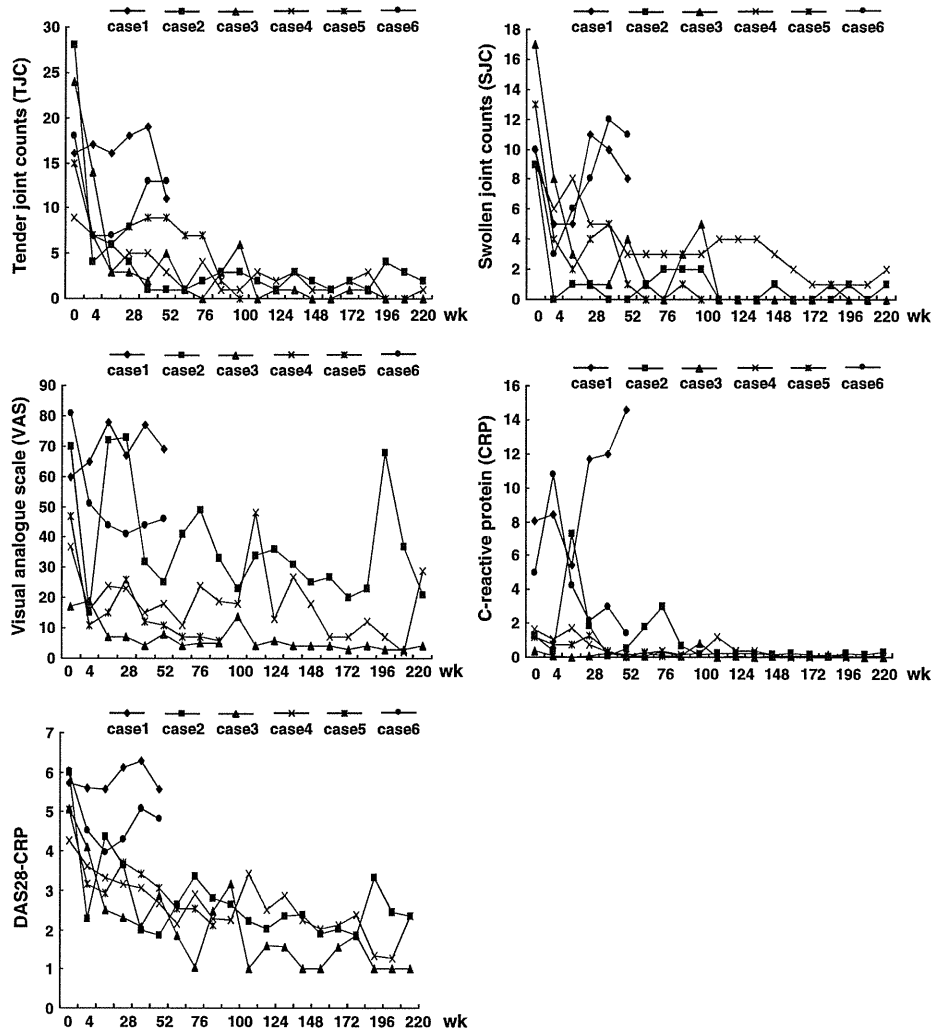

Fig. 1 Effectiveness of adalimumab for 6 RA patients was assessed at 4, 16, and 28 weeks, and every 12 weeks in the extension phase using tender joint counts (TJC), swollen joint counts (SJC), the visual analog scale (VAS), C-reactive protein (CRP) level, and DAS28-CRP (calculated using CRP concentration and evaluation of 28 joints)

Table 2 Clinical course and response of 6 RA patients during a 220-week follow-up

Case	1	2	3	4	5	6
Dose (mg)	20–40	20–40	40	40	40–80	40–80
Course	Discontinued	Ongoing	Ongoing	Ongoing	Discontinued	Discontinued
DAS28-CRP						
Before (treatment)	5.72 (46 weeks)	5.99 (220 weeks)	5.09 (220 weeks)	4.25 (220 weeks)	5.05 (88 weeks)	6.01 (48 weeks)
After	5.58	2.33	1.02	2.33	2.11	4.78
AAA	Negative	Negative	Negative	Negative	Negative	Positive
Adverse event	None	Naso-pharyngitis	Common cold	Injection site reaction	Non-Hodgkin lymphoma	Injection site reaction

DAS28-CRP the 28 joint count Disease Activity Score using C-reactive protein, *AAA* anti-adalimumab antibody

48), and one patient withdrew because of adverse events (case 5 discontinued at week 88 because of non-Hodgkin lymphoma) (Table 2).

We report the effectiveness of adalimumab monotherapy for 6 Japanese RA patients followed up for 220 weeks. In this study, we found its effectiveness last long time even in 220 weeks. The efficacy and safety of adalimumab as monotherapy in 544 RA patients for 26 weeks were previously reported. In the previous study, patients were treated with monotherapy of adalimumab

20 mg every other week, 20 mg weekly, 40 mg every other week, or 40 mg weekly, or with a placebo. After 26 weeks, patients were treated with adalimumab 40 mg every other week or 40 mg weekly. The groups achieved ACR20 in 46 and 53.4% of patients, respectively. These response rates were significantly better than that in the placebo group (19.1%) [8]. Recently, Miyasaka et al. [6] reported that in the Clinical investigation in Highly disease-affected rheumatoid Arthritis patients in Japan with Adalimumab applying staNdard and General Evaluation (CHANGE) study, adalimumab 20, 40, and 80 mg were safe and effective in Japanese patients. All adalimumab treatment groups achieved statistically significantly higher ACR20 responses (28.7% in the 20 mg group, 44.0% in the 40 mg group, and 50.6% in the 80 mg group) compared with the placebo group (13.8%) during 24-week follow-up. In our patients, the median score of DAS28-CRP was decreased from 5.35 ± 0.69 to 3.52 ± 1.61 at week 24. Afterward the efficacy continued, and the median score on DAS28-CRP had decreased to 1.89 ± 0.75 by week 220. We demonstrated 220 week long-term follow-up data about the efficacy of adalimumab monotherapy for the first time in Japan. The efficacies at week 220 were better than those at week 24. Four of 6 patients achieved DAS28 <2.7, meaning clinical low disease activity at 4 weeks after starting treatment in case 2, at 16 weeks in case 3, and at 64 weeks in cases 4 and 5. Three patients continued this treatment under remission state until week 220; one patient (case 5) discontinued the treatment because of suffering from non-Hodgkin lymphoma.

The Anti-TNF Research Study Program of the Monoclonal Antibody D2E7 in Rheumatoid Arthritis (ARMADA) trial [9], a 6-month, placebo-controlled, phase II/III study, demonstrated significant reductions in signs and symptoms of RA, and improvement in physical function in response to combined therapy with MTX and adalimumab. In their study, the clinical response and remission were sustained in patients with RA during 4 years of treatment with adalimumab [10]. Although our patients were not treated with MTX, 3 patients showed a good response to adalimumab monotherapy. Three patients were discontinued because of an adverse event in one and no response in two. One (case 6) of two non-responder patients was anti-adalimumab antibody (AAA)-positive. Bartelds et al. [11] reported that lack of response to adalimumab is probably caused by the formation of AAA. AAA normally appears at low levels but at high frequency, especially in Japanese RA patients.

Regarding adverse events, 2 of 6 patients (33%) experienced injection site reaction, and 2 of 6 patients had infectious adverse events, including nasopharyngitis and common colds (33%). Although the number of patients was small in our study, the occurrence of injection site reaction and infectious adverse events were almost the same as in a previous report [6], even during a 220-week follow-up. We did not experience tuberculosis or opportunistic infections. There were no reports of tuberculosis in the adalimumab treatment groups in the 24-week follow-up CHANGE study in Japan [6], but there was a 4- to 7-fold increased risk of reactivation of latent tuberculosis in patients using TNF inhibitors [12, 13]. Case 5 was forced to discontinue treatment because of non-Hodgkin lymphoma in week 88. Recent reports have suggested a slight increase in the risk of lymphoma, particularly in patients with RA [14]. In the CHANGE study, malignancies were reported in two patients only in the placebo group during the double-blinded period. On the other hand, a recent metanalysis involving infliximab and adalimumab demonstrated an increased risk of lymphoproliferative disease and malignancies in patients treated with these agents [15]. Therefore, we have to consider both the risk and the benefit when we use TNF antagonists for RA patients.

In conclusion, among patients with RA for whom previous anti-rheumatic treatment had failed, adalimumab monotherapy achieved significant and sustained improvements in disease activity for a long time and improved physical function while being safe and well tolerated. Our study has a limitation, because the number of patients is small. Next, we plan to increase the number of patients and examine when these patients should discontinue the treatment, which needs further investigation.

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Conflict of interest None.

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ORIGINAL ARTICLE

Contribution of an adenine to guanine single nucleotide polymorphism of the matrix metalloproteinase-13 (*MMP-13*) −77 promoter region to the production of anticyclic citrullinated peptide antibodies in patients with HLA-DRB1*shared epitope-negative rheumatoid arthritis

Naoki Iwamoto · Atsushi Kawakami · Kazuhiko Arima · Mami Tamai · Hideki Nakamura · Shin-ya Kawashiri · Junko Kita · Akitomo Okada · Tomohiro Koga · Makoto Kamachi · Satoshi Yamasaki · Kunihiro Ichinose · Hiroaki Ida · Tomoki Origuchi · Katsumi Eguchi

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Abstract We examined whether matrix metalloproteinase-13 (MMP-13) contributes to disease susceptibility or severity of rheumatoid arthritis (RA). Eighty-seven patients with RA whose disease duration was <2 years and 71 healthy controls were enrolled in the study. Adenine (A) to guanine (G) single nucleotide polymorphism (SNP) of the −77 *MMP-13* promoter region in RA and healthy controls was determined by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique. Human leukocyte antigen (HLA)-DRB1 genotyping was also performed using the same populations. Anticyclic citrullinated peptide (anti-CCP) antibodies from RA patients at entry were studied, and their relationships were examined. The genotype and allele frequency of SNP of *MMP-13* at −77 did not differ between RA patients and healthy controls. We focused on the RA patients who were negative for HLA-DRB1*shared epitope (SE) alleles and found that the seropositivity of anti-CCP antibodies with a titer >25 U/ml was high in the A/A genotype compared with the G/G genotype. The same characteristic was also found in HLA-DRB1*0405 allele-negative patients. Our data suggest that SNP of the −77 *MMP-13* promoter region acts as a surrogate marker of anti-CCP antibody production in HLA-DRB1*SE allele-negative RA patients, which may reflect RA severity.

Keywords MMP-13 · Rheumatoid arthritis · Polymorphism · Anti-CCP antibodies

N. Iwamoto (✉) · A. Kawakami · K. Arima · M. Tamai · H. Nakamura · S. Kawashiri · J. Kita · A. Okada · T. Koga · M. Kamachi · S. Yamasaki · K. Ichinose · H. Ida · K. Eguchi
Unit of Translational Medicine, Department of Immunology and Rheumatology, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
e-mail: naoki_iwa@hotmail.com

T. Origuchi
Nagasaki University School of Health Sciences, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by joint destruction. Although the etiology of the disease remains unknown, recent evidence suggests that both genetic and environmental factors contribute to RA susceptibility and severity [1]. The most prominent genetic component in RA heritability is the human leukocyte antigen (HLA) locus on chromosome 6, which accounts for one third of RA genetic susceptibility [2]. In addition, several non-HLA genes are also considered to contribute to RA development [3].

Joint destruction in RA occurs by the degradation of type I, II, and III collagen, which constitutes cartilage and bone. Matrix metalloproteinases (MMPs) play an important role in this process. One such metalloproteinase is MMP-13, which degrades type II collagen of the interstitial collagens. MMP-13 expression is recognized in the synovial lining layer, vascular endothelial cells, fibroblast-like synoviocytes, monocytes, osteoblasts, and cartilage cells in rheumatoid synovial tissues. The intensity of MMP-13 messenger RNA (mRNA) expression in rheumatoid synovial tissues correlates with a severe clinical course of RA [4]. Serum MMP-13 concentrations in early-stage RA

patients are high, but decrease in response to anti-rheumatic treatment [5]. These data indicate that MMP-13 is one of the most important MMPs involved in the development of RA.

In a study of allele-specific effects on the regulation of MMP-13 expression in relation to the −77 A>G polymorphism, it was found that the *MMP-13* −77 A allele has higher promoter activity than the *MMP-13* −77 G allele [6]. A previous study also found that the functional disability assessed by the Steinbrocker Index in patients with RA is high in the *MMP-13* −77 A/A genotype compared with the −77 G/G genotype [7]. Based on these previous findings, we investigated whether the *MMP-13* −77 A>G polymorphism is associated with anticyclic citrullinated (anti-CCP) peptide antibody production in early-stage RA from the Japanese population.

Patients and methods

Study population

Eight-seven patients with RA diagnosed according to the 1987 revised criteria of the American College of Rheumatology for classification of the disease [8] and who had visited our early arthritis clinic as previously reported [9, 10] were enrolled in the study. The Early Arthritis Clinic opened in 2001 as part of the Unit of Translational Medicine, Department of Immunology and Rheumatology, Graduate School of Biomedical Sciences, Nagasaki University. Patients were referred from an area in the western part of Japan, Nagasaki Prefecture, which has approximately 450,000 inhabitants. The control samples were 60 unrelated healthy Japanese individuals. Each individual provided a signed consent form to participate in the study, which was approved by the Institutional Review Board of Nagasaki University. Baseline clinical manifestations and variables included gender; age; localization of arthritis; duration of morning stiffness; number of tender joints; number of swollen joints; C-reactive protein level (CRP) measured by latex turbidimetric immunosorbent assay (Daiichi Pure Chemicals, Fukuoka, Japan); immunoglobulin M (IgM)-rheumatoid factor (RF) positivity measured by latex-enhanced immunonephelometric assay, cutoff value 14 IU/ml (Dade Behring, Marburg, Germany); positive status for anti-CCP antibodies measured by enzyme-linked immunosorbent assay (ELISA), cutoff value 4.5 U/ml (DIASTAT Anti-CCP; Axis-Shield, Dundee, UK); and MMP-3 measured ELISA, cutoff value 59.7 ng/ml for women and 121.0 ng/ml for men (Daiichi, Japan). We summarize some of this information in Table 1.

Table 1 Patient characteristics

Characteristics	Total cohort ($n = 87$)
Sex, no. female/male	70/17
Age, mean ± SD (years)	53.26 ± 14.14
Mean disease duration, months ± SD	5.66 ± 5.91
No. (%) seropositive for anti-CCP antibodies[a]	54 (62)
No. (%) seropositive for anti-CCP antibodies with a titer >25 U/ml	39 (45)
No. (%) positive for HLA-DRB1*SE alleles	47 (54)
No. (%) positive for HLA-DRB1*0405 allele	38 (44)

SD standard deviation, *SE* shared epitope, *anti-CCP* anti-CCP anticyclic citrullinated peptide

[a] Anti-CCP antibodies cutoff value 4.5 U/ml

DNA isolation and genotyping

Genomic DNA was extracted from peripheral blood samples by a standard procedure. The *MMP-13* −77 A>G polymorphism was examined according to the published method [6]. In brief, a 445-bp DNA fragment, including the polymorphic site, was amplified by polymerase chain reaction (PCR) using a set of oligonucleotide primers: sense 5′-GATACGTTCTTACAGAAGGC-3′; antisense 5′-GACAAATCATCTTCATCACC-3′. The PCR products were digested with 1 U of *Bsr*I (New England Biolabs Inc., Beverly, MA, USA), which claves the G allele, generating two fragments 197 and 248 bp in size. The digests were analyzed on 3% agarose gels. HLA-DRB1 genotyping was also performed, as we previously described [11].

Statistical analysis

Distributions of the *MMP-13* −77 A>G polymorphism in RA patients and healthy controls were determined using Fisher's exact test. The Chi-square test was used for comparison. A p value <0.05 was considered statistically significant.

Results

Baseline characteristics and distribution of MMP-13 −77 A>G polymorphism between early-stage RA patients and healthy controls

Baseline characteristics of the 87 patients are given in Table 1. As the mean disease duration from the onset of articular symptoms to entry was 5.66 ± 5.91 months, our population is considered to be early-stage RA patients. Fifty-four of 87 patients (62%) were seropositive toward

Table 2 Genotype and allele frequency of the −77 polymorphism of the matrix metalloproteinase-13 (*MMP-13*) gene in patients and controls

	Patients [*n* (%)]	Controls [*n* (%)]	*P* value	Odds ratio	95% CI
Genotype					
A/A	23 (26)	18 (30)	0.64	0.84	0.40–1.74
A/G	39 (45)	27 (45)	0.98	0.99	0.51–1.92
G/G	25 (29)	15 (25)	0.62	1.21	0.57–2.55
Presence of allele					
A	62 (71)	45 (75)	0.62	0.83	0.39–1.74
G	64 (74)	42 (70)	0.64	1.19	0.58–2.47
Allele frequency					
A	85 (49)	63 (53)	0.54	0.86	0.54–1.38
G	89 (51)	57 (48)	0.54	1.16	0.73–1.84

CI confidential interval

anti-CCP antibodies. The carriership of HLA-DRB1*shared epitope (SE) alleles and the HLA-DRB1*0405 allele was 54 and 44%, respectively, which is similar to previous reports from Japanese populations [11, 12]. We compared genotype, presence of the allele, and allele frequency of *MMP-13* −77 A>G polymorphism between RA patients and healthy controls. As shown in Table 2, there was no statistically significant difference between the two populations.

Percentage of RA patients with anti-CCP antibodies >25 U/ml is high in the *MMP-13* −77 A/A genotype compared with the −77 G/G genotype among the HLA-DRB1*SE allele-negative population in patients with RA

We next tried to examine the relationship between the *MMP-13* −77 A>G polymorphism and RA severity. Syversen et al. [13] recently revealed that titers of anti-CCP antibodies in early-stage RA patients at baseline reflect the further radiographic bone destruction. They set the anti-CCP antibody titers as 25 U/ml [13]. Thus, we examined the distribution of anti-CCP antibodies based on the cutoff value of 25 U/ml, but we found no statistically significant difference among *MMP-13* −77 A/A, −77 A/G, and −77 G/G polymorphisms (data not shown). To exclude the influence of HLA-DRB1*SE alleles on the *MMP-13* −77 A>G polymorphism, we carried out the same analysis in HLA-DRB1*SE allele-negative RA patients and found that the percentage of patients with anti-CCP antibody titers >25 U/ml was statistically high in the *MMP-13* −77 A/A genotype compared with the *MMP-13* −77 G/G genotype (Fig. 1). A similar result was also obtained in HLA-DRB1*0405 allele-negative RA patients [patients with anti-CCP antibody titer >25 U/ml was 7 of 12 in *MMP-13* −77 A/A genotype (58%) and 3 of 16 in *MMP-13* −77 G/G genotype (19%); $p = 0.039$, −77 A/A genotype vs. −77 G/G genotype].

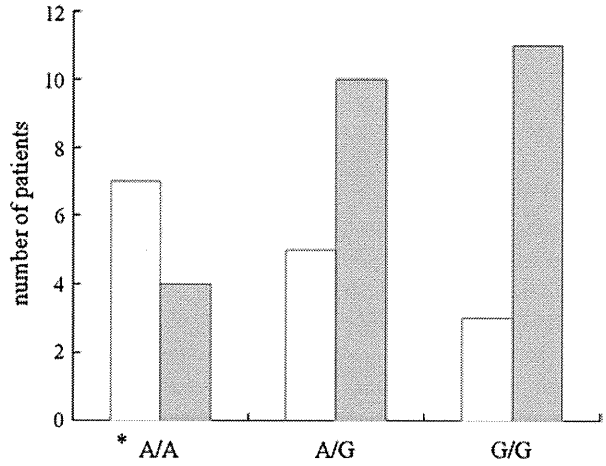

Fig. 1 Association of the −77 polymorphism of the matrix metalloproteinase-13 (*MMP-13*) gene with levels of anticyclic citrullinated peptide (anti-CCP) antibodies in the human leukocyte antigen (HLA)-DRB1*shared epitope (SE) allele-negative population in patients with rheumatoid arthritis (RA). *Open bars* show the number of RA patients with anti-CCP antibody titer >25 U/ml, whereas *gray bars* indicate those with anti-CCP antibody titer <25 U/ml. The percentage of RA patients with anti-CCP antibody titer >25 U/ml was statistically significantly high in the *MMP-13* −77 A/A genotype compared with the *MMP-13* −77 G/G genotype. Data were calculated by chi-square test, as described in "Patients and methods"

Discussion

RA is a multifactorial disorder with an estimated heritability of 60% [14]. As the mean disease duration from onset to entry of our 87 RA patients was <6 months, the study population were considered to be early-stage RA patients. Early-stage patients reflect the disease process well compared with established patients with RA. The *MMP-13* −77 A>G polymorphism did not differ between RA patients and healthy controls, suggesting that *MMP-13* is not an RA-susceptible gene.

We next investigated whether *MMP-13* determines RA severity. We focused on the relationship between anti-CCP antibodies with the *MMP-13* −77 A>G polymorphism in patients with RA. Anti-CCP may be directly involved in RA pathogenesis. Locally produced anti-CCP generates immune complexes and may contribute to initiating and sustaining synovial inflammation by triggering monocyte and granulocyte activation and cytokine production [15, 16]. Therefore, patients with high levels of anti-CCP might have severe progression compared with patients with low levels. Actually, the presence of anti-CCP antibodies

in RA is considered to be a prognostic factor toward further radiographic progression [13]. Patients with anti-CCP antibody levels >25 U/ml are especially more likely to develop radiographic progression [13]. Susceptibility to and severity of the disease has been associated with variations in *HLA* genes. Both radiographic progression and anti-CCP antibodies have proved to correlate positively with the presence of HLA-DRB1*SE alleles. Probably due to the influence of HLA-DRB1*SE alleles, we found no association between the *MMP-13* −77 A>G polymorphism and anti-CCP antibodies in our entire population. However, the distribution of anti-CCP antibody-positive patients, defined as having antibody levels >25 U/ml, differs between −77 A/A and −77 G/G genotypes of the *MMP-13* polymorphism in HLA-DRB1*SE allele-negative patients with RA. Considering that MMP-13 expression in the rheumatoid synovial tissues of patients with the *MMP-13* −77 A/A genotypes might be high, the production of anti-CCP antibodies as well as joint destruction might become obvious in these patients. Accordingly, our data appear to support the previous finding [7].

In summary, our data indicate an association between the *MMP-13* −77 A>G polymorphism and production of anti-CCP antibodies in patients with HLA-DRB1*SE allele-negative early-stage RA. Recently, strong combined gene–environment effects in anti-CCP antibody-positive RA have been identified, such as in the interactions of HLA-DRB1*SE alleles with smoking and drinking coffee [17]. As we did not include environmental factors in this report, the more precise consideration of environmental factors with the *MMP-13* −77 A>G polymorphism, HLA-DRB1*SE alleles, and anti-CCP antibodies might lead to new insights into the gene–environment effects of RA.

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Conflict of interest All authors declared no conflict of interest.

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CD4+CD25^{high}CD127$^{low/-}$ Treg Cell Frequency from Peripheral Blood Correlates with Disease Activity in Patients with Rheumatoid Arthritis

SHIN-YA KAWASHIRI, ATSUSHI KAWAKAMI, AKITOMO OKADA, TOMOHIRO KOGA, MAMI TAMAI, SATOSHI YAMASAKI, HIDEKI NAKAMURA, TOMOKI ORIGUCHI, HIROAKI IDA and KATSUMI EGUCHI

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CD4+CD25^{high}CD127$^{low/-}$ Treg Cell Frequency from Peripheral Blood Correlates with Disease Activity in Patients with Rheumatoid Arthritis

SHIN-YA KAWASHIRI, ATSUSHI KAWAKAMI, AKITOMO OKADA, TOMOHIRO KOGA, MAMI TAMAI, SATOSHI YAMASAKI, HIDEKI NAKAMURA, TOMOKI ORIGUCHI, HIROAKI IDA, and KATSUMI EGUCHI

ABSTRACT. ***Objective.*** To investigate whether the frequency of peripheral blood (PB) regulatory T cells (Treg) correlates with the clinical disease activity of rheumatoid arthritis (RA).

Methods. PB Treg cells, defined as the CD4+CD25^{high}CD127$^{low/-}$ population, were examined by flow cytometry in 48 patients with RA, including 13 who had never received disease-modifying antirheumatic drugs (DMARD), 19 with active disease who were receiving (n = 14) or had received (n = 5) DMARD, and 16 receiving DMARD whose disease was in remission. The clinical disease activity of the patients was defined by the 28-joint Disease Activity Score (DAS28). The association of DAS28, C-reactive protein (CRP), or erythrocyte sedimentation rate (ESR) with the frequency of PB Treg cells was examined.

Results. The frequency of PB Treg cells in patients with RA was significantly low compared with that of healthy controls (n = 14). Among the 3 populations of patients with RA, Treg cell frequency was lowest in patients with active RA. In contrast, the Treg cell frequency of patients with RA in remission was similar to that of healthy controls. Accordingly, the frequency of CD4+CD25^{high}CD127$^{low/-}$ Treg cells negatively correlated with DAS28, CRP, and ESR in patients with RA.

Conclusion. The data suggest that Treg cells, defined as the CD4+CD25^{high}CD127$^{low/-}$ population, may contribute to the pathogenesis of RA and be an indicator of disease activity. (First Release Sept 15 2011; J Rheumatol 2011;38:2517–21; doi:10.3899/jrheum.110283)

Key Indexing Terms:
REGULATORY T CELL
RHEUMATOID ARTHRITIS
CD127 (INTERLEUKIN 7Rα)
DISEASE ACTIVITY

CD4+ regulatory T cell (Treg)-cell deficiency or absence is known to correlate with the development or exacerbation of autoimmune diseases, implying a crucial role for Treg cells in maintaining immunological self-tolerance[1,2]. In recent years, Treg cell counts and function have also been examined in patients with rheumatoid arthritis (RA)[3,4,5,6,7,8]. Treg cell function in patients with active RA is assumed to be impaired, a trend that seems to be reversed by tumor necrosis factor (TNF) antagonist therapy[6,7]; however, Treg cell counts in peripheral blood (PB) have varied across studies[3,4]. These discrepancies can probably be ascribed to differences in the labeling and definition of CD4+CD25+ T cells[9]. Among CD4+CD25+ T cells, those exerting suppressive effects[9,10] are only those expressing large amounts of CD25, e.g., CD4+CD25^{high} T cells, which highly express forkhead box P3 (FOXP-3). The intracellular staining process for FOXP-3 is somewhat time-consuming as compared with cell surface staining in clinical practice; thus, a more convenient marker on the cell surface closely correlating with FOXP-3 expression is awaited. In this regard, Saleem, *et al* recently reported that the frequency of CD62L^{+} Treg cells in PB from RA is associated with sustained remission during TNF antagonist therapy[11].

Another candidate cell surface molecule for the identification of Treg cells is CD127. Two recent studies have demonstrated that downregulation of the interleukin (IL)-7 receptor α chain, CD127, distinguishes Treg cells from activated T cells, demonstrating a significant correlation between the FOXP-3 and CD127$^{low/-}$ phenotype at the same time that it functionally suppresses the CD127$^{low/-}$ population[12,13].

We examined whether the frequency of Treg cells correlates with the clinical disease activity of RA by staining cells

From the Unit of Translational Medicine, Department of Immunology and Rheumatology, and the Department of Health Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki; and the Division of Respirology, Neurology, and Rheumatology, Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan.

S-Y. Kawashiri, MD, PhD; A. Kawakami, MD, PhD; A. Okada, MD; T. Koga, MD, PhD; M. Tamai, MD, PhD; S. Yamasaki, MD, PhD; H. Nakamura, MD, PhD, Unit of Translational Medicine, Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences; T. Origuchi, MD, PhD, Department of Health Sciences, Nagasaki University Graduate School of Biomedical Sciences; H. Ida, MD, PhD, Division of Respirology, Neurology, and Rheumatology, Department of Medicine, Kurume University School of Medicine; K. Eguchi, MD, PhD, Unit of Translational Medicine, Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences.

Address correspondence to Dr. S-Y. Kawashiri, Unit of Translational Medicine, Department of Immunology and Rheumatology, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. E-mail: shin-ya@hotmail.co.jp

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with CD4, CD25, and CD127. The frequency of CD4+CD25^{high}CD127$^{low/-}$ Treg cells negatively correlated with the 28-joint Disease Activity Score (DAS28), C-reactive protein (CRP), and the erythrocyte sedimentation rate (ESR). This is a more convenient method of detecting Treg cells in clinical practice and may follow the data of Saleem, *et al*[11], suggesting that Treg cells contribute to the pathogenesis of RA.

MATERIALS AND METHODS

Study population. Patients with RA (n = 48) and healthy controls (n = 14) were included in our study. All of the patients fulfilled the 1987 criteria of the American College of Rheumatology for RA[14]. All patients were seropositive for rheumatoid factor and/or anticitrullinated protein antibodies. Informed consent was obtained from all patients and controls. The Institutional Review Board of Nagasaki University approved the study. Clinical response to the therapy was evaluated by DAS28 (high disease activity > 5.1, moderate disease activity < 5.1 and > 3.2, low disease activity < 3.2, remission < 2.6). Patients were divided into 3 groups: (1) those naive to disease-modifying antirheumatic drugs (DMARD; n = 13); (2) those with active RA (n = 19) whose disease activity was moderate (DAS28 > 3.2), including both those who were receiving (n = 14) or had received (n = 5) DMARD; and (3) those whose disease was in clinical remission (DAS28 < 2.6) with concomitant use of DMARD (n = 16). All patients in the group with active RA and the remission group were on stable therapy. Patient characteristics are shown in Table 1. For the controls, the median (range) of age was 34.5 (27-50) years and the sex ratio was 4:10 (men:women). They were statistically younger than patients with RA. We examined a correlation of age and each population of T cell frequency among 14 controls by Spearman's rank correlation and did not find any association (data not shown).

Cell isolation and analysis by flow cytometry. Peripheral blood samples were collected in heparin. Peripheral blood mononuclear cells (PBMC) were isolated by standard Ficoll-Hypaque density centrifugation and used for flow cytometry. Freshly isolated PBMC were stained with 3 colors: FITC-labeled CD4; PE-Cy5-labeled CD25 and PE-labeled CD127; or PE-Cy5-labeled CD4, PE-labeled CD25, and FITC-labeled FOXP-3 (clone: PCH101, intracellular staining) by the standard protocol. All antibodies were products of eBioscience (San Diego, CA, USA). For flow cytometric analysis, lymphocytes were gated morphologically.

Statistical analyses. Within-group comparisons were made using the Mann-Whitney U test. Changes from the baseline were compared using Wilcoxon's signed-rank test. Correlations were assessed with Spearman's correlation coefficient test. The overall significance level for statistical analysis was 5% (2-sided). P values < 0.05 were considered statistically significant.

RESULTS

Patient characteristics are shown in Table 1. CRP, ESR, and DAS28 in the remission group were significantly lower than in the other 2 groups. CRP and ESR in the patients with active RA who were receiving or had received DMARD were significantly higher than those of DMARD-naive patients with RA, but DAS28 was not significantly different between the 2 groups.

Although most CD4+CD25+CD127$^{low/-}$ T cells were positive for FOXP-3, a portion of this population was negative for FOXP-3 (Figure 1A). On the other hand, almost all CD4+CD25^{high}CD127$^{low/-}$ T cells were positive for FOXP-3 (Figure 1A).

Phenotypes of peripheral blood CD4+ T cells of patients with RA and controls were compared (Table 2). There were no significant differences in the frequencies of CD4+CD25– T cells and CD4+CD25+ T cells between DMARD-naive patients with RA and healthy volunteers. The frequency of CD4+CD25+CD127$^{low/-}$ T cells (Figure 1A) was significantly lower in the patients with active RA who were receiving or had received DMARD than in controls ($p < 0.05$).

We counted the frequency of CD4+CD25^{high}CD127$^{low/-}$ T cells, using the cutoff value of < 5% CD127 expression among CD4+ T cells (Figure 1B). We have adopted this method to identify the CD4+CD25^{high}CD127$^{low/-}$ T cells accurately in each individual. The frequency of this population was lower in the DMARD-naive RA patient group than in the healthy controls ($p < 0.01$), and was lower in the active RA group taking DMARD than in the DMARD-naive RA group ($p < 0.01$). Further, the frequency of this population was higher in the remission group than in the active RA group with DMARD ($p < 0.0001$).

We investigated the correlation between the phenotype of

Table 1. Patient characteristics. Within-group comparisons were made using the Mann-Whitney U test and the chi-squared test (Fisher's exact probability test when appropriate).

Characteristics	Healthy Controls	DMARD-naive RA Group	Active RA Group	Remission Group
Patient, no.	14	13	19	16
Age, yrs, median (range)	34.5 (27–50)	59 (39–81)	57 (19–79)	58 (26–76)
Sex (men/women)	4/10	3/10	1/18	4/12
Disease duration, yrs, median (range)	—	0.25 (0.15–1.5)	3.5* (0.5–28)	2.5* (0.33–22)
CRP, mg/dl, median (range)	—	0.54** (0.04–5.35)	2.59**# (0.16–10.08)	0.07 (0.01–0.12)
ESR, mm/h, median (range)	—	48** (12–120)	57.4**# (1–127)	13.5 (5–26)
DAS28, median (range)	—	4.68** (3.54–7.75)	5.70** (3.21–8.16)	1.83 (1.13–2.54)
Therapy	—	—	MTX: 13, SASP: 1	MTX: 11, SASP: 2, BU: 1, ETN: 1, IFX: 3
Concomitant glucocorticoid, n (dose < 7.5 mg daily)	—	2	10***#	0

* $p < 0.0001$ vs DMARD-naive RA group. ** $p < 0.0001$ vs remission group. *** $p < 0.001$ vs remission group. # $p < 0.05$ vs DMARD-naive RA group. DMARD: disease-modifying antirheumatic drugs; RA: rheumatoid arthritis; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS28: 28-joint Disease Activity Score; BU: bucillamine; ETN: etanercept; IFX: infliximab; MTX methotrexate; SASP: salazosulfapyridine.

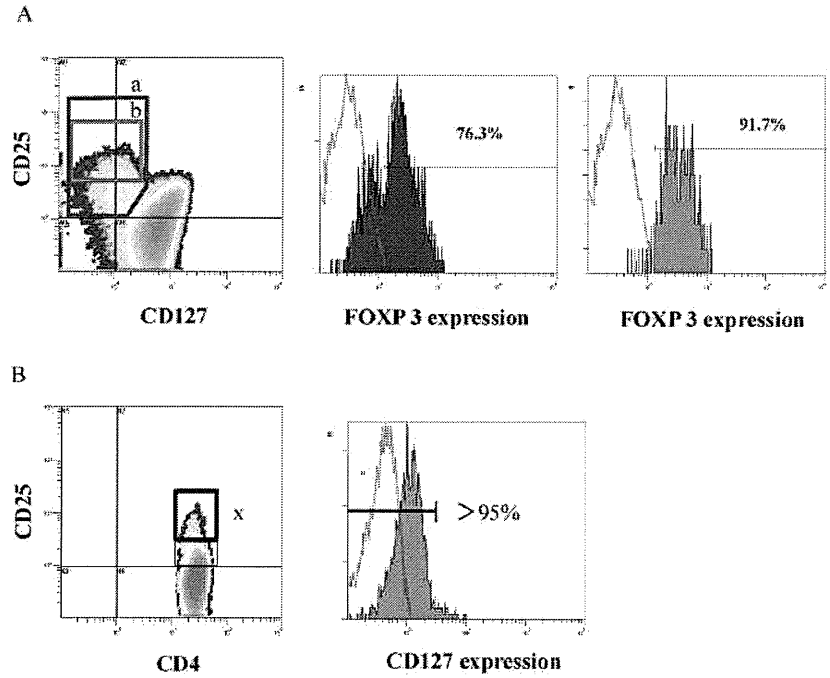

Figure 1. The CD4+CD25^{high}CD127$^{low/-}$ population as the phenotype of Treg cells. The graphs show representative data of several healthy samples. A. Plots are gated for CD4+ T cells. CD25+CD127$^{low/-}$ cells and CD25^{high}CD127$^{low/-}$ cells are found in box a and box b. Expressions of FOXP-3 in boxes a and b are shown in the second and third panels. B. Mononuclear cells were stained for CD4, CD25, and CD127. Plots are gated for CD4+ T cells. The CD4+CD25^{high} population, with the cutoff of CD127 expression at < 5% among CD4+ T cells (right side), is boxed as CD4+CD25^{high}CD127$^{low/-}$ T cells (left side, box x). Box x is individually adjusted.

Table 2. Phenotype of peripheral blood CD4+ T cells in patients with rheumatoid arthritis (RA) and in healthy controls. Data are percentage of CD4+ T cells; median (range). Within-group comparisons were made using the Mann-Whitney U test.

Study Participants	CD4+CD25–	CD4+CD25+	CD4+CD25+CD127$^{low/-}$	CD4+CD25^{high}CD127$^{low/-}$
Healthy controls, n = 14	84.7 (72.4–87.2)	15.3 (12.8–27.6)	3.63 (2.34–7.54)	3.76 (2.11–9.80)
DMARD-naive RA group, n = 13	78.3 (63.9–88.3)	21.7 (11.7–36.1)	3.12 (1.72–5.73)	2.23** (0.57–5.18)
Active RA group, n = 19	79.9 (63.9–87.2)	20.1 (12.8–36.1)	2.76* (1.25–5.31)	1.35***# (0.41–2.21)
Remission group, n = 16	80.6 (66.6–90.1)	19.4 (9.9–33.4)	3.35 (2.13–7.21)	2.98$ (1.34–4.89)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$ vs HC, # $p < 0.01$ vs DMARD-naive RA group, $ $p < 0.0001$ vs active RA group. DMARD: disease-modifying antirheumatic drugs.

peripheral blood Treg cells and the markers of disease activity such as CRP, ESR, and DAS28 in the 48 patients with RA (Table 3). The frequencies of CD4+CD25+ T cells and CD4+CD25+CD127$^{low/-}$ T cells were not correlated with disease activity. However, the frequency of CD4+CD25^{high}-CD127$^{low/-}$ T cells was negatively correlated with CRP, ESR, and DAS28, respectively ($p < 0.0001$).

As mentioned, CD4+ T cells were almost all positive for CD127 (Figure 2A); however, a large CD127$^{low/-}$ population was detected among CD4+CD25+ T cells in healthy individuals (Figure 2B). In patients with RA, the expression of this population was lower than in healthy individuals (Figure 2C), but it recovered after the disease went into clinical remission (Figure 2D).

DISCUSSION

Recent data obtained from patients with RA during TNF-antagonist therapy have suggested that TNF downmodulates the function of human CD4+CD25+ Treg cells[6,7,8]. Therefore, Treg cells may dynamically fluctuate, depending on the disease status of RA, and reflect the disease activity of RA. We have focused on a convenient cell surface staining method to identify Treg cells and tried to investigate the association of Treg cell frequency with the disease activity of RA.

CD25 and CD127 were used to identify the Treg cell population in our study. Since FOXP-3 is strongly expressed in CD4+CD25^{high}CD127$^{low/-}$ population, CD4+CD25^{high}- CD127$^{low/-}$ cells can be estimated as Treg

Table 3. The correlations between regulatory T cells (Treg) and rheumatoid arthritis (RA) disease activity in 48 patients with RA. The correlations were assessed using Spearman's correlation coefficient test.

	CRP		ESR		DAS28	
	r	p	r	p	r	p
CD4+CD25+	0.03	NS	0.12	NS	0.04	NS
CD4+CD25+CD127$^{low/-}$	–0.21	NS	–0.20	NS	–0.17	NS
CD4+CD25^{high}CD127$^{low/-}$	–0.65	< 0.0001	–0.58	< 0.0001	–0.61	< 0.0001

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS28: 28-joint Disease Activity Score; NS: not significant.

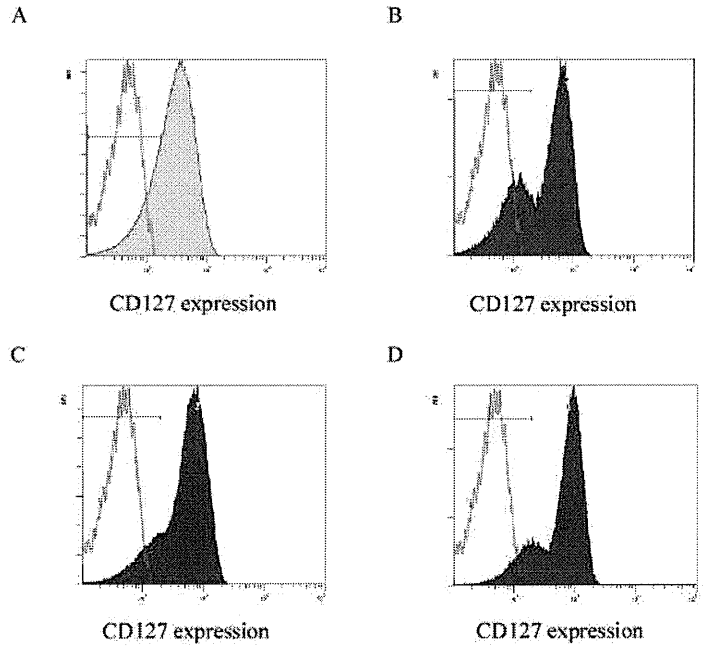

Figure 2. Changes in the proportion of the CD127$^{low/-}$ population among CD4+CD25+ T cells. Expression of CD127 among CD4+ T cells (A) and CD4+CD25+ T cells (B-D) are shown. Panels A and B describe peripheral blood mononuclear cell (PBMC) samples collected from healthy individuals. Although CD4+ T cells are likely almost all positive for CD127 (A), the expression of CD127 among CD4+CD25+ T cells is differential, with both CD127$^{low/-}$ cells and CD127^{+} cells in healthy controls (B). Panels C and D describe PBMC samples collected from a patient with early RA (male, disease duration 3 months). The proportion of CD127$^{low/-}$ cells among CD4+CD25+ T cells decreased before this patient was treated (28-joint Disease Activity Score 4.27, panel C). After this patient's disease went into clinical remission by treatment with bucillamine, the expression of CD127 among CD4+CD25+ T cells recovered to nearly the same level as that in healthy controls (D). The frequencies of CD4+CD25^{high}CD127$^{low/-}$ cells were 5.73% before therapy and 7.21% after clinical remission.

cells[12,13]. Additionally, the clinical differences between patients with RA and controls as well as the clinical measures among patients with RA were most predominantly found in the CD4+CD25^{high}CD127$^{low/-}$ population. We have set the cutoff of CD127 expression at < 5% in the individual case; thus, our definition may correctly identify the frequency of naturally arising Treg cells (Figure 3). The controls were younger than the patients with RA in our study. Although there was no correlation between Treg cell frequency and age of the control, a previous study[4] demonstrated a weak negative correlation between age and Treg cell frequency. The use of glucocorticoids was more frequent in the active RA group as compared with the DMARD-naive RA group, as well as the remission group. The influence of glucocorticoids regarding the function or number of Treg cells might be controversial[15,16]. Therefore, age-matched studies involving glucocorticoid-naive patients are necessary to confirm our results.

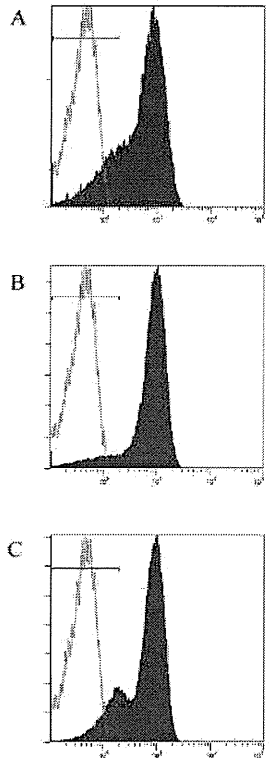

Figure 3. The proportion of the CD127$^{low/-}$ population among CD4+CD25+ T cells in the 3 RA groups. A. The group naive to disease-modifying antirheumatic drugs. B. The group with active RA. C. The remission group. Expressions of CD127 among CD4+CD25+ T cells are shown. The frequencies of CD4+CD25+CD127$^{low/-}$ cells were 3.28%, 2.24%, and 5.77% in panels A, B, and C, respectively.

DMARD may alter the function of T cells in patients with RA. However, we have found that Treg cell frequency may depend not on the use of DMARD but on the RA disease activity. The Treg cell frequency was lowest in patients with active RA, while that of patients with RA in remission was similar to that of controls, despite the administration of DMARD in both groups. In addition, the Treg cell frequency was not statistically different between DMARD-naive patients with RA and patients receiving DMARD whose RA was in remission, and an inverse correlation was found between the disease activity of RA and the Treg cell frequency. In fact, we found fluctuation of Treg cell frequency depending on the disease activity. The difference of our study as compared with previous reports is to estimate Treg cells as FOXP-3^{bright} cells. As shown in previous reports[3,4], the difference of CD4+CD25+ T cell frequency between the patients with RA and controls was not significant. In addition to CD127, a similar result is obtained when estimating Treg cells as CD62 ligand^{+} FOXP-3^{bright} cells[11]. Since we have not performed followup analysis of Treg cell frequency in each case, a prospective followup study should be performed to establish that the CD4+CD25^{high}CD127$^{low/-}$ Treg cell population does in fact reflect changes in the disease activity of RA. Further examinations, including studies with a larger number and with followup observation, are needed to confirm our findings.

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ORIGINAL ARTICLE

Treatment discontinuation in patients with very early rheumatoid arthritis in sustained simplified disease activity index remission after synthetic disease-modifying anti-rheumatic drug administration

Junko Kita · Mami Tamai · Kazuhiko Arima · Yoshikazu Nakashima · Takahisa Suzuki · Shin-ya Kawashiri · Naoki Iwamoto · Akitomo Okada · Tomohiro Koga · Satoshi Yamasaki · Hideki Nakamura · Tomoki Origuchi · Hiroaki Ida · Kiyoshi Aoyagi · Masataka Uetani · Katsumi Eguchi · Atsushi Kawakami

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Abstract We aimed to identify whether drug-free remission could be achieved in patients with very early rheumatoid arthritis (RA) with poor prognosis factors by treatment with synthetic disease-modifying antirheumatic drugs (DMARDs). Thirteen patients with very early RA, whose disease was considered to have highly erosive potential, were included. Magnetic resonance imaging (MRI)-proven bone edema and autoantibodies were determined in these patients. A treat-to-target strategy initiated with synthetic DMARDs was employed for 12 months. If the patients achieved simplified disease activity index (SDAI) remission along with a reduction of the RA MRI scoring bone edema score to <33% as compared with baseline at 12 months, DMARD treatment was stopped and the clinical status was further observed for the following 12 months. Synthetic DMARDs were stopped at 12 months in 5 patients. One of the 5 was lost to follow-up because of sustaining an injury that required orthopedic surgery. Three of the remaining 4 patients showed continued SDAI remission that was DMARD-free without any evidence of radiographic progression for the following 12 months. Although this was a small clinical trial, we have shown–for the first time–that true remission of very early RA with poor prognosis factors can be achieved by treatment with synthetic DMARDs.

Keywords Very early RA · Synthetic DMARDs · SDAI · Remission · RAMRIS bone edema score

J. Kita and M. Tamai contributed equally to this work.

J. Kita · Y. Nakashima · T. Suzuki · S. Kawashiri · N. Iwamoto · A. Okada · T. Koga · S. Yamasaki · H. Nakamura · A. Kawakami (✉)
Unit of Translational Medicine, Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
e-mail: atsushik@nagasaki-u.ac.jp

M. Tamai
Center for Health and Community Medicine, Nagasaki University, Nagasaki, Japan

K. Arima
Department of Medical Gene Technology, Atomic Bomb Disease Institute, Nagasaki, Japan

T. Origuchi
Department of Rehabilitation Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

H. Ida
Division of Respirology, Neurology, and Rheumatology, Department of Medicine, Kurume University School of Medicine, Kurume, Japan

K. Aoyagi
Department of Public Health, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

M. Uetani
Department of Radiology and Radiation Research, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

K. Eguchi
Sasebo City General Hospital, Sasebo, Japan

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Abbreviations

ACR	American College of Rheumatology
Anti-CCP antibodies	Anti-cyclic citrullinated peptide antibodies
BeSt study	Behandelstrategieen study
CRP	C-reactive protein
DAS	Disease activity score
DMARDs	Disease-modifying antirheumatic drugs
IgM-RF	Immunoglobulin M-rheumatoid factor
MMP-3	Matrix metalloproteinase 3
MRI	Magnetic resonance imaging
MTX	Methotrexate
PROMPT study	Probable RA: methotrexate versus placebo treatment study
PIP joint	Proximal interphalangeal joint
RRR study	Remission induction by Remicade in RA study
RA	Rheumatoid arthritis
RAMRIS	RA MRI scoring
SASP	Salazosulfapyridine
SDAI	Simplified disease activity index
Tac	Tacrolimus
T2T	Treat to target

Introduction

Early diagnosis and the treat-to-target (T2T) strategy are now indispensable for the management of rheumatoid arthritis (RA) [1–4]. In particular, these therapeutic options significantly improve the outcome of early-stage RA [1]. The European League Against Rheumatism (EULAR) Task Force has recently developed a consensus on recommendations for the management of RA with synthetic and biological disease-modifying antirheumatic drugs (DMARDs) [5]. These recommendations indicate that for the vast majority of patients with RA, the first treatment approach should include synthetic DMARDs, especially methotrexate (MTX) [5].

As a result of the increasing percentage of patients achieving remission with the introduction of early intensive goal-steered therapy, more often the dilemma is whether a patient with RA in prolonged remission should discontinue DMARDs or whether the treatment should be continued. The Japanese remission induction by Remicade in RA (RRR) study has indicated that approximately half of RA patients were able to discontinue infliximab after attaining low disease activity, as defined by the disease activity score (DAS) 28 [6]. The Behandelstrategieen (BeSt) study has shown that nearly 11% of patients with early-stage RA treated with DMARDs achieve drug-free remission, as defined by the DAS44, even those treated with synthetic DMARDs alone [7]; however, none of the evidence from Japanese RA patients has determined whether drug-free remission can be achieved with synthetic DMARDs.

In the present study, we selected patients with very early RA whose joints, we suspected, would become highly erosive in the later stages of the disease; we used the following criteria for selection: the subjects showed magnetic resonance imaging (MRI)-proven bone edema and tested positive for autoantibodies [8]. None of the patients met the 1987 criteria of the American College of Rheumatology (ACR) for RA [9], but all fulfilled the 2010 RA classification criteria [2, 3] at entry. A tight control approach through the T2T strategy initiated with synthetic DMARDs was adopted in these patients, and we found that drug-free simplified disease activity index (SDAI) remission in the absence of radiographic progression was successfully induced after the patients had been treated with synthetic DMARDs for 12 months.

Patients and methods

Patients

The present study was an investigator-initiated clinical study that attempted to examine the efficacy of the T2T strategy for patients with very early RA with poor prognosis factors. Patients with very early RA were defined in the present study as those who did not meet the 1987 criteria of the ACR for RA [9] but who fulfilled the 2010 RA classification criteria [2, 3] at entry. We recently reported that patients with early undifferentiated arthritis with MRI-proven bone edema and autoantibodies at entry later developed 1987-criteria-fulfilling RA with erosive radiographic changes [8]. Thus, MRI-proven bone edema and serologic autoantibodies are thought to be poor prognostic factors in early arthritis. Accordingly, patients with very early RA who did not meet the 1987 criteria of the ACR for RA but who fulfilled the 2010 RA classification criteria at entry, in addition to having MRI-proven bone edema and serologic autoantibodies, were selected for the present study. We excluded patients who met the international criteria of rheumatic diseases other than RA at baseline. Thirteen patients who met our inclusion criteria were serially recruited from the Early Arthritis Clinic opened in 2001 as part of the Unit of Translational Medicine, Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences. Patients were referred from an area in the western part of

Japan, Nagasaki Prefecture, which has approximately 450,000 inhabitants. These 13 patients were recruited from 2008 to 2009.

Baseline clinical manifestations and variables examined included gender, age, disease duration from onset to study entry, morning stiffness, use of DMARDs, use of glucocorticoids, SDAI, CRP (measured by latex turbidimetric immunosorbent assay; Daiichi Pure Chemicals, Fukuoka, Japan), matrix metalloproteinase 3 (MMP-3; measured by enzyme-linked immunosorbent assay, with cut-off values of 59.7 ng/ml for females and 121.0 ng/ml for males, Daiichi Pure Chemicals), anti-cyclic citrullinated peptide antibodies (anti-CCP Abs) (measured by enzyme-linked immunosorbent assay, cut-off value 4.5 U/ml; DIASTAT Anti-CCP; Axis-Shield, Dundee, UK), IgM-rheumatoid factor (IgM-RF) (measured by latex-enhanced immunonephelometric assay, cut-off value 14 IU/ml; Dade Behring, Marburg, Germany), MRI-proven synovitis, MRI-proven bone edema, MRI-proven bone erosion, and plain radiographs of both hands and both feet. All variables were examined on the same day, as we recently reported [10–13]. Each patient provided a signed consent form to participate in the study, which was approved by the Institutional Review Board of Nagasaki University. All of the above variables except for MRI were also measured every 3 months. MRI and plain radiographs were examined every 6 months, and we followed the above variables for 24 months.

T2T strategy for the treatment of very early RA

In accordance with findings in previous reports, we have employed the T2T strategy for the treatment of very early RA in an attempt to induce SDAI remission [4, 14]. In brief, synthetic DMARDs were initially introduced, and SDAI was evaluated every 3 months. If the SDAI showed moderate disease activity at 3 months or low disease activity after 6 months, the anti-rheumatic therapies were basically modulated. MTX was initiated in 10 of the 13 patients, salazosulfapyridine (SASP) in 2 patients, and tacrolimus (Tac) in 1 patient. SASP and Tac were used in 3 patients because of the presence of interstitial lung disease. Because the officially approved maximum weekly dosage of MTX in Japan was limited to 8 mg, which is much less than that in Europe and the United States [15], the maximum dosage of MTX in our patients was 8 mg per week. After 12 months of treatment with the T2T strategy, if the patients achieved SDAI remission along with a reduction of the RA MRI scoring (RAMRIS) bone edema score to <33% as compared with baseline at 12 months, DMARD treatment was stopped and the clinical status was still observed, as described above.

Radiographic examinations during the treatment

Plain radiographs of both hands and both feet were taken every 6 months and evaluated using Genant-modified Sharp scores. Plain MRIs of both wrists and all finger joints were also examined every 6 months, as we described previously [10–13]. In brief, MRIs of wrists and finger joints were acquired using a 1.5 T system (Sigma, GE Medical Systems, Milwaukee, WI, USA) with an extremity coil. T1-weighted spin echo (TR 450, TE 13) images and short-time inversion recovery (STIR; TR 3,000, TE 12, T1 160) images were simultaneously acquired on the same day. The images were evaluated for synovitis, bone edema, and bone erosion at 15 sites in each finger and wrist: the distal radioulnar joint, the radiocarpal joint, the midcarpal joint, the first carpometacarpal joint, the second-fifth carpometacarpal joints (together), the first-fifth metacarpophalangeal joints (separately), and the first-fifth proximal interphalangeal joints (PIP joints) separately (for a total of 30 sites in both hands), as we have reported recently [10–13]. MRI was evaluated by an experienced radiologist (M.U.), and the severity of MRI-proven joint injury was evaluated by RAMRIS according to the standard method as used for the RAMRIS total score, RAMRIS synovitis score, RAMRIS bone edema score, and RAMRIS erosion score [16, 17]. The RAMRIS score is a semi-quantitative score from 0 to 3 that is used to assess the severity of MRI-proven joint injury [16, 17]. In our experience, the complete resolution of MRI-proven bone edema is a rare event during treatment even if these patients achieve a clinical remission. Therefore, in the present study, we defined a significant improvement of bone edema to be if the RAMRIS bone edema score declined to <33% as compared with the baseline. When this RAMRIS bone edema score was obtained, synthetic DMARD administration was stopped at 12 months, as described above.

Statistical assessment

We used Fisher's exact probability test and the Mann–Whitney *U*-test to assess differences statistically. Variables with a P value of <0.05 were considered to be significant.

Results

Baseline variables and therapeutic response in the 13 patients

Table 1 summarizes the baseline variables in the 13 patients. The mean disease duration from the onset of symptoms to the initiation of synthetic DMARDs was 13.7 weeks, which is considered to be very early RA. All

of the patients were seropositive for anti-CCP Abs and/or IgM-RF, as described in "Patients and methods". Plain radiographic injury at entry was minimal, as evidenced by the mean Genant-modified Sharp score of 1.8, but all of the 13 patients showed MRI-proven bone edema, as described in "Patients and methods". The RAMRIS scores in the present study appeared to be more severe than those in a similar clinical study–the Ciclosporine, Methotrexate, Steroid in RA (CIMESTRA) trial [18, 19]; in the CIMESTRA trial the mean RAMRIS synovitis score was 10.9, the mean RAMRIS bone edema score was 1.6, and the mean RAMRIS bone erosion score was 1.7. After 12 months' treatment with the T2T strategy, 6 of the 13 patients fulfilled our criteria for quitting the synthetic DMARDs. All 6 of these patients were treated with synthetic DMARDs only, without the addition of biological DMARDs. At 12 months, one patient did not agree to quit the DMARD; however, the synthetic DMARDs were stopped at 12 months in the other 5 patients (in 3 patients treated with MTX, 1 patient treated with SASP, and 1 patient treated with Tac). We compared the baseline variables in the 2 groups (i.e., those quitting and those not quitting synthetic DMARDs), but we were not able to identify any significant intergroup differences (Table 2).

Table 1 Baseline variables in 13 patients

Baseline variables	% Positive or mean ± SD
Gender (female %)	69.20%
Age (years)	59.2 ± 11.0
Disease duration (weeks)	13.7 ± 12.8
Morning stiffness (min)	97.7 ± 133.7
MTX use during 12 months (%)	76.90%
Glucocorticoid use during 12 months (%)	30.80%
SDAI	20.2 ± 10.9
CRP (mg/dl)	1.1 ± 1.0
MMP-3 (ng/ml)	148.7 ± 72.4
Anti-CCP Abs and/or IgM-RF (%)	100%
Genant-modified Sharp score	1.8 ± 2.1
RAMRIS score	
Total	35.8 ± 29.0
Synovitis	12.6 ± 5.2
Bone edema	16.2 ± 17.3
Bone erosion	6.9 ± 10.8

Presentation of the 3 patients who stopped treatment with synthetic DMARDs for 12 months and maintained sustained remission

Of the 5 patients who stopped the synthetic DMARD treatment at 12 months, 1 patient was lost to follow-up (this patient suffered from a femoral bone fracture and was transferred to another hospital at 3 months after stopping the DMARD (SASP); we therefore obtained follow-up data for 4 patients. One patient was required to restart synthetic DMARD treatment (MTX) because of an increase in clinical disease activity at 3 months after stopping MTX. The other 3 patients continued to have a sustained SDAI remission 12 months after quitting the synthetic DMARDs. We have summarized the data for these 3 patients in Table 3. In addition to maintaining the SDAI remission, none of the patients showed progression of radiographic

Table 2 Comparison of baseline variables in patients quitting and those not quitting disease-modifying anti-rheumatic drugs (DMARDs)

Variables	Quitting DMARDs ($n = 5$)	Not quitting DMARDs ($n = 7$)	P value
Gender (female %)	100	57.1	0.2
Age (years)	62.0 ± 11.5	59.7 ± 9.8	1
Disease duration (weeks)	14.4 ± 9.5	13.5 ± 16.3	0.52
Morning stiffness (min)	24.0 ± 25.1	160.0 ± 159.7	0.19
MTX use during 12 months (%)	60	85.7	0.52
Glucocorticoid use during 12 months (%)	20	28.6	1
SDAI	15.9 ± 4.2	24.3 ± 13.4	0.14
CRP (mg/dl)	1.4 ± 1.22	0.97 ± 0.86	0.63
MMP-3 (ng/ml)	179.3 ± 90.0	120.4 ± 55.0	0.26
Genant-modified Sharp score	2.6 ± 2.8	0.84 ± 0.88	0.35
RAMRIS score			
Total	24.2 ± 9.4	39.3 ± 36.5	0.68
Synovitis	13.0 ± 6.0	12.9 ± 5.2	1
Bone edema	9.6 ± 5.9	18.7 ± 22.2	0.81
Bone erosion	1.6 ± 1.1	7.7 ± 11.6	0.46

Table 3 Presentation of the 3 patients in whom DMARDs were stopped for the 12 months following 12-month treatment and who maintained remission

Variables	0 months	Quitting MTX at 12 months	12 Months after quitting MTX (i.e., at 24 months after starting MTX
A. Case 1			
SDAI	22.44	0.04	0.03
CRP (mg/dl)	2.44	0.04	0.03
Genant-modified Sharp score	2	2	2
RAMRIS score			
Total	27	10	6
Synovitis	11	8	4
Bone edema	14	0	0
Bone erosion	2	2	2
B. Case 2			
SDAI	14.26	1.1	0.05
CRP (mg/dl)	0.26	0.1	0.05
Genant-modified Sharp score	0	0	0
RAMRIS score			
Total	9	7	5
Synovitis	4	5	5
Bone edema	3	1	0
Bone erosion	2	1	0
C. Case 3			
SDAI	14.99	0.11	1.31
CRP (mg/dl)	2.69	0.11	1.31
Genant-modified Sharp score	4.4	4.4	4.4
RAMRIS score			
Total	22	6	6
Symmetrical synovitis	18	6	6
Bone edema	3	0	0
Bone erosion	0	0	0

damage, as evaluated by the Genant-modified Sharp score and the RAMRIS bone edema score (Table 3).

Discussion

The clinical efficacy of synthetic DMARDs, especially MTX, in early-stage RA has been established [1, 4, 5]. Stopping DMARDs while maintaining remission would be beneficial with respect to adverse events and costs, but there are few data on the efficacy and safety of quitting synthetic DMARDs in RA [7, 20, 21]. The BeSt study is an intriguing clinical trial that has found that drug-free remission is a realistic goal in patients with early-stage RA treated with synthetic DMARDs [7, 20, 21]. In the BeSt study the patients were treated with synthetic DMARDs for 2 years, and the rate of drug-free remission was 11% [7]. Also, the probable RA: methotrexate versus placebo treatment (PROMPT) study has shown in the earlier stage of disease that patients who had been treated with MTX for 12 months continued with their good disease status after stopping MTX [22]. However, the efficacy of MTX in the PROMPT study may not have been as obvious, because the radiographic progression tended to increase after the cessation of MTX [22]. Therefore, although drug-free remission induced by synthetic DMARDs can be achieved, its precise nature has, until now, remained to be elucidated.

Our present study differs from the BeSt study and the PROMPT study in several ways. First, the definition of RA in our study is based on the 2010 RA classification criteria. This definition appears to be similar to that used in the PROMPT study, but quite different from that used in the BeSt study, which targeted patients fulfilling the 1987 criteria of the ACR at entry [7, 20–22]. None of the patients in the present study fulfilled the 1987 ACR criteria at entry. Second, all of the subjects in our study were seropositive for anti-CCP Abs or IgM-RF. In the BeSt study, 65% of the subjects were seropositive for both antibodies [20, 21]. In the PROMPT study, the prevalence of anti-CCP Abs was 24.5% and that of RF was 35.5% [22]. Third, all of our patients were defined as being positive for MRI-proven bone edema. Previous studies have identified that MRI-proven bone edema reflects a severe disease condition in RA [10, 17]. These data indicate that patients with very early but, nevertheless, severe RA were the subjects of our study, as compared with patients in the BeSt and PROMPT studies. Fourth, we defined the criteria for quitting synthetic DMARDs as achieving SDAI remission as well as a significant improvement in MRI-proven bone edema. Fifth, the doses of the synthetic DMARDs used in our study, especially that of MTX, was quite low as compared with the doses used in Europe and the United States [15]. Therefore, this is the first clinical trial attempting to determine whether the discontinuation of synthetic DMARDs is a realistic goal in Japanese patients with severe but very early RA.

Although the number of patients in the present study was very small, 3 out of 4 patients successfully achieved drug-free SDAI remission for 12 months after the cessation of synthetic DMARDs. Plain radiographic progression as well as increases in the RAMRIS bone edema score were completely suppressed in these 3 patients. These data are excellent as compared with those from the BeSt study and the PROMPT study. Our criteria for quitting synthetic DMARDs, i.e., achieving SDAI remission along with a

significant improvement in MRI-proven bone edema, may have led to the better results. Accordingly, SDAI remission is believed to be a more stringent requirement than DAS remission [14]. We and other investigators have found that MRI-proven bone edema is a very strong predictor of further plain radiographic progression [8, 18, 19]. However, as the sample size of the present study was small, our criteria for quitting synthetic DMARDs could be changed if the present patients show radiographic progression in a further follow-up period. Of note, biomarkers reflecting the clinical characteristics might be present in the patients' serum or plasma, and such biomarkers may be of value in making the decision to quit DMARD treatment. Future prospective studies will be necessary to guide the rationale for quitting DMARDs in patients in whom clinical remission has been achieved.

Synthetic DMARD use in Japan is quite different from that in western countries; the present study, however, has shown–for the first time–that, in patients with very early RA with poor prognosis factors, true remission can be achieved by treatment with synthetic DMARDs. Evaluation of disease activity by the SDAI and MRI-proven bone edema is thought to be very valuable for identifying whether sustained remission has been achieved.

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Conflict of interest None.

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