Table 4 Steinbrocker's rheumatoid arthritis (RA) stage-specific mean ΔDAS28 stratified by prior biologic and concomitant MTX use

Steinbrocker's RA stage	Week 4	Week 12	Week 24
All patients			
Stages I and II			
No MTX			
Number	118	134	153
ΔDAS28 ± SD	−1.00 ± 1.14	−0.92 ± 1.36	−1.14 ± 1.52
With MTX			
Number	351	404	444
ΔDAS28 ± SD	−1.24 ± 1.12	−1.39 ± 1.27	−1.51 ± 1.35
Student's *t* test[a]	$p = 0.053$	$p < 0.001$	$p = 0.005$
Stages III and IV			
No methotrexate			
Number	268	324	378
ΔDAS28 ± SD	−0.90 ± 1.19	−0.95 ± 1.38	−1.04 ± 1.41
With MTX			
Number	605	706	808
ΔDAS28 ± SD	−1.26 ± 1.05	−1.34 ± 1.19	−1.46 ± 1.28
Student's *t* test[a]	$p < 0.001$	$p < 0.001$	$p < 0.001$
Biologic-naïve			
Stages I and II			
No MTX			
Number	66	74	83
ΔDAS28 ± SD	−1.32 ± 1.15	−1.29 ± 1.31	−1.48 ± 1.37
With MTX			
Number	226	265	288
ΔDAS28 ± SD	−1.45 ± 1.01	−1.58 ± 1.13	−1.68 ± 1.23
Student's *t* test[a]	$p = 0.377$	$p = 0.063$	$p = 0.202$
Stages III and IV			
No MTX			
Number	134	152	174
ΔDAS28 ± SD	−1.28 ± 1.05	−1.45 ± 1.23	−1.51 ± 1.28
With MTX			
Number	309	343	403
ΔDAS28 ± SD	−1.55 ± 1.01	−1.72 ± 1.09	−1.85 ± 1.16
Student's *t* test[a]	$p = 0.012$	$p = 0.015$	$p = 0.001$
Prior biologic			
Stages I and II			
No MTX			
Number	52	60	70
ΔDAS28 ± SD	−0.60 ± 1.01	−0.46 ± 1.28	−0.74 ± 1.60
With MTX			
Number	125	139	156
ΔDAS28 ± SD	−0.85 ± 1.19	−1.03 ± 1.43	−1.19 ± 1.49
Student's *t* test[a]	$p = 0.193$	$p = 0.008$	$p = 0.042$
Stages III and IV			
No MTX			
Number	134	172	204
ΔDAS28 ± SD	−0.52 ± 1.19	−0.50 ± 1.35	−0.63 ± 1.39
With MTX			

Table 4 continued

Steinbrocker's RA stage	Week 4	Week 12	Week 24
Number	296	363	405
ΔDAS28 ± SD	−0.96 ± 1.00	−0.97 ± 1.17	−1.07 ± 1.28
Student's *t* test[a]	$p < 0.001$	$p < 0.001$	$p < 0.001$

ΔDAS28 change in 28-joint Disease Activity Score from baseline, *MTX* methotrexate, *SD* standard deviation

[a] *P* values comparing concomitant MTX use to no MTX use (Student's *t* test)

with RA emphasizes that certain genetic and environmental factors may predispose Japanese patients to serious risks associated with biologic therapies: namely, bacterial pneumonia, tuberculosis, PCP, and interstitial pneumonia [16]. The 0.1% incidence of tuberculosis in our population is consistent with the incidence observed in a PMS for etanercept and the latter 2,000 patients in the infliximab PMS study cohort after the percentage of patients given chemoprophylaxis for tuberculosis increased [1, 2]. Comparison of the incidence of tuberculosis with other countries is difficult because of differences in epidemiologic factors influencing the incidence of tuberculosis and different regional guidelines. These factors lead to differing screening and chemoprophylaxis practices worldwide. Even though the incidence of tuberculosis in Japan is higher than in Western countries, intensive screening and chemoprophylaxis in Japanese patients receiving biologic therapy appear to be effective. Evidence indicates a >70% reduction in the risk of tuberculosis with screening and chemoprophylaxis [14].

Several studies have characterized the small but clinically important increased risk for serious infection in RA patients receiving anti-TNF therapy [17–23]. In our study, the incidence of infectious ADRs, including serious infections, was not changed by concomitant DMARD or MTX use. Our results are consistent with the study by the Consortium of Rheumatology Researchers of North America (CORRONA) in which the rate of infections did not increase in patients treated with combination anti-TNF plus MTX therapy compared with monotherapy with these agents [24]. Infection rates between biologic-naïve and biologic-experienced patients were also similar. Consistent with prior studies [24–28], we found that concomitant glucocorticoid therapy is associated with greater risk of developing infections. In particular, serious infections were significantly more common in patients receiving glucocorticoid dosages >5 mg/day. The incidences of bacterial/bronchial pneumonia, PCP, and interstitial pneumonia among adalimumab-treated patients in this study were generally similar to those of infliximab and etanercept PMS studies [1, 2]. These finding are supported by an analysis of serious infections in the British Society for Rheumatology Biologics Register (BSRBR) study, which found similar overall risk among adalimumab, etanercept, and infliximab treatment [22].

Risk factors for developing serious infections included age ≥65 years, diabetes mellitus history or comorbidity, interstitial pneumonitis history or comorbidity, and advanced Steinbrocker's RA class. Similar risk factors for pneumonia or serious infection were reported from PMS programs for infliximab [1] or etanercept [29], respectively. In addition, older age, functional disability, and concomitant use of glucocorticoid were reported as risk factors for infection in RA patients in general [25]. When adalimumab is administrated to patients who have multiple risk factors described above, risk–benefit balance should be carefully considered after proper evaluation of these risk factors.

Because nonresponse and loss of response are factors in biologic therapy for RA, our database provided an opportunity to assess response in patients treated with another anti-TNF therapy before receiving adalimumab. Biologic-naïve patients responded better to adalimumab therapy than did biologic-experienced patients, especially patients who had received etanercept. The concomitant use of anti-TNF therapies and MTX is recommended by the updated Japanese guidelines for use of infliximab and etanercept in RA patients [9]. We found that patients receiving concomitant adalimumab and MTX have more improvement in ΔDAS28 than patients receiving adalimumab alone. Importantly, there were no clinically important differences in the incidence of AEs with increasing MTX dosage, indicating a positive risk–benefit profile for adalimumab plus MTX, even at dosages >8 mg/week.

A limitation of this PMS registry study is the relatively short duration of follow-up; however, our data were rigorously collected, and the 24-week follow-up period should be sufficient to capture most serious infections. Support for this view comes from Galloway et al. [22], who found the risk of serious infection in patients with RA is greatest during the first 6 months of anti-TNF therapy [22]. One advantage of PMS registry studies is that they can be applied more generally than clinical trials because they typically enroll larger numbers of patients under less-restrictive inclusion/exclusion criteria, and patients are evaluated in an actual clinical practice setting [30]. In addition, the patient population in our study is similar to

those in large US and European RA registries. The mean age among the biologic cohorts in the Western registries ranged from 50 to 64 years; 72–86% were women, with the exception of the one registry that targeted US veterans, which had only 11% women; duration of RA ranged from 9 to 19 years, with the exception of one registry targeting early RA in which patients had an average disease duration of 1.5 years; baseline DAS28 scores ranged from 3.5 to 6.6; and 38–93% of patients used concomitant glucocorticoids [21].

In conclusion, this interim analysis of postmarketing data for the first 3,000 patients treated with adalimumab following its approval in Japan supports the safety and effectiveness demonstrated in clinical trials. No new safety concerns were identified, and the incidence of clinically important AEs with this anti-TNF therapy was similar to those of other biologic agents approved for RA treatment. The use of adalimumab was most favorable for RA patients naïve to biologic therapy and who were treated concomitantly with MTX.

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Conflict of interest Doctors T. Koike, M. Harigai, N. Ishiguro, S. Inokuma, S. Takei, T. Takeuchi, H. Yamanaka and Y. Tanaka are members of the Postmarketing Surveillance (PMS) Committee of the Japan College of Rheumatology. It is the belief of the authors that this does not constitute a conflict of interest. The doctors participated in review and analysis of the PMS data in their capacity as committee members and are so listed. The financial relationships of the authors with manufacturers of biological products used in the management of RA are listed as: #1, a research grant to the institute to which they are affiliated; #2 a consulting fee; and #3 membership of a speakers' bureau. T. Koike, Abbott Japan, 1; Bristol-Myers Squibb, 1; Chugai Pharmaceutical Co. Ltd., 1; Eisai Co. Ltd., 1; Mitsubishi Tanabe Pharma, 1; Takeda Pharmaceutical Co. Ltd., 1; Wyeth KK, 1; Otsuka Pharmaceutical Co. Ltd., 2; Abbott Japan, 3; Bristol-Myers Squibb, 3; Chugai Pharmaceutical Co. Ltd., 3; Eisai Co. Ltd., 3; Mitsubishi Tanabe Pharma, 3; Takeda Pharmaceutical Co. Ltd., 3; Wyeth KK, 3; M. Harigai, Abbott Japan, 1; Astellas, 1; Bristol-Myers Squibb, 1; Chugai Pharmaceutical Co. Ltd., 1; Eisai Co. Ltd., 1; Mitsubishi Tanabe Pharma, 1; Pfizer Japan Inc., 1; Takeda Pharmaceutical Co. Ltd., 1; Abbott Japan, 2; Bristol-Myers Squibb, 2; Chugai Pharmaceutical Co. Ltd., 2; Jansen Pharma, 2; Mitsubishi Tanabe Pharma, 2; Abbott Japan, 3; Bristol-Myers Squibb, 3; Chugai Pharmaceutical Co. Ltd., 3; Eisai Co. Ltd., 3; Mitsubishi Tanabe Pharma, 3; Pfizer Japan Inc., 3; Takeda Pharmaceutical Co. Ltd., 3; N. Ishiguro, Abbott Japan, 1; Chugai Pharmaceutical Co. Ltd., 1; Daiichi-Sankyo Pharmaceutical Co. Ltd., 1; Eisai Co. Ltd., 1; Mitsubishi Tanabe Pharma, 1; Takeda Pharmaceutical Co. Ltd., 1; Wyeth KK, 1; Abbott, 3; Bristol-Myers Squibb, 3; Chugai Pharmaceutical Co. Ltd., 3; Daiichi-Sankyo Pharmaceutical Co. Ltd., 3; Eisai Co. Ltd., 3; Mitsubishi Tanabe Pharma, 3; Takeda Pharmaceutical Co. Ltd., 3; Wyeth KK, 3; S. Inokuma, None; S. Takei, None; T. Takeuchi, Abbott Japan, 3; Bristol-Myers Squibb, 3; Mitsubishi Tanabe Pharma, 3; Novartis, 3; Chugai Pharmaceutical Co. Ltd., 3; Eisai Pharma, 3; Janssen Pharmaceutica, 3; Takeda Pharmaceutical Co. Ltd., 3 Pfizer Japan Inc., 3; H. Yamanaka, Abbott Japan, 1; Chugai Pharmaceutical Co. Ltd., 1; Eisai Co. Ltd., 1; Mitsubishi Tanabe Pharma, 1; Takeda Pharmaceutical Co. Ltd., 1; Bristol-Myers Squibb, 3; Y. Tanaka, Abbott, 1; Astellas Pharma Inc., 1; Chugai Pharmaceutical Co. Ltd., 1; Eisai Co. Ltd., 1; Mitsubishi Tanabe Pharma, 1; MSD KK, 1; Pfizer Inc., 1; Takeda Pharmaceutical Co. Ltd., 1; Mitsubishi Tanabe Pharma, 2; Abbott, 3; Astellas Pharma Inc., 3; Chugai Pharmaceutical Co. Ltd., 3; Eisai Co. Ltd., 3; Mitsubishi Tanabe Pharma, 3; Takeda Pharmaceutical Co. Ltd., 3.

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Inhibition of plasma IL-6 in addition to maintenance of an efficacious trough level of infliximab associated with clinical remission in patients with rheumatoid arthritis: analysis of the RISING Study

Many clinical studies have reported the excellent clinical efficacy of infliximab (IFX), an antitumour necrosis factor α (anti-TNFα) monoclonal antibody, in the treatment of rheumatoid arthritis (RA).[1] IFX is also reported to induce a rapid and marked reduction in circulating interleukin 6 (IL-6) levels, suggesting that its efficacy may result from the suppression of IL-6 as well as TNF.[2–5] In the RISING Study (NCT00691028),[6 7] we observed patients who showed no response to IFX therapy, despite maintaining a serum IFX level higher than the threshold level for clinical response. Here, we examined data on clinical response to better understand the mechanism of action of IFX.

In this study, patients with methotrexate-refractory RA treated with 3 mg/kg of IFX at weeks 0, 2 and 6 were randomly assigned to receive 3, 6 or 10 mg/kg of IFX every 8 weeks from week 14 to 46 in combination with methotrexate. A total of 271 patients were classified into four groups based on serum IFX, which was used as a surrogate marker of TNF suppression since the TNF level could not be accurately measured in the presence of IFX, and plasma IL-6 levels at week 54 (Group 1, IFX-high/IL-6-low; Group 2, IFX-high/IL-6-high; Group 3, IFX-low/IL-6-low; Group 4, IFX-low/IL-6-high), with cut-off values of IFX and IL-6 of 1.0 μg/ml[6 8 9] and 10 pg/ml (lower 80% confidence limit with one tail at baseline), respectively.

The median IL-6 level at baseline was 28.9 pg/ml, which rapidly decreased at week 10 and remained at 2.4 pg/ml at week 54 with a median suppression rate of 87.2%.

Clinical characteristics such as TNF and C reactive protein (CRP) levels and rheumatoid factor (RF) values showed significant differences among groups at baseline (table 1). At week 54, however, significant differences were observed in each of the disease activity indices, with the lowest disease activity in Group 1, intermediate in Groups 2 and 3 and highest in Group 4. In particular, remission rates were significantly higher in Group 1 (figure 1). A difference was also observed in the improvement of physical function. By contrast, the progression of joint damage was inhibited in the majority of patients, with no significant difference between groups.

We speculate that the efficacy of treatment seen in Group 1 resulted from the simultaneous suppression of TNF and IL-6, that in Group 2 from the direct action of TNF neutralisation and that in Group 3 via IL-6 suppression. TNF served as a main modulator of IL-6 in Group 1, as proposed by Feldman *et al*,[2] but exerted only partial modulation in Group 2. RF and

Table 1 Clinical characteristics at baseline and week 54 of the four groups classified by serum infliximab and plasma IL-6 levels at week 54

	Group 1 (n=134) IFX≥1.0 mg/ml, IL-6<10 pg/ml	Group 2 (n=31) IFX≥1.0 mg/ml, IL-6≥10 pg/ml	Group 3 (n=48) IFX<1.0 mg/ml, IL-6<10 pg/ml	Group 4 (n=58) IFX<1.0 mg/ml, IL-6≥10 pg/ml	p Value
At baseline (week 0)					
Plasma tumour necrosis factor level, pg/ml	0.88 (<0.55, 1.22)	0.87 (<0.55, 1.30)	1.01 (<0.55, 1.26)	1.07 (0.68, 1.90)	0.023
Plasma IL-6 level, pg/ml	27.7 (12.3, 61.6)	44.1 (12.1, 95.5)	28.7 (11.2, 54.8)	33.2 (19.5, 72.1)	0.328
DAS28-ESR	6.2 (5.6, 6.8)	6.2 (5.5, 6.7)	6.0 (5.4, 6.8)	6.5 (5.8, 7.0)	0.146
DAS28-CRP	5.5 (5.0, 6.1)	5.4 (4.9, 6.0)	5.1 (4.6, 5.9)	5.7 (5.3, 6.4)	0.045
SDAI	34.6 (27.6, 43.9)	34.0 (27.4, 44.2)	32.4 (25.8, 45.3)	38.3 (31.8, 46.0)	0.199
CDAI	32.0 (25.3, 40.7)	30.2 (24.5, 39.7)	30.4 (24.8, 41.5)	34.9 (28.3, 40.0)	0.395
TJC, 68 joints	15.0 (11.0, 23.0)	13.0 (9.0, 22.0)	15.0 (11.0, 22.0)	16.0 (10.0, 28.0)	0.389
SJC, 66 joints	12.0 (9.0, 17.0)	10.0 (6.0, 14.0)	12.5 (8.0, 16.0)	13.5 (10.0, 18.0)	0.367
CRP, mg/dl	2.0 (1.1, 4.1)	3.0 (1.9, 5.2)	1.7 (0.6, 3.2)	2.9 (2.0, 4.1)	<0.001
ESR, mm/hr	49.5 (32.0, 67.0)	49.0 (43.0, 68.0)	46.5 (33.5, 70.0)	63.5 (38.0, 87.0)	0.057
Patients global VAS, mm	53.0 (34.0, 71.0)	60.0 (36.0, 75.0)	51.0 (34.5, 65.5)	63.5 (46.0, 78.0)	0.067
Physicians global VAS, mm	61.0 (47.0, 75.0)	72.0 (55.0, 85.0)	59.5 (48.0, 76.0)	68.5 (53.0, 79.0)	0.056
TSS, point	40.9 (12.0, 84.9)	33.0 (11.5, 66.5)	20.8 (9.0, 56.5)*	35.5 (14.0, 86.0)	0.139
Estimated yearly progression of TSS, point/year	6.7 (3.2, 11.7)	5.5 (3.3, 12.4)	5.0 (2.5, 8.5)*	7.5 (4.0, 11.4)	0.134
HAQ score, unit	1.06 (0.63, 1.63)	1.25 (0.88, 1.63)	1.19 (0.75, 1.63)	1.38 (0.88, 1.75)	0.266
RF value, IU/ml	78.5 (33.0, 194.0)	81.0 (22.0, 128.0)	165.5 (51.0, 319.5)	130.5 (74.0, 280.0)	0.005
ACPA value, IU/ml	≥100 (24.6, ≥100)	≥100 (28.4, ≥100)	≥100 (32.9, ≥100)	≥100 (47.6, ≥100)	0.128
MMP3 value, ng/ml	191 (101, 388)	271 (183, 523)	174 (71, 364)	257 (109, 415)	0.075
At week 54					
Serum IFX level, μg/ml	4.6 (2.6, 8.3)	2.5 (1.7, 3.5)	<0.1 (<0.1, 0.5)	<0.1 (<0.1, 0.2)	-
Plasma IL-6 level, pg/ml	1.0 (0.6, 1.8)	23.7 (12.9, 42.8)	2.7 (1.1, 6.5)	37.0 (19.2, 68.5)	-
DAS28-ESR	2.6 (2.0, 3.4)	3.4 (2.5, 4.5)	3.6 (2.7, 4.4)	5.1 (4.0, 6.0)	<0.001
DAS28-CRP	1.9 (1.4, 2.7)	2.9 (2.4, 3.7)	2.7 (1.8, 3.7)	4.3 (3.6, 5.3)	<0.001
SDAI	3.6 (1.6, 8.1)	8.8 (4.1, 12.0)	7.0 (2.6, 13.0)	19.0 (10.4, 29.1)	<0.001
CDAI	3.3 (1.4, 8.0)	6.8 (3.8, 10.1)	6.8 (2.3, 12.4)	15.6 (8.0, 25.9)	<0.001
TJC, 68 joints	1.0 (0.0, 3.0)	2.0 (1.0, 5.0)	2.0 (1.0, 5.5)	6.0 (3.0, 15.0)	<0.001
SJC, 66 joints	1.0 (0.0, 2.0)	1.0 (0.0, 4.0)	1.0 (0.0, 4.5)	4.0 (2.0, 8.0)	<0.001
CRP, mg/dl	0.1 (0.0, 0.2)	0.6 (0.2, 2.5)	0.3 (0.1, 0.7)	2.1 (1.0, 4.0)	<0.001
ESR, mm/hr	14.0 (8.0, 24.0)	26.0 (15.0, 41.0)	29.0 (16.5, 44.0)	58.0 (41.0, 84.0)	<0.001
Patients global VAS, mm	9.0 (3.0, 21.0)	16.0 (6.0, 25.0)	13.5 (5.0, 36.0)	29.5 (18.0, 66.0)	<0.001
Physicians global VAS, mm	6.0 (2.0, 12.0)	15.0 (6.0, 26.0)	9.5 (3.0, 23.0)	32.5 (12.0, 53.0)	<0.001
Delta-TSS, point	0.0 (-1.0, 0.5)	0.0 (-1.5, 1.5)	0.0 (-0.5, 1.0)*	0.5 (-0.5, 3.0)	0.056
HAQ score, unit	0.25 (0.00, 0.75)	0.38 (0.13, 1.00)	0.38 (0.00, 0.94)	0.88 (0.38, 1.38)	<0.001
RF value, IU/ml	20.5 (5.0, 60.0)	20.0 (7.0, 44.0)	64.5 (16.5, 170.5)	88.5 (45.0, 238.0)	<0.001
ACPA value, IU/ml	38.2 (10.6, ≥100)	43.3 (10.2, ≥100)	≥100 (23.5, ≥100)	≥100 (31.8, ≥100)	0.009
MMP3 value, ng/ml	69 (37, 113)	185 (114, 387)	55 (38, 106)	220 (131, 390)	<0.001

Data values are median (IQR). Statistics were analysed using the Kruskal–Wallis test.
*n=46.
ACPA, anticyclic citrullinated peptide antibody; CDAI, clinical disease activity index; CRP, C reactive protein; DAS, disease activity score; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; IFX, infliximab; IL, interleukin; MMP, matrix metalloproteinase; RF, rheumatoid factor; SDAI, simplified disease activity index; SJC, swollen joint count; TJC, tender joint count; TSS, total modified-Sharp score; VAS, visual analogue scale.

anticyclic citrullinated peptide antibody titres were lower in Groups 1 and 2 (IFX-high) than in Groups 3 and 4 (IFX-low). Meanwhile, CRP and matrix metalloproteinase 3 levels were lower in Groups 1 and 3 (IL-6-low) than in Groups 2 and 4 (IL-6-high). However, we found no clear difference in evaluations when using the simplified disease activity index, which includes acute-phase reactants, or the clinical disease activity index, which does not include these reactants. We also analysed the proportion of primary or secondary non-responders in each group, as defined previously,[7] and found no obvious difference indicating a primary or secondary failure pattern.

Several limitations of our study warrant mention. First, we set the cut-off values for IFX and IL-6 levels ourselves because there are no standard reference values. Second, serum IFX level was used as a surrogate marker of TNF suppression, which cannot be accurately measured in the presence of IFX. Third, we had no data on other cytokines, so we could not explore their influence on clinical response.

This study demonstrates that the profound suppression of TNF and IL-6 in IFX treatment results in better clinical response, in addition to clinical remission.

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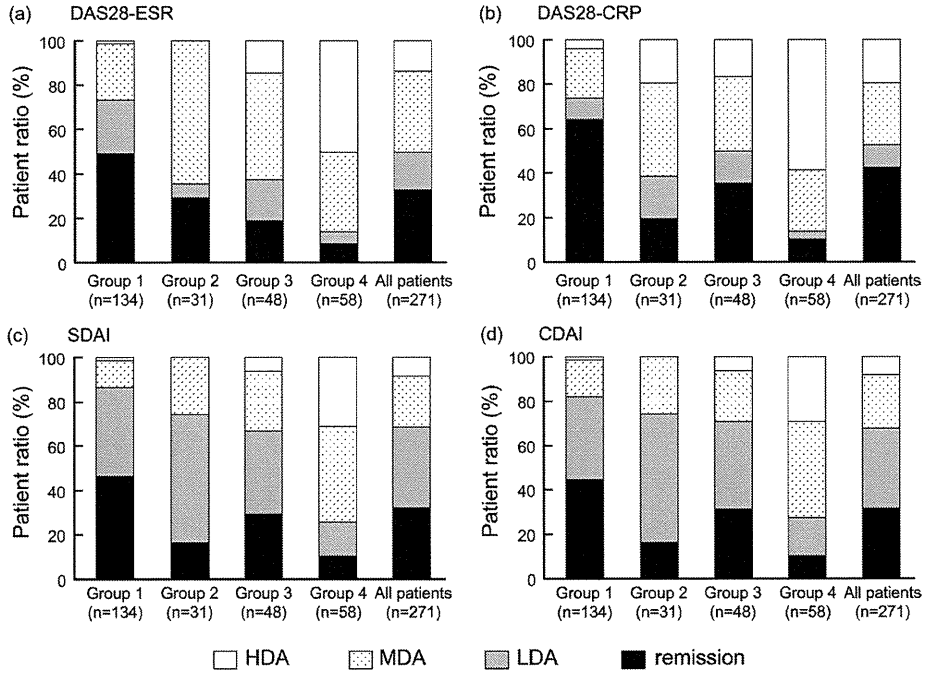

Figure 1 Efficacy of infliximab therapy at week 54 among the four groups classified by serum infliximab and plasma interleukin 6 levels at week 54. Clinical responses were analysed by disease activity score 28 (DAS28)-erythrocyte sedimentation rate (ESR) (A), DAS28-C reactive protein (CRP) (B), simplified disease activity index (SDAI) (C) and clinical disease activity index (CDAI) (D) at week 54. HDA, high disease activity; MDA, moderate disease activity; LDA, low disease activity but no remission. Cut-off values for disease activity in DAS28-CRP were used as reported by Inoue *et al* (HDA: >4.1; MDA: ≥2.7 to ≤4.1; LDA: ≥2.3 to <2.7; remission: <2.3).[10]**Figure 1** Efficacy of infliximab therapy at week 54 among the four

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Contributors TT: manuscript preparation, study design, advice for statistics. NM: advice for study design. YT: manuscript preparation, data management. T Yano: statistics. T Yoshinari: data collection. TA: advice for study design. TK: management of the study.

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Patients consent Obtained.

Ethics approval This study was conducted with the approval of the local ethics committees of every participant centre.

Competing interests TT has received lecture fees from Abbott Japan, Bristol-Myers Squibb, Chugai Pharmaceutical, Eisai, Janssen Pharmaceutical, Mitsubishi Tanabe Pharma, Novartis, Pfizer Japan and Takeda Pharmaceutical. NM has received grant support from Abbott Japan, Astellas Pharma, Chugai Pharmaceutical, Daiichi Sankyo, Mitsubishi Tanabe Pharma and Takeda Pharmaceutical; and lecture fees from Benesis and Otsuka Pharmaceutical. YT, T Yano and T Yoshinari are employees of Mitsubishi Tanabe Pharma. TA has no conflicts of interest. TK has received lecture fees from Abbott Japan, Bristol-Myers Squibb, Chugai Pharmaceutical, Eisai, Mitsubishi Tanabe Pharma, Otsuka Pharmaceutical, Pfizer and Takeda Pharmaceutical.

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arthritis research&therapy

EDITORIAL

What is the future of CCR5 antagonists in rheumatoid arthritis?

Tsutomu Takeuchi* and Hideto Kameda

See related research by Fleishaker *et al.*, http://arthritis-research.com/content/14/1/R11

Abstract

Fleishaker and colleagues reported on a double-blind placebo controlled clinical trial of a C-C chemokine-receptor type 5 (CCR5) antagonist, maraviroc, in rheumatoid arthritis (RA) patients with inadequate response to methotrexate, showing that it was ineffective. Two additional CCR5 antagonists, SCH351125 and AZD5672, also failed to demonstrate clinical efficacy. In addition, CCR5-blocking antibodies could not inhibit synovial fluid-induced monocyte chemotaxis. Thus, CCR5 appears not to be a desirable target in RA treatment. Given the multiple functions of CCR5, redundancies in the chemokine system, and patient selection in the trial, we overview the recent understanding for chemokine receptor blockade in the treatment of RA

Rheumatoid arthritis (RA) is an autoimmune disease characterized by both the presence of autoantibodies (either rheumatoid factor or those against citrullinated protein/peptide) and the chronic infiltration of leukocytes into synovial tissue and fluid. The latter is thought to be driven by interactions between chemokines and their G-protein-coupled receptors. Chemokines are known to play important roles in angiogenesis and lymphoid organization, and their expression patterns have been used as markers to identify a subset of lymphocytes and monocytes. As such, chemokines and their receptors have been deemed reasonable targets for the development of new RA treatments.

In a recent article in *Arthritis Research & Therapy*, Fleishaker and colleagues [1] reported on the results of a clinical trial of a chemokine receptor antagonist in the treatment of patients with RA. Chemokines are classified into CXC, CC, C, and CX3C supergene families according to the number and spacing of conserved cysteines. C-C chemokine-receptor type 5 (CCR5) is abundantly expressed in the RA synovium and T helper-cell type 1 inflammatory infiltrates, and is bound by macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4), and RANTES (regulated upon activation, normal T cell expressed, and secreted; CCL5) [2]. A CCR5-deficient mouse model showed reduced bacterial clearance and was protected against endotoxin-induced systemic inflammation and other enhanced immune reactions [3]. Further, although still controversial, a single nucleotide polymorphism resulting in the production of a non-functional receptor (CCR5-Δ32) protected against RA.

These findings have spurred the development of several CCR5 inhibitors. However, in a randomized, double-blind, placebo-controlled clinical trial, Fleishaker and colleagues [1] reported that a CCR5 antagonist (maraviroc), approved for use in HIV patients because CCR5 is the major co-receptor for HIV-1 entry into cells, was ineffective in treating patients with RA who had shown inadequate responses to methotrexate (MTX). Given that their study found no significant clinical efficacy as evaluated based on American College of Rheumatology responder rates or changes from baseline in Disease Activity Score 28-4 C-reactive Protein (DAS28-4 (CRP)), the study was terminated [1]. Similarly, two additional CCR5 antagonists, SCH351125 and AZD5672, respectively tested on RA [4] and MTX-refractory RA patients [5], also failed to demonstrate clinical efficacy. Moreover, neither CCR2- nor CCR5-blocking antibodies were able to inhibit synovial fluid-induced monocyte chemotaxis [6]. Therefore, CCR5 appears not to be a desirable target in RA treatment.

The above-described failures in using CCR5 inhibitors to treat RA may be explained by the multiple functions of CCR5 and redundancies in the chemokine system. However, although the expression of chemokines and their receptors has been believed to be redundant for decades, evidence shows that this may not be the case [7]. Instead, like most developmental processes, a strict temporal and spatial control of their expression could be

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critical in RA pathogenesis. A more fundamental understanding of the pathogenesis and pathophysiology of each RA patient may be needed in order to achieve precise control of the disease through manipulation of the chemokine system. Thus, whether or not drugs are administered to the right patients, at the right time, and with a sufficient dosing regimen in clinical trials is critical.

As for the dosing regimen, that based on conventional pharmacokinetics/pharmacodynamics methods may not have been sufficient to block targeting receptors more than 95% of the time. A recent review underscored the importance of maintaining dose levels sufficiently above coverage levels for serum A10-receptors [7]. The maximum dosing may be partly limited by non-specific toxicity, such as liver dysfunction, of low molecular weight chemicals and the recycling of CCR5 molecules via the trans-Golgi network [8]. Although the clinical trials targeting inflammatory cytokines such as tumor necrosis factor or interleukin-6 have been consistently positive, while those targeting chemokines have seldom been positive, this may be partly attributable to the differences between biological agents and low molecular weight chemicals, in addition to those between inflammatory cytokines and chemokines. Even with biological agents against tumor necrosis factor, we recently demonstrated via a sub-analysis of the RISING study (a prospective, randomized, double-blind study to compare the efficacy and safety of 10 mg/kg infliximab with those of 3 mg/kg infliximab treatment in MTX-refractory RA patients) that the dose of infliximab required to sufficiently neutralize tumor necrosis factor dramatically differs among RA patients [9]. Therefore, even when CCR5 inhibitors may be potentially effective for a small fraction of RA patients, the appropriate dosing regimen of CCR5 inhibitors to achieve sustained receptor occupancy above a sufficient level might be crucial in the successful treatment of RA, but not of HIV infection.

While findings regarding RA treatment with CCR5 inhibitors have thus far proven negative, these results still represent an important milestone in the development of target therapies for RA and other systemic autoimmune and inflammatory diseases.

Abbreviations

CCR5, C-C chemokine-receptor type 5; MIP, macrophage inflammatory protein; MTX, methotrexate; RA, rheumatoid arthritis.

Competing interests

The authors declare that they have no competing interests.

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CD3 ζ defects in systemic lupus erythematosus

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ABSTRACT

The prototype autoimmune disease, systemic lupus erythematosus (SLE), has been known to be associated with deficiency of ζ chain, a component of the T-cell receptor–CD3 complex. Comprehensive analysis has shown that expression of the CD3 ζ chain is attenuated or absent in over half of SLE patients. Furthermore, aberrant transcripts of the CD3 ζ chain, including spliced variants lacking exon 7 or having a short 3′-untranslated region, have been detected in SLE T cells. Although attenuated expression of the CD3 ζ chain is also observed in cancer patients, infections and other autoimmune diseases, sustained attenuation of the CD3 ζ expression accompanied with aberrant transcripts are only observed in SLE. In this study, the authors review the unique features of CD3 ζ defects observed in SLE and discuss the molecular basis of the defects by recent findings in animal models, single-nucleotide polymorphisms and genome-wide association studies.

Systemic lupus erythematosus (SLE) is a prototype autoimmune disease with a wide spectrum of clinical manifestations.[1] While abundant production of autoantibodies and subsequent formation of immune complexes lead to tissue damage such as glomerulonephritis,[2 3] a particularly crucial role in SLE pathogenesis is played by SLE T cells.[2 4] In addition, comprehensive messenger RNA expression analysis has revealed that type I interferon (IFN)-related genes are upregulated in peripheral blood cells, partly through the increased production of IFN.[5–7] Although all of these events may interact with each other, the detailed molecular characterisation of SLE T cells is required. Here, we focus on the abnormal expression of the ζ chain of T-cell receptor (TCR)–CD3 complexes (CD3 ζ chain, TCR ζ chain, CD247) in patients with SLE, and discuss how the molecular basis of the defects affect developing autoimmune diseases.

DEFECTIVE EXPRESSION OF THE CD3 ζ CHAIN IN SLE PATIENTS

When SLE T cells are stimulated in vitro or in vivo through TCR–CD3 complexes, the response is usually attenuated in SLE T cells, compared with normal T cells.[8 9] However, the direct activation of protein kinase C with phorbol ester and ionomycin leads to normal or enhanced response in SLE T cells, raising a hypothesis that defects might reside in the proximal signal transduction molecules between the TCR–CD3 complexes and protein kinase C[9] (figure 1). Indeed, the tyrosine phosphorylation of cellular proteins, particularly the CD3 ζ chain, was diminished in SLE T cells in response to anti-CD3 and anti-CD4 treatment.[10–12] Surprisingly, the protein expression of the CD3 ζ chain was diminished in SLE patients.[10 11] While reduced protein expression of the CD3 ζ chain in cancer patients, infections and other autoimmune diseases such as rheumatoid arthritis has been reported to be transient,[13] the reduction was maintained throughout the course of the disease in more than half of the SLE patients.[12 14 15]

MECHANISM OF REDUCED PROTEIN EXPRESSION OF THE CD3 ζ CHAIN IN SLE PATIENTS

Several mechanisms are responsible for the decreased expression of the CD3 ζ chain, including low transcription activity,[11] generation of spliced variants,[10 16 17] increased ubiquitination,[18] increased caspase-3-dependent proteolysis,[19] redox status,[20] oxidative stress,[21] heat stress,[22] chronic exposure to pro-inflammatory cytokines[23] and direct contact with activated macrophages.[24] No mutations or deletions have been identified in the 5′-flanking region of the CD3 ζ gene in patients with SLE,[25] while other researchers have found those in the 5′-flanking region.[16] On the other hand, we and others have detected abnormal transcripts of the CD3 ζ chain, such as the splice variants including those lacking exon 7 and with the 3′-untranslated region (UTR).[10 16 26 27] The η (exon 1–7 plus exon 9) and the ι (exon 1–7 plus exon 10) variants have been shown to be generated by alternative splicing of the CD3 ζ chain on human and mouse chromosome 1q22–23[28–32] (figure 2); however, the functions of these splice variants are not fully understood. The CD3 ζ chain lacking exon 7 and short 3′-UTR variants, which are exclusively observed in SLE patients, are new class spliced variants[9] (figure 2).

ROLE OF UNIQUE SPLICE VARIANTS IN DEFECTIVE EXPRESSION IN THE CD3 ζ CHAIN

A 562-bp region containing the consensus sequence for mRNA stabilisation and a 31-nucleotide conserved sequence is missing from the short 3′-UTR splice variant.[17] As this conserved region is important for stabilisation, transportation and localisation of the CD3 ζ chain,[33] we speculated that this short 3′-UTR splice variant accounts for the downregulation of protein expression. To test this hypothesis, mRNA from the spliced variants was transfected into mouse T-cell hybridomas lacking CD3 ζ, resulting in instability of CD3 ζ mRNA and thereby leading to reduced protein expression.[27]

The 3′-UTR region of mRNA is known to control the turnover rate of presynthesised mRNA through interactions with trans-acting factors by altering mRNA stability and affecting its transportation and localisation.[34] mRNA 3′-UTR contains cis-acting, adenosine–uridine-rich elements that bind to trans-acting proteins and participate in either the stabilisation or destabilisation of transcripts. Adenosine–uridine-rich elements are located at positions +735, +803 and +1646 of the CD3 ζ mRNA,

and the second element is found within the deleted sequence in the short 3′-UTR variant (figure 3). This notable absence may affect the stability of the short 3′-UTR variant mRNA. Using deletion mutants we found that the regions +871 to +950 and +1070 to +1136, which contain conserved regions one and two, respectively, are necessary to maintain the stability of CD3 ζ mRNA (figure 3). Similar transcript instability has been shown in another variant lacking exon 7,[35] suggesting that exon deletion and exon skipping also lead to the downregulation of protein expression through mRNA instability.

DEFECTS OF PROXIMAL SIGNAL TRANSDUCTION MOLECULES IN T CELLS IN MODEL ANIMALS

The SKG mouse, which models human autoimmune arthritis, exhibits a ζ-associated protein 70-kDa loss-of-function mutation, suggesting that one cause of the disease is defective proximal signalling molecules in T cells.[36] It is speculated that the thymic selection process has been altered by defective signal transduction, resulting in positive, but not negative, selection of autoreactive clones. More comprehensive data have been obtained by a study that tested whether the loss of tyrosine residues in each immunoreceptor tyrosine-based activation motif (ITAM) domain of a TCR–CD3 complex leads to autoimmunity.[37] That study demonstrated that scalable defects in signalling capability of the TCR–CD3 complex lead to multi-organ systemic autoimmune diseases such as interstitial pneumonitis, bowel inflammation and liver inflammation. Interestingly, a defect in two ITAM domains is sufficient to allow the development of autoimmune diseases and produces skewed cytokine production from interleukin (IL) 2 to IFNγ.

CONSEQUENCES OF EXPRESSING THE UNIQUE CD3 ζ CHAIN SPLICE VARIANTS

The evidence obtained from the model animal introduced an attractive hypothesis that defective signal transduction in T cells can be a cause of autoimmunity, partly through the altered thymic selection. One may raise the question as to whether other mechanisms may be responsible for developing skewed cytokine production and multi-organ disease. In this regard, it is interesting to know the common upregulated and downregulated genes after introducing two unique spliced variants of the CD3 ζ chain. After transfecting the short 3′-UTR and exon 7-lacking spliced variants from SLE patients into mouse T-cell hybridoma defective for CD3 ζ, DNA microarray analysis has been performed in two transfectants.[38] While only 16 common genes were upregulated in both the short 3′-UTR and exon 7-lacking variants, 36 shared genes were downregulated. We further supported that these results using real-time PCR, showing that expression levels of IL-2, IL-13, IL-15, IL-18 and transforming growth factor β-2 were significantly reduced in both spliced variants compared with those found after transfection with wild-type CD3 ζ.[38] In contrast, levels of Gsta4, Gzma, Lcn2, Mad3, Pmm1, Ptp4a3, Pvrl2, Sdc1, Selenbp1, Slc4a8, Tcf7 and Wasl were significantly increased. The possibility of whether these molecules may be involved in tissue inflammation or damage in SLE remains to be elucidated in the future.

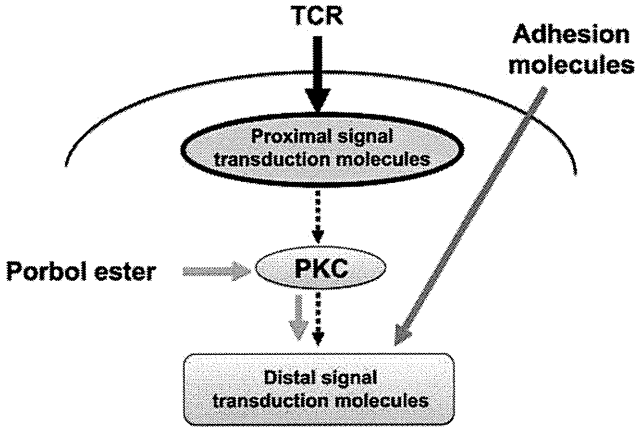

Figure 1 Functional defects of systemic lupus erythematosus (SLE) T cells. Phorbol ester can restore defects associated with SLE, indicating that proximal signal transduction molecules may be responsible for these defects. In contrast, adhesion molecules and their downstream signalling molecules are upregulated, raising the possibility that signals via adhesion molecules can bypass the proximal transduction molecules. PKC, protein kinase C; TCR, T-cell receptor.

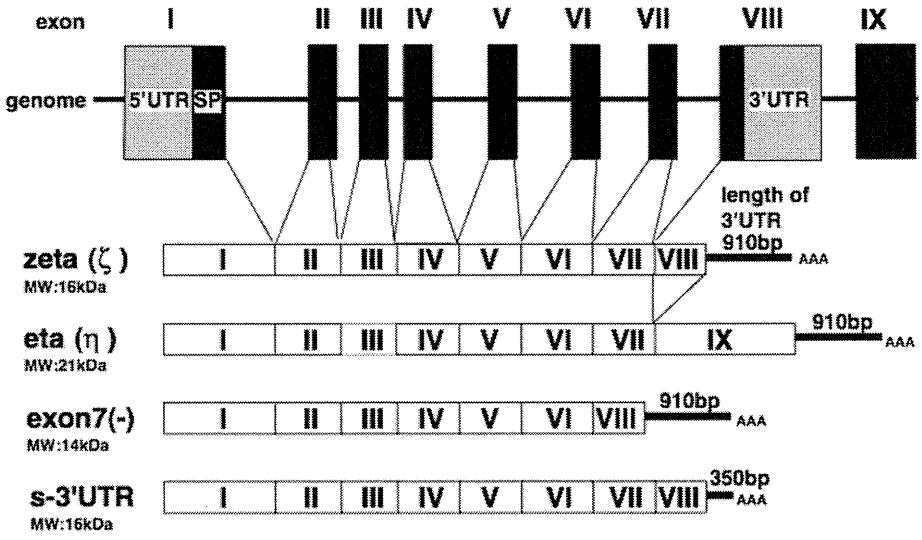

Figure 2 Structure of spliced variants of the human CD3 ζ chain. Exon–intron organisation of CD3 ζ chain genes and their transcripts for wild-type and spliced variants found in systemic lupus erythematosus (lacking exon 7 and short 3′-untranslated region; UTR).

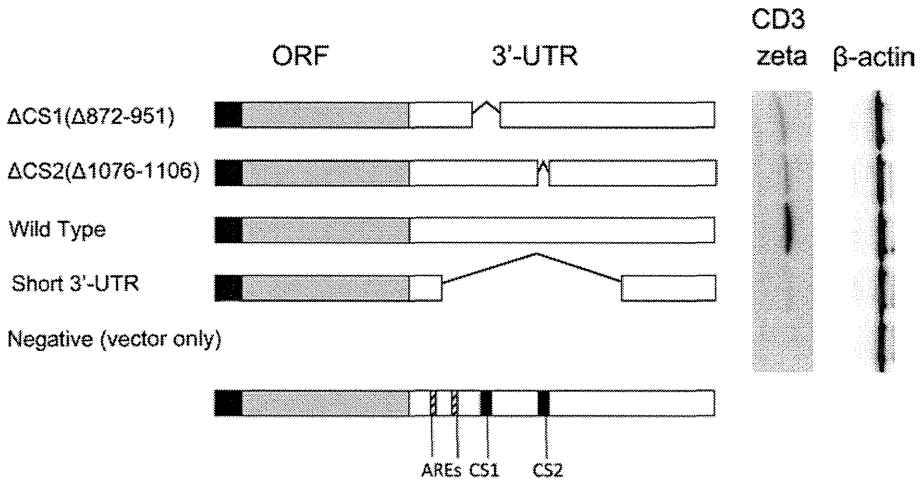

Figure 3 CD3 ζ protein expression by deleting conservative regions of the 3′-untranslated region (UTR). Structure of wild-type and deletion mutant constructs (left). CD3 ζ protein expression after transfection of mRNA from the spliced variants into mouse T-cell hybridomas lacking CD3 ζ (right). Deletion of conservative regions 1 (ΔCS1 (Δ position 872–951)), 2 (ΔCS2 (Δ position: 1076–1106)) and short 3′-UTR are indicated. ARE, adenosine–uridine-rich elements; CS, conservative sequence; ORF, open reading frame.

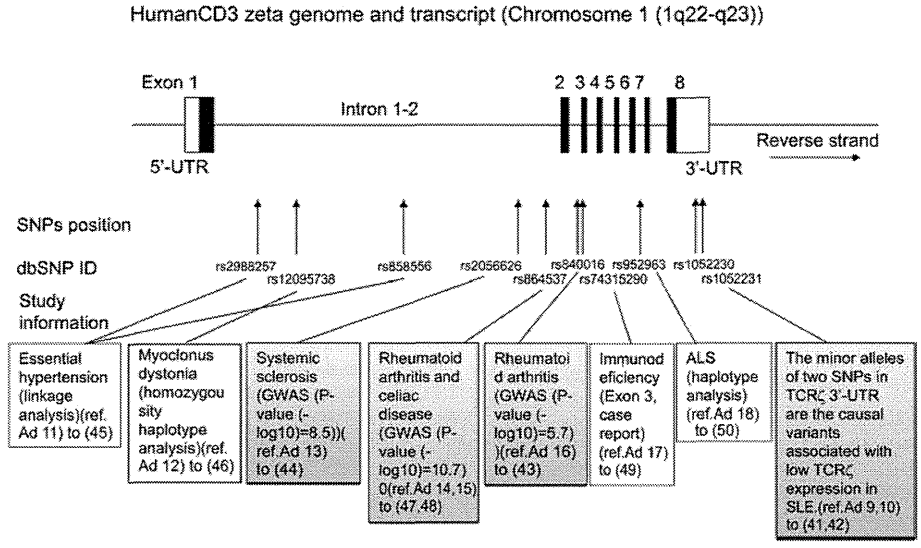

Figure 4 Single-nucleotide polymorphisms (SNP) of CD3 ζ related to systemic lupus erythematosus (SLE) and other diseases. Schemata of human CD3 ζ genome and transcripts are shown with summary information of diseases related to 10 reported SNP (position, dbSNP ID and study information) registered in the National Center for Biotechnology Information database. ALS, amyotrophic lateral sclerosis; GWAS, genome-wide association study; TCR, T-cell receptor; UTR, untranslated region.

mRNA EXPRESSION PROFILE OF PERIPHERAL BLOOD CELLS FROM PATIENTS WITH SLE

Large-scale microarray technology has been used to study global gene expression patterns of peripheral blood cells from lupus patients and control subjects in heterogeneous populations. These experiments demonstrated upregulated expression of genes in the IFN pathway in peripheral blood from SLE patients.[3 5–7] Furthermore, this signature gene expression served as an indicator for more severe diseases of the kidneys, haematopoietic cells and the central nervous system. IFN are known to have protean effects on the immune system and may therefore account for many of the immune system alterations that characterise SLE and contribute to autoimmunity.[3] As noted above, mice carrying reduced ITAM domains, such as mutated CD3 ζ, produced a substantial amount of cytokines, including IFNγ,[37] thereby prompting the attractive hypothesis that CD3 ζ defects are linked to IFN signature expression.

In fact, the level of CD3 ζ chain expression in SLE patients is inversely correlated with both in-vitro and serum levels of IFNγ,[39] whereas microarray analysis of transfectants with the spliced variant did not detect any IFN signature.[38] Recent proof-of-concept trials in SLE patients using monoclonal antibody against IFNα may provide a clue to understand the relationship between CD3 ζ and IFN in SLE patients.

SINGLE-NUCLEOTIDE POLYMORPHISMS AND GENOME-WIDE ASSOCIATION STUDIES OF THE TCR ζCD3 ζ CHAIN

The detailed mechanism of generating spliced variants of the CD3 ζ chain in SLE patients is potentially interesting, but is not fully

understood. While no genomic mutations or deletions in either the splicing donor or acceptor sites have been reported in SLE,[40] two groups recently noted single-nucleotide polymorphisms (SNP) in the 3′-UTR region[41 42] (figure 4), reporting that the minor alleles of two SNP were causal variants associated with low TCR ζ expression and that one third of the mRNA was identical to that of the major alleles. It has been shown that the haplotype carrying the low-expression variants predisposes carriers to develop SLE.[41]

At present, 10 SNP found in the CD3 ζ gene are associated with autoimmune diseases, and have been registered in the National Center for Biotechnology Information database.[41–50] The genome-wide association study of SLE patients from several ethnic groups is spreading rapidly across the world (figure 4). Although no study of SLE has yet shown specific association for the CD3 ζ chain, a meta-analysis for rheumatoid arthritis[43] and a study for systemic sclerosis[44] have reported interesting findings with regard to the possible association between CD3 ζ SNP and rheumatoid arthritis and systemic sclerosis, both of which are located in the 78-kilo-base-long intron 1–2 region. Future analyses should focus on the functional consequences of these SNP on CD3 ζ expression.

Competing interests None.

Provenance and peer review Commissioned; externally peer reviewed.

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ORIGINAL ARTICLE

Discontinuation of adalimumab treatment in rheumatoid arthritis patients after achieving low disease activity

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Abstract
Objective We implemented a retrospective study to explore discontinuation of therapy with adalimumab (ADA) without exacerbation in rheumatoid arthritis (RA) patients who had achieved low disease activity (LDA) with the biological agent.
Methods We enrolled 46 RA patients who had completed open extension of a double-blind, placebo-controlled trial of ADA monotherapy in Japan and who had LDA (DAS28-CRP <2.7) at the last administration of ADA in the extension trials; this date was defined as week 0 in the present study. Treatment of RA was at the discretion of the attending physician after week 0. The primary endpoint of this study was the percentage of patients who maintained discontinuation of biological agents and LDA for 52 weeks.
Results Twenty-four of the enrolled patients continued ADA while the rest discontinued ADA after the administration of the drug at week 0. Fourteen of the 22 patients did not restart biological agents, and 4 (18.2%) of these maintained LDA through week 52. All 4 of these patients had received ADA monotherapy before week 0.
Conclusion Some RA patients who have achieved LDA with ADA monotherapy can discontinue the biologic without incurring increased disease activity. A prospective randomized study is required to confirm the results of our study.

Keywords Adalimumab · Biological disease-modifying antirheumatic drug · Low disease activity · Rheumatoid arthritis

For the BRIGHT Study Investigators Group.

The members of the BRIGHT Study Investigators Group are listed in the "Appendix."

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Introduction

Recent advances in treatment strategies for rheumatoid arthritis (RA) have enabled us to target remission, especially in patients with early disease [1, 2]. Clinical trials of tumor necrosis factor (TNF) inhibitors have shown their excellent efficacy for alleviating the signs and symptoms of RA, as well as for inhibiting the progression of structural damage to affected joints [3, 4]. Despite this recognized efficacy, some drawbacks to the use of these drugs have been found, including adverse drug reactions, such as serious infections, high drug costs, and moderate drug retention rates. One solution to these problems would be to discontinue TNF inhibitors when the treatment target is achieved.

Several investigators have reported promising results from studies of discontinuation of biologic therapies, including the TNF20 study [5], the Behandel Strategieen (BeSt) study [6–11], and the Remission Induction by Remicade in RA (RRR) study [12]. The TNF20 and BeSt studies enrolled RA patients with early disease, while the RRR study enrolled established RA patients; all three studies used infliximab (IFX). A similar attempt has been reported in RA patients using the anti-interleukin-6 receptor antibody tocilizumab, with successful discontinuation of the biological agent in a subset of the enrolled patients without incurring significant elevation of disease activity [13].

Adalimumab (ADA), a fully human monoclonal anti-human TNF antibody, has shown good clinical efficacy and tolerability in clinical trials both in Japan [14] and worldwide [15–20]. ADA was approved for treatment of RA in the United States in 2002, in Europe in 2003, and in Japan in 2008. In a randomized, double-blind, placebo-controlled phase II/III trial of ADA in Japan (M02-575 or CHANGE; Clinical Investigation in Highly Disease-Affected Rheumatoid Arthritis Patients in Japan with Adalimumab Applying Standard and General Evaluation) [14], RA patients ($n = 352$) with a mean disease duration of 9.8 years were enrolled and allocated to either ADA monotherapy or placebo arms for 24 weeks. After completing the trial, 309 patients were enrolled into open extension trials, one requiring the administration of ADA by medical staff (M03-651), and one requiring self-injection by patients (M03-775). Taking advantage of the termination of these extension trials (M03-651 and M03-775) and the launch of ADA into the market in Japan, we implemented a retrospective study, the Biologics-free RemIssion and low disease activity after stoppinG adalimumab in Japanese patients with rHeumatoid arThritis (BRIGHT) study, to explore the possibility of discontinuing ADA without incurring exacerbation in RA patients who had achieved a low disease activity (LDA) with the biological agent.

Materials and methods

Patients

We identified 61 RA patients who had completed the open extension trials (M03-651 or M05-775) and had LDA at the last administration of ADA. Disease activity was assessed using DAS28-CRP, a formula requiring a tender joint count of 28 joints (TJC28), a swollen joint count of 28 joints (SJC28), and C-reactive protein (CRP) serum levels, and a general health visual analog scale assessed by patients (GH-VAS) [21]. LDA was defined as DAS28-CRP <2.7, as established by a large-scale cohort study in Japan [21]. Invitation letters to the BRIGHT study were sent to the investigators from M03-651 and M05-775 who had treated those 61 patients. Forty-six patients from 29 facilities were enrolled in the BRIGHT study. Among the 46 patients enrolled in the BRIGHT study, 34/46 (73.9%) were treated with ADA alone, while the remaining 12 had received ADA plus disease-modifying antirheumatic drugs (DMARDs) during the extension trials.

Therapeutic regimes

Among the 46 patients enrolled in BRIGHT, at the attending physicians' discretion, 24 continued ADA (the continued group), while the remaining 22 discontinued the biological agent after the last administration of ADA in the M05-775 and M03-651 open extension trials (the stopped group). The patients were assessed in a clinical practice setting and treatments were adjusted accordingly. The protocol for the BRIGHT study required no treatment change or modification.

Data collection

The date of the last administration of ADA in each patient in the M05-775 or M03-651 trials was defined as week 0 of this study. We evaluated TJC28, SJC28, CRP, GH-VAS, the Health Assessment Questionnaire—Disability Index (HAQ-DI), the use of DMARDs, and the use of corticosteroid (CS) 26 and 13 weeks before the ends of those trials (week −26 and week −13) and at the ends of those trials (week 0 of the BRIGHT study). TJC28, SJC28, CRP, GH-VAS, and treatments at weeks 26 and 52 of the BRIGHT study were retrospectively evaluated from medical records in the participating facilities (Fig. 1).

Statistical analysis

The primary endpoint of this study was the percentage of patients who maintained discontinuation of ADA for

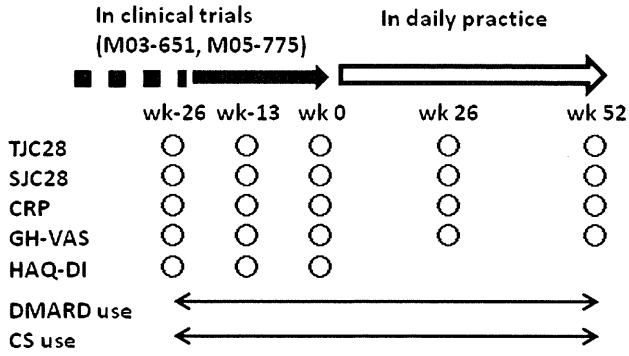

Fig. 1 Design of the BRIGHT study. Patients with RA who participated in the M03-651 or M05-775 open extension trials and had low disease activity (DAS28-CRP <2.7) at the last administration of adalimumab in the clinical trials (i.e., week 0 of the BRIGHT study) were enrolled. These 46 patients continued ($n = 24$) or discontinued ($n = 22$) adalimumab after the last administration of the drug in the trials at their attending physicians' discretion, and were followed up in daily practice. Data at weeks −26, −13, and 0 were collected from the trial databases, and the data at weeks 26 and 52 were collected retrospectively from medical records. *TJC28* tender joint count 28, *SJC28* swollen joint count 28, *CRP* C-reactive protein, *GH-VAS* general health Visual Analog Scale by patients, *HAQ-DI* Health Assessment Questionnaire—Disability Index, *DMARD* disease-modifying antirheumatic drug, *CS* corticosteroid

52 weeks without incurring elevation of DAS28-CRP to >2.7. Because this was a retrospective study, we anticipated that some patients would have missing data. Therefore, the DAS28-CRP of patients with missing data for TJC, SJC, CRP or GH-VAS at weeks 26 or 52 was regarded as ≥2.7. In some analyses, we replaced the missing data with 0 in order to calculate the theoretical minimum DAS28-CRP at that time point. Demographic data and baseline data at week 0 were compared between the two groups using Fisher's direct probability test for categorical variables and Student's *t* test or the Mann–Whitney test for continuous variables, depending on the data distribution. Changes in disease activity over time were compared visually between the two groups using observed data without statistical assessment. Treatments for RA between week 0 and 52 were compared using Fisher's direct probability test. We used SPSS 17.0 (Tokyo, Japan) for statistical analyses.

Ethics

The Helsinki Declaration (revised in 2008) and the ethical guidelines for epidemiologic research in Japan were followed. The ethical committee of the Tokyo Medical and Dental University Hospital and those of the participating facilities approved the study. Written informed consent was obtained from each patient enrolled in the BRIGHT study.

Table 1 Clinical characteristics of the patients enrolled in the BRIGHT study at week 0

Characteristics	Continued group ($n = 24$)	Stopped group ($n = 22$)
Age (years)	60.1 ± 12.7	55.7 ± 14.2
Female (%)	79.2	63.6
Disease duration[a] (years)	10.3 ± 7.3	10.3 ± 9.0
Steinbrocker's stage 1/2/3/4[a]	1/5/5/13	2/6/7/7
Steinbrocker's class 1/2/3/4[a]	7/11/5/0	3/14/5/0
Rheumatoid factor positive (%)[a]	87.5	81.8
DAS28-CRP	1.8 ± 0.5	1.6 ± 0.3
TJC28 (number)	0.4 ± 0.7	1.5 ± 2.1
SJC28 (number)	0.4 ± 1.1	0.6 ± 0.9
CRP (mg/dl)	0.4 ± 0.8	0.1 ± 0.1
GH-VAS by patients (mm)	11.9 ± 10.8	9.9 ± 8.6
HAQ-DI	0.4 ± 0.5	0.2 ± 0.5
Dosage of ADA		
40 mg/2 weeks	23	20
80 mg/2 weeks	1	2
Treatment duration of ADA (months)	46.0 ± 4.2	45.8 ± 3.3
Use of MTX between weeks −26 and 0 (%)	29.2	*13.6*
ADA monotherapy between weeks −26 and 0 (%)	62.5	*86.4*
Dosage of MTX (mg/week)	6.9 ± 1.6	*6.0 ± 2.0*
Use of CS between weeks −26 and 0 (%)	62.5	40.9
Dosage of CS (mg/day) (PSL equivalent)	4.3 ± 1.6	3.7 ± 1.3

Data are expressed as the mean ± standard deviation unless otherwise mentioned. Mean dosages of methotrexate and corticosteroid are calculated among the users of each drug at the last administration of ADA in the extension trials

DAS28-CRP disease activity score 28 with C-reactive protein, *TJC28* tender joint counts of 28 joints, *SJC28* swollen joint counts of 28 joints, *CRP* C-reactive protein, *GH-VAS* general health Visual Analog Scale, *HAQ-DI* Health Assessment Questionnaire—Disability Index, *ADA* adalimumab, *MTX* methotrexate, *CS* corticosteroid, *PSL* prednisolone

There was no statistical difference between the two groups. *P* ($P > 0.05$) values of continuous variables were calculated using the Mann–Whitney test or Student's *t* test according to the distribution of the data, and those of categorical variables were calculated using Fisher's direct probability test

[a] Data at the start of adalimumab in the extension trials (M03-651 or M05-775)

Results

Baseline characteristics of the enrolled patients

Demographic and clinical characteristics for the continued group and the stopped group are compared in Table 1. The initial trial (CHANGE) compared ADA monotherapy and

placebo in RA patients who showed an inadequate response to DMARDs, but the open extension trials of ADA therapy also allowed investigators to use DMARDs at their discretion. However, 86.4% of the continued group and 62.5% of the stopped group were still treated without nonbiological DMARDs and methotrexate (MTX) between weeks −26 and 0 (the beginning of the BRIGHT study). There was no significant difference in baseline characteristics between the two groups.

Maintenance of LDA after stopping adalimumab

Fourteen patients in the stopped group did not restart any biological agents, but 3 started ADA, 3 etanercept, 1 tocilizumab, and 1 ocrelizumab before week 52. Four out of the 14 patients who maintained discontinuation of ADA without starting other biological DMARDs for 52 weeks had DAS28-CRP <2.7 at weeks 26 and 52, and achieved the primary endpoint of this study. Among the remaining 10 patients who maintained discontinuation of ADA for 52 weeks, 4 had DAS28-CRP ≥2.7 at week 26 or 52, while the rest of the patients ($n = 6$) did not report either GH-VAS or CRP at week 26 or 52, and were therefore deemed to have DAS28-CRP ≥2.7 at those times. Among the 24 patients who continued ADA after week 0, 2 had discontinued ADA by week 52: 1 patient discontinued because of remission of RA, and the other developed cerebellar infarction and discontinued ADA. Sixteen of the 22 RA patients who continued ADA for 52 weeks maintained DAS28-CRP <2.7 at weeks 26 and 52 (Fig. 2).

Disease activity of RA after discontinuing adalimumab

Changes in the DAS28-CRP score and those of its individual components (TJC, SJC, CRP, GH-VAS) over time are compared between the two groups in Fig. 3. It should be noted that the mean DAS28-CRP in both groups was <2.0 from week −26 to the end (week 0) of the extension trials, suggesting that these patients were well controlled by ADA, which was the monotherapy in the majority (73.9%) of the patients. All components of the DAS28-CRP of the stopped group were numerically higher than those of the continued group at weeks 26 and 52, as was the DAS28-CRP itself.

Treatment of RA after stopping adalimumab

We next compared treatment modification between weeks 0 and 52. MTX was started or the dosage of MTX was increased in 6 patients in the continued group and 12 in the stopped group, ($P = 0.040$, Fisher's direct probability test). Percentages of patients who started new DMARDs, except for MTX, and those of patients who started or increased the dosage of CS were numerically, but not significantly, higher in the stopped group compared to the continued group (Table 2).

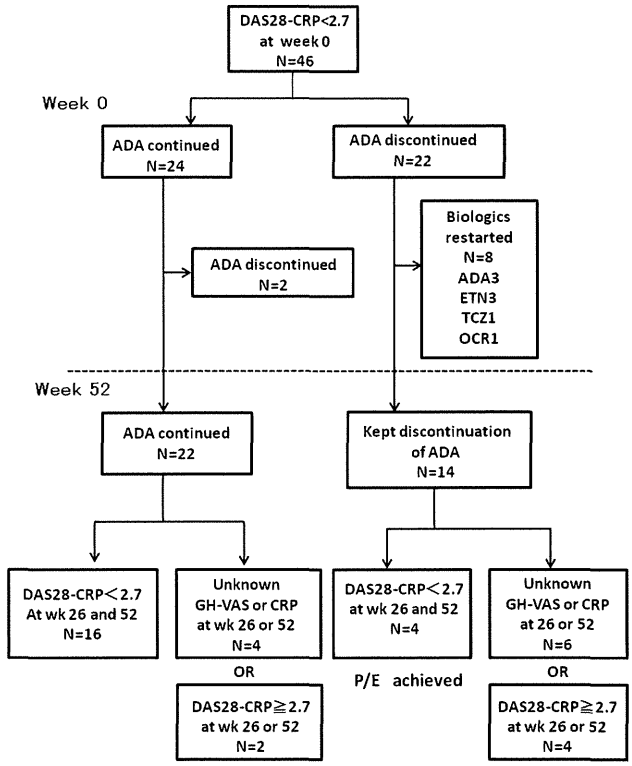

Fig. 2 BRIGHT study patient disposition through week 52. Adalimumab was continued ($n = 24$) or discontinued ($n = 22$) after the last administration of the drug in the open extension studies (i.e., week 0 of the BRIGHT study) and the patients were followed for 52 weeks. The patients were categorized according to the use of adalimumab and DAS28-CRP values. Four patients who had DAS28-CRP <2.7 at week 26 and 52 achieved the primary endpoint. *ADA* adalimumab, *ETN* etanercept, *TCZ* tocilizumab, *OCR* ocrelizumab, *CRP* C-reactive protein, *GH-VAS* general health Visual Analog Scale by patients, *P/E* primary endpoint

None of the patients who achieved the primary endpoint used MTX between weeks −26 and 0, but 3 patients used low-dose PSL (2.5–5 mg/day). The concomitant drugs used by these patients during weeks 0–52 were as follows: the first patient did not start any DMARDs, tapered off PSL at week 32, and was in drug-free remission from week 32 to week 52; the second and third patients started MTX (6 and 4 mg/week, respectively) at week 0 and continued both MTX and PSL until week 52; the fourth patient started PSL (7.5 mg/day) at week 0 and MTX (8 mg/week) at week 8, and continued both drugs until week 52. There was 1 patient who did not use DMARDs (including MTX, biologics, and CS) for 52 weeks. The patient, however, did not achieve the primary endpoint because of a lack of CRP data at week 26. There was no statistical difference in the use of MTX and CS at week 52 between the two groups (Table 2).

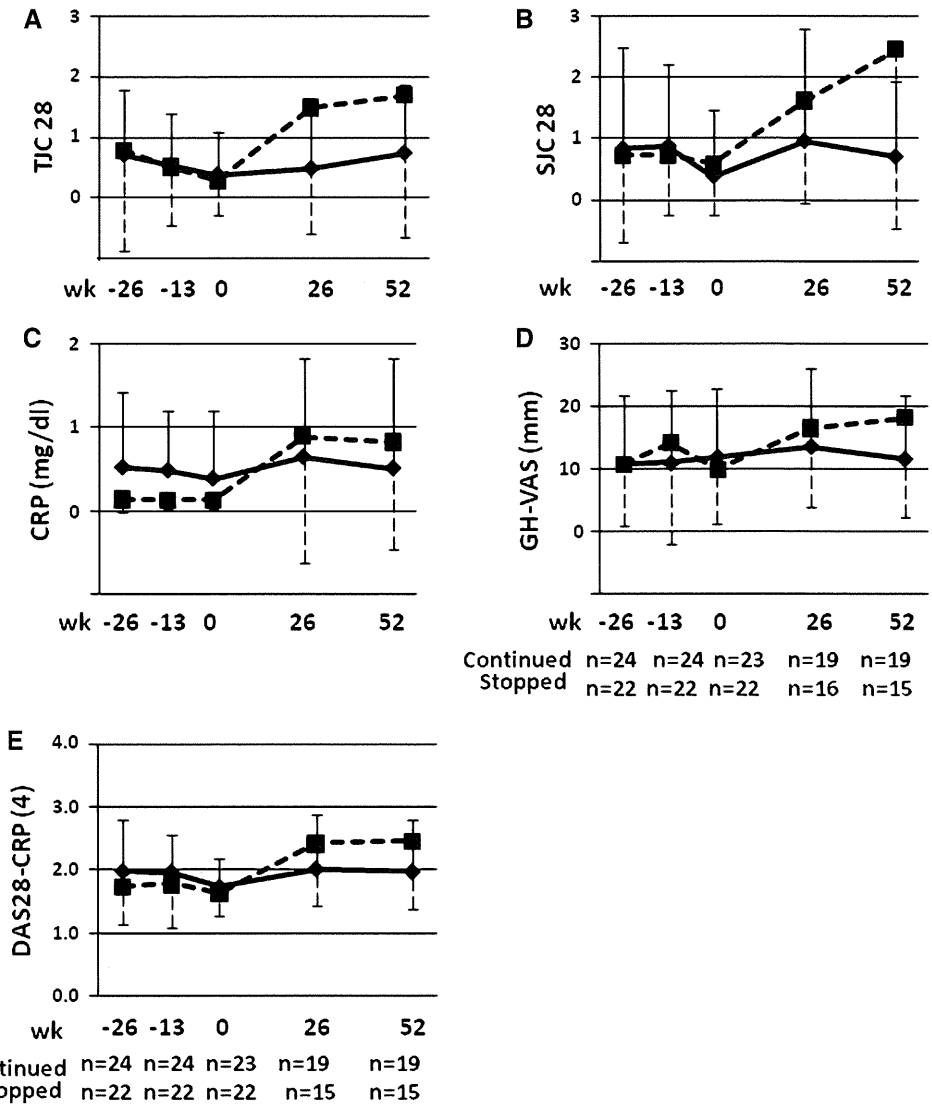

Fig. 3 Changes in the rheumatoid arthritis disease activity of patients enrolled in the BRIGHT study from week −26 to week 52. Tender joint count 28 (*TJC28*) (**a**), swollen joint count 28 (*SJC28*) (**b**), C-reactive protein (*CRP*) serum levels (**c**), general health Visual Analog Scale by patients (*GH-VAS*) (**d**), and disease activity score 28-CRP (*DAS28-CRP*) (**e**) were measured from week −26 to week 52 for the continued group ($n = 24$, *diamonds with solid line*) and the stopped group ($n = 22$, *squares with dashed line*). The mean and standard deviation at each time point are shown. The number of patients is shown when there were patients with missing data (**d**, **e**)

Comparison between patients who did and did not achieve the primary endpoint

To explore the characteristics of patients who maintained LDA after stopping ADA for 52 weeks, we compared the baseline data of patients who did and did not achieve the primary endpoint. Although 6 patients who had missing data for GH-VAS or CRP at week 26 or 52 were deemed to have not achieved the primary endpoint (Fig. 2), 3 of them had none or only one TJC28 or SJC28 and did not seem to be appropriate for this comparison. We therefore calculated the theoretical minimum DAS28-CRP for the 6 patients with missing data by replacing the missing values with 0. Patients who restarted biologics ($n = 8$), who had DAS28-CRP >2.7 at week 26 or 52 ($n = 4$), who had missing data for CRP or VAS-GH, and had a theoretical minimum DAS28-CRP of >2.7 at week 26 or 52 ($n = 3$) were included in this analysis. Because we had only 4 patients who achieved the primary endpoint, we deliberately did not perform statistical analyses (Table 3). Patients who achieved the primary endpoint had a longer duration of RA and used CS more frequently at week 0, and they had higher mean and median titers of rheumatoid factor at the beginning of the M03-651 or M05-775 trials.

Discussion

In the BRIGHT study, we demonstrated that 4 of the 22 RA patients (18.2%) who discontinued ADA after achieving LDA maintained the same disease activity status for at least 1 year without restarting biological agents. The majority (73.9%) of the patients who enrolled onto the BRIGHT study had received ADA monotherapy in the extension trials. The stopped group had a higher DAS28-CRP at

Table 2 Use of DMARDs and corticosteroid from week 0 to week 52 of the BRIGHT study

Treatment	Continued group ($n = 24$)	Stopped group ($n = 22$)	P value
Change of MTX dosage between week 0 and 52			
Increased or started	6 (25.0)	12 (54.5)	0.040*
No change	3 (12.5)	2 (9.1)	
Decreased	3 (12.5)	0 (0.0)	
Not used	12 (50.0)	8 (36.4)	
Use of MTX at week 52	11 (45.8)	13 (59.1)	NS†
Dosage of MTX at week 52 (mg/week)	6.8 ± 2.8	6.3 ± 2.4	NS
Starting DMARD except for MTX	1 (4.2)	5 (22.7)	NS†
Use of DMARD except for MTX at week 52	1 (4.2)	5 (22.7)	NS†
CS dosage			
Increased or started	3 (12.5)	8 (36.4)	NS*
No change	10 (41.7)	4 (18.2)	
Decreased	5 (20.8)	3 (13.6)	
Not used	6 (25.0)	7 (31.8)	
Use of CS at week 52	15 (62.5)	15 (68.2)	NS
Dosage of CS at week 52 (mg/day) (PSL equivalent)	4.7 ± 1.5	4.9 ± 2.0	NS

Percentages are shown in parentheses. Mean dosages of methotrexate and corticosteroid and their standard deviation at week 52 are calculated among the users of each drug

P values of continuous variables were calculated using the Mann–Whitney test or Student's *t* test according to the distribution of the data

MTX methotrexate, *DMARD* disease-modifying antirheumatic drug, *CS* corticosteroid

* Percentages of patients who increased or started methotrexate or corticosteroid were compared between the two groups using Fisher's direct probability test

† Percentages of patients who used MTX at week 52, who started new DMARDs except for MTX, and who used DMARDs except for MTX at week 52 were compared between the two groups using Fisher's direct probability test

Table 3 Comparison of patients who did not did not achieve the primary endpoint in the stopped group

Characteristics	Patients who achieved the primary endpoint ($n = 4$)	Patients who did not achieve the primary endpoint ($n = 15$)
Age (years)	58.3 ± 12.3	54.9 ± 13.6
Female (%)	75.0	60.0
Disease duration[a] (years)	4.4 ± 4.3	18.1 ± 9.8
Steinbrocker's stage 1/2/3/4[a]	0/0/3/1	4/2/4/5
Steinbrocker's class 1/2/3/4[a]	0/3/1/0	2/9/4/0
Rheumatoid factor titer[a], mean ±SD (median)	78.8 ± 69.4 (73.0)	259.8 ± 423.6 (90.0)
DAS28-CRP	1.5 ± 0.1	1.7 ± 0.4
HAQ-DI	0.2 ± 0.3	0.3 ± 0.6
Use of MTX between weeks −26 and 0 (%)	0	13.3
Use of CS between weeks −26 and 0 (%)	75	33.3
Dosage of CS (mg/day) (PSL equivalent)	3.8 ± 1.3	3.4 ± 1.5

Four patients who maintained discontinuation of ADA without starting other biological DMARDs for 52 weeks and had a DAS28-CRP of <2.7 at weeks 26 and 52 were included in the "Patients who achieved the primary endpoint" group. Fifteen patients who restarted biologics ($n = 8$), who had a DAS28-CRP of >2.7 at week 26 or 52 ($n = 4$), and who had missing data for CRP or VAS-GH and had a theoretical minimum DAS28-CRP of >2.7 ($n = 3$) were included in the "Patients who did not achieve the primary endpoint" group. Because we had only 4 patients who achieved the primary endpoint, we deliberately did not perform statistical analyses. Data are expressed as the mean ± standard deviation unless otherwise mentioned. Mean dosages of corticosteroid are calculated among the users of each drug at the last administration of ADA in the extension trials

DAS28-CRP disease activity score 28 with C-reactive protein, *HAQ-DI* Health Assessment Questionnaire—Disability Index, *MTX* methotrexate, *CS* corticosteroid, *PSL* prednisolone

[a] Data at the start of adalimumab in the extension trials (M03-651 or M05-775)

week 26 and 52 than the continued group, despite the increased use or start of MTX therapy in this group.

The first clinical trial that evaluated discontinuation of biological agents was the TNF20 study [5]. Six of 10 early RA patients who received MTX + IFX for the first 52 weeks and who discontinued IFX thereafter showed DAS28-ESR <2.6 at week 104 [5]. The sustained benefits of IFX therapy for early RA following withdrawal of the drug was confirmed in the BeSt study, which enrolled a larger number of patients with a longer study period [6–11]. Among 128 RA patients who received MTX + IFX as initial therapy, 54 (42%) of them were in clinical remission (DAS <1.6) at year 4, and 23 (18%) stopped all antirheumatic drugs without incurring an increase in disease activity and progression of structural joint damage [11]. Recently, Tanaka et al. [12] reported that 56% of RA patients with a mean disease duration of 5.9 years who achieved LDA (DAS28-ESR <3.2) for more than 24 weeks with IFX + MTX maintained DAS28-ESR <3.2 for 1 year after discontinuing IFX. These results show that there is substantial evidence that the benefits of IFX are sustained after the withdrawal of the drug in RA patients who had achieved remission or LDA with the biological agent and concurrent MTX therapy.

Emery et al. [22] recently reported the results of their study of the discontinuation of ADA, the Optimal Protocol for Treatment Initiation with Methotrexate and Adalimumab (OPTIMA). Early RA patients who responded (DAS28-CRP <3.2) to treatment with MTX + ADA at weeks 22 and 26 were randomly allocated into placebo + MTX ($n = 102$) or ADA + MTX ($n = 105$) groups and followed for an additional 52 weeks. The percentage of patients who achieved DAS28-CRP <3.2 was 81%, DAS28-CRP <2.6 was 66%, and simplified disease activity index (SDAI) <3.3 was 51% at week 78 in the placebo + MTX group, similar to the results reported in the BeSt study [5], but apparently better than the corresponding figure for the BRIGHT study. Three important differences in the characteristics of the enrolled patients between the OPTIMA and the BRIGHT study should be mentioned. First, the mean disease durations of the enrolled patients were 3.9 months for the OPTIMA and 10.3 years for the BRIGHT study. A significant association of disease duration with the discontinuation of IFX has been reported in the RRR study [12], while an association of symptom duration with drug-free remission has been reported in the BeSt study [11]. The longer mean disease duration of our patients may be relevant to the lower rate of discontinuation of ADA without exacerbation in the BRIGHT study compared to the OPTIMA study. This possibility may be supported by the finding that the disease durations of the four RA patients who met the primary endpoint were 0.8, 1.3, 5.6, and 9.9 years, and the mean value was numerically smaller than that of the patients who did not achieve the primary endpoint of our study (Table 3). Second, OPTIMA had predefined criteria for the discontinuation of biologics, just as the BeSt study and the RRR study did, while discontinuation was determined at the discretion of the attending physicians and/or according to patient preference in the BRIGHT study. It is plausible that lack of criteria for discontinuation affected the success rate upon the discontinuation of biologics. Third, the concomitant use of non-biological DMARDs before discontinuing ADA should be discussed. In the stopped group of the BRIGHT study, only 3 out of 22 patients received MTX before ADA discontinuation (Table 1), and no patients who achieved the primary endpoint used concomitant DMARDs, including MTX, before discontinuing ADA, a marked difference from the OPTIMA study, where all patients received concomitant MTX. Because only 3 patients received MTX before ADA discontinuation, we could not analyze the possible effect of MTX on realizing the primary endpoint in this study.

Stringency of disease control was associated with the successful discontinuation of IFX without incurring an RA flare in the RRR study [12], but we could not find a difference in DAS28-CRP at week 0 between those who did and those who did not achieve the primary endpoint (Table 3). It is difficult to analyze the association between stringency of disease control and discontinuation of treatment with ADA in the BRIGHT study because of the small number of patients enrolled.

Among the 14 patients who maintained discontinuation of biological agents for 1 year without restarting biological agents, there were 6 patients with missing data in components of the DAS28-CRP. Based on our predefined criteria, these patients were deemed to have DAS28-CRP ≥2.7 at the corresponding time point. In actuality, 3 of these patients had very low disease activity; 2 patients had TJC28 ≤1, SJC28 ≤1, and CRP ≤1 without GH-VAS at weeks 26 and 52, and 1 patient had TJC28 = 0, SJC28 = 0, and GH-VAS = 10/100 mm without CRP at week 26 and a DAS28-CRP of 1.92 at week 52. These were the patients who had a theoretical minimum DAS28-CRP of <2.7 and were excluded from the analyses in Table 3. These data suggest that we can expect a higher probability of ADA discontinuation without RA disease activity elevation than the 18.2% resulting from our study.

Some limitations of this study should be mentioned. The study design was retrospective and open, the number of patients enrolled was small, and no data were collected to evaluate structural changes in joints. These limitations should be considered when interpreting the results of this study.

In conclusion, this study suggests that once continuous good control is achieved with ADA monotherapy, it is