Table 2 Comparison of disease activity between patients who completed the 52-week TCZ-free period and those who restarted anti-rheumatic treatment

	Before first TCZ infusion (baseline of previous studies)	*n*	At cessation of TCZ treatment (baseline of this study)	*n*	Last observation point of this study	*n*	Difference[a] (95 % CI)
DAS28-ESR, median (range)							
Completed	6.3 (2.2–7.5)	24	1.0 (0.0–2.7)	24	2.4 (0.5–3.3)	23	1.05 (0.75–1.35)
Restarted treatment	6.2 (3.2–8.8)	160	1.5 (0.1–3.2)	161	4.3 (0.8–7.8)	161	2.85 (2.67–3.03)
Tender joint count, median (range), 28-joint count							
Completed	9 (0–19)	24	0 (0–2)	24	0 (0–3)	23	0.2 (−0.1 to 0.4)
Restarted treatment	9 (1–28)	160	0 (0–7)	161	3 (0–27)	161	3.9 (3.2–4.5)
Swollen joint count, median (range), 28-joint count							
Completed	8 (0–26)	24	0 (0–2)	24	0 (0–4)	23	0.3 (−0.1 to 0.7)
Restarted treatment	9 (1–25)	160	0 (0–6)	161	2 (0–16)	161	2.8 (2.3–3.3)
CRP, median (range), mg/dL							
Completed	4.9 (0.5–9.3)	24	0.0 (0.0–0.7)	24	0.1 (0.0–2.3)	23	0.23 (0.02–0.45)
Restarted treatment	3.1 (0.3–20.1)	161	0.0 (0.0–5.2)	161	0.8 (0.0–13.5)	161	1.52 (1.18–1.86)
ESR, median (range), mm/h							
Completed	62 (16–123)	24	4 (1–28)	24	14 (2–53)	23	13.7 (8.0–19.3)
Restarted treatment	57 (11–165)	161	5 (1–26)	161	36 (2–115)	161	34.3 (30.7–37.8)
MHAQ scores, median (range)							
Completed	0.3 (0.0–2.0)	24	0.0 (0.0–0.4)	24	0.0 (0.0–0.5)	23	0.02 (−0.01 to 0.05)
Restarted treatment	0.8 (0.0–2.0)	161	0.0 (0.0–1.4)	161	0.3 (0.0–2.1)	161	0.29 (0.23–0.35)
MMP-3, median (range), ng/mL							
Completed	262.0 (38–800)	17	47.7 (32–225)	24	57.1 (13–109)	23	5.7 (0.6–10.8)
Restarted treatment	365.0 (38–800)	88	58.7 (23–697)	157	98.9 (36–800)	142	86.9 (64.4–109.5)
Percentage of patients whose MMP-3 levels were within normal range (%)							
Completed	5.9		91.7		73.9		–
Restarted treatment	3.4		56.7		24.6		–

[a] Difference: mean of the difference between the value at cessation of TCZ treatment (baseline of this study) and at the last observation point of this study

DAS28 28-joint disease activity score, *ESR* erythrocyte sedimentation rate, *MHAQ* modified health assessment questionnaire, *MMP-3* matrix metalloproteinase 3, *Completed* patients who completed the 52-week observational period without anti-rheumatic treatment, *Restarted treatment* patients who restarted anti-rheumatic treatment

were within normal range was lower in the group of patients who restarted anti-rheumatic therapy (56.7 %) than in the group who completed the 52-week study period (91.7 %).

Even though the median DAS28 was slightly increased from 1.0 at baseline of this study to 2.4 at the last observation point of this study (Table 2), tender joint count and swollen joint count at week 52 did not meaningfully worsen from the baseline of this study in the patients who completed the 52-week study period. MMP-3 concentration at week 52 was also almost stable during the study period in these patients.

In the patients who restarted anti-rheumatic therapy, disease activity and MMP-3 levels had worsened compared to the baseline of this study. Nevertheless, the values of those parameters were no worse than they had been before the initiation of TCZ treatment in previous studies (Table 2).

Factors contributing to the prolongation of duration of DAS28 remission and LDA

Univariate Cox regression analysis showed the following variables to be associated with the rate of continued efficacy: negative RF at baseline of the previous study and low serum IL-6 level (<35 pg/mL), under upper limit of the normal MMP-3 level, no concomitant corticosteroid use, DAS28-ESR <median, and an MHAQ score of zero at TCZ discontinuation.In contrast, disease duration, gender, functional class, and RA stage were not associated with continued efficacy (Fig. 2a). Multivariate Cox regression analysis showed that low serum IL-6 (<35 pg/mL) and normalisation of MMP-3 levels at TCZ cessation were independently associated with continued efficacy (Fig. 2b).

Based on this result, we examined the effects that IL-6 and MMP-3 levels at cessation of TCZ treatment had on the rate of continued efficacy. We found that the rate of continued efficacy in the patients with low serum IL-6 (<35 pg/mL) was 39.3 % (95 % CI 31.1–47.4) at 24 weeks and 15.9 % (95 % CI 9.7–22.0) at 52 weeks (Fig. 3a). In contrast, 69.7 % of the patients with serum IL-6 levels ≥35 pg/mL met the criteria for loss of efficacy within 12 weeks, and in none was efficacy maintained until 52 weeks. Analysis of the ROC curve identified the most sensitive and specific cut-off value for the serum IL-6 level to be 12.9 pg/mL. The rate of continued efficacy in patients whose serum IL-6 levels were less than 12.9 pg/mL was 63.2 % (95 % CI 48.8–77.5) at 24 weeks and 30.2 % (95 % CI 16.4–44.0) at 52 weeks (Fig. 3b).

The rate of continued efficacy in those with normalised MMP-3 levels was 50.9 % (95 % CI 41.6–60.2) at 24 weeks and 20.3 % (95 % CI 12.8–27.8) at 52 weeks (Fig. 3c), compared with 11.8 % at 24 weeks and 3.0 % at 52 weeks in patients with abnormal MMP-3 levels.

In patients with both serum IL-6 <12.9 pg/mL and normalised MMP-3 level, the rate of continued efficacy reached 70.6 % (95 % CI 55.3–85.9) at 24 weeks and 38.0 % (95 % CI 21.6–54.4) at 52 weeks (Fig. 3d).

Discussion

This study indicated that, in about 13 % of patients who achieve LDA (70.8 % of them were DAS28 remission) during long-term TCZ monotherapy, efficacy can be sustained for 1 year after cessation of TCZ treatment without concomitant use of synthetic DMARDs or immune suppressants; and in 79 % of them (19 patients), efficacy was maintained without concomitant use of corticosteroids or NSAIDs. To the best of our knowledge, this is the first report to show evidence that anti-IL-6 therapy can induce drug-free remission/LDA for 1 year in RA patients. The treatment recommendations of the EULAR state that, in patients who achieve remission with biological products, it may be possible to taper off the biological product after tapering off the corticosteroid. However, at present,

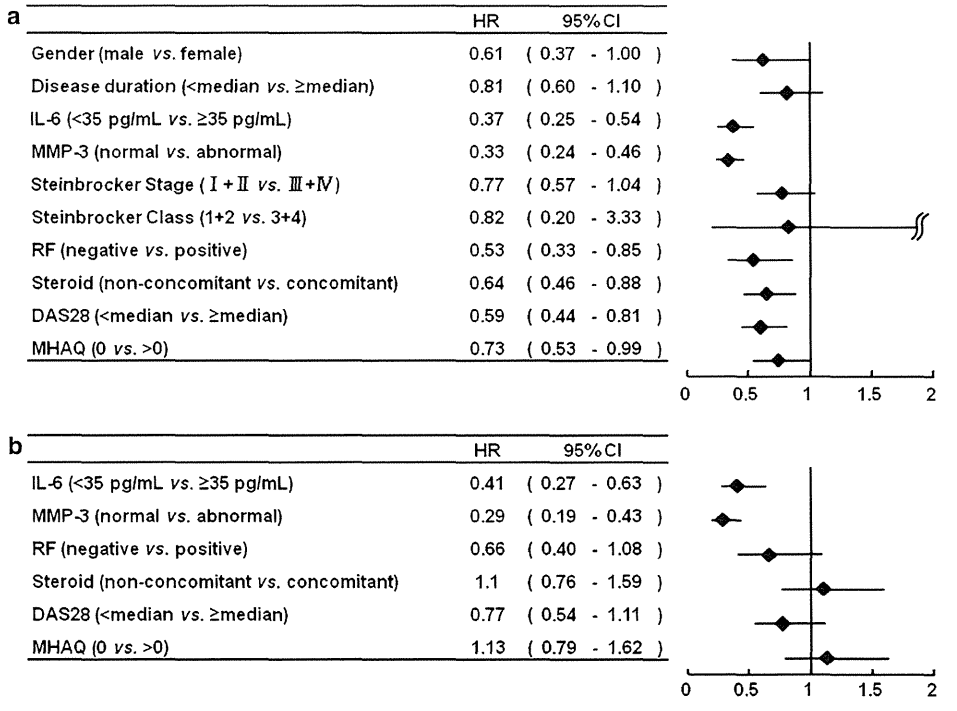

Fig. 2 Factors associated with continued LDA. **a** Univariate Cox regression analysis, **b** multivariate Cox regression analysis. *HR* hazard ratio, *CI* confidence interval, *RF* rheumatoid factor, *DAS28* 28-joint disease activity score, *MHAQ* modified health assessment questionnaire, *IL-6* interleukin 6, *MMP-3* matrix metalloproteinase 3

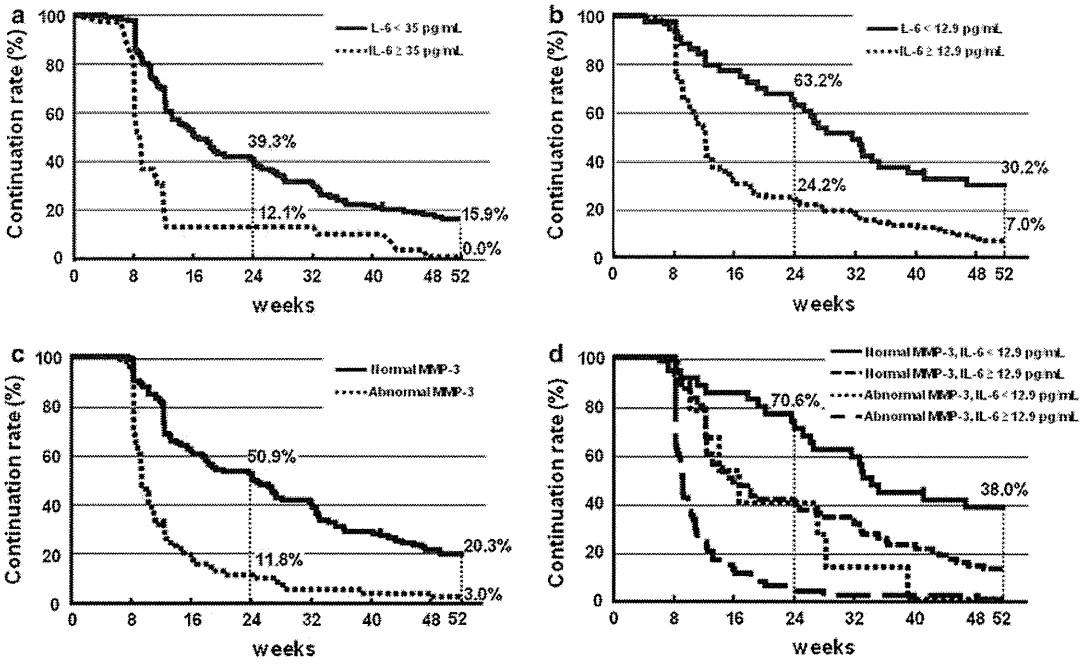

Fig. 3 Effects of serum IL-6 and MMP-3 levels on rate of continued LDA after cessation of tocilizumab treatment estimated by Kaplan–Meier method over 52 weeks. Contributing factors: **a** serum IL-6 level (cut-off level: 35 pg/mL), **b** serum IL-6 level (cut-off level: 12.9 pg/mL), **c** MMP-3 level (normal vs abnormal), **d** combinations of MMP-3 (normal vs abnormal) and serum IL-6 levels (cut-off level: 12.9 pg/mL). Space between *curves* represents the contribution of each factor to rate of continued efficacy

evidence in support of this conjecture is insufficient [26]. We believe that this report supports the possibility of discontinuing biological products as per the ACR/EULAR recommendations.

In a previous study (the BeST study), van der Kooij et al. [27] indicated that 18 % of patients could discontinue infliximab and synthetic DMARDs. Even though the characteristics of the patients in our study differed from those in BeSt study, the success rate of discontinuing TCZ without synthetic DMARDs in our study is comparable to that of the BeSt study. Moreover, with the use of synthetic DMARDs including MTX, a high rate of continued efficacy was shown after discontinuation of infliximab in the BeSt study [27]. A similar result was shown in the Japanese RRR study [28]. Therefore, it can be expected that introducing the use of synthetic DMARDs would similarly result in an increased rate of continued DAS28 remission or LDA after cessation of TCZ.

Because multivariate Cox regression analysis identified low serum IL-6 and normalised MMP-3 levels at the start of cessation of TCZ to be factors associated with continued efficacy, it can be considered that these factors may predict continued efficacy of a preceding TCZ treatment. With long-term TCZ treatment, reduced serum IL-6 levels are observed in some patients although TCZ does not directly inhibit IL-6 production but blocks IL-6R. We previously reported that, during blockade of IL-6R by TCZ, the serum IL-6 level represents the true IL-6 production *in vivo* and correlates well with true disease activity in RA patients [21, 22]. Therefore, TCZ treatment may improve not only inflammation-related symptoms but also the underlying cause of RA in patients whose serum IL-6 levels decrease. This implies that TCZ could be discontinued without acute disease flare in patients with normalised serum IL-6 levels. IL-6, as such a biomarker, is available only for anti-IL-6R antibody therapy but for anti-IL-6 neutralizing antibody therapies.

MMP-3 is deeply involved in cartilage destruction in RA and is also correlated with disease activity [29]. Since normalisation of the MMP-3 level is thought to reflect inhibition of excessive cartilage and bone destruction in the joints, normalisation of the MMP-3 level may indicate an improvement in the underlying cause of RA as well as synovial inflammation. In this study, we did not examine the progression of joint damage by imaging after cessation of TCZ. However, since the MMP-3 level during the TCZ-free period did not increase in the majority of the patients showing continued efficacy, it can be inferred that there was no sudden progression of joint destruction during the cessation of TCZ treatment. Further study will be necessary to evaluate this question.

In conclusion, these results showed that TCZ monotherapy can induce biologics-free remission/LDA without concomitant use of conventional DMARDs. Serum levels

of IL-6 and MMP-3 are useful markers for identifying patients who could possibly discontinue TCZ without acute disease flare. This evidence has also encouraged us to taper and adjust the interval of TCZ treatment in patients who show good response and normalisation of serum IL-6 and MMP-3 levels.

Acknowledgments The authors wish to thank all members of the MRA study group for RA for treating the patients. This study was funded by Chugai Pharmaceutical Co., Ltd.

Conflict of interest N. Nishimoto has served as a consultant to and received honoraria from Chugai Pharmaceutical Co., Ltd. N.N. also works as a scientific advisor to F. Hoffmann–La Roche, which is developing TCZ in collaboration with Chugai Pharmaceutical Co., Ltd. N.N. also has received research grants from Chugai Pharmaceutical Co. Ltd., Bristol–Myers Japan, and Pfizer Japan Inc. K. Amano has received research grants from Chugai Pharmaceutical Co. Ltd., Astellas Pharm Inc., and Mitsubishi Tanabe Pharma. Y. Hirabayashi has received speakers' bureau honoraria from Chugai Pharmaceutical Co. Ltd. M. Iwamoto has received a Royalty from Chugai Pharmaceutical Co. Ltd. H. Kohsaka has received research grants, consultant fees, and/or speakers' bureau honoraria from, Bristol-Myers Japan, Pfizer Japan Inc., and Takeda Pharmaceutical Co. Ltd. T. Mimura received research grants from Abbott Japan, Chugai Pharmaceutical Co. Ltd., Mitsubishi Tanabe Pharma, and Takeda Pharmaceutical Co. Ltd. T. Takeuchi has received research grants, consultant fees, and/or speakers' bureau honoraria from Abbott Japan, Bristol-Myers Squibb, Chugai Pharmaceutical Co. Ltd., Eisai Co. Ltd., Janssen Pharmaceutical KK, Mitsubishi Tanabe Pharma, Novartis, Pfizer Japan Inc., and Takeda Pharmaceutical Co. Ltd. S. Tohma has received a research grant from Pfizer Japan Inc. and has received subsidies or donations from Health and Labour Sciences Research Grants for Research on Allergic Disease and Immunology, and Chugai Pharmaceutical Co. Ltd. N. Takagi is a full-time employee of Chugai Pharmaceutical Co., Ltd. All other authors have declared no conflicts of interest.

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Japan College of Rheumatology
MODERN RHEUMATOLOGY

http://informahealthcare.com/mor
ISSN 1439-7595 (print), 1439-7609 (online)

Mod Rheumatol, 2014; 24(1): 26–32
© 2013 Japan College of Rheumatology
DOI 10.3109/14397595.2013.854080

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ORIGINAL ARTICLE

Retreatment efficacy and safety of tocilizumab in patients with rheumatoid arthritis in recurrence (RESTORE) study

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Received: 27 September 2011 / Accepted: 15 April 2013

Abstract

Objectives To evaluate the safety and efficacy of retreatment with tocilizumab (TCZ) in patients who had participated in the DREAM study (*Drug free* remission/low disease activity after cessation of tocilizumab [Actemar] monotherapy study) and had experienced loss of efficacy.

Methods Patients were retreated with TCZ or other disease modifying antirheumatic drugs (DMARDs). Disease activity was measured using the 28-joint disease activity score (DAS28) for 12 weeks.

Results A total of 164 eligible patients, including 161 who experienced loss of efficacy within 52 weeks of the DREAM study, resumed treatment: 157 with TCZ and 7 with DMARDs and/or infliximab. Of TCZ-treated patients, 88.5 % (139 patients) achieved DAS28 <2.6 within 12 weeks, whereas among patients treated with DMARDs and/or infliximab only 14.3 % (1 patient) achieved DAS28 <2.6. Adverse events were observed in 70 TCZ-treated patients (44.0 %), but no serious infusion reactions were observed.

Conclusions Retreatment with TCZ was well-tolerated and effective in patients who had responded to the preceding TCZ monotherapy but had experienced loss of efficacy after cessation of TCZ.

Keywords Interleukin 6 · Retreatment · *Drug free* · Rheumatoid arthritis · Tocilizumab

For the MRA study group for RA.

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Published online: 17 May 2013

DOI 10.3109/14397595.2013.854080

Introduction

Tocilizumab (TCZ) treatment frequently achieves remission in patients with rheumatoid arthritis (RA) as measured by the 28-joint disease activity score (DAS28) [1–12]. We have demonstrated in the DREAM study (*Drug free* remission/low disease activity (LDA) after cessation of TCZ [Actemra] monotherapy study) [13] that in some cases the efficacy of TCZ is sustained for more than 1 year after cessation of TCZ and without the use of other disease modifying antirheumatic drugs (DMARDs). However, the majority of patients experienced loss of efficacy, and needed to restart treatment for RA. In this study we evaluate the safety and efficacy of TCZ retreatment at recurrence of disease activity after cessation of TCZ.

Methods

Patients

All patients who participated in the DREAM study and had experienced loss of efficacy were enrolled. Criteria for loss of efficacy in the DREAM study was defined as DAS28-erythrocyte sedimentation rate (ESR) >3.2 at 2 consecutive observations, initiation of additional RA treatments including increase in oral corticosteroid dose, the patient's request for retreatment, or the treating physician judging that retreatment was necessary.

Study protocol

The study protocol was approved by the Ministry of Health, Labour and Welfare of Japan and by the local ethical committees. This study is registered with http://clinicaltrials.gov (NCT00661284). Patients were treated with biologic DMARDs including TCZ and infliximab (IFX), and/or conventional synthetic DMARDs including methotrexate (MTX). If the patient received TCZ retreatment, TCZ was administered intravenously (8 mg/kg) every 4 weeks. Other biologic DMARDs and/or synthetic DMARDs were administered based on the dosage and regimen in the package insert. The concomitant use of corticosteroids and non-steroidal anti-inflammatory drugs was allowed during the study period.

Anti-tocilizumab antibodies

Serum anti-TCZ antibody levels were determined by ELISA. Serum was added to the wells coated with 100 μl of Fab fragment of TCZ (0.2 μg/ml) and incubated for 2 h. After washing, biotin-conjugated TCZ was added and developed with alkaline phosphatase conjugated to streptavidin.

IgE-type anti-TCZ antibodies were also measured by ELISA. In this case, whole TCZ was used because an antigen coated each cup, and enzyme-linked anti-IgE antibodies were used as second antibodies.

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Statistical analysis

Clinical response was measured by DAS28-ESR. Remission was defined, in accordance with the European League Against Rheumatism (EULAR) definition, as DAS28 <2.6 [14]. The rates of remission under the new EULAR/American College of Rheumatology (ACR) remission criteria (Boolean definition) were also considered [15]. Adverse events (AEs) and serious adverse events (SAEs) were tabulated after converting the verbatim event names to MedDRA Ver. 8.0 System Organ Class (SOC) terms.

The factors contributing to the resumption of DAS28-ESR remission after retreatment were estimated from univariate and multivariate logistic regression analyses using the following patient baseline data for this study: DAS28-ESR, tender joint count (TJC), swollen joint count (SJC), patient's global assessment (Pt-GA), modified health assessment questionnaire (MHAQ) score, serum C-reactive protein (CRP) concentration, erythrocyte sedimentation rate (ESR), serum IL-6 concentration, serum matrix metalloproteinase (MMP)-3 concentration, and the duration of TCZ cessation. In the multivariate logistic analysis, stepwise selection with a level of significance of 0.05 was used for entry or removal of variables. Logistic regression analysis was also conducted to analyse the relationship between the TCZ treatment interval and development of AEs during this study.

Results

Characteristics of patients

In total, 166 patients were enrolled and resumed treatments. Of the patients who received TCZ retreatment, 2 were ineligible and were excluded from the analysis of efficacy. The 164 remaining patients eligible for analysis of efficacy included 161 patients who had experienced loss of efficacy by week 52 of the DREAM study, and 3 patients who had experienced loss of efficacy after completion of the DREAM study (an interval of >1 year).

In the 164 eligible patients, 73 patients (44.5 %) resumed treatment due to DAS28-ESR >3.2 at 2 consecutive visits, 66 patients (40.2 %) to investigator's judgement, 11 patients (6.7 %) to patients' request, and 14 patients (8.5 %) to addition of RA treatments including increase in oral corticosteroid dose. The major reason investigators judged retreatment was necessary was a DAS28-ESR >3.2 score at one visit in 55/66 patients (83.3 %). Four out of eleven patients who requested treatment were also DAS28-ESR >3.2. Therefore, 146/164 patients were DAS28-ESR >3.2 at the baseline of the RESTORE study (the mean DAS28-ESR[95 %CI] was 4.6 [4.5–4.8]).

A total of 159 patients received at least 1 infusion of TCZ (including 2 ineligible patients), and 7 patients received other DMARDs, including MTX, tacrolimus, and/or IFX. In the TCZ-treated patients, 133 patients received TCZ monotherapy and 26 received TCZ therapy in combination with synthetic DMARDs (25 patients with MTX; 1 patient with salazosulfapyridine). The median treatment interval between the last TCZ infusion and restarting the TCZ treatment in this study was 13.1 weeks (min–max, 6.14–60.4 weeks). Corticosteroids were used concomitantly in 57 of the patients treated with TCZ and in 4 of the patients treated with other DMARDs. The median corticosteroid dose in TCZ-treated patients at baseline of this RESTORE study was 3.0 mg/day, which was comparable with the median dose in patients treated with other DMARDs (2.3 mg/day). Other baseline characteristics of the patients who received TCZ were comparable with those of patients treated with other DMARDs (Table 1).

Efficacy of TCZ retreatment

The mean (±SD) DAS28-ESR before initial treatment using TCZ in the previous clinical studies (i.e. Japanese phase I/II open-label dose escalation study, a phase II double-blind dose finding study, a phase III open-label randomized study (SAMURAI), a phase III double-blind study (SATORI), a drug–drug interaction study, and a renal failure study) was 6.2 (±1.0) and improved with 12 weeks of TCZ treatment to 2.8 (±1.2). The mean (±SD) DAS28-ESR at the last observation point of the previous TCZ treatment studies (i.e., baseline of the DREAM study) was 1.5 (±0.7) (Fig. 1a).

In this study, the mean (±SD) DAS28-ESR in patients who restarted TCZ treatment decreased from 4.4 (±1.1) (95 % CI: 4.2–4.6) before restarting treatment to 1.8 (±0.8) (95 % CI: 1.6–1.9) after 12 weeks of treatment. In contrast, the mean (±SD) DAS28-ESR in patients treated with DMARDs and/or IFX was 4.2 (±1.1) (95 % CI: 3.2–5.2) before restarting treatment and 3.3 (±1.0) (95 % CI: 2.5–4.2) after 12 weeks of treatment (Fig. 1a).

Of the TCZ-retreated patients, 95.5 % (150/157 patients, 95 % CI: 91.0–98.2 %) achieved DAS28-ESR ≤3.2 and 88.5 % (139/157 patients, 95 % CI: 82.5–93.1 %) achieved DAS28-ESR <2.6 within 12 weeks as compared to only 28.6 % of the other DMARD-treated patients (2/7 patients, 95 % CI 3.7–71.0 %) achieving DAS28-ESR ≤3.2 and 14.3 % (1/7 patients, 95 % CI: 0.4–57.9 %) achieving DAS28-ESR <2.6.

The percentage of TCZ-retreated patients who reached DAS28-ESR <2.6 within 12 weeks in the TCZ monotherapy group (87.9 %, 116/132 patients, 95 % CI: 81.1–92.9 %) was comparable to the percentage in the TCZ plus synthetic DMARDs therapy group (92.0 %, 23/25 patients, 95 % CI: 74.0–99.0 %).

Table 1 Demographic and clinical characteristics of patients at baseline of RESTORE study

No. of patients	Total	Patients treated with TCZ	Patients treated with other DMARDs
	166	159[a]	7
Age, years (median [range])	57 (26–78)	56 (26–78)	65 (42–74)
Gender, female (%)	149 (89.8)	144 (90.6)	5 (71.4)
Disease duration, years (median [range])	7.8 (3.7–24.0)	7.7 (3.7–24.0)	8.6 (6.9–18.9)
No. (%) of patients using concomitant corticosteroids	61 (36.7)	57 (35.8)	4 (57.1)
Dose, mg/day (prednisolone equivalent) (median [range])	3.0 (0.5–10.0)	3.0 (0.5–10.0)	2.3 (2.0–7.0)
DAS28-ESR (median [range])	4.3 (0.8–7.8)	4.4 (0.8–7.8)	4.1 (2.9–5.9)
(Mean ± SD)	4.4 ± 1.1	4.4 ± 1.1	4.2 ± 1.1
Tender joint count (28-joint count) (median [range])	3.0 (0–27)	3.0 (0–27)	3.0 (1–5)
(Mean ± SD)	4.3 ± 4.3	4.4 ± 4.4	2.6 ± 1.4
Swollen joint count (28-joint count) (median [range])	2.0 (0–16)	2.0 (0–16)	2.0 (0–7)
(Mean ± SD)	3.3 ± 3.1	3.3 ± 3.2	2.4 ± 2.2
CRP, mg/dl (median [range])	0.8 (0.0–13.5)	0.9 (0.0–13.5)	0.8 (0.1–4.7)
(Mean ± SD)	1.6 ± 2.1	1.6 ± 2.1	1.2 ± 1.6
ESR, mm/h (median [range])	36 (2–115)	37 (2–115)	32 (16–113)
(Mean ± SD)	41 ± 24	40 ± 23	49 ± 39
MHAQ score (median [range])	0.3 (0.0–2.1)	0.4 (0.0–2.1)	0.0 (0.0–0.8)
(Mean ± SD)	0.5 ± 0.5	0.5 ± 0.5	0.2 ± 0.3
MMP-3, ng/ml (median [range])	95 (34–800)	96 (34–800)	77 (44–319)
(Mean ± SD)	167 ± 167	169 ± 169	129 ± 112

DAS28 28-joint disease activity score, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *MHAQ* modified health assessment questionnaire, *MMP-3* matrix metalloproteinase-3, *TCZ* tocilizumab, *DMARDs* disease modifying antirheumatic drugs

[a] Two ineligible patients who did not meet the eligible criteria of DREAM study were included

The mean (±SD) tender joint count (TJC) in 28 joints in TCZ-retreated patients improved from 4.4 (±4.4) before restarting treatment to 0.8 (±1.6) after 12 weeks. The mean (±SD) swollen joint count (SJC) in 28 joints also improved from 3.3 (±3.2) to 0.8 (±1.6) (Fig. 1b). Moreover, 63.1 % of patients (99/157) had no tender and/or swollen joints after 12 weeks retreatment with TCZ (Fig. 1c). Under the Boolean remission criteria, the remission rate by TCZ treatment was 43.9 % (69/157 patients, 95 % CI: 36.0–52.1 %) at week 12 (Fig. 1d). The mean (±SD) MMP-3 values in TCZ-retreated patients improved from 166.5 (±164.5) ng/ml at baseline in this study, i.e. prior to TCZ retreatment, to 77.4 (±64.8) ng/ml at week 12. Univariate logistic regression analysis showed the following variables to be associated with the resumption of DAS28-ESR remission: lower DAS28-ESR, lower TJC, lower SJC and lower MHAQ at baseline. On the other hand, duration of TCZ cessation in the DREAM study was not associated with resumption of DAS28-ESR remission (Fig. 2). Multivariate logistic regression analysis showed that lower DAS28-ESR at baseline was the contribution factor for resumption efficacy.

At baseline, 17 patients had DAS28-ESR ≤3.2. Thus, we further analysed efficacy in the 140 patients who had DAS28-ESR >3.2 at the baseline (the mean DAS28-ESR [95 % CI] was 4.6 [4.5–4.8]) and restarted TCZ in this study. Out of these patients, 87.1 % (122/140 patients, 95 % CI: 80.4–92.2 %) achieved DAS28-ESR <2.6 and 42.9 % (60/140 patients, 95 % CI: 34.5–51.5 %) achieved Boolean remission within 12 weeks. In addition, univariate and multivariate logistic regression analysis also identified lower DAS28-ESR value at baseline to be the factor contributing the resumption of DAS28-ESR remission by 12 weeks of TCZ treatment in these patients. These results are not significantly different from those including the patients with DAS28-ESR ≤ 3.2 at baseline.

Safety of TCZ retreatment

AEs were reported in 44.0 % (70/159) of the patients who were retreated with TCZ and in 42.9 % (3/7) of the patients treated with other DMARDs. All AEs reported in the TCZ-treated group were mild and tolerable relative to the benefit provided. The incidence rate of AEs in the TCZ monotherapy group (42.9 %, 57/133 patients, 95 % CI: 34.3–51.7) was comparable to the incidence rate in the TCZ plus synthetic DMARDs therapy group (50.0 %, 13/26 patients, 95 % CI: 29.9–70.1). There was no

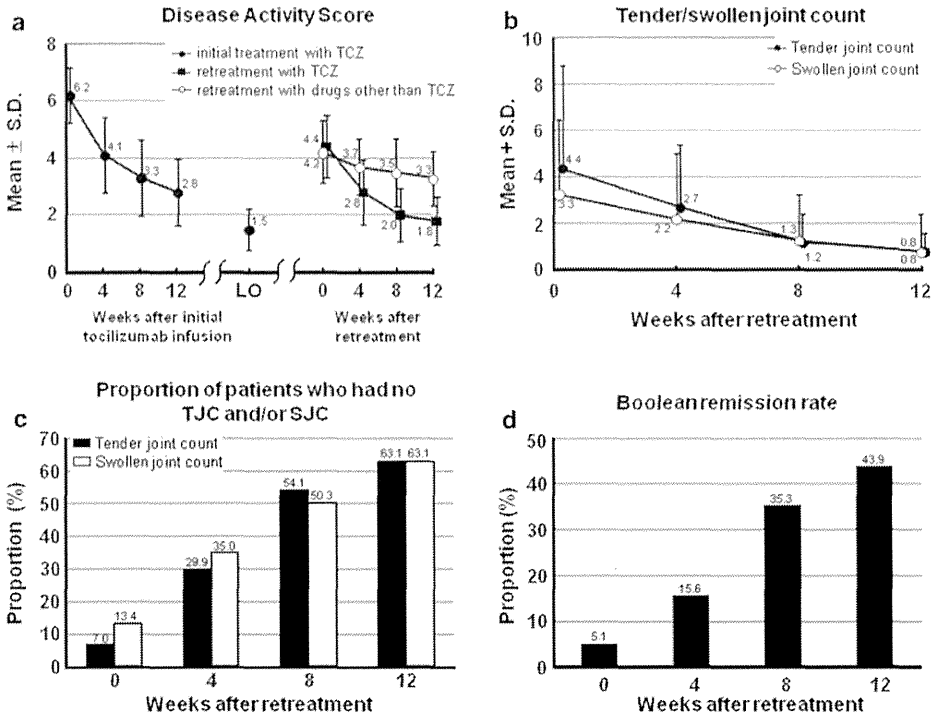

Fig. 1 Changes in DAS28-ESR, tender joint count, swollen joint count, and Boolean remission rate after resumption of treatment. **a** Mean (±SD) change in DAS28-ESR: from baseline of the initial tocilizumab (TCZ) treatment to week 12 and last observation point of the long-term extension studies (*closed circles*), and from the baseline of this study to week 12 in patients retreated with TCZ (*closed squares*) and in patients treated with other DMARDs (*open circles*). *Error bars* show SD. **b** Mean (+SD) tender joint count in 28 joints (*closed circles*), and mean (+SD) swollen joint count in 28 joints in TCZ-retreated patients (*open circles*). *Error bars* show SD. **c** Proportion of TCZ-retreated patients with no tender joints (*solid bars*) and those with no swollen joints (*open bars*). **d** Remission rates under the new EULAR/ACR remission criteria in the TCZ-retreated patients. *TJC* tender joint count, *SJC* swollen joint count, *LO* last observation point

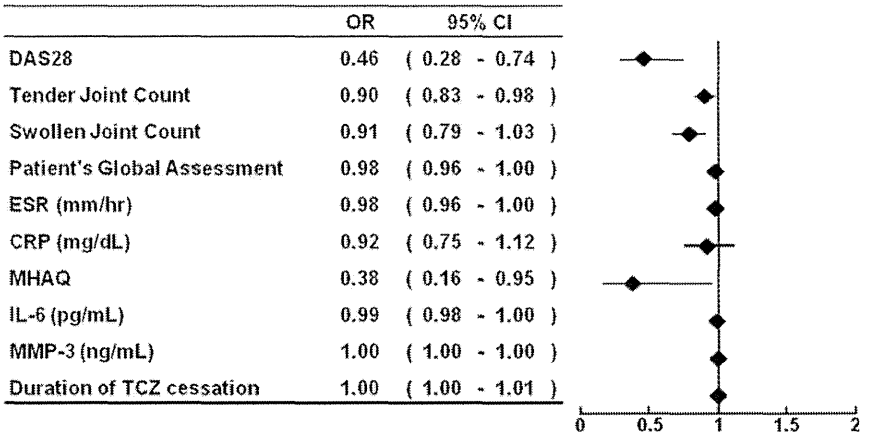

Fig. 2 Factors associated with resumption of DAS28-ESR remission by 12 weeks of TCZ retreatment after cessation of TCZ therapy. Factors contributing to the resumption of DAS28-ESR remission by 12 weeks of TCZ treatment were estimated by univariate and multivariate logistic regression analyses. *OR* odds ratio, *CI* confidence interval, *DAS28* 28-joint disease activity score, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *MHAQ* modified health assessment questionnaire, *IL-6* interleukin 6, *MMP-3* matrix metalloproteinase 3, *TCZ* tocilizumab

relationship between the development of AEs and the duration of TCZ cessation in the DREAM study. Infections were the most common AEs in the TCZ-treated group (27 patients, 17.0 %) (Table 2). None of the patients in this study were positive for anti-TCZ IgE antibodies. Only 1 patient who discontinued TCZ treatment for 35 weeks

Table 2 Adverse events observed after restarting TCZ treatment

Adverse event (SOC)	No. patients (%)
Total	70 (44.0)
Infections and infestations	27 (17.0)
Investigations	17 (10.7)
Gastrointestinal disorders	14 (8.8)
Skin and subcutaneous tissue disorders	12 (7.5)
Injury, poisoning and procedural complications	8 (5.0)
Respiratory, thoracic and mediastinal disorders	5 (3.1)
Nervous system disorders	3 (1.9)
General disorders and administration site conditions	3 (1.9)
Neoplasms benign, malignant and unspecified	2 (1.3)
Eye disorders	2 (1.3)
Vascular disorders	2 (1.3)
Musculoskeletal and connective tissue disorders	2 (1.3)
Blood and lymphatic system disorders	1 (0.6)
Immune system disorders	1 (0.6)
Ear and labyrinth disorders	1 (0.6)
Cardiac disorders	1 (0.6)
Reproductive system and breast disorders	1 (0.6)

SOC MedDRA Ver. 8.0 System Organ Class

became positive for anti-TCZ IgG antibodies 12 weeks after restarting TCZ treatment, and no decrease in the efficacy or any infusion reaction was observed in this patient. Moreover, no serious allergic reactions were reported in any patient.

One patient who discontinued TCZ treatment for 24 weeks experienced an infusion reaction 8 weeks after restarting TCZ therapy. The reactions included eruption, fatigue, and hypertension following the third infusion, but were mild and transient and did not require any treatment.

Three SAEs (1.9 %) were reported during retreatment with TCZ: appendicitis, wrist fracture, and chronic sinusitis. Causal relationships with TCZ were ruled out in the wrist fracture and chronic sinusitis.

Discussion

This study demonstrated that retreatment with TCZ was well-tolerated and effective in patients who had previously withdrawn from TCZ treatment. None of the patients in this study developed anti-TCZ IgE antibodies and only 1 patient tested positive for anti-TCZ IgG antibodies after restarting the TCZ treatment. Moreover, no serious allergic reactions were reported in any patient, including 3 patients retreated with TCZ after a long-term interval of more than 1 year. Our results confirm the results reported by Sagawa [16]. On the other hand, the development of serious infusion reactions was reported in patients who had restarted IFX treatment after long-term cessation of IFX [17]. This difference between TCZ and IFX can be attributable to the fact that, whereas IFX is a chimeric monoclonal antibody, TCZ is humanised, which reduces the content of foreign protein and thus the potential for the development of neutralising antibodies or IgE antibodies.

Regarding the efficacy of restarting TCZ at recurrence of disease activity after the cessation of TCZ treatment, the DAS28-ESR remission rate at 12 weeks after restarting TCZ was 88.5 %, which is comparable to the remission rate at the last observation point before cessation of initial TCZ treatment in the DREAM study (90.4 %). This improvement in DAS28-ESR was induced not only by improvement in acute-phase reactions, but also by improvement in TJC and SJC: over 60 % of the TCZ-retreated patients had complete improvement in terms of TJC or SJC or both (TJC or SJC or both was zero) within 12 weeks of treatment. Moreover, the Boolean remission rate as newly recommended by ACR/EULAR [15] reached 43.9 % (69/157 patients) at week 12. This value was extremely high.

The ACR/EULAR treatment recommendations state that, in patients who achieve remission with biological products, it may be possible to taper off the biological product after tapering off the corticosteroid [18]. However, in the majority of patients who discontinue treatment with biologics, it is found that efficacy cannot be sustained without use of the biologics and that disease activity may increase [16, 19]. This fact indicates that after attempting discontinuation of treatment with a biologic DMARD, it is necessary to guarantee safety and the ability to resume efficacy when restarting treatment with the same DMARD. Our results clearly indicate that TCZ was well-tolerated and effective in the patients who resumed TCZ treatment.

MMP-3 is deeply involved in cartilage destruction in RA and is also correlated with disease activity [20]. Since normalisation of the MMP-3 level is thought to reflect inhibition of excessive cartilage and bone destruction in the joints, normalisation of the MMP-3 level may indicate an improvement in the underlying cause of RA as well as synovial inflammation. In this study, we did not examine the progression of joint damage by imaging after restarting TCZ. However, since the MMP-3 levels were quickly improved after TCZ retreatment, TCZ retreatment should be considered to control disease activities and potentially prevent joint destruction once disease activity increased after the cessation of TCZ treatment. Further study of changes in radiological progression will be necessary to validate the modality of TCZ treatment investigated in the DREAM/RESTORE studies.

In conclusion, our results indicate that TCZ retreatment was effective and well tolerated in patients in whom disease activity recurred after cessation of TCZ monotherapy. Our

results also indicate that, together with the results of the DREAM study, the treatment interval of TCZ can also be adjusted flexibly without attenuation of efficacy.

Acknowledgments The authors wish to thank all members of the MRA study group for RA for treating the patients. This study was funded by Chugai Pharmaceutical Co. Ltd.

Conflict of interest N. Nishimoto has served as a consultant to and received honoraria from Chugai Pharmaceutical Co. Ltd. NN also works as a scientific advisor to F. Hoffmann-La Roche, which is developing TCZ in collaboration with Chugai Pharmaceutical Co. Ltd. NN also has received research grants from Chugai Pharmaceutical Co. Ltd., Bristol-Myers Japan, and Pfizer Japan Inc. K. Amano has received research grants from Chugai Pharmaceutical Co. Ltd., Astellas Pharm Inc., and Mitsubishi Tanabe Pharma. Y. Hirabayashi has received speakers' bureau honoraria from Chugai Pharmaceutical Co. Ltd. M. Iwamoto has received royalties from Chugai Pharmaceutical Co. Ltd. H. Kohsaka has received research grants, consultant fees, and/or speakers' bureau honoraria from Bristol-Myers Japan, Pfizer Japan Inc., and Takeda Pharmaceutical Co. Ltd. T. Mimura has received research grants from Abbott Japan, Chugai Pharmaceutical Co. Ltd., Mitsubishi Tanabe Pharma, and Takeda Pharmaceutical Co. Ltd. T. Takeuchi has received research grants, consultant fees, and/or speakers' bureau honoraria from Abbott Japan, Bristol-Myers Squibb, Chugai Pharmaceutical Co. Ltd:, Eisai Co. Ltd., Janssen Pharmaceutical KK, Mitsubishi Tanabe Pharma, Novartis, Pfizer Japan Inc., and Takeda Pharmaceutical Co. Ltd. S. Tohma has received a research grant from Pfizer Japan Inc. and has received subsidies or donations from the Health and Labour Sciences Research Grants for Research on Allergic Disease and Immunology and from Chugai Pharmaceutical Co. Ltd. N. Takagi is a full-time employee of Chugai Pharmaceutical Co. Ltd. All other authors have declared no conflicts of interest.

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susceptible locus in European subjects [6]. We did not show statistical significant difference in the distribution of polymorphism rs394581 and rs182429 between RA patients and controls as previously reported [6,7]. Consequently, we suggest that polymorphism rs212389 better predicts the association of TAGAP locus with RA.

Given that TAGAP is involved in T-cell activation, polymorphisms in TAGAP gene may be very important in autoimmune diseases' pathogenesis. TAGAP locus has been associated to many autoimmune diseases with opposite effects in many disorders. Therefore, more genetic association studies are needed in larger groups of patients and of different ethnicities in order to fine map the risk polymorphisms in each inflammatory disease, while functional studies of TAGAP protein could help to extend our limited knowledge over its role in cells.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Accepted 16 January 2013

Available online 1 March 2013

doi:10.1016/j.jbspin.2013.01.008

Osteolytic change of distal interphalangeal joints and sacroiliac joints in subluxing arthropathy associated with anti-Jo-1 antibody

ARTICLE INFO

Keywords:
Osteolytic change
Arthropathy
Anti-Jo-1 antibody
Sacroiliac joint

Inflammatory joint symptoms are often seen in idiopathic inflammatory myopathies, including polymyositis (PM). Destructive articular changes are uncommon in PM, but patients with anti-Jo-1 antibody occasionally develop a deforming arthritis. It was first described by Bunch et al. [1] and Oddis et al. [2], and its characteristics are quite different from those of rheumatoid arthritis (RA) in terms of subluxation of the distal interphalangeal (DIP) joints, especially of the thumb IP joints. We describe a case of severe osteolytic changes in DIP joints and sacroiliac joints (SIJ) in subluxing arthropathy with anti-Jo-1 antibody.

A 66-year-old woman was admitted to our hospital due to polyarthralgia and muscle pain. She had pain in her fingers for 20 years, considered as having RA in another hospital. Her physical examination revealed symmetric arthritis of the DIP, proximal IP, metacarpophalangeal, wrist, and metatarsopharangeal joints,

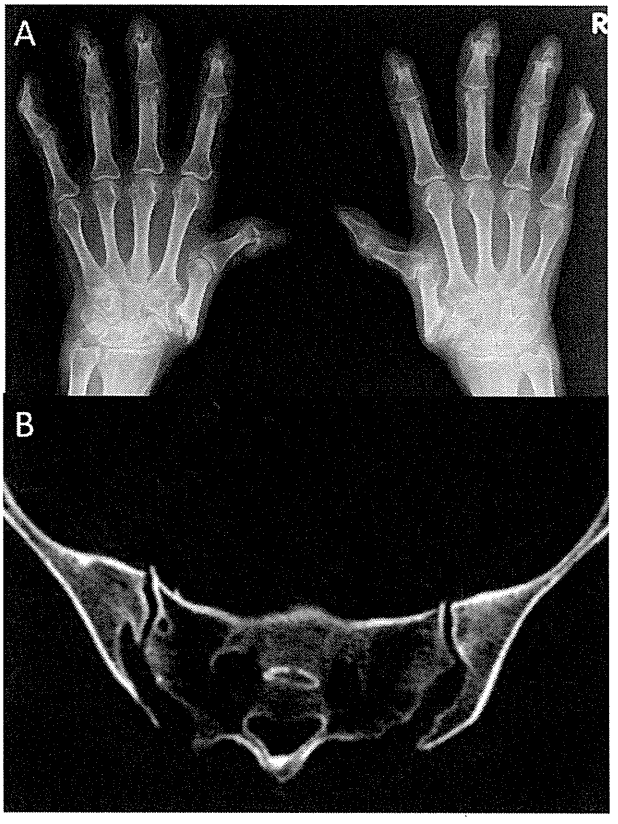

Fig. 1. A. Plain radiograph of hands. Osteolytic changes and subluxation of several distal interphalangeal joints in addition to the subluxation of interphalangeal joints and carpometacarpal joints of the thumbs were to be seen. B. Computed tomography scan of sacroiliac joints. Osteolytic change on the iliac side with widening of the joint space was seen without no typical features of spondyloarthropathy including sclerosis, joint space narrowing or ankylosis.

and proximal muscle weakness. Laboratory findings showed a C-reactive protein of 5.9 mg/dL (normal < 0.35 mg/dL), creatine kinase 1306 IU/L (normal range 60–250 IU/L), negative rheumatoid factor and anti-citrullinated peptide antibody, and positive anti-Jo-1 autoantibody. Radiographic findings demonstrated osteolytic change and subluxation of several DIP joints in addition to the subluxation of IP and carpometacarpal joints of the thumbs without apparent apatite deposits (Fig. 1A). ^{67}Gallium scintigraphy revealed expected findings of uptake in several peripheral joints and unexpected uptake in both SIJs. CT evaluation of the SIJs showed osteolytic change on the iliac side with widening of the joint space (Fig. 1B). There were no typical features of spondyloarthropathy (SpA), including sclerosis, joint space narrowing or ankylosis, and syndesmophytes were not seen on spinal radiographs. She was HLA-B27 negative and had no history of psoriasis, uveitis, inflammatory low back pain, family history, or injury. She was diagnosed as having PM and subluxing arthropathy associated with anti-Jo-1 antibody accompanied by DIP joints and SIJs osteolytic change.

While Oddis et al. reported that the radiographic changes of the deforming arthropathy with anti-Jo-1 antibody were primarily those of joint subluxation rather than erosion [2], there were some case reports presenting as osteolysis of DIP joints [3,4]. Meyer et al. reported that joint manifestations of antisynthetase syndrome fell into three categories, including subluxing arthropathy affecting DIP joints, polyarthritis, and arthralgia alone [5]. Our case was compatible with the subluxing arthropathy group and had additional osteolysis in SIJs. Coexistence of SpA is highly unlikely because she had no sign of SpA, and SpA is very rare in Japanese population with an estimated prevalence as low as 0.0095% [6]. We regard those osteolytic changes in DIP joints and SIJs as associated with anti-Jo-1 antibody and encourage further studies focusing on SIJ involvement in patients with anti-Jo-1 antibody.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

The corresponding author certifies that all authors approved the entirety of the material and contributed actively to the study.

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Accepted 24 January 2013
Available online 30 July 2013
doi:10.1016/j.jbspin.2013.01.012

Widespread psoriasis induced by rituximab in a patient with rheumatoid arthritis: An unexpected adverse reaction

ARTICLE INFO

Keywords:
Rituximab
Psoriasis
Seropositive rheumatoid arthritis

Rituximab (RTX) is a chimeric B-cell-depleting monoclonal antibody used for a range of immune-mediated diseases with good efficacy profile. However, RTX is also able to induce autoimmune phenomena such as psoriasis [1–4].

A 50-year-old woman was diagnosed as rheumatoid arthritis (RA) after presenting with symmetric erosive polyarthritis of the hand, wrist and elbow joints in 1995. She was positive for rheumatoid factor (RF) (167, [0–10 IU/mL]), negative for anticyclic citrullinated peptide (anti-CCP) antibodies. Over the next 14 years, she received disease-modifying antirheumatic drugs in combination including methotrexate, leflunomide, hydroxychloroquine and corticosteroids, thereafter tumor necrosis factor-α inhibitors (TNFi) infliximab, for 3 years, and etanercept, for 1 year, sequentially. As she had persistent synovitis with 28-joint Disease Activity Score (DAS28) of 7.35, RTX therapy (two infusions of 1 g, 2 weeks apart with 100 mg intravenous methylprednisolone) was initiated. RTX improved symptoms with decrease in DAS28 score. Five months after 3rd cycle, 25 months after 1st infusion, she presented with erythematous patch-plaque lesions on extremities (Fig. 1a). B-cell count was low, gammaglobulin levels were normal at that time. Biopsy showed neutrophilic perivascular dermatitis with extravasated erythrocytes, marked hyperkeratosis, Munro microabscesses and minimal spongiosis consistent with early psoriasis, guttate psoriasiform pattern (Fig. 1b). Absence of lichenoid infiltration or dyskeratotic cells within the epidermis excluded drug eruption. Biopsy was free of both fungi with Periodic Acid Schiff stain and signs of vasculitis. The body surface area (BSA) involvement and Psoriasis Area Severity Index (PASI) score were 6% and 3.8, respectively. While lesions were stable with topical agents one more RTX infusion deteriorated lesions, BSA involvement 15%, PASI score 8.6.

RTX and psoriasis have a conflicting relationship that either worsening [5] or improvement [6] or new-onset of psoriasis have been reported [1–4]. Features of RTX induced psoriasis cases are summarized in Table 1. In our patient, neither personal/family history, other risk factors nor clinically obvious infectious trigger known to induce psoriasis were present. Although RF/anti-CCP positivity can also be seen in psoriatic arthritis (PsA) [7], this patient's diagnosis of RA was certain in terms of typical distribution of structural damage. The patient did not fulfill the Classification Criteria for PsA with absence of dactylitis, typical radiological changes, distal interphalangeal joint, axial and enthesseal involvement.

A single nucleotide polymorphism of TRAF1 predicts the clinical response to anti-TNF treatment in Japanese patients with rheumatoid arthritis

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Abstract

Objective

Recent genome-wide association studies disclosed that several single nucleotide polymorphisms (SNPs), including tumour necrosis factor (TNF) receptor-associated factor 1 (TRAF1) (+16860A/G), are associated with the pathophysiology of rheumatoid arthritis (RA). We assessed the usefulness of TRAF1 genotyping as a genetic predictor of the response to anti-TNF treatment in Japanese RA patients.

Methods

TRAF1 (+16860A/G) was genotyped using the TaqMan SNP genotyping assay in 101 Japanese RA patients treated with anti-TNF drugs for >24 weeks. We retrospectively analysed the association between SNP and the clinical response to treatment. TRAF1 mRNA and protein expression was also evaluated in $CD4^+$, $CD8^+$, $CD14^+$, or $CD19^+$ cells from 25 healthy subjects using quantitative polymerase chain reaction and intracellular staining flow cytometry, respectively.

Results

No statistical difference in DAS28-ESR at baseline was observed between the patient groups with the AA, AG, or GG genotype. The GG genotype was more frequent in non-responders than in good or moderate responders [odds ratio (OR) 7.4, 95% confidence interval (CI) 1.5–37.5]. The non-responders possessed the G allele more frequently than the good or moderate responders (OR 3.5, 95% CI 1.4–9.0). TRAF1 protein expression increased significantly in $CD14^+$ monocytes from healthy subjects with the GG genotype compared with that in subjects with the AA or AG genotype.

Conclusion

TRAF1 (+16860A/G) may be useful for predicting the clinical response to anti-TNF treatment and may contribute to resistance to treatment in RA patients with the GG genotype by increasing the TRAF1 expression in circulating inflammatory cells.

Key words

rheumatoid arthritis, tumour necrosis factor, TRAF1, polymorphism

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Received on May 30, 2013; accepted in revised form on November 12, 2013.

Funding: this work was supported by a Grant-in-Aid for Scientific Research (C) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

Competing interests: none declared.

Introduction

Rheumatoid arthritis (RA) is a progressive inflammatory disorder resulting in joint damage and disability (1). Abnormalities in circulating immune cells and inflammatory cytokines are known to be involved in the pathogenesis of RA (2). Recent genome-wide association studies have shown that several genetic factors, including single nucleotide polymorphisms (SNPs), are associated with the pathophysiology of RA and that genetic variants may contribute to 50–60% of the etiology of the disorder (3-5).

Conventional disease-modifying antirheumatic drugs (DMARDs) such as methotrexate (MTX) remain the standard treatment for RA, although efficacy and safety issues of these drugs and the heterogeneous nature of RA patients may necessitate additional treatment strategies (6). Increased understanding of the immunological processes associated with the pathophysiology of RA has led to the development of biological agents that target signal transduction molecules and proinflammatory cytokines responsible for inflammation and structural damage. Although the most commonly used biological agents in clinical practice, such as drugs against tumour necrosis factor (TNF [including infliximab (IFX), adalimumab (ADA), and etanercept (ETN)], have excellent efficacy against RA, a substantial number of patients still show inadequate responses. Several clinical and genomic predictors of the response to anti-TNF treatments have been determined. It has been reported that the response to anti-TNF treatment factors is influenced by factors such as the level of disability at the onset of treatment as measured by the Health Assessment Questionnaire, current smoking, concurrent therapy with MTX, and autoantibody status, including rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (ACPA) (7-10). A number of studies have also evaluated the usefulness of genetic polymorphisms mainly in genes encoding TNF-α and TNF receptors as genetic predictors of the response to anti-TNF treatment (11-15). On the other hand, it has been reported that there is no association between the FcGRIIIa polymorphism and the response to anti-TNF treatments, although the FcGRIIIa polymorphism is shown to be associated with the development of RA (16). However, even when these factors are combined, the usefulness of these predictions still remains insufficient.

It has been recently reported that SNPs in the TNF receptor-associated factor 1 (TRAF1) gene are associated with the pathophysiology of RA in Asians, Caucasians, and the North Africa population (17, 18). In particular, TRAF1 (+16860A/G) has been shown to be associated with RA susceptibility. TRAF1 binds several protein kinases and adaptor proteins and possesses multiple functions in signalling networks through the TNF receptor superfamily (19). This suggests that the TRAF1 polymorphism may be associated with the pathophysiology of RA as a consequence of modulation of TNF signalling. This study assessed the usefulness of TRAF1 (+16860A/G) genotyping as a novel genetic predictor of the response to anti-TNF treatments in Japanese patients with RA. The study also examined the underlying mechanism of the association between TRAF1 polymorphisms and the clinical response to anti-TNF treatment.

Materials and methods

Patients and healthy subjects

A total of 364 unrelated Japanese adult patients with RA treated at Keio University Hospital were reviewed retrospectively using the database of the SAKURA study, a single center cohort study on RA. The patients eligibility for the study was based on the following criteria: treated for more than 24 weeks with anti-TNF drugs such as IFX, ADA, and ETN, as the first biological agent; started anti-TNF treatment between July 2009 and June 2012; and had available complete medical records. All patients enrolled in the study fulfilled the American College of Rheumatology 1987 revised criteria for RA. The clinical response to anti-TNF treatments was based on the EULAR response criteria (20) and was evaluated using the relative change in disease

activity score in 28 joints (DAS28-ESR) from baseline to 24 weeks. Patients with a good or moderate response were defined as responders, while patients with no response were classified as non-responders. To evaluate the expression level of TRAF1, 25 healthy subjects with the AA (n=10), AG (n=10), or GG (n=5) genotype were also recruited. All the samples from the patients and healthy subjects were obtained after they provided written informed consent to participate in the study. The study protocol was approved by the Institutional Review Board of Keio University.

Cell preparation

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinised venous blood samples using Lymphoprep (Fresenius Kabi Norge AS, Oslo, Norway) density-gradient centrifugation. In some experiments, $CD4^+$, $CD8^+$, $CD14^+$, or $CD19^+$ cells were separated from the PBMCs obtained from healthy subjects with either the AA, AG, or GG genotype using magnetic cell sorting column separation (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. All these sorted fractions consistently had >90% purity, as assessed by flow cytometric analysis.

TRAF1 (+16860) SNP genotyping

Genomic DNA was extracted from the PBMCs using the QIAamp DNA Blood Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. TRAF1 (+16860A/G) was determined using a TaqMan Real-Time PCR System and TaqMan SNP Genotyping Assay C_29005978_10 (Applied Biosystems, Inc., Foster City, CA, USA) according to the manufacturer's instructions.

Analysis of mRNA expression for TRAF1

The mRNA expression for TRAF1 was examined using the reverse transcription (RT)–polymerase chain reaction (PCR) as described previously with some modifications (21). In brief, total RNA was extracted from $CD4^+$, $CD8^+$, $CD14^+$, or $CD19^+$ cells using the RNeasy Kit (Qiagen, Valencia, CA, USA). First-strand cDNA was synthesised from total RNA using avian myeloblastosis virus reverse transcriptase (Takara, Kyoto, Japan) with oligo-dT priming. The cDNA was then subjected to a quantitative TaqMan Real-Time PCR System and TaqMan Gene Expression Assay Hs01090170_m1 (Applied Biosystems). The expression levels of the TRAF1 gene were normalised to the expression level of GAPDH.

Intracellular staining of TRAF1 by flow cytometry

The protein expression level of TRAF1 was evaluated using flow cytometry to detect intracellular staining in combination with staining for CD4, CD8, CD14, or CD19. In brief, the PBMCs were stained with fluorescein-conjugated anti-CD4 (clone 13B8.2; Beckman-Coulter, Fullerton, CA, USA), anti-CD8 (clone B9.11; Beckman-Coulter), anti-CD14 (clone RMO52; Beckman-Coulter), or anti-CD19 monoclonal antibody (clone SJ25C1; Becton Dickinson, San Jose, CA, USA). The cells were then permeabilised and fixed using the BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit (Becton Dickinson), followed by incubation with fluorescein-conjugated anti-TRAF1 monoclonal antibody (clone H-3; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The cells were analysed on a FACS® Calibur Flow Cytometer (Becton Dickinson) using CellQuest software. The TRAF1 expression level was quantified as the mean fluorescence index (MFI), calculated as the ratio of cells treated with anti-TRAF1 antibody to those treated with isotype-matched control antibody.

Statistical analysis

Statistical power was calculated using IBM SPSS Statics, version 18 (International Business Machines Corporation, Armonk, NY, USA). Differences were considered significant if $p<0.05$. Continuous values were expressed as the mean ± standard deviation (SD). Baseline characteristics were compared across TRAF1 (+16860A/G) genotypes using the Kruskal–Wallis test for continuous data and the 2-tailed Yates chi square test or Fisher's test for qualitative variables. The associations between the EULAR response at 24 weeks and explanatory variables, including TRAF1 (+16860A/G) genotypes, patient and disease characteristics, and concurrent treatments at baseline, were analysed by the 2-tailed Yates chi square test, Fisher's test, or univariate logistic regressions. Significant variables in the univariate analyses were then entered into a forced entry multivariate model. The results were expressed as the odds ratios (ORs) and 95% confidence interval (CI). The association between the TRAF1 (+16860A/G) genotypes and TRAF1 expression level were examined by the Kruskal-Wallis test, followed by the non-parametric Mann-Whitney U-test between the 2 groups.

Results

Clinical characteristics of the RA patients

Of the 364 patients, 116 were treated with anti-TNF drugs as the first biological agent, with 112 continuing this treatment for more than 24 weeks. Of these 112 patients, 11 did not have full clinical information, leaving a total of 101 patients enrolled in the study (Fig. 1). The demographic and clinical characteristics of these patients are summarised in Table I. The age (Mean ± SD) of the patients was 56±16 years, 85% were female, 81% were RF-positive, and 77% were ACPA-positive. The proportion of patients treated with IFX, ETN, and ADA as the first biological agent was 65%, 22%, and 13%, respectively. During the anti-TNF treatment, 89% of patients also received methotrexate. There was no difference in the clinical baseline characteristics of the RA patients grouped according to their TRAF1 (+16860) genotype (AA, AG, or GG).

Association between a TRAF1 SNP and clinical response to anti-TNF treatment

The frequencies of the TRAF1 (+16860A/G) genotypes in the patients were AA 50%, AG 42%, and GG 8%. This genotype distribution was consistent with the Hardy-Weinberg equilibrium and similar to the HapMap-JPT

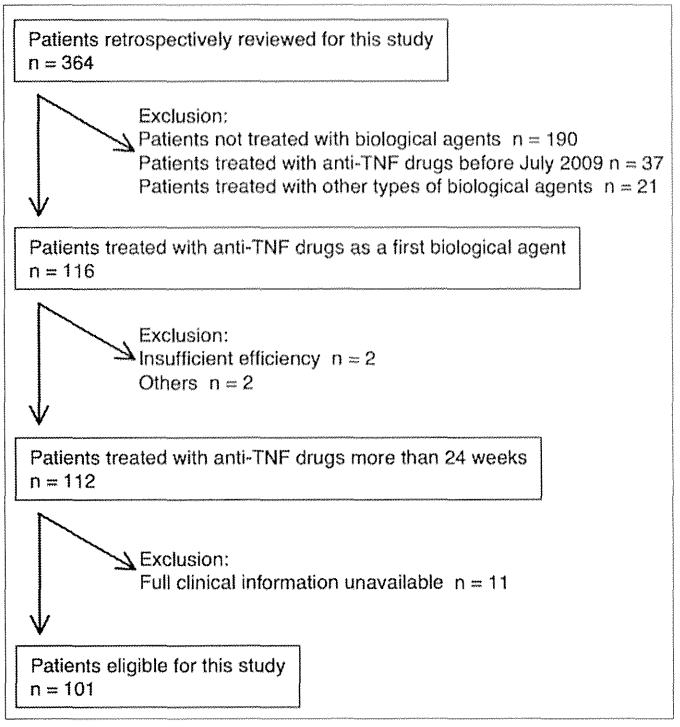

Fig. 1. Flow diagram of the study selection process. Patients eligible for the study were selected using the following criteria: treated for more than 24 weeks with anti-TNF drugs such as IFX, ADA, and ETN as a first biological agent; started anti-TNF treatment between July 2009 and June 2012; and had available complete medical records. Of the 364 RA patients followed at Keio University Hospital, 101 fulfilled these criteria, with their data being analysed in the study.

Table I. Baseline characteristics of the 101 patients with RA treated with anti-TNF agents*.

	Total	Genotype			*p* value**
	n=101	AA n=51	AG n=42	GG n=8	
Mean age (SD)	55.5 (15.7)	55.6 (15.4)	55.9 (16.5)	52.4 (14.2)	0.82
Female, n. (%)	86 (85.1)	42 (82.4)	36 (85.7)	8 (100.0)	0.42
DAS28-ESR, mean (SD)	4.9 (1.1)	5.0 (1.1)	4.9 (1.0)	4.4 (0.7)	0.39
SDAI, mean (SD)	21.1 (10.6)	21.6 (11.9)	21.3 (9.7)	17.1 (5.5)	0.48
RF positive, n. (%)	82 (81.2)	38 (74.5)	37 (88.1)	7 (87.5)	0.22
ACPA positive, n. (%)	78 (77.2)	37 (72.5)	35 (83.3)	6 (75.0)	0.46
Concurrent treatments					
Methotrexate, n. (%)	90 (89.1)	44 (86.3)	39 (92.9)	7 (87.5)	0.59
Other DMARDs, n. (%)	29 (28.7)	15 (29.4)	12 (28.6)	2 (25.0)	0.97
Prednisolone, n. (%)	18 (17.8)	8 (15.7)	7 (16.7)	3 (37.5)	0.32
Anti-TNF treatments					
Infliximab, n. (%)	66 (65.3)	34 (66.7)	30 (71.4)	4 (50.0)	0.75
Etanercept, n. (%)	22 (21.8)	12 (23.5)	8 (19.0)	2 (25.0)	
Adalimumab, n. (%)	13 (12.9)	5 (9.8)	6 (14.3)	2 (25.0)	

*RA: rheumatoid arthritis; TNF: tumour necrosis factor; SD: standard deviation; DAS-28: disease activity score in 28-joint count; SDAI: simplified disease activity index; RF: rheumatoid factor; ACPA: anti-cyclic citrullinated peptide antibody; DMARDs: disease-modifying anti-rheumatic drugs.
**p-values were calculated using the 2-tailed Yates chi square test or Fisher's test for dichotomous variables or the Kruskal-Wallis test for continuous variables.

(Japanese) frequencies [AA 57%, AG 38%, and GG 5%] and previous reports on other Asian populations (17, 22).

In the 101 RA patients who received anti-TNF treatment for 24 weeks, 63 (62.4%), 28 (27.7%), and 10 (9.9%) patients achieved a good, moderate, or no response, respectively. A summary of the EULAR response in the patients is shown in Figure 2. The non-responders to anti-TNF treatment were mainly patients with the GG genotype than those with the AA or AG genotype (37.5% *vs.* 7.5%, p=0.031, OR 7.4, 95% CI 1.5–37.5). On the other hand, the responders to anti-TNF treatments were mainly patients with the AA genotype than those with the AG or GG genotypes (96.1% *vs.* 84.0%, p=0.051, OR 4.7, 95% CI 0.9–23.2). The absolute change in DAS28-ESR from baseline to 24 weeks after initiation of anti-TNF treatment tended to decrease in patients with the GG genotype compared with that in patients with the AA or AG genotype (p=0.058) (data not shown). According to the allele frequency analysis, the non-responders to anti-TNF treatments more frequently possessed the G allele than the responders (55.0% *vs.* 25.8%, p=0.006, OR 3.5, 95% CI 1.4–9.0).

To investigate whether the TRAF1 (+16860A/G) polymorphism was an independent factor of the clinical response to anti-TNF treatment, explanatory variables including the TRAF1 (+16860A/G) genotype, clinical characteristics, and concurrent treatment were analysed in univariate and multivariate analyses (Table II). The univariate analysis showed that the GG genotype and prednisolone treatment (50% *vs.* 14.3%, p=0.015, OR 6.0, 95% CI 1.5–23.7) were more frequent in the non-responders than in the responders. Multivariate analysis confirmed that the GG genotype was independently associated with no response to anti-TNF treatment (p<0.001, OR 16.9, 95% CI 6.7–41.7).

Association between a TRAF1 SNP and gene expression levels of TRAF1

We next investigated the potential association between TRAF1 (+16860A/G) and the expression levels of TRAF1 using $CD4^+$, $CD8^+$, $CD14^+$, or $CD19^+$ cells obtained from healthy subjects. Quantitative RT-PCR showed that there was no significant difference in the expression levels of mRNA for TRAF1 in healthy subjects with either the AA, AG, or GG genotype (data not shown). However, flow cytometry showed that the protein expression levels of TRAF1 increased significantly in $CD14^+$ cells with the GG genotype compared with those in the cells with the AA or AG genotype (p=0.044) (Fig. 3). This finding indicates that subjects with the GG genotype have increased expression of TRAF1 in circulating monocytes.

Discussion

The present study examined the clinical features of 101 RA patients who received 24 weeks of anti-TNF treatment.

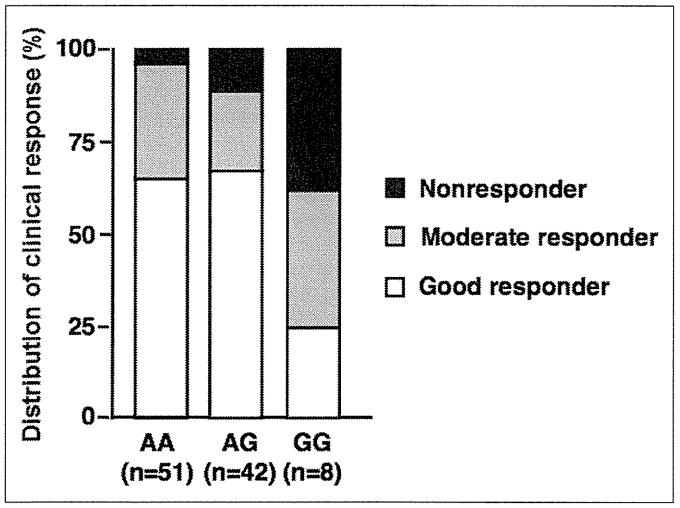

Fig. 2. Distributions of the EULAR response to anti-TNF treatment in the 101 patients with RA.

Table II. Association between the clinical response to anti-TNF treatment and explanatory variables in the univariate and multivariate analyses*.

	Univariate analysis**		Multivariate analysis	
Variable	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)
GG genotype	0.031	7.4 (1.5–37.5)	<0.001	16.9 (6.7–41.7)
Age	0.695	1.0 (1.0–1.1)		
Females	0.351	1.1 (1.0–1.2)		
DAS28-ESR at baseline	0.791	0.9 (0.5–1.7)		
SDAI at baseline	0.824	1.0 (0.9–1.1)		
Positive RF	0.201	0.8 (0.7–1.9)		
Positive ACPA	0.448	2.9 (0.3–23.9)		
Methotrexate	1.000	1.1 (0.1–9.7)		
Prednisolone	0.015	6.0 (1.5–23.7)	0.073	3.3 (0.9–14.3)

*TNF: tumour necrosis factor; DAS-28: disease activity score in 28-joint count; SDAI: simplified disease activity index; RF: rheumatoid factor; ACPA: anti-cyclic citrullinated peptide antibody.
**p-values were calculated using the 2-tailed Yates chi square test or Fisher's test for dichotomous variables or the logistic regression test for continuous variables.

We found that a TRAF1 (+16860A/G) SNP in intron 3 of the TRAF1 gene was significantly associated with the clinical response to anti-TNF treatment and that the GG genotype was more frequent in patients who did not respond to treatment. The G allele was also associated with increased expression levels of TRAF1 in CD14$^+$ monocytes from healthy subjects.

Although the role of TRAF1 in the TNF-α signalling network has not been fully elucidated, TRAF1 mainly inhibits TNF-α-mediated signalling through the TNF receptor II (18). Furthermore, TRAF1 knockout mice are hypersensitive to TNF-induced stimulation through the NF-κB and JNK pathways (23). In addition, several studies have shown that CD14$^+$ monocytes play an important role in the pathophysiology of RA (24-28). Of note, TNF-α is known to increase bone resorption by monocyte-derived osteoclasts, resulting in joint inflammation and damage in RA (28). These earlier reports and our results suggest that TNF-α-mediated stimulation is possibly inhibited by increased TRAF1 expression in circulating monocytes from subjects with the G allele and that other inflammatory cytokines such as IL-6 and IL-1, but not TNF-α, play a pivotal role in the pathophysiology of RA in patients with the GG genotype rather than those with the AA or AG genotypes. Therefore, RA patients with the GG genotype may be refractory to anti-TNF treatment. Recently, several studies demonstrated the association between SNPs for candidate genes encoding for several cytokines including TNF, and bone erosion evaluated by musculoskeletal ultrasound (MSUS) (29). Since TRAF1 is also involved in the TNF-mediated signalling pathway, the association between the three TRAF1 genotypes and MSUS-detected bone erosion should be evaluated in future.

The TRAF1 (+16860A/G) SNP (rs7021206) has been reported to be in linkage disequilibrium with other SNPs associated with RA susceptibility, such as rs2416806, rs2900180, and rs3761847 (17, 30). Although further experiments are necessary to determine which of these SNPs directly affects TRAF1 expression, the present study is the first to report an association between SNPs within the TRAF1 gene and expression levels of TRAF1.

Autoantibody status, including RF and ACPA, and concurrent therapy with MTX are associated with the response to anti-TNF treatment (7-10). However, these associations were not detected in our study. The lower OR in previous studies and the small number of samples in this study may explain this absence of a statistically significant difference.

There were 2 methodological limitations in our study. First, the patients were treated with structurally different types of anti-TNF drugs, including IFX, ADA, and ETN. IFX and ADA are both monoclonal antibodies specifically reactive to TNF-α, the first being a chimeric human–murine antibody and the latter a fully human antibody. On the other hand, ETN is a fusion protein consisting of the extracellular domain of the p75 TNF receptor and the hinge and Fc domains of human IgG_1 (31). However, the 3 drugs have a similar mechanism of action and their efficacy was comparable in our study. When only RA patients treated with IFX were analysed, the association between the TRAF1 (+16860A/G) SNP and clinical response was consistent (data not shown). Second, only patients treated with anti-TNF drugs for more than 24 weeks were selected for this study. This was necessary as the primary endpoint of the study was assessment of the clinical response after 24 weeks of treatment. Some patients had discontinued or changed treatment prior to the 24-

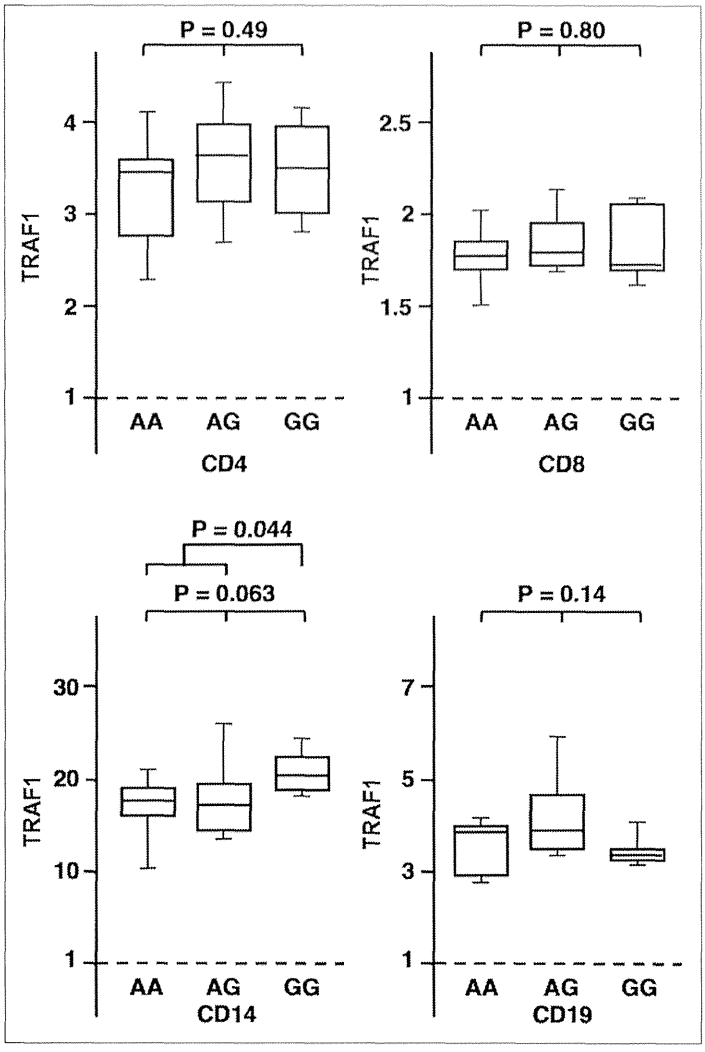

Fig. 3. Expression levels of TRAF1 in $CD4^+$, $CD8^+$, $CD14^+$, or $CD19^+$ cells. Expression levels of TRAF1

week follow-up because of inefficiency of the therapy. It is therefore necessary to perform further prospective investigations including patients who withdraw from the study.

In conclusion, TRAF1 (+16860A/G) genotyping may be useful for predicting the clinical response to anti-TNF treatment. This polymorphism may contribute to resistance to anti-TNF treatments in RA patients with the GG genotype by increasing the expression of TRAF1 in circulating inflammatory cells.

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