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CASE REPORT

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# Two cases of adult Still's disease with abnormally high level of telomerase activity in peripheral blood mononuclear cells

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**Abstract** We report two patients with adult Still's disease with an abnormally high level of telomerase activity. The first patient was a 61-year-old woman. The mean telomerase activity value for peripheral blood mononuclear cells of healthy adults measured by our method was 0.13 ± 0.03, whereas that in this patient during the active phase was abnormally high, at more than 27.56. The patient was treated by steroid therapy and successfully brought into remission, during which the telomerase activity value for peripheral blood mononuclear cells was reduced to 2.22. The second patient was a 19-year-old man. Although he stayed in remission after steroid therapy, a reduction in the steroid dose resulted in recrudescence, at which time the telomerase activity value peripheral blood mononuclear cells was high, at 11.76. Elevated levels of telomerase activity have been reported in patients with various pathological conditions other than malignant tumors. However, our literature search failed to reveal a report on such a high level of telomerase activity in association with a benign disease.

**Key words** Adult Still's disease · Peripheral blood mononuclear cells (PBMCs) · Telomerase

## Introduction

The telomere is a nucleoprotein complex that is indispensable for stabilization of chromosomes.[1] Telomere DNA is found at the ends of chromosomes and is not replicated by ordinary DNA polymerase; without telomerase, the chromosome shortens with each cell division.[2] Telomerase is a reverse transcriptase that adds telomere sequences at the ends of chromosomes and is thought to counteract telomere shortening to maintain telomere structure.[2] In recent years, telomerase has attracted attention because its activity was reported to be associated with the malignant transformation and aging of cells.[2,3] Telomerase activation has occurred in cancer cells, resulting in the maintenance of telomere length with cell divisions and allowing infinite cell divisions.[2,3]

A recent report of telomerase activation in lymphocytes and other cells in the absence of malignant transformation has attracted attention.[4] Lymphocyte activation is associated with elevated telomerase activity. These observations are beginning to call attention to the importance of telomerase in immune diseases.[5]

We previously reported high telomerase activity in peripheral blood mononuclear cells (PBMCs) of systemic lupus erythematosus (SLE) patients.[6,7] In this study, we measured telomerase activity in patients with adult Still's disease and obtained interesting results.

## Methods

Informed consent based on the Declaration of Helsinki was obtained from two patients with adult Still's disease, and 20 ml of peripheral blood was collected from each using heparin. Telomerase activity was measured by the method used in our previous analysis of SLE patients.[7] Briefly, mononuclear cells were isolated from 20 ml of heparinized blood by Ficoll-Paque density gradient centrifugation, and $1 \times 10^6$ cells were analyzed for telomerase activity by the telomerase repeat amplification protocol (TRAP) assay using a TRAPeze Telomerase Detection Kit (Intergen, Purchase, NY, USA). The TRAP assay products obtained were subjected to 12% polyacrylamide gel electrophoresis. Telomerase activity was expressed as the ratio of the total TRAP assay product ladder to the internal control bands. This band ratio was defined as the telomerase activity value. In all assays, heat-inactivated controls served as negative controls. As positive controls, telomerase activity was mea-

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sured in Ramos human B lymphoma cells. Before specimen measurement, a calibration curve was constructed, and quantitative accuracy in the range of 0.06–27.56 was confirmed. The mean telomerase activity value measured by this method in the PBMCs of 45 healthy individuals was 0.13 ± 0.03.

## Case reports

Case 1

A 61-year-old woman had fever, malaise, and knee joint pain in September 2001. She visited a local doctor but had no symptomatic improvement and was admitted to our hospital on September 28. She had a history of hypertension and hyperlipidemia and a family history of rheumatoid arthritis (her mother). On admission, she had remittent fever between 39° and 40°C. Physical examination showed small erythematous lesions chiefly on the abdomen and back and extending to all extremities.

Laboratory tests showed an elevated white blood cell (WBC) count of 23 700/μl, high C-reactive protein (CRP) of 15.7 mg/dl, elevated aspartate aminotransaminase (AST) of 121 IU/l and alanine aminotransaminase (ALT) of 72 IU/l), and an abnormally high serum ferritin of 98 380 ng/ml. Bone marrow examination showed no abnormalities including blood cell phagocytosis. After excluding a malignant tumor and infection, adult Still's disease was diagnosed.

The patient received steroid therapy including pulse therapy, after which the intermittent fever, skin rashes, and arthralgia disappeared, and the transaminases, CRP, and ferritin returned to normal (Fig. 1A). Telomerase activity in PBMCs was measured at two time points: during the active stage before the start of steroid therapy and during the remission period after treatment. The PBMC telomerase activity value was abnormally high (>27.56) during the active stage but was reduced (to 2.22) during the remission period (Figs. 1A, 2).

Case 2

A 19-year-old man who had remittent fever of 40°C or higher, polyarthritis, and generalized small erythematous lesions in December 2000 was admitted to this hospital. Detailed examination led to a diagnosis of adult Still's disease. Treatment with 40 mg of prednisolone per day was started and resulted in symptomatic improvement, allowing tapering of the steroid dose. When the steroid was tapered to 10 mg, the fever and rashes reappeared beginning in May 2001 and did not respond to antibiotics. The patient was readmitted on May 26.

His past history was unremarkable, but there was a family history of rheumatoid arthritis (his grandmother). On readmission, he had remittent fever between 39° and 40°C. Physical examination showed erythema on the cheek and small erythematous lesions involving the trunk and extremities that tended to coalesce. Laboratory tests showed a high CRP level of 9.8 mg/dl and elevated transaminases (AST 73 IU/l and ALT 125 IU/l). The condition was regarded as a recrudescence of adult Still's disease, and 500 mg of methylprednisolone was administered for 3 days. The after-treatment was 30 mg of prednisolone, which was later tapered. The PBMC telomerase activity value measured at the time of recrudescence was high, at 11.76 (Figs. 1B, 2).

## Discussion

We report two patients with adult Still's disease in whom abnormally high PBMC telomerase activity was detected. Rheumatic diseases in which abnormal telomerase activity has been reported include systemic lupus erythematosus,[6–8] mixed connective tissue disease,[8] Sjögren's syndrome,[8] scleroderma,[8] and rheumatoid arthritis.[9] However, because these reports used different methods and standards for the assay, a simple comparison of data on telomerase activity is inappropriate.

We expressed telomerase activity as the ratio of the total TRAP assay product to the internal control and obtained a mean PBMC telomerase activity value of 0.13 ± 0.03 for the normal control. Using the same method, we previously measured and reported PBMC telomerase activity in SLE patients.[6,7] At that time, we found that PBMC telomerase activity was elevated in patients in the active stage of SLE, and that the PBMC telomerase activity value was well correlated with the SLE activity. It was less than 2.0 in most patients, although some had abnormally high values, which were still less than 3.5. In contrast, the two patients in the active stage of adult Still's disease reported here had PBMC telomerase activity values of 11.76 and >27.56, respectively, which were considerably higher than those of the SLE patients.

Katayama et al. reported high PBMC telomerase activity values for SLE, mixed connective tissue disease, Sjögren's syndrome, and scleroderma, but the values were similar to those in our SLE patients in comparison with those of the normal controls.[8] Similarly, high PBMC telomerase activity values have also been reported for rheumatoid arthritis but were similar to those in our SLE patients in comparison with those of the normal controls.[9] Abnormal telomerase activity has also been reported in various specimens from patients with benign diseases other than rheumatic diseases. However, compared with those of the normal controls, the values appeared to be similar to those observed in the SLE patients.[10–16]

On the other hand, in malignant tumor patients, levels of telomerase activity similar to those described here have been reported. Trentin et al. reported that the mean ± SD peripheral blood B lymphocyte telomerase activity value in seven patients with advanced hairy-cell leukemia was 9.16 ± 7.25, with the highest 17.24, which was nearly 60 times as high as the mean ± SE of 0.29 ± 0.13 for normal B-lymphocytes.[17] The values of ≥27.56 and 11.76 reported for our two patients were, respectively, 211 and 90 times as high as the mean ± SD 0.13 ± 0.16 for the normal controls. Because

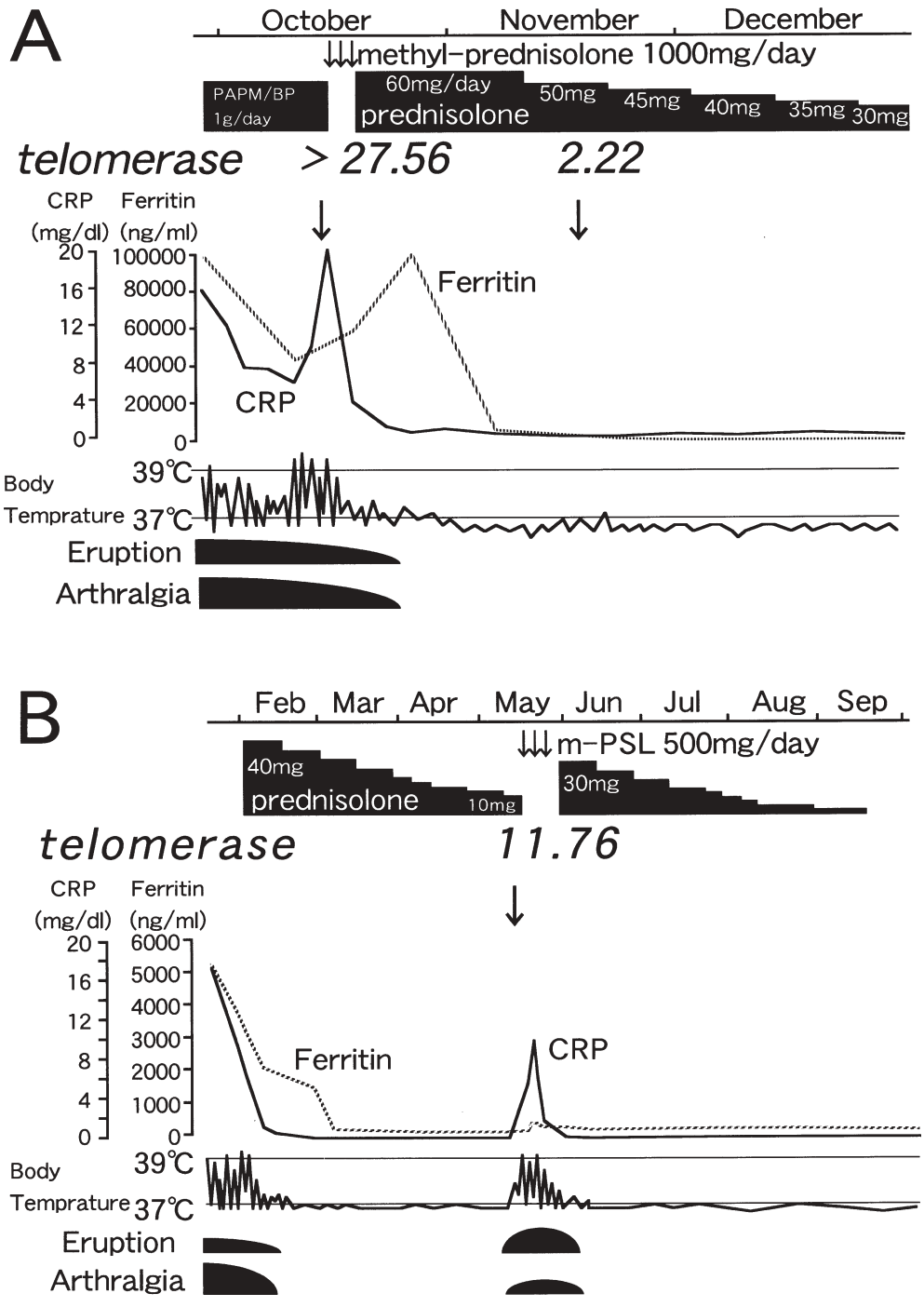


**Fig. 1.** Clinical course and telomerase activity in peripheral blood mononuclear cells. **A** A 61-year-old woman with adult Still's disease. The telomerase activity value for peripheral blood mononuclear cells was abnormally high at more than 27.56. Steroid therapy improved the laboratory test results and the symptoms and lowered the telomerase activity in peripheral blood mononuclear cells to 2.22. **B** A 19-year-old man with adult Still's disease. Although steroid therapy induced remission, a reduction in the steroid dose resulted in recrudescence. The telomerase activity value for peripheral blood mononuclear cells at the time of recrudescence was high at 11.76. *m-PSL*, methylprednisolone; *PAPM/BP*, panipnem/betamipron; *CRP*, C-reactive protein

their measurement method was similar to, but slightly different, from ours, a direct comparison of the results is not appropriate. However, considering the normal values in their and our measurements, the PBMC telomerase activity described here was similar to, or slightly higher than, the telomerase activity reported by them.

More interestingly, PBMC telomerase activity paralleled the clinical symptoms associated with the activity of the disease in the two patients. In case 1, we were able to measure PBMC telomerase activity at two time points: before and after treatment. Symptomatic improvement with decreased PBMC telomerase activity followed treatment. In this case, the effects of steroids as therapeutic agents must be considered. However, when we analyzed the SLE patients, the steroid dose was not correlated with PBMC telomerase activity.[7] Furthermore, in case 2, steroid was administered to some extent, but PBMC telomerase activity was elevated. These observations suggest that in patients with adult Still's disease as well as SLE patients PBMC telomerase activity may reflect the activity of the disease. However, this possibility requires further study in more patients.

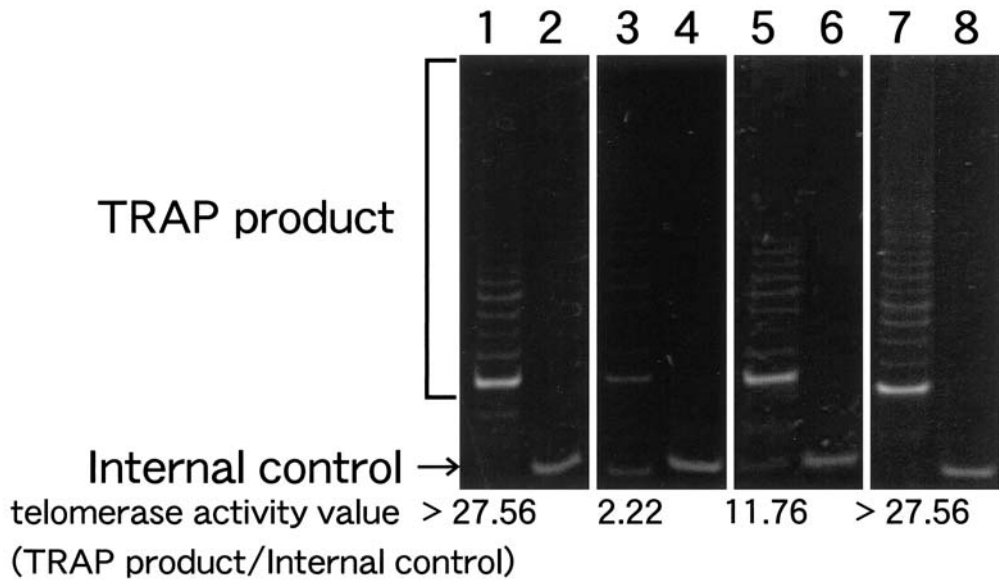


**Fig. 2.** Polyacrylamide gel electrophoresis of telomerase repeat amplification protocol (*TRAP*) assay products. Mononuclear cells ($1 \times 10^6$ cells) were measured for telomerase activity by TRAP assay. The TRAP assay products obtained were subjected to 12% polyacrylamide gel electrophoresis. The telomerase activity value was expressed as the ratio of the total TRAP product ladder to the internal control bands. For all measurements, heat-inactivated controls served as negative controls. As positive controls, telomerase activity was measured in Ramos cells ($1 \times 10^6$). Lane *1*, pretreatment telomerase activity value for peripheral blood mononuclear cells in case 1. Lane *3*, posttreatment telomerase activity value for peripheral blood mononuclear cells in case 1. Lane *5*, telomerase activity value for peripheral blood mononuclear cells at recrudescence in case 2. Lane *7*, telomerase activity value for Ramos cells ($1 \times 10^6$). Lanes *2*, *4*, *6*, and *8* are the negative controls

Further studies are needed to determine the type of cells from which the abnormal PBMC telomerase activity in this study was derived. With the assay method used in this study, the PBMC fraction contains lymphocytes and monocytes. There have been various reports since Hiyama et al.[4] reported that normal peripheral blood lymphocytes have telomerase activity. It is also known that activation of lymphocytes by various stimuli is accompanied by an increase in their telomerase activity.[4] We have recently reported that, in SLE patients, abnormal PBMC telomerase activity is chiefly derived from B cells.[18] Another study noted that lymphocyte activation also occurs in adult Still's disease.[19] The above observations suggest that the abnormal PBMC telomerase activity reported here was derived from lymphocytes.

Next, the possibility that abnormal PBMC telomerase activity is derived from monocytes should be considered. Recently, adult Still's disease has been thought to be a pathological condition based on macrophage activation.[20] Therefore, it is possible that abnormal telomerase activation has occurred in monocytes. There have been no reports of monocyte telomerase activity being detected in benign disease without malignant transformation. Many studies in healthy individuals have reported that no telomerase activity is detected in the monocyte fraction.[21] Therefore, detection of abnormal telomerase activity in this fraction would be an interesting finding.

## Conclusions

This report of adult Still's disease patients with abnormally high PBMC telomerase activity is interesting when considering the pathogenesis of adult Still's disease.

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