[1] TOSHIHIKO HIDAKA,[2] SHUNICHI MIYAUCHI,[1] SHIRO UENO,[1] KAZUYOSHI KUBO,[1] ICHIRO TAKAJO,[1] YAYOI HASHIBA,[2] YASUFUMI KAI,[2] YASUHIRO NAGATOMO,[1] AND AKIHIKO OKAYAMA[1]

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

Methods. **Therapeutic response was evaluated in 10 HTLV-I–positive and 20 HTLV-I–negative patients with RA (sex and age matched) at 3 months after the beginning of anti-TNF therapy using the European League Against Rheumatism improvement criteria. As secondary end points, the discontinuation rate of anti-TNF therapy and its 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-I–positive patients than in HTLV-I–negative patients ($P = 0.0003$). The response rate to anti-TNF therapy was lower in HTLV-I–positive patients than in HTLV-I–negative patients. The median CRP level, erythrocyte sedimentation rate, and Disease Activity Score in 28 joints at 3 months after anti-TNF treatment in HTLV-I–positive patients were significantly higher than in HTLV-I–negative patients ($P = 0.003$, $P = 0.03$, and $P = 0.003$, respectively). The discontinuation rate due to insufficient response was significantly higher in HTLV-I–positive patients than in HTLV-I–negative patients ($P = 0.013$). During the 2-year observation period, no patients developed ATL.**

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

Introduction

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

Human T lymphotropic virus type I (HTLV-I) is a causative agent of adult T cell leukemia (ATL). The number of HTLV-I carriers within the global population is estimated at 20 million. HTLV-I is endemic to Japan, and a recent study reported the number of carriers to be 1 million (2). Chronic inflammatory diseases, such as myelopathy, uveitis, Sjögren's syndrome, arthritis, bronchoalveolitis, and polymyositis, have been reported to be related to HTLV-I infection (3,4). A study in Nagasaki, Japan showed the HTLV-I positive rate in patients with RA was higher than in blood donors (5). There are studies that have reported HTLV-I–associated arthropathy, which has unique clinical

[1]Kunihiko Umekita, MD, PhD (current address: University Hospital Zurich and Zurich Center of Integrative Human Physiology, Zurich, Switzerland), Shunichi Miyauchi, MD, Shiro Ueno, MD, PhD, Kazuyoshi Kubo, MD, Ichiro Takajo, MD, PhD, Yasuhiro Nagatomo, MD, Akihiko Okayama, MD, PhD: University of Miyazaki, Miyazaki, Japan; [2]Toshihiko Hidaka, MD, PhD, Yayoi Hashiba, MD, Yasufumi Kai, MD: Zenjinkai Shimin-no-Mori Hospital, Miyazaki, Japan.

Address correspondence to Kunihiko Umekita, MD, PhD, Division of Rheumatology, Infectious Diseases and Laboratory Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan. E-mail: Kunihiko_Umekita@med.miyazaki-u.ac.jp.

Submitted for publication April 3, 2013; accepted in revised form October 8, 2013.

Significance & Innovations

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

characteristics, such as the involvement of large joints, marked inflammation, and extraarticular symptoms (4,6). Recently, we reported 2 HTLV-I–positive patients with RA treated with anti-TNF agents showing lower effectiveness (7).

These data suggest the possibility that RA patients with HTLV-I infection may have clinical features and responses to antirheumatic treatment that differ from HTLV-I–negative RA patients. Therefore, we performed a small retrospective study to evaluate the clinical response of 10 HTLV-I–positive and 20 HTLV-I–negative RA patients treated with anti-TNF agents. Moreover, as secondary end points, the discontinuation rate of anti-TNF therapy and its safety (development of HTLV-I–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 American College of Rheumatology (ACR) diagnostic criteria (8). It was already known that 3 patients were positive for HTLV-I antibody before the beginning of anti-TNF treatment. Serum samples from the other 121 patients were tested for HTLV-I antibody using Lumipulse HTLV-1 (Fujirebio) after obtaining informed consent, and 7 of these samples tested positive. Therefore, a total of 10 patients with RA were positive for HTLV-I antibody. Subsequently, 2 age- (within 5 years) and anti-TNF agent–matched HTLV-I–negative patients were selected for each HTLV-I–positive patient as controls in this cohort. Therefore, 10 HTLV-I–positive and 20 HTLV-I–negative patients with RA were included in this study. The study protocol was approved by the Institutional Review Board of the University of Miyazaki.

The characteristics of these patients before anti-TNF therapy are shown in Table 1. All of the patients were women. Only 1 HTLV-I–positive patient was negative for

Table 1. Patient characteristics before anti-TNF therapy*

	HTLV-I positive (n = 10)	HTLV-I negative (n = 20)	*P*
Age, median (IQR) years	70.0 (8.5)	68.5 (11.7)	0.98
Disease duration, median (IQR) years	5.0 (5.0)	9.0 (19.5)	0.21
Disease activity markers			
CRP level, median (IQR) mg/dl	4.1 (4.2)	0.7 (1.3)	0.0003
ESR, median (IQR) mm/hour	74.5 (37.5)	65.0 (34.5)	0.15
TJC28, median (IQR)	4.5 (4.3)	4.5 (4.0)	0.72
SJC28, median (IQR)	4.5 (3.8)	2.0 (5.0)	0.35
DAS28, median (IQR)	5.8 (0.8)	5.2 (0.8)	0.18
Disease activity according to EULAR criteria, %			
High disease activity (DAS28 >5.1)	80	70	–
Moderate disease activity (DAS28 3.2–5.1)	20	25	–
Low disease activity (DAS28 <3.2)	0	5	–
Serologic markers, %			
RF positive	80	80	> 0.99
ACPA positive	90	100	0.33
Treatment			
DMARDs, %	33	45	> 0.99
Methotrexate, median (IQR [%]) mg/week	10 (2.0 [50])	8.0 (2.5 [75])	0.23
Prednisolone, median (IQR [%]) mg/day	5.5 (2.0 [90])	3.5 (3.0 [70])	0.37

* Anti-TNF = anti–tumor necrosis factor; HTLV-I = human T lymphotropic virus type I; IQR = interquartile range; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; TJC28 = tender joint count in 28 joints; SJC = swollen joint count in 28 joints; DAS28 = Disease Activity Score in 28 joints; EULAR = European League Against Rheumatism; RF = rheumatoid factor; ACPA = anti–citrullinated protein antibody; DMARDs = disease-modifying antirheumatic drugs.

anti–citrullinated protein antibody (ACPA); however, she had polyarthritis, and radiographs showed progressive bone erosion. Her clinical features fulfilled the 1987 ACR criteria for RA. In HTLV-I–positive patients, the anti-TNF agents IFX, ETN, and ADA were administered in 3 patients, 6 patients, and 1 patient, respectively.

Methods. The differences in background characteristics and clinical outcomes after anti-TNF treatment were evaluated between HTLV-I–positive and HTLV-I–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 disease activity, moderate disease activity, low disease activity, and remission when the Disease Activity Score in 28 joints (DAS28) calculated using the erythrocyte sedimentation rate (ESR) was >5.1, ≥3.2 to ≤5.1, ≥2.6 to <3.2, and <2.6, respectively. At 3 months after the beginning of anti-TNF therapy, DAS28 scores were evaluated and the patients were categorized into good responders, moderate responders, or nonresponders based on changes in the DAS28 and the level of DAS28 reached. Good responders were defined as patients who had a decrease in DAS28 from baseline (ΔDAS28) of >1.2 and a DAS28 at 3 months of <3.2, moderate responders were defined as having either a ΔDAS28 of >1.2 and a DAS28 at 3 months of ≥3.2 or a ΔDAS28 of 0.6–1.2 and a DAS28 at 3 months of ≤5.1, and nonresponders were defined as having either a ΔDAS28 of <0.6 or a DAS28 at 3 months of >5.1.

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

Statistical analysis. The results are expressed as the median with the interquartile range (IQR). A nonparametric test (Mann-Whitney U test) was used to compare disease activity markers, such as C-reactive protein (CRP) level, elevated ESR, tender joint count in 28 joints (TJC28), swollen joint count in 28 joints (SJC28), and DAS28 between HTLV-I–positive and HTLV-I–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, those of ACPA, and frequency of prednisolone and methotrexate use between HTLV-I–positive and HTLV-I–negative patients. A nonparametric test (Wilcoxon's signed rank test) was also used to compare the change in disease activity markers before and after anti-TNF therapy. The log rank test was used to compare the difference of the continuation periods of anti-TNF treatment between HTLV-I–positive and HTLV-I–negative patients. P values less than 0.05 were considered statistically significant. The data were analyzed by GraphPad Prism 5 for Windows, version 5.04.

Results

Background characteristics of patients prior to anti-TNF therapy. The level of serum CRP was higher in HTLV-I–positive patients than in HTLV-I–negative patients (median 4.1 mg/dl [IQR 4.2] versus 0.7 mg/dl [IQR 1.3]; $P = 0.0003$) (Table 1). TJC28, SJC28, and DAS28 did not differ between HTLV-I–positive and HTLV-I–negative patients. There were no differences in disease activity, including low disease activity/remission rate according to the EULAR improvement criteria between HTLV-I–positive and HTLV-I–negative patients.

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-I–positive patients was lower than that in HTLV-I–negative patients (10% versus 50%) and the rate of no response in HTLV-I–positive patients was higher than that in HTLV-I–negative patients (30% versus 5%) (Figure 1A). The rate of low disease activity/remission in HTLV-I–negative patients was higher than that in HTLV-I–positive patients (50% versus 10%) (Figure 1B).

The CRP level, ESR, and DAS28 at 3 months after anti-TNF therapy were significantly decreased in HTLV-I–negative patients (CRP level: median 0.7 mg/dl [IQR 1.3] versus 0.1 mg/dl [IQR 0.3]; $P = 0.0002$, ESR: median 65.0 mm/hour [IQR 34.5] versus 34.5 mm/hour [IQR 25.7]; $P = 0.0004$, and DAS28: median 5.2 [IQR 0.8] versus 3.2 [IQR 0.8]; $P < 0.0001$). Conversely, in HTLV-I–positive patients, the CRP level and ESR at 3 months after anti-TNF therapy were lower than before therapy (CRP level: median 4.1 mg/dl [IQR 4.2] versus 1.3 mg/dl [IQR 3.4]; $P = 0.0645$ and ESR: median 74.5 mm/hour [IQR 37.5] versus 62.0 mm/hour [IQR 44.5]; $P = 0.425$); however, these values did not reach statistical significance. The DAS28 in HTLV-I–positive patients after therapy was significantly lower than before treatment (median 5.8 [IQR 0.8] versus 4.4 [IQR 1.1]; $P = 0.0137$). The median CRP level, ESR, and DAS28 at 3 months after anti-TNF treatment in HTLV-I–positive patients were significantly higher than in HTLV-I–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-I–positive patients (2 cases due to adverse reactions and 4 cases due to lack of efficacy). Conversely, anti-TNF therapy was discontinued in only 3 HTLV-I–negative patients (2 cases due to adverse reactions and 1 case due to lack of efficacy). The discontinuation rates due to any reason and due to an insufficient effect were significantly higher in HTLV-I–positive patients (60% and 40%, respectively) than in HTLV-I–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-I–positive patients during the 2-year observation period.

Discussion

The background levels of CRP in HTLV-I–positive patients with RA were higher than those in HTLV-I–negative patients with RA in the present study. The HTLV-I Tax protein has been reported to promote the production of IL-6 (9,10). Production of IL-6 from synovial cells has been reported to be up-regulated in HTLV-I–positive patients

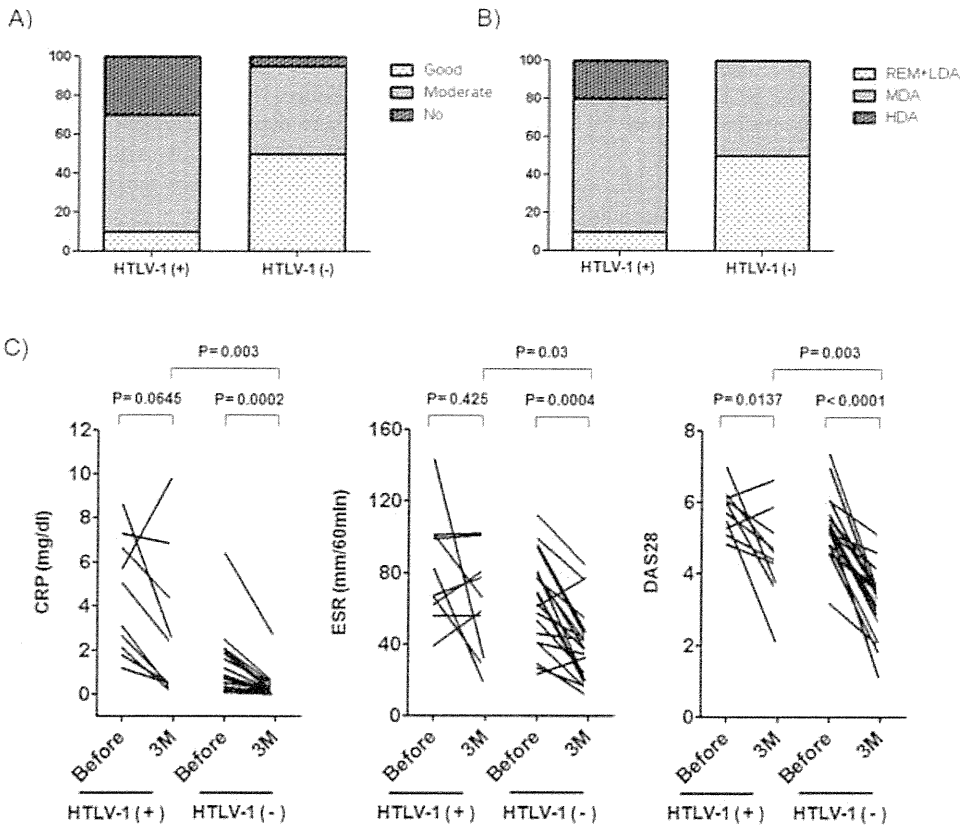


Figure 1. Efficacy of anti–tumor necrosis factor (anti-TNF) therapy 3 months after the beginning of treatment. **A**, Response rate of human T lymphotropic virus type I (HTLV-1)–positive (n = 10) and –negative (n = 20) patients with rheumatoid arthritis (RA) according to the European League Against Rheumatism (EULAR) improvement criteria. **B**, Disease activity of HTLV-1–positive (n = 10) and –negative (n = 20) patients with RA according to the EULAR improvement criteria. **C**, Changes in the C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR), and Disease Activity Score in 28 joints (DAS28) at 3 months after anti-TNF therapy. REM = remission; LDA = low disease activity; MDA = moderate disease activity; HDA = high disease activity.

with osteoarthritis (11). These data suggest that production of IL-6 could be up-regulated by HTLV-I 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 (12–14). In the present study, the response rate (moderate or better) to anti-TNF treatment in HTLV-I–negative RA patients was 95%, which is consistent with the rates reported in previous studies. In contrast, in HTLV-I–positive patients, the decrease in CRP level and ESR at 3 months after anti-TNF therapy did not reach statistical significance. The CRP level, ESR, and DAS28 in HTLV-I–positive patients were significantly higher than in HTLV-I–negative patients. According to the EULAR improvement criteria, the rate of low disease activity and remission in HTLV-I–positive patients was much lower than that in HTLV-I–negative patients (10% versus 50%). Therefore, we suggest that HTLV-I–positive patients with RA are resistant to anti-TNF therapy.

High serum levels of CRP have been reported as a factor in insufficient response to anti-TNF treatment in RA patients (15). Therefore, it is still not clear whether the low response to anti-TNF therapy in HTLV-I–positive patients was due to high inflammation or due to the HTLV-I posi-

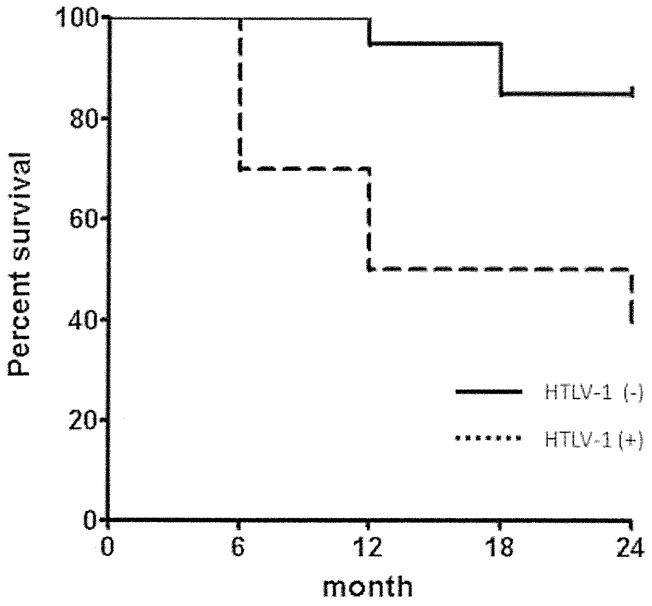


Figure 2. Continuation rate of anti–tumor necrosis factor therapy in human T lymphotropic virus type I (HTLV-1)–positive (n = 10) and –negative (n = 20) patients with rheumatoid arthritis.

tivity itself. To clarify this question, a greater number of HTLV-I patients with RA must be classified according to CRP level to examine the response to anti-TNF therapy based on the level of CRP. It was also not clear whether HTLV-I–positive patients with RA showed insufficient response only to anti-TNF agents. Future studies to clarify these questions are necessary.

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

This retrospective study has a number of limitations. The numbers of HTLV-I–positive and HTLV-I–negative patients were only 10 and 20, respectively, and therefore were too small to reach a conclusion about the difference in response to anti-TNF therapy. Because the incidence of ATL among HTLV-I carriers has been reported as only 1 case per 1,000 person-years, a prospective study including a greater number of HTLV-I–positive patients and with longer observation periods would be necessary to clarify the risk of ATL. At the same time, proviral loads and clonality of HTLV-I–infected cells should be measured.

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

ACKNOWLEDGMENTS

The authors thank Dr. Yoshiaki Ohkuwa, Social Medicine Course Mathematical Principle Science Field, School of Medicine, Faculty of Medicine, University of Miyazaki, for his helpful advice on the statistical analysis. The authors also thank Ms Yuki Kaseda (University of Miyazaki) and Michiyo Kofuku (Zenjinkai Shimin-no-Mori Hospital) for their technical assistance.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Umekita had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Umekita, Hidaka, Miyauchi, Ueno, Kubo, Takajo, Hashiba, Kai, Nagatomo, Okayama.

Acquisition of data. Umekita, Hidaka, Miyauchi, Ueno, Kubo, Takajo, Hashiba, Kai, Nagatomo, Okayama.

Analysis and interpretation of data. Umekita, Hidaka, Miyauchi, Ueno, Kubo, Takajo, Hashiba, Kai, Nagatomo, Okayama.

REFERENCES

1. Moot RJ, Naisbett-Groet B. The efficacy of biologic agents in patients with rheumatoid arthritis and an inadequate response to tumour necrosis factor inhibitors: a systematic review. Rheumatology (Oxford) 2012;51:2252–61.
2. Satake M, Yamaguchi K, Tadokoro K. Current prevalence of HTLV-1 in Japan as determined by screening of blood donors. J Med Virol 2012;84:327–35.
3. Watanabe T. HTLV-1 associated diseases. Int J Hematol 1997; 66:257–78.
4. Nishioka K, Sumida T, Hasunuma T. Human T lymphotropic virus type I in arthropathy and autoimmune disorders. Arthritis Rheum 1996;39:1410–8.
5. Eguchi K, Origuchi T, Takashima H, Iwata K, Katamine S, Nagataki S. High seroprevalence of anti–HTLV-I antibody in rheumatoid arthritis. Arthritis Rheum 1996;39:463–6.
6. Sato K, Maruyama I, Maruyama Y, Kitajima I, Nakajima Y, Higaki M, et al. Arthritis in patients infected with human T lymphotropic virus type I: clinical and immunopathologic features. Arthritis Rheum 1991;34:714–21.
7. Umekita K, Umeki K, Miyauchi S, Ueno S, Kusumoto N, Kubo K, et al. Use of anti-tumor necrosis factor biologics in the treatment of rheumatoid arthritis does not change human T-lymphotropic virus type 1 markers: a case series. Mod Rheumatol 2013. E-pub ahead of print.
8. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
9. Mori N, Shirakawa F, Shimizu H, Murakami S, Oda S, Yamamoto K, et al. Transcriptional regulation of the human interleukin-6 gene promoter in human T-cell leukemia virus type I-infected T-cell lines: evidence for the involvement of NF-κ B. Blood 1994;84:2904–11.
10. Mori N, Shirakawa F, Abe M, Kamo Y, Koyama Y, Murakami S, et al. Human T-cell leukemia virus type I tax transactivates the interleukin-6 gene in human rheumatoid synovial cells. J Rheumatol 1995;22:2049–54.
11. Yoshihara Y, Tsukazaki T, Osaki M, Nakashima M, Hasui K, Shindo H. Altered expression of inflammatory cytokines in primary osteoarthritis by human T lymphotropic virus type I retrovirus infection: a cross-sectional study. Arthritis Res Ther 2004;6:R347–54.
12. Tanaka Y, Takeuchi T, Inoue E, Saito K, Sekiguchi N, Sato E, et al. Retrospective clinical study on the notable efficacy and related factors of infliximab therapy in a rheumatoid arthritis management group in Japan: one-year clinical outcomes (RECONFIRM-2). Mod Rheumatol 2008;18:146–52.
13. Kameda H, Ueki Y, Saito K, Nagaoka S, Hidaka T, Atsumi T, et al. Etanercept (ETN) with methotrexate (MTX) is better than ETN monotherapy in patients with active rheumatoid arthritis despite MTX therapy: a randomized trial. Mod Rheumatol 2010;20:531–8.
14. Kameda H, Kanbe K, Sato E, Ueki Y, Saito K, Nagaoka S, et al. Continuation of methotrexate resulted in better clinical and radiographic outcomes than discontinuation upon starting etanercept in patients with rheumatoid arthritis: 52-week results from the JESMR study. J Rheumatol 2011;38:1585–92.
15. Yamanaka H, Tanaka Y, Sekiguchi N, Inoue E, Saito K, Kameda H, et al. Retrospective clinical study on the notable efficacy and related factors of infliximab therapy in a rheumatoid arthritis management group in Japan (RECONFIRM). Mod Rheumatol 2007;17:28–32.

Plants **2014**, *3*, 18-26; doi:10.3390/plants3010018

OPEN ACCESS

plants

ISSN 2223-7747

www.mdpi.com/journal/plants

Article

Identification of a Bioactive Compound against Adult T-cell Leukaemia from Bitter Gourd Seeds

Hisahiro Kai [1,2,*], Ena Akamatsu [2], Eri Torii [2], Hiroko Kodama [2,3], Chizuko Yukizaki [3], Isao Akagi [2,4], Hisatoshi Ino [5], Yoichi Sakakibara [6], Masahito Suiko [6], Ikuo Yamamoto [7], Akihiko Okayama [7], Kazuhiro Morishita [8], Hiroaki Kataoka [9] and Koji Matsuno [1]

1 Department of Pharmaceutical Health Sciences, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1 Yoshino-machi, Nobeoka, Miyazaki 882-8508, Japan; E-Mail: kjmtsn@phoenix.ac.jp

2 Research Promotion Bureau for Collaboration of Regional Entities, Miyazaki Prefectural Industrial Support Foundation, 16500-2 Higashi-Kaminaka, Sadowara-cho, Miyazaki, Miyazaki 880-0303, Japan; E-Mails: rojopino@mac.com (E.A.); ushio16@fc.miyazaki-u.ac.jp (E.T.); hiroyoko33@hotmail.com (H.K.); akagi046@chem.agri.kagoshima-u.ac.jp (I.A.)

3 Miyazaki Prefectural Food Research and Development Center, 16500-2 Higashi-Kaminaka, Sadowara-cho, Miyazaki, Miyazaki 880-0303, Japan; E-Mail: yukizaki@iri.pref.miyazaki.jp

4 Department of Biochemical Science and Technology, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima, Kagoshima 890-0065, Japan

5 Miyazaki Agricultural Experiment Station, 5805 Shimonaka, Sadowara-cho, Miyazaki, Miyazaki 880-0212, Japan; E-Mail: ino-hisatoshi@pref.miyazaki.lg.jp

6 Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuenkibanadai-nishi, Miyazaki, Miyazaki 889-2192, Japan; E-Mails: ysakaki@cc.miyazaki-u.ac.jp (Y.S.); msuiko@cc.miyazaki-u.ac.jp (M.S.)

7 Department of Rheumatology, Infectious Diseases and Laboratory Medicine, Faculty of Medicine, University of Miyazaki, 5200 Kihara Kiyotake, Miyazaki, Miyazaki 889-1692, Japan; E-Mails: yamamoto@fc.miyazaki-u.ac.jp (I.Y.); okayama@med.miyazaki-u.ac.jp (A.O.)

8 Division of Tumor and Cellular Biochemistry, Department of Medical Sciences, Faculty of Medicine, University of Miyazaki, 5200 Kihara Kiyotake, Miyazaki, Miyazaki 889-1692, Japan; E-Mail: kmorishi@fc.miyazaki-u.ac.jp

9 Section of Oncopathology and Regenerative Biology, Department of Pathology, Faculty of Medicine, University of Miyazaki, 5200 Kihara Kiyotake, Miyazaki, Miyazaki 889-1692, Japan; E-Mail: mejina@med.miyazaki-u.ac.jp

* Author to whom correspondence should be addressed; E-Mail: hkai@phoenix.ac.jp; Tel.: +81-982-23-5704; Fax: +81-982-23-5705.

Received: 31 October 2013; in revised form: 18 December 2013 / Accepted: 19 December 2013 / Published: 27 December 2013

Abstract: In our previous report, an 80% ethanol bitter gourd seed extract (BGSE) was found to suppress proliferation of adult T-cell leukemia (ATL) cell lines. The present study aimed to identify the bioactive compounds from BGSE specific against ATL. From the result of an HPLC-MS analysis, α-eleostearic acid (α-ESA) was present in BGSE at 0.68% ± 0.0022% (±SD, n = 5). In the cell proliferation test, α-ESA potently suppressed proliferation of two ATL cell lines (ED and Su9T01; IC_{50} = 8.9 and 29.3 μM, respectively) more than several other octadecanoic acids. However, α-ESA moderately inhibited phytohemagglutinin-activated human peripheral blood mononuclear cells (PBMC; IC_{50} = 31.0 μM). These results suggest that BGSE-derived α-ESA has potential as a functional food constituent because of its activity against ATL, particularly against ED cells. Moreover, α-ESA might be effective for the prevention of moderate adverse effects of ATL on normal T cells.

Keywords: adult T-cell leukemia; bitter gourd seed extract; α-eleostearic acid; phytohemagglutinin-activated human peripheral blood mononuclear cell

1. Introduction

Adult T-cell leukemia (ATL) occurs in a small population of human T-cell leukemia virus type I (HTLV-I) infected individuals. After transmission of HTLV-I, 2%–5% of carriers are likely to develop ATL after a long latency period (30–50 years) [1]. These patients have been frequently identified as being from a restricted area of tropical regions [2]. It is currently very difficult to effectively treat patients with ATL using existing therapeutic methods, and most clinical trials focus on chemotherapeutic treatment and allogeneic hematopoietic stem cell transplantation. Therefore, it is important to find appropriate therapeutic methods to prevent the development of ATL or to prolong survival after its occurrence.

In our previous report, we screened 52 agricultural plant samples for their ability to inhibit proliferation in seven kinds of ATL related cell lines to start structure of a study for finding potential drug candidates with the prevention of ATL. We found that an 80% ethanol bitter gourd (*Momordica charantia* L.) seed extract (BGSE) showed an inhibitory effect on the proliferation of ATL-related human leukemia cells [3].

Bitter gourd belongs to the Cucurbitaceae family and is cultivated worldwide as a vegetable crop. The fruit is not only used as a food, but also for its medicinal properties, such as anti-microbial, anti-diabetic, anti-HIV and anti-tumor activities, which were described in a recent review [4]. BGSE has also been reported to have anti-leukemic potential on human acute myelogenous leukemia cells (HL-60) [5]. However, HL-60 and ATL cell lines comprise different types of leukemia cells, and as there have been no reports about bioactive compounds from BGSE active against ATL, the effect of

BGSE on ATL cell proliferation requires elucidation. The aim of the present study was to identify bioactive compounds in BGSE exhibiting activity against ATL.

2. Results and Discussion

2.1. Identification of Active Compounds in BGSE

Figure 1a,b show the HPLC-DAD chromatogram (a) and HPLC-MS-total ion chromatogram (TIC) (b) of BGSE. As shown in Figure 1a, a major peak (peak 1: retention time (RT) = 8.570 min, λ = 270 nm) was detected for BGSE. The TIC showed several peaks (Figure 1b). Figure 1c shows the MS spectrum of the peak with the RT: 8.570 min. in Figure 1b, which shows a deprotonated molecular ion signal at *m/z* 277. This compound was estimated to be $C_{18}H_{29}O_2$ and was proposed to be α-ESA on the basis of reference data [6]. α-ESA was analyzed using the same method. Figure 1d–f shows the HPLC-DAD chromatogram (d), TIC (e) and MS spectrum (f) of α-ESA. α-ESA (50 μg/mL) showed a clear peak at 270 nm (Figure 1d, peak 2) and the same RT of peak 1 in Figure 1a (BGSE). The RT (8.570 min.) of the highest peak in TIC for α-ESA (Figure 1e, peak 2) was the same as peak 1 in Figure 1b. The MS spectrum of α-ESA (Figure 1f) showed the same result as the analysis of BGSE (Figure 1c). From these results, peak 1 in Figure 1 was determined to be α-ESA, and α-ESA is the main compound in BGSE.

We also attempted to quantitatively determine α-ESA in BGSE. Figure 2 shows the α-ESA calibration curve. The calibration curve showed good linearity (R^2 = 0.9977). α-ESA was contained in the concentration range of 34.0 and 34.2 μg/5 mg of freeze-dried bitter gourd seeds (0.68% ± 0.0022%, ±SD, *n* = 5). In our previous report, BGSE suppressed the proliferation of ATL cell lines [3]. The current study shows the presence of α-ESA in BGSE. Tsuzuki *et al.* reported that α-ESA accounted for about 60% of the total fatty acid composition of bitter gourd seed oil [7]. Therefore, α-ESA might have greatly contributed to suppressing the proliferation of ATL cell lines.

2.2. The Inhibitory Effects of Octadecanoic Acid Analogs on ATL Cell Lines

Of the octadecanoic acids, conjugated linoleic acids (CLA), which includes α-ESA, has been acknowledged to have numerous biological activities, such as anti-obesity, anti-diabetes, anti-cancer, anti-arthritis, anti-asthma, and anti-cardiovascular disease effects [8]. However, no study has yet reported the anti-leukemic effects of α-ESA on ATL cell lines. We examined the inhibitory effects of related compounds, seven octadecanoic acid groups, on the proliferation of two types of ATL cell lines (ED and Su9T01). As shown in Table 1, α-ESA (C18:3, n-5), γ-linolenic acid (C18:3, n-6), and α-linolenic acid (C18:3, n-3), which belong to the triunsaturated fatty acid group, substantially inhibited ED cell growth (IC_{50} values of 8.9, 61.3 and 129.9 μM, respectively) and Su9T01 cell growth (IC_{50} values of 29.3, 174.3 and 167.4 μM, respectively). Linoleic acid (C18:2, n-6), which belongs to the diunsaturated fatty acid group, inhibited ED and Su9T01 cell growth (IC_{50} values of 100.7 and 180.1 μM, respectively). In ED cells, these compounds exhibited higher activity than EGCG, which was used as the positive control [9] (IC_{50} = 152.7 μM). On the other hand, in Su9T01 cells, only α-ESA exhibited higher inhibitory activity than EGCG (IC_{50} = 166.0 μM). We cannot calculate the exactly IC_{50} values of monounsaturated fatty acid group (oleic acid (C18:1, n-9) and elaidic acid

(C18:1, n-9)) and saturated fatty acid (stearic acid (C18:0)) in both cell lines. This assay was employed to compare the effects of octadecanoic acids on ED and Su9T01 cell growth; α-ESA showed the highest inhibitory activity. IC_{50} values decreased roughly in proportion to the number of double bonds; therefore, the number of double bonds was an important determinant of anti-proliferation activity. Indeed, the triunsaturated fatty acid group was more potent than EGCG.

Figure 1. HPLC-DAD and MS chromatograms of BGSE and α-ESA. (**a**,**d**) HPLC-DAD chromatograms (270 nm) of BGSE (**a**) and α-ESA (**d**). (**b**,**e**) Total ion chromatograms (TIC) of BGSE (**b**) and α-ESA (**e**). (**c**) MS spectrum (negative-ion spectra) of peak 1 of BGSE in Figure 1b. (**f**) MS spectrum (negative-ion spectra) of peak 2 of α-ESA in Figure 1e.

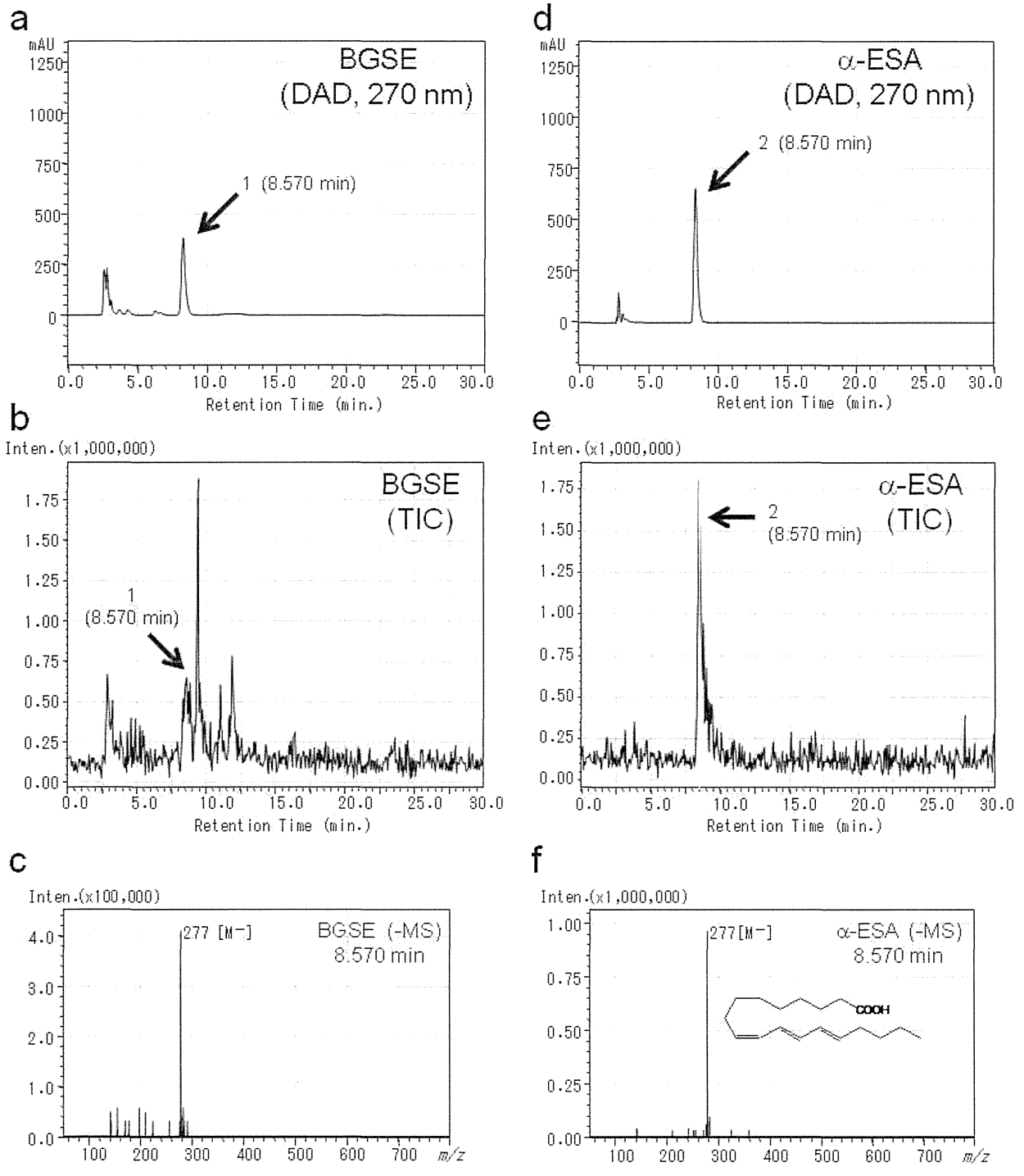


Figure 2. Calibration curve of α-ESA.

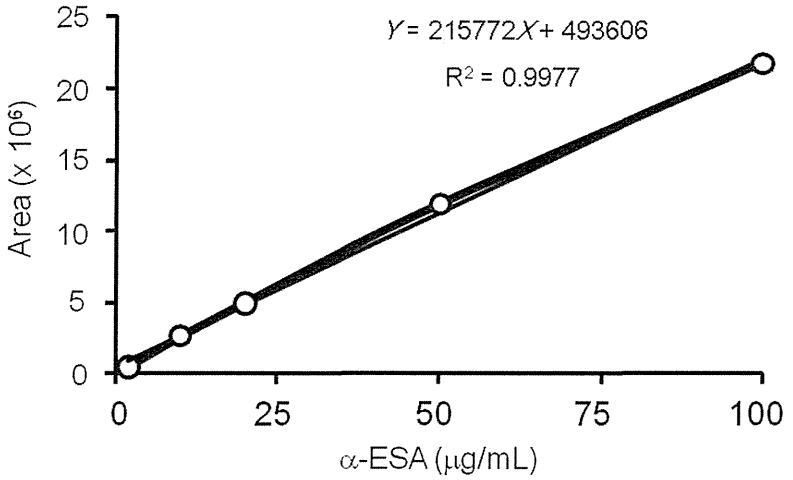


Table 1. Relationship between octadecanoic acid structure and inhibition of adult T-cell leukemia (ATL) cell line proliferation.

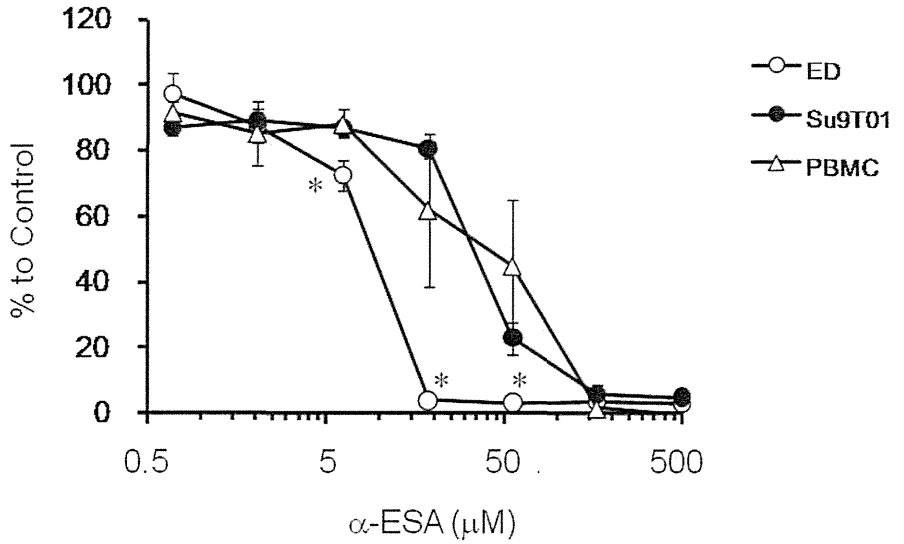
Compounds		IC_{50} (μM)	
		ED	**Su9T01**
α-ESA	(C18:3, n-5)	8.9	29.3
γ-linolenic acid	(C18:3, n-6)	61.3	174.3
α-linolenic acid	(C18:3, n-3)	129.9	167.4
linoleic acid	(C18:2, n-6)	100.7	180.1
oleic acid	(C18:1, n-9)	500.0–166.7	500.0–166.7
elaidic acid	(C18:1, n-9)	>500.0	>500.0
stearic acid	(C18:0)	500.0–166.7	>500.0
EGCG	(Positive Control)	152.7	166.0

ATL cells (ED and Su9T01) were incubated for 72 h in RPMI-1640 medium containing each compound. Viable cells were detected using a WST-8 assay kit. The concentration at which cell proliferation is inhibited by 50% compared to untreated control is expressed as IC_{50}.

2.3. The Effect of α-ESA on ATL Cell Line and Phytohemagglutinin-Activated Human Peripheral Blood Mononuclear Cell (PBMC) Proliferation

As shown in Figure 3, we compared the suppressive effect of α-ESA on ED, Su9T01 cells and PBMCs. PBMCs are commonly used as the healthy/normal cell model in comparison to cancer cell lines. Significant differences were observed between ED cells and PBMCs treated at 6, 19 and 56 μM α-ESA ($p < 0.05$). Su9T01 cells and PBMCs treated with between 1 and 500 μM α-ESA were not significantly different. These results confirmed that the α-ESA strongly and selectively inhibited ED cells and moderately inhibited PBMCs, which are healthy normal T cells. α-ESA at 166 and 500 μM significantly decreased proliferation of all cell types. α-ESA showed inhibitory effects on ED, Su9T01 cells and PBMCs (IC_{50} of 8.9, 29.3 and 31.0 μM, respectively).

Figure 3. Effect of α-ESA on ATL cell and PBMC proliferation.



A comparison of the dose-dependent effects of α-ESA on ED, Su9T01 cells and PBMCs. Each mark represents the mean ± SD of three independent tests (Student's *t*-test). Significantly different between ED cells and PBMCs: $p < 0.05$ (*).

Tsuzuki *et al.* and Kobori *et al.* reported that bitter gourd seed oil and its constituent α-ESA had anti-leukemic potential in HL-60 cells [5,10]. While HL-60 and ATL (ED and Su9T01) are leukemia cell lines, they differ in origin and whether they are a result of viral infection. The antiproliferative properties of unsaturated fatty acids are well known. For example, Wendel *et al.* reported that the unsaturated fatty acids (mainly omega-3 fatty acids and derivatives) like conjugated eicosapentaenoic acid as important nutritional adjuvant therapeutics in the management of various human cancer diseases and the impact of nutritional omega-3 fatty acids on cancer prevention [11]. α-ESA also may have similar potential with nutritional function for cancer prevention. The present study is the first to show the inhibitory effects of α-ESA on ATL cells *in vitro*. Specifically, α-ESA showed an inhibitory effect in the rank order: ED cells > Su9T01 cells ≥ PBMCs. Sasaki *et al.* reported that tumor suppressor in lung cancer 1 (*TSLC1*) gene expression was different between ED and Su9T01 cells [12,13]. Therefore, using gene expression analysis, future studies should investigate the regulatory mechanism of *TSLC1* and its DNA methylation, as well as the possible role of α-ESA in the inhibition of ED and Su9T01 proliferation and *TSLC1* expression.

3. Experimental

3.1. Chemicals

α-Eleostearic acid (α-ESA) was obtained from Larodan Fine Chemicals AB, Malmö, Sweden and Cayman Chemical, Ann Arbor, MI, USA. γ-Linolenic acid, linoleic acid, α-linolenic acid, and elaidic acid were purchased from Cayman Chemical. Stearic acid and oleic acid were purchased from Wako, Osaka, Japan. Epigallocatechin-3-gallate (EGCG) was purchased from Nagara Science Co., Gifu, Japan. Ficoll was purchased from GE Healthcare, Uppsala, Sweden. Phytohemagglutinin (M Form) was purchased from Invitrogen, Carlsbad, CA, USA. IL-2 was purchased from R&D Systems,

Minneapolis, MN, USA. A 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt (WST-8) assay kit was purchased from Dojindo, Kumamoto, Japan.

3.2. Identification of Compounds in BGSE

The freeze-dried powder of bitter gourd seeds (5 mg) was extracted with 80% EtOH (0.5 mL) by vortexing for 30 s, followed by centrifugation at 1,500 rpm for 3 min. The supernatant was used for high performance liquid chromatography-diode array detector (HPLC-DAD) and mass spectrometry (MS) analysis. The α-ESA HPLC analysis method was a modification of the methods of Amakura *et al.* and Řezanka *et al.* [6,14]. The HPLC-DAD and MS analysis consisted of a Shimadzu HPLC System (LC-20A Prominence, Shimadzu, Kyoto, Japan) coupled to a SPD-20A (DAD; Shimadzu, Kyoto, Japan) and an LC/MS-ion trap-time of flight (LC/MS-IT-TOF, Shimadzu, Kyoto, Japan) fitted with an atmospheric pressure chemical ionization (APCI) source. HPLC separation was performed on a reverse-phase column (Atlantis T3, 2.1 mm I.D. ϕ100 mm, 3 μm; Waters, Milford, MA, USA). The column was maintained at 40 °C. The mobile phase consisted of eluent A (0.1% acetic acid and MeOH)/eluent B (0.1% acetic acid and 10% MeOH aq.) = 90:10 at a flow rate of 0.10 mL/min. The injection volume was 10 μL. APCI conditions were recorded from m/z = 50 to 400 in negative ion mode. The other MS conditions were as follows: nebulizer N_2 gas, 2.5 L/min; APCI interface temperature, 400.0 °C; curved desolvation line (CDL) temperature, 250.0 °C; heat block temperature, 200.0 °C; detector voltage, 1.80 kV.

3.3. α-ESA Calibration Curve

The α-ESA standard was dissolved in 80% ethanol and serial dilutions were analyzed by HPLC-DAD. α-ESA content was calculated using the following linear equation based on the calibration curve: $Y = 215772X + 493606$, $R^2 = 0.9977$. Y is the area detected by DAD (270 nm), and X is the α-ESA content in μg/mL.

3.4. ATL Cell Proliferation Assay

We used two ATL cell lines (ED and Su9T01) that are highly sensitive to inhibition of cell proliferation, as determined in our previous study [3]. ED cells were kindly provided by Dr. M. Maeda (Kyoto University, Kyoto, Japan) and Su9T01 cells were kindly provided by Dr. N. Arima (Kagoshima University, Kagoshima, Japan). The test compounds were dissolved in dimethyl sulfoxide and subjected to assay screening. The method of ATL assay is described in a previous report [3]. IC_{50} calculation was some curve fitted onto the determined proliferation inhibition points.

3.5. Isolation and Culture of PBMCs

The method of isolation and culture of PBMCs is as follows. Heparinised blood (5 mL) was diluted by adding 5 mL of PBS. The diluted blood samples were divided into four equal parts, loaded on 4 mL of Ficoll and centrifuged at 400 × *g* for 30 min. The PBMC layer was located within the interphase between the Ficoll and plasma. The Ficoll contained the erythrocytes and most of the granulocytes. The plasma was removed using a pipette until ~5 mL above the PBMC interphase. The cells were

washed three times with PBS (centrifuged at 200 × *g* for 15 min) and resuspended in RPMI 1640 medium supplemented with 10% foetal bovine serum containing 100 U/mL penicillin G, 100 μg/mL streptomycin, 2 ng/mL IL-2 and 128-fold dilution of phytohemagglutinin to a final cell density of 1×10^6 cells/mL. The PBMC proliferation assay was conducted using the same method as for the ATL proliferation assay [3].

3.6. Statistics

Each experiment was conducted at least three times. All data are expressed as the mean ± standard deviation (SD) of three independent experiments. Statistically significant differences were calculated by Student's *t*-test.

4. Conclusions

α-ESA was shown to be the main bioactive compound in BGSE, and contributes to the inhibition of ED cell differentiation and proliferation without damaging normal cells, leading to the disruption of ATL pathogenesis.

Acknowledgments

We thank Michiyuki Maeda (Kyoto University) and Naomichi Arima (Kagoshima University) for supplying the cell lines. We also thank Yuuki Maeda and Naomi Makisumi for their excellent technical assistance. This work was supported by a Grant-in-Aid from the Collaboration of Regional Entities for the Advancement of Technological Excellence (CREATE) from the Japanese Science and Technology Agency.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Arisawa, K.; Soda, M.; Endo, S.; Kurokawa, K.; Katamine, S.; Shimokawa, I.; Koba, T.; Takahashi, T.; Saito, H.; Doi, H.; *et al.* Evaluation of adult T-cell leukemia/lymphoma incidence and its impact on non-Hodgkin lymphoma incidence in southwestern Japan. *Int. J. Cancer* **2000**, *85*, 319–324.
2. Proietti, F.A.; Carneiro-Proietti, A.B.; Catalan-Soares, B.C.; Murphy, E.L. Global epidemiology of HTLV-I infection and associated diseases. *Oncogene* **2005**, *24*, 6058–6068.
3. Kai, H.; Akamatsu, E.; Torii, E.; Kodama, H.; Yukizaki, C.; Sakakibara, Y.; Suiko, M.; Morishita, K.; Kataoka, H.; Matsuno, K. Inhibition of proliferation by agricultural plant extracts in seven human adult T-cell leukaemia (ATL)-related cell lines. *J. Nat. Med.* **2011**, *65*, 651–655.
4. Fang, E.F.; Ng, T.B. Bitter gourd (*Momordica charantia*) is a cornucopia of health: A review of its credited antidiabetic, anti-HIV, and antitumor properties. *Curr. Mol. Med.* **2011**, *11*, 417–436.

5. Soundararajan, R.; Prabha, P.; Rai, U.; Dixit, A. Antileukemic Potential of *Momordica charantia* Seed Extracts on Human Myeloid Leukemic HL60 Cells. *J. Evid.-Based Complement. Alternat. Med.* **2012**, doi:10.1155/2012/732404.
6. Amakura, Y.; Kondo, K.; Akiyama, H.; Ito, H.; Hatano, T.; Yoshida, T.; Maitani, T. Characteristic long-chain fatty acid of *Pleurocybella porrigens*. *Shokuhin Eiseigaku Zasshi* **2006**, *47*, 178–181.
7. Tsuzuki, T.; Tokuyama, Y.; Igarashi, M.; Miyazawa, T. Tumor growth suppression by alpha-eleostearic acid, a linolenic acid isomer with a conjugated triene system, via lipid peroxidation. *Carcinogenesis* **2004**, *25*, 1417–1425.
8. Ruiz, R.A.; Reglero, G.; Ibañez, E. Recent trends in the advanced analysis of bioactive fatty acids. *J. Pharm. Biomed. Anal.* **2012**, *51*, 305–326.
9. Li, H.C.; Yashiki, S.; Sonoda, J.; Lou, H.; Ghosh, S.K.; Byrnes, J.J.; Lema, C.; Fujiyoshi, T.; Karasuyama, M.; Sonoda, S. Green tea polyphenols induce apotosis *in vitro* in peripheral blood T lymphocytes of adult T-cell leukemia patients. *Jpn. J. Cancer Res.* **2000**, *91*, 34–40.
10. Kobori, M.; Ohnishi-Kameyama, M.; Akimoto, Y.; Yukizaki, C.; Yoshida, M. α-eleostearic acid and its dihydroxy derivative are major apoptosis-inducing components of bitter gourd. *J. Agric. Food Chem.* **2008**, *56*, 10515–10520.
11. Wendel, M.; Heller, A.R. Anticancer actions of omega-3 fatty acids—Current state and future perspectives. *Anticancer Agents Med. Chem.* **2009**, *9*, 457–470.
12. Sasaki, H.; Nishikata, I.; Shiraga, T.; Akamatsu, E.; Fukami, T.; Hidaka, T.; Kubuki, Y.; Okayama, A.; Hamada, K.; Okabe, H.; *et al.* Overexpression of a cell adhesion molecule, TSLC1, as a possible molecular marker for acute-type adult T-cell leukemia. *Blood* **2005**, *105*, 1204–1213.
13. Watanabe, M.; Nakahata, S.; Hamasaki, M.; Saito, Y.; Kawano, Y.; Hidaka, T.; Yamashita, K.; Umeki, K.; Taki, T.; Taniwaki, M.; *et al.* Downregulation of CDKN1A in adult T-cell leukemia/lymphoma despite overexpression of CDKN1A in human T-lymphotropic virus 1-infected cell lines. *J. Virol.* **2010**, *84*, 6966–6977.
14. Řezanka, T.; Votruba, J. Analysis of Fatty Acids by APCI-MS. In *Modern Methods for Lipid Analysis by Liquid Chromatography: Mass Spectrometry and Related Techniques*; William, C.B., Ed.; AOCS Press: Urbana, IL, USA, 2005; pp. 242–275.

血液内科，68(1)：12-17，2014

特集 ATL/HTLV-1研究の最近の進展

ATLとHAM以外のHTLV-1感染関連疾患に関する研究の現状*

岡山昭彦**

Key Words：HTLV-1, chronic inflammatory diseases, immuno-suppressive therapy

はじめに

ヒトT細胞白血病ウイルス1型(HTLV-1)は血液悪性腫瘍である成人T細胞白血病(ATL)や神経疾患であるHTLV-1関連脊髄症(HAM)の原因である．ATL, HAM以外にも本邦ではHTLV-1高浸淫地域においてぶどう膜炎，関節炎，膠原病，慢性肺疾患など種々の慢性炎症性疾患に本ウイルスキャリアがみられ，関連が示唆されてきた．また海外においても慢性皮膚疾患などをふくめ同様の報告が散見される．しかしながらこれら疾患におけるHTLV-1感染の頻度や，HTLV-1陽性患者では陰性患者と病態が異なるか否かなどは明らかになっていない．また岩永らにより，診療の過程で発見されたHTLV-1キャリアはそれ以外の機会で発見されたキャリアに比べてATL発症の頻度が高いことが報告されている[1]．このことは上記疾患の合併や治療がATL発症リスクとなる可能性を示唆している．特に近年関節リウマチ(RA)を中心とした慢性炎症性疾患では，抗サイトカイン薬を中心としたいわゆる生物学的製剤やカルシニューリン阻害薬などの免疫抑制剤が積極的に使用されている．このため，HTLV-1感染がこれら疾患の病態や治療に影響を与えているかどうかを明らかにすることは重要な問題となってきた．

この問題の解明を目的として，われわれは平成23年度から厚生労働科学研究費補助金による助成を受けて，HTLV-1感染に関連する非ATL非HAM慢性炎症疾患の実態把握と病態解明を目的とした研究を行ってきた．本稿においてはその知見も含めてATLとHAM以外のHTLV-1感染関連疾患に関する研究の現状について考察する．

ATLとHAM以外でHTLV-1感染との関連が示唆される疾患(表1)

1．ぶどう膜炎

1992年望月らによる疫学調査により，九州における原因不明のぶどう膜炎ではその他の眼疾患に比べてHTLV-1抗体陽性率が高いことが報告され，ATL細胞の眼内浸潤とは異なる無症候性キャリアに発症する新たな疾患概念，HTLV-1関連ぶどう膜炎(HU)が提唱された[2]．女性に多く，発症年齢のピークは男性では40歳代，女性では50歳代である．主な症状は飛蚊症，霧視，眼の充血，視力の低下などで，一般に急性に発症し，両眼性と片眼性がある．HUはHAM患者との合併がみられ，バセドウ病の既往の頻度が高いことが特徴の一つとされている．副腎皮質ステロイド薬の局所治療(点眼，局所注射)あるいは経口投与が奏効するが，再発がありうる．HU患者の

* HTLV-1-associated diseases except ATL and HAM/TSP.

** Akihiko OKAYAMA, M.D., Ph.D.: 宮崎大学医学部内科学講座免疫感染病態学分野〔〒889-1692 宮崎県宮崎市清武町木原5200〕; Department of Rheumatology, Infectious Diseases and Laboratory Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki 889-1692, JAPAN

表1 ATL，HAM以外にHTLV-1感染との関連が示唆されている主な疾患

1. 眼科的疾患
   HTLV-1関連ぶどう膜炎(HU)
2. 関節炎，膠原病
   多発関節炎
   HAAP
   シェーグレン症候群
   多発性筋炎
3. 呼吸器疾患
   肺胞隔炎・気管支炎
   間質性肺炎
   気管支拡張症
   気管支肺胞上皮がん
4. 皮膚疾患
   infective dermatitis
   seborrheic dermatitis
   尋常性乾癬

末梢血液中のプロウイルス量は高く，眼内にポリクローナルなHTLV-1感染細胞の浸潤と前房水中のIL-6高値が報告され，病態と関連していると考えられている[3][4]．

2．関節炎

1989年西岡らにより，HTLV-1キャリアにおいて特徴的な慢性炎症性関節症が存在すると報告され，HTLV-1 associated arthropathy(HAAP)として提唱された[5]．この概念では比較的少数の大関節に炎症が起こりやすく，関節液からHTLV-1抗体が，また滑膜細胞からHTLV-1が検出されるなど，ウイルスの関与を示唆する所見が報告されている．一方，同じグループによる対馬における疫学研究でHTLV-1キャリアでは関節症状の訴えやRAの頻度が高いことが報告されている[6]．HTLV-1抗体陽性率がRA患者において一般献血者集団よりも高いことは江口らによっても報告されている[7]．またHTLV-1トランスジェニックマウスにおいてリウマチ類似の関節炎がみられることも報告された[8]．しかしHAAPとHTLV-1陽性RAの異同，病態や治療などに関するその後の研究報告は少ない．

3．シェーグレン症候群・その他の膠原病

1989年にはHTLV-1トランスジェニックマウスにおいてシェーグレン症候群様の唾液腺炎が生じることが報告されていた[9]．1992年江口らによって行われた長崎県の疫学調査において，原発性シェーグレン症候群患者でのHTLV-1抗体陽性率が一般献血者集団よりも高いことが報告された[10]．別のグループからも，HTLV-1陽性シェーグレン患者では病変部である唾液腺に感染細胞の浸潤があることが示された[11]．さらに最近中村らによって，唾液腺の組織におけるケモカインレセプターの発現がHTLV-1陽性シェーグレン患者において陰性患者とは異なることが報告されている[12]．

シェーグレン症候群以外の膠原病とHTLV-1感染の関連については，本邦における報告は少ないが，ジャマイカなどの海外においては多発性筋炎がHTLV-1感染と関連しているという報告がある[13]．また関節炎を含めた膠原病において末梢血液中のプロウイルス量が増加しているという報告もある[14]．しかしHTLV-1感染と膠原病の関連については否定的な報告もあり，また本邦においてまとまった症例を解析した報告もない．

4．呼吸器疾患

1987年杉本らによって，HTLV-1感染者における肺胞隔炎が報告された[15]．その後もHTLV-1感染者に種々の慢性炎症性肺病変がみられることが報告されている．その特徴として症状や画像所見に乏しいが，気管支鏡検査を行うと気管支肺胞洗浄液中のTリンパ球の増加がみられ，気管支・肺胞隔壁などの間質を中心に病変が存在するとされている．このほか近年，間質性肺炎，気管支拡張症，さらには気管支肺胞上皮がんなどとの関連も報告されている[16]～[18]．また肺胞や気管上皮由来細胞株にHTLV-1を感染させると，サイトカインやケモカイン産生の変化が起こることが報告されている[19]．

5．皮膚疾患そのほか

中南米においてはHTLV-1陽性の小児でブドウ球菌などの感染によるinfective dermatitisやseborrheic dermatitisなどの皮膚病変が高頻度にみられることが報告されている[20]．またHTLV-1感染者ではアトピーなどのアレルギー性疾患が少なく，抗原刺激に対する皮膚反応も弱いという報告もみられている[21]．一方同じHTLV-1高浸淫地域である本邦では，尋常性乾癬等との関連を示唆する報告はあるが[22]，HTLV-1感染と(ATLを除く)皮膚疾患との関連についてのまとまった報告はみられていない．国内外で異なった皮膚病変が報告されている背景にはHTLV-1以外の要因

表2　HTLV-1感染との関連が示唆されている疾患の特徴

①慢性炎症性疾患である．
②病変部にT細胞の浸潤が認められ，HTLV-1プロウイルスが検出されるが，感染細胞の増殖はモノクローナルではない．
③病変部のサイトカインやケモカイン異常が報告されている．
④末梢血液のプロウイルス量(感染細胞数)が多い．
⑤HAMとの合併の報告が多い．
⑥同一患者に稀ならず複数の疾患が認められる．
⑦HTLV-1トランスジェニックマウスなどの動物モデルで同様の病変がみられる．

との関連が考えられる．

HTLV-1関連疾患の特徴

HTLV-1との関連が示唆されている上記の疾患群の主な特徴をまとめると次のようになる(表2)．

・慢性炎症性疾患である．
・病変部にT細胞の浸潤，HTLV-1プロウイルスが検出される．しかし，感染細胞の増殖はモノクローナルではない．
・病変部のサイトカインやケモカイン異常が報告されている．
・末梢血液のプロウイルス量(感染細胞数)が多い．
・HAMとの合併の報告が多い．
・同一患者に稀ならず複数の疾患が認められる．
・HTLV-1トランスジェニックマウスなどの動物モデルで同様の病変がみられる．

このような特徴からATL，HAM以外のHTLV-1関連疾患は，HTLV-1感染細胞数の多いキャリアから発症し，感染Tリンパ球がなんらかの機序で特定の臓器に浸潤し，サイトカイン異常などの共通の病態を介して，局所的な慢性炎症をひき起こしていることが考えられる．またその病像形成にはHAMと共通のプロセスをシェアしている可能性がある．HAM患者では$CD4^+CD25^+CCR4^+Foxp3^-$ T細胞にインターフェロンγ産生能があり病態形成に関与していると報告されており，慢性炎症性疾患の病態を考える上でも興味深い[23]．また海外と本邦の間で報告に差のある疾患群もあり，発症にはHTLV-1感染以外の環境，並存する感染症，人種などの差が影響している可能性が高い．

しかしながら，ATLやHAMとは異なり，ぶどう膜炎，関節炎，膠原病，慢性肺疾患のような疾患群はHTLV-1陽性者のみにみられるわけではなく，一般集団における頻度も稀ではない．このため，HTLV-1陽性の慢性炎症性疾患患者を発見した場合，HTLV-1感染が疾患に直接関与している場合とHTLV-1キャリアにその疾患が偶然合併している場合の両者が考えられる．今後HTLV-1関連疾患を一つのclinical entityとして考えるかどうかを検討する場合や，HTLV-1陽性患者が特別な症状や病態を呈するか否かを分析していく上では，その点について特に注意を払う必要がある．

HTLV-1関連疾患と治療

HTLV-1との関連が示唆されている疾患群においては，HUにおいて副腎皮質ステロイド薬による治療が確立されているほかは，HTLV-1感染を考慮した特別な治療はない．またそれ以前の問題として，HTLV-1高浸淫地域の一部を除いて，関節炎，膠原病，慢性肺疾患，皮膚疾患等の治療開始時にHTLV-1のスクリーニングを行うことは，少なくとも現時点では勧奨されていない．このため，たとえばRAにおいてHTLV-1陽性患者と陰性患者では異なった治療を行うべきか否かについては報告やエビデンスがなかったのが現状である．

HTLV-1感染を考慮して治療を行う必要があるとすれば，HTLV-1陽性患者と陰性患者の間で，ある治療法に対する反応性が異なる，あるいは副作用・安全性に問題がある場合である．治療反応性が異なるか否かの解析においては，先に述べたように偶然の合併も考慮する必要があるため，HTLV-1陽性患者すべてが共通した傾向を示さない可能性が高い．

しかし安全性，特に治療によってATLの発症リスクが上昇するか否かについてはすべてのHTLV1陽性患者に共通して考えておく必要がある問題と思われる．近年，特にRA，そのほかの膠原病，尋常性乾癬などの疾患においてはTNFやIL-6阻害作用のある生物学的製剤やTリンパ球を標的と

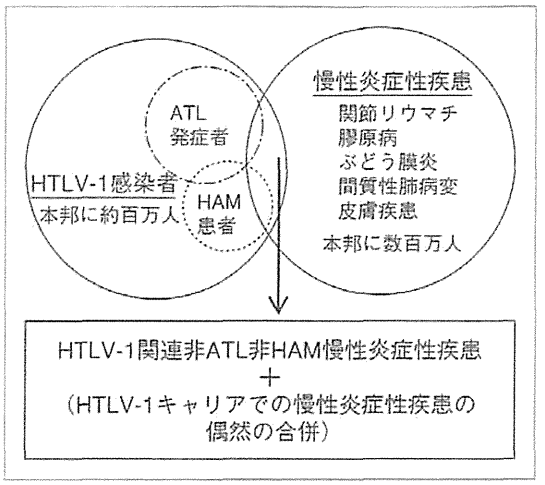


図1　HTLV-1感染者と慢性炎症性疾患患者の位置づけ

する免疫抑制剤が積極的に用いられるようになっている．さらにRAについては細胞内シグナル伝達阻害薬，血管炎ではCD20抗体製剤も使用可能となった．このような治療の際，B型肝炎ウイルス感染ではすでにde novo肝炎予防のため，治療開始前にウイルススクリーニングを行い，感染があればウイルス量のモニタリングや抗ウイルス薬治療を行うことが勧奨されている．しかしながらHTLV-1感染に関しては，陽性患者の治療に際してなんらかの配慮を行うべきか否かについての定見はない．さらに重要な疑問は強力な免疫関連治療開始時にHTLV-1のスクリーニングを行うべきか否かということである．

HTLV-1感染に関連する非ATL非HAM希少疾患の実態把握と病態解明を目的とした研究班

上記HTLV-1関連慢性炎症性疾患の病態や治療反応性などの解明を目的として，平成23年度から厚生労働省科学研究助成を得て，宮崎大学，長崎大学，琉球大学，東京医科歯科大学の研究者により，RAその他の膠原病，慢性呼吸器疾患，慢性皮膚疾患，ぶどう膜炎についてHTLV-1感染の有無を検討した患者のコホートを構築し，あわせてHTLV-1感染が慢性炎症性疾患をひき起こすメカニズム解明のための研究を行っている．これまでに，RAにおいてはHTLV-1陽性患者の炎症反応（CRP）が陰性患者に比べて高く，抗TNF生物学的製剤の効果が陰性患者よりも低いことが示された[24]．安全性に関する調査では，IL-6レセプター阻害薬を使用中のRA患者から1例ではあるがATLの発症が確認された[25]．同様のATL発症例については海外からも抗TNF製剤において報告された[26]．一方でプロウイルス量の少ないRA患者において抗TNF製剤を使用した場合，少数例・短期間の観察ではあるが，ATL発症の危険因子とされているプロウイルス量や感染細胞クローナリティには変化がみられなかった[27]．HTLV-1陽性患者に対する生物学的製剤の有効性と安全性の確認には，さらに多くの患者をリクルートし，長期にわたる観察が必要である．またその他の疾患に関しても解析が進行中である．平成25年度はさらに無症候性キャリアとの比較などを含めた研究を継続中であり，HTLV-1陽性慢性炎症性疾患患者の特徴を疫学的，基礎的な面から明らかにし，今後の診療を行うのに有用な情報を提供したいと考えている．

ま と め

以上，ATLとHAM以外でHTLV-1感染との関連が示唆されている疾患に関する研究の現状について述べた．本邦におけるHTLV-1感染者は約108万人と推定されており，その一部と慢性炎症性疾患患者にオーバーラップがある（図1）．病態を説明する仮説としてはHTLV-1プロウイルス量の多いキャリアにおいて，感染細胞が特定の臓器に浸潤し，サイトカインなどの異常を介して，慢性炎症をひき起こしていることが考えられる．このプロセスはHAMで想定されているものと類似している可能性がある．しかしそれぞれの慢性炎症性疾患患者の中でHTLV-1感染がどの程度のインパクトを与えているのかについての理解はいまだ不十分である．

また慢性炎症性疾患とHTLV-1感染の関係は一方向のものではない（図2）．HTLV-1感染が慢性炎症性疾患の一部の原因となっている可能性，あるいはHTLV-1感染が病態の悪化や治療反応性に影響を与えている可能性に加えて，逆に慢性炎症性疾患を有するHTLV-1感染者ではプロウイルス量（感染細胞数）が多いことが報告されており，患者におけるATL発症リスク上昇の懸念があ

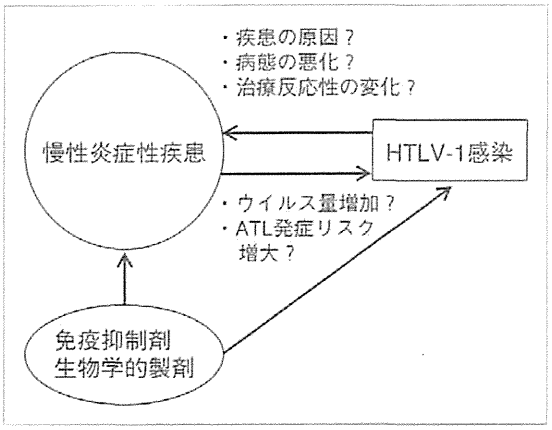


図2　慢性炎症性疾患，その治療とHTLV-1感染

る．さらにこれら疾患で使用される免疫抑制あるいは抗サイトカイン作用のある薬剤がこのリスク上昇を助長しないかどうかも重要な疑問である．

今後，十分な母集団の長期にわたる疫学的解析およびHTLV-1陽性慢性炎症性疾患の病態の基礎的研究を行い，これらの疑問に答えていく必要がある．このような研究はHTLV-1感染の頻度の高い唯一の先進工業国であるわが国でのみ可能と思われ，国際貢献の視点からもぜひ進めていく必要がある．

文　献

1) Iwanaga M, Watanabe T, Utsunomiya A, et al ; Joint Study on Predisposing Factors of ATL Development investigators. Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers : a nationwide prospective study in Japan. Blood 2010 ; 116 : 1211.
2) Mochizuki M, Yamaguchi K, Takatsuki K, et al. HTLV-I and uveitis. Lancet 1992 ; 339 : 1110.
3) Ono A, Mochizuki M, Yamaguchi K, et al. Increased number of circulating HTLV-1 infected cells in peripheral blood mononuclear cells of HTLV-1 uveitis patients : a quantitative polymerase chain reaction study. Br J Ophthalmol 1995 ; 79 : 270.
4) Ono A, Mochizuki M, Yamaguchi K, et al. Immunologic and virologic characterization of the primary infiltrating cells in the aqueous humor of human T-cell leukemia virus type-1 uveitis. Accumulation of the human T-cell leukemia virus type-1-infected cells and constitutive expression of viral and interleukin-6 messenger ribonucleic acids. Invest Ophthalmol Vis Sci 1997 ; 38 : 676.
5) Nishioka K, Maruyama I, Sato K, et al. Chronic inflammatory arthropathy associated with HTLV-I. Lancet 1989 ; 1 : 441.
6) Motokawa S, Hasunuma T, Tajima K, et al. High prevalence of arthropathy in HTLV-I carriers on a Japanese island. Ann Rheum Dis 1996 ; 55 : 193.
7) Eguchi K, Origuchi T, Takashima H, et al. High seroprevalence of anti-HTLV-I antibody in rheumatoid arthritis. Arthritis Rheum 1996 ; 39 : 463.
8) Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. Science 1991 ; 253 : 1026.
9) Green JE, Hinrich SH, Vogel J, Jay G. Exocrinopathy resembling Sjögren's syndrome in HTLV-I tax transgenic mice. Nature 1989 ; 341 : 72.
10) Terada K, Katamine S, Eguchi K, et al. Prevalence of serum and salivary antibodies to HTLV-I in Sjögren's syndrome. Lancet 1994 ; 344 : 1116.
11) Ohyama Y, Nakamura S, Hara H, et al. Accumulation of human T lymphotropic virus type I-infected T cells in the salivary glands of patients with human T lymphotropic virus type I-associated Sjögren's syndrome. Arthritis Rheum 1998 ; 41 : 1972.
12) Nakamura H, Kawakami A, Hayashi T, et al. Low prevalence of ectopic germinal centre formation in patients with HTLV-I-associated Sjogren's syndrome. Rheumatology (Oxford) 2009 ; 48 : 854.
13) Morgan OS, Rodgers-Johnson P, Mora C, Char G. HTLV-1 and polymyositis in Jamaica. Lancet 1989 ; 2 : 1184.
14) Yakova M, Lézin A, Dantin F, et al. Increased proviral load in HTLV-1-infected patients with rheumatoid arthritis or connective tissue disease. Retrovirology 2005 ; 2 : 4.
15) Sugimoto M, Nakashima H, Watanabe S, et al. T-lymphocyte alveolitis in HTLV-I-associated myelopathy. Lancet 1987 ; 2 : 1220.