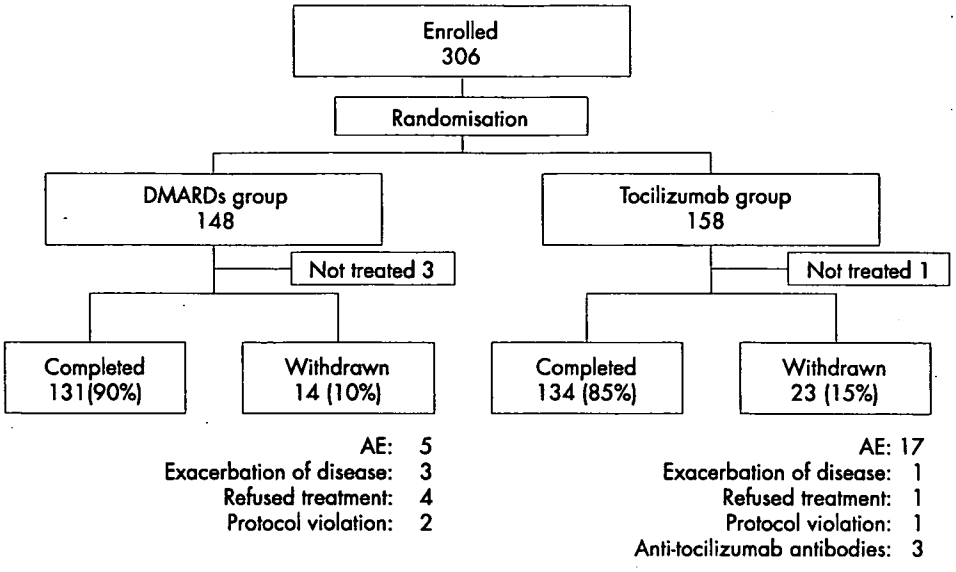

Figure 1 Randomisation, reasons for withdrawal, and numbers of patients who completed the trial. DMARDs, disease-modifying antirheumatic drugs; Tocilizumab, humanised anti-interleukin-6 receptor antibody.

Welfare of Japan, and by the local ethical committee, and patients gave their written informed consent. This trial was registered with http://clinical trials.gov (NCT00144508).

Patients were randomly assigned to receive either tocilizumab monotherapy at 8 mg/kg intravenously every 4 weeks or conventional DMARD therapy for 52 weeks. A long-term placebo-controlled study in RA patients with highly active disease was not acceptable from an ethical point of view, and therefore DMARDs were used for the controls. The randomisation was performed by registering of patients at the patient registration centre with a centralised allocation method. For the tocilizumab group, DMARDs and/or immunosuppressants were discontinued from the start of the study. Oral corticosteroids ($\leqslant$10 mg prednisolone per day) were allowed, but the dosage could not be increased during the study. Intra-articular corticosteroid injections were not allowed. Use of one non-steroidal anti-inflammatory drug (NSAID), including switching to another NSAID, was allowed. For the conventional DMARD group, the dose, type and combination of DMARDs and/or immunosuppressants, except for anti-TNF agents and leflunomide, could be varied according to disease activity at the discretion of the treating physician. Variations of NSAIDs and/or corticosteroids including intra-articular corticosteroid injections were also allowed. Surgical treatment and use of bisphosphonates was not allowed in either group. Safety was assessed through recording of adverse events, physical examinations, and standard laboratory tests for both groups.

Radiographic and clinical assessment

Posteroanterior radiographs of hands and anteroposterior radiographs of feet were performed at baseline, week 28 and week 52 or at the last visit for patients who withdrew from the study prior to week 52. For patients who dropped out before week 52, missing radiograph values at week 52 were estimated by linear extrapolation using data at baseline, week 28 and the early termination visit. Radiographs were scored using van der Heijde's modified Sharp method[24 25] independently by two readers who were well trained and competent to score radiographs. The readers were blinded to the treatment group, chronologic order of the films and clinical response of each patient. Ten percent of the patients' films were re-read for the analysis of intra-reader variability.

ACR20, 50 and 70 responses, and disease activity score in 28 joints (DAS28) were assessed for clinical improvement of RA using an intent-to-treat (ITT) analysis.

Statistical analysis

A sample size of 120 patients per treatment group was estimated to provide 80% power for detecting a significant ($p<0.05$) difference in mean change score of radiographic findings between the tocilizumab and DMARD groups. We decided to recruit 150 patients per treatment group to allow for anticipated withdrawals. Radiographic endpoints, such as TSS, erosion score and joint space narrowing score, were assessed with a rank transformed analysis of covariance (ANCOVA) on the change scores that included factors for baseline score and baseline disease duration. The incidences of clinical improvements were analysed by the chi-square test.

All statistical analyses were two-sided and p values less than 0.05 were considered significant. All patients receiving at least one dose of study drug were included in the efficacy and safety analysis.

RESULTS

Characteristics of the patients

This study enrolled 306 patients in total (Figure 1). Four patients were withdrawn before treatment either due to their ineligibility or at the patients' request. A total of 302 patients received study drugs. A total of 134 patients in the tocilizumab group and 131 patients in the DMARDs group completed 52 weeks treatment. Discontinuation occurred in 23 patients in the tocilizumab group and 14 patients in the DMARDs group. The reported reasons for withdrawal are shown in Figure 1.

Demographics and baseline disease characteristics did not differ between the two groups (Table 1).

Mean disease duration was 2.3 years. Patients had active disease, indicated by a DAS28 score of 6.5 and CRP of 48 mg/L at baseline. Moreover, TSS at baseline was 29.4, which was very high despite the relatively short disease duration. The mean estimated yearly progression rate, calculated from the baseline TSS divided by disease duration for each patient, was 13.3 Sharp units.

Treatment in the conventional DMARD group

At baseline, 67% of the patients in the DMARDs group received methotrexate (MTX): 37% received a combination of MTX and DMARDs, 30% received MTX monotherapy, and 22% received DMARDs and/or immunosuppressants other than MTX, besides corticosteroids. The dose of MTX was 7.1 ± 1.9 mg/week (mean $\pm$ SD) in patients treated with MTX. During the study, 123 patients (85%) received MTX: 81 (56%) received a

Table 1 Patient demographics and clinical characteristics at baseline*

	Conventional DMARDs (n=145)	8 mg/kg Tocilizumab (n=157)
Demographics		
Age, years	53.1±12.5	52.9±11.6
Male:female ratio	26:119	32:125
Clinical characteristics		
RA duration, years	2.4±1.3	2.2±1.4
No. of failed DMARDs, mean (range)	2.8 (1–7)	2.7 (1–7)
Functional Class†, I:II:III:IV	11:114:20:0	12:126:19:0
RA Stage†, I:II:III:IV	18:57:51:19	14:77:46:20
Tender joint count, 0–49 scale	14.4±7.2	15.3±7.3
Swollen joint count, 0–46 scale	11.9±5.5	12.5±6.4
ESR, mm/h	71.0±25.2	70.8±27.9
CRP, mg/L	49±29	47±29
DAS28	6.4±0.9	6.5±0.8
Radiographic findings		
Modified TSS, 0–448 scale	30.6±42.0	28.3±43.9
Erosion score, 0–280 scale	13.9±21.7	13.8±24.6
Joint space narrowing score, 0–168 scale	16.7±21.8	14.5±20.8
Estimated annual TSS progression	12.3±16.2	14.1±26.9
Treatment classification		
MTX and at least one DMARD, patients (%)	53 (37)	43 (27)
MTX monotherapy, patients (%)	44 (30)	73 (46)
DMARDs/immunosuppressants, patients (%)	32 (22)	30 (19)
MTX dose, mg/week	7.1±1.9	6.9±2.0
Prednisolone equivalent corticosteroid dose, mg/day	5.4±3.2	5.4±3.1

*Except where indicated otherwise, values are the mean ± SD.
†RA functional status determined by American College of Rheumatology criteria. RA stage determined by Steinbrocker's criteria.
DMARDs, disease-modifying antirheumatic drugs; Tocilizumab, humanised anti-interleukin-6 receptor antibody; RA, rheumatoid arthritis; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS28, Disease Activity Score in 28 joints; TSS, total Sharp score; MTX, methotrexate.

combination of MTX and DMARDs, 42 (29%) received MTX monotherapy, and 20 (14%) received DMARDs and/or immunosuppressants other than MTX, besides corticosteroids. The dose of MTX was 8.0±2.1 mg/week in patients treated with MTX (Japanese government recommends 6–8 mg/week of MTX based on the evidence from the Japanese clinical trial of MTX for RA).[26 27] Besides MTX, salazosulfapyridine (41%), bucillamine (23%), mizoribine (8%) and D-penicillamine (8%) were frequently used in more than 5% of the patients.

Reliability of radiographic scoring

Intra-reader intraclass correlation coefficients for erosion, joint space narrowing and TSS were all 0.99 for both readers. Inter-reader intraclass correlation coefficients for erosion, joint space narrowing and TSS were 0.98, 0.96 and 0.98, respectively.

Radiographic evaluation of joint damage

Figure 2 shows the cumulative probability plots of the change from baseline to week 52 in the TSS. The space between the curves indicates different treatment effects with a considerable difference in favour of the tocilizumab monotherapy group. The plots representing the TSS changes in the tocilizumab group clearly shifted to the right compared with those in the conventional DMARDs, indicating that fewer patients in the tocilizumab group showed radiographic progression and also a smaller amount of progression than those in the DMARDs group. At week 52, 56% of patients receiving tocilizumab had no radiographic progression (i.e., change from baseline in the TSS ≤ 0.5) compared with 39% of patients receiving conventional DMARDs ($p<0.01$). Moreover, more patients receiving

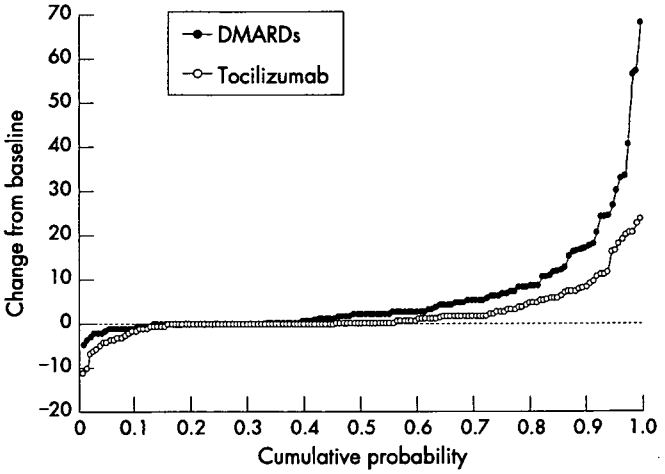

Figure 2 Cumulative probability distribution of radiographic changes in total Sharp/van der Heijde scores from baseline to week 52 for patients treated with tocilizumab or with conventional DMARDs. The space between the curves indicates the different treatment effects with a considerable difference in favour of the tocilizumab group.

tocilizumab monotherapy had negative TSS scores than those receiving conventional DMARDs (24 patients and 18 patients at week 52, respectively).

The mean changes in the TSS as well as erosion scores at week 28 were statistically significantly less in the tocilizumab group than in the DMARDs group with an ANCOVA model (Table 2).

The efficacy was more evident at week 52. In addition to the TSS and erosion score, joint space narrowing scores also showed significantly less change in the tocilizumab group than in the DMARD group. In the tocilizumab group, patients who achieved a higher ACR response showed less radiological progression at week 52 (in the patients with ACR70 response (n = 73), mean TSS 1.6; 95% CI 0.3 to 2.8). A similar effect was observed in the DMARDs group (in the patients with ACR70 response (n = 8), mean TSS 1.5; 95% CI −0.6 to 3.6).

Table 2 Change in radiographic scores

	Conventional DMARDs (n=143)	8 mg/kg Tocilizumab (n=157)
Week 28		
Total Sharp score		
Mean (95% CI)	4.5 (3.1 to 6.0)	1.9 (1.2 to 2.6)*
Median (IQR)	1.0 (0.0 to 5.0)	0.5 (0.0 to 2.0)
Erosion score		
Mean (95% CI)	2.4 (1.6 to 3.2)	0.8 (0.4 to 1.2)†
Median (IQR)	0.5 (0.0 to 2.5)	0.0 (0.0 to 1.0)
Joint space narrowing score		
Mean (95% CI)	2.2 (1.4 to 2.9)	1.1 (0.7 to 1.6)
Median (IQR)	0.0 (0.0 to 2.0)	0.0 (0.0 to 1.0)
Week 52		
Total Sharp score		
Mean (95% CI)	6.1 (4.2 to 8.0)	2.3 (1.5 to 3.2)†
Median (IQR)	2.5 (0.0 to 7.0)	0.5 (0.0 to 3.0)
Erosion score		
Mean (95% CI)	3.2 (2.1 to 4.3)	0.9 (0.3 to 1.4)‡
Median (IQR)	1.0 (0.0 to 3.5)	0.0 (0.0 to 1.0)
Joint space narrowing score		
Mean (95% CI)	2.9 (2.0 to 3.8)	1.5 (0.9 to 2.1)*
Median (IQR)	1.0 (0.0 to 4.0)	0.0 (0.0 to 1.7)

*$p<0.05$, †$p<0.01$, ‡$p<0.001$.
P values were analysed with a rank transformed analysis of covariance (ANCOVA) on the change scores that included factors for baseline score and baseline disease duration. DMARDs, disease-modifying antirheumatic drugs; Tocilizumab, humanised anti-interleukin-6 receptor antibody; 95% CI, 95% confidence interval; IQR, interquartile range.

Clinical efficacy

At week 52, proportions of the patients achieving ACR20, ACR50, and ACR70 response were 78%, 64%, and 44% in the tocilizumab group and 34%, 13%, and 6% in the DMARD group, respectively, indicating the superiority of tocilizumab monotherapy to conventional DMARD therapy ($p<0.001$, for each comparison) although clinical efficacy was assessed unblinded (Figure 3A).

Greater reduction in DAS28 scores and higher remission rates were also observed in the tocilizumab group than in the DMARDs group (Figure 3B). At week 52, clinical remission (defined as DAS28 <2.6)[28] was achieved in 59% of patients receiving tocilizumab, but only in 3% of patients receiving DMARDs ($p<0.001$). Major clinical response (ACR70 response for 6 consecutive months) was achieved in 24% of patients receiving tocilizumab compared with only 2% of patients receiving DMARDs during the study period of 52 weeks.

Physical function and health-related quality of life

Tocilizumab monotherapy significantly improved MHAQ scores compared to conventional DMARDs (Figure 3C). A decrease of >0.22 units in HAQ scores represents significant clinical improvement and the minimum clinically important difference.[29] Such improvement was seen in 40% of the patients treated with tocilizumab as early as week 4, the first scheduled study visit, and was even more evident at week 52 (68% in the tocilizumab group and 40% in the DMARDs group, $p<0.001$).

Safety

The percentages of patients with adverse events were 89% and 82% in the tocilizumab and DMARD groups, respectively. Most of adverse events were mild or moderate. Table 3 shows frequent adverse events observed in at least 5% of the patients.

Nasopharyngitis was the most common adverse event, but the incidences were similar in both groups.

Serious adverse events were reported in 18% and 13% in the tocilizumab group and DMARDs group, respectively. In the tocilizumab group, 12 serious infections were reported: 3 (1.9%) patients with pneumonia, 2 (1.3%) with upper respiratory tract infection, 2 (1.3%) with cellulitis, 1 (0.6%) each with gastroenteritis, herpes zoster, herpes simplex, perianal abscess and an unidentified infection. In the DMARD group, 8 serious infections were reported: 3 (2.1%) patients with gastroenteritis, 2 (1.4%) with pneumonia, and 1 (0.7%) each with upper respiratory tract infection, herpes zoster and sepsis. All the serious adverse events improved with appropriate treatment. There was no significant prolongation of infection by the tocilizumab treatment. Tuberculosis was not observed in this 1-year study without required screening or prophylactic use of any antituberculous drug.

Three malignancies were reported in the tocilizumab group: 2 patients with breast cancer (including 1 lobular carcinoma in situ) and 1 with colon cancer, which were improved or resolved by appropriate treatment (including surgery). No malignancies were reported in the DMARD group.

Drug-related infusion reactions were reported 14 times in 11 (7.0%) patients of the tocilizumab group: 3 with transient increase in blood pressure, 2 with injection site redness, 2 with headache, 2 with nausea, 2 with skin eruption, and 1 each with vomiting, pruritus, and malaise. All the infusion reactions were mild, and no patient withdrew from the study as a consequence.

Laboratory test abnormalities were reported in 61% and 31% of patients in the tocilizumab and DMARD groups, respectively. In the tocilizumab group, lipid metabolism-related reactions were common. Anomalous increases in total cholesterol (TC), triglycerides, and low-density lipoprotein cholesterol were reported in 38%, 17%, and 26% of the patients, respectively, and most of them were grade 1 according to the National Cancer Institute Common Toxicity Criteria. Twenty-seven patients were treated (HMG-CoA reductase inhibitor, 26 cases; fenofibrate, 1 case) and their cholesterol levels improved during the study. Tocilizumab monotherapy also raised high-density lipoprotein cholesterol (HDLC) levels to above the normal range

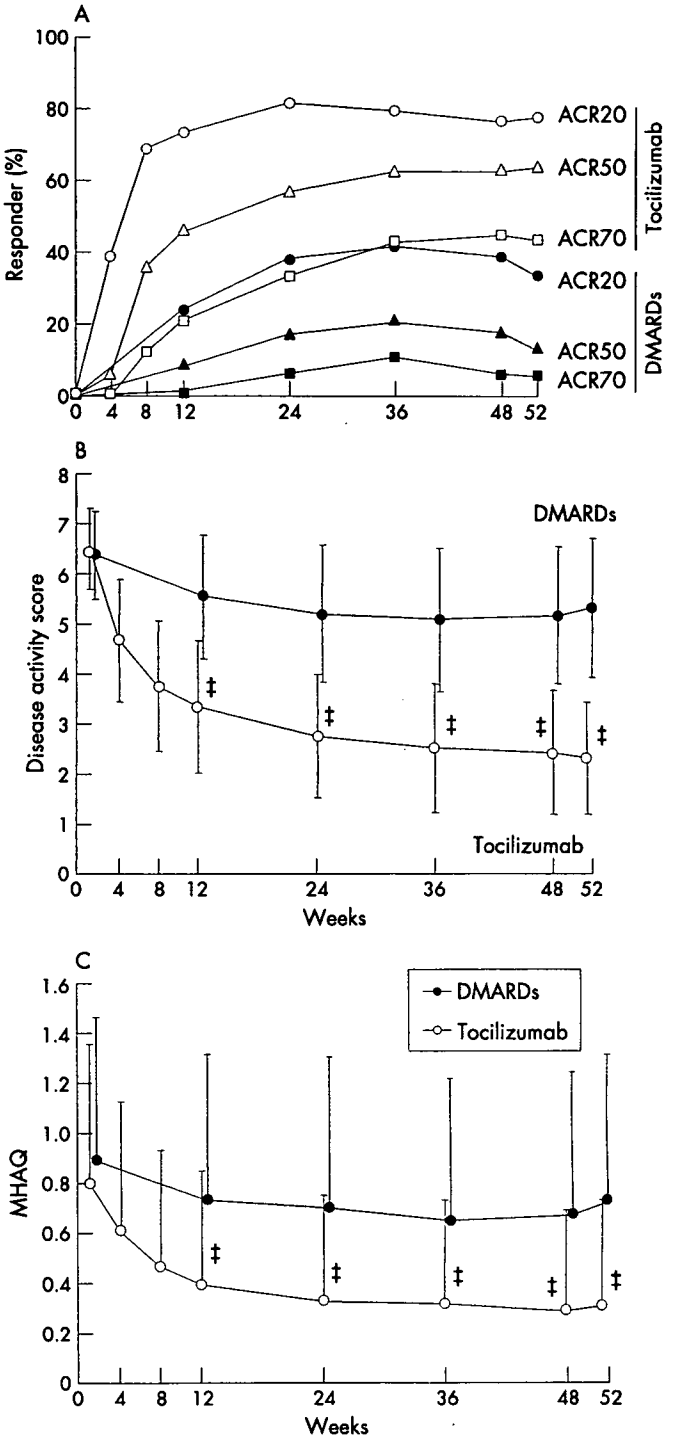

Figure 3 Percentage of responders according to the American College of Rheumatology (ACR) improvement criteria and the Disease Activity Score in 28 joints (DAS28) as well as mean change in Modified Health Assessment Questionnaire (MHAQ) scores. Percentage of responders according to the ACR improvement criteria (A) and the DAS28 (B) according to the ITT analysis over 52 weeks. Mean change in MHAQ scores from baseline to week 52 (C). ‡$p<0.001$ versus DMARDs by paired t-test.

in 24% of patients. The atherogenic index, calculated by (TC–HDLC)/HDLC, did not change during the study period of 52 weeks. No cardiovascular complications were observed in association with abnormal lipid profile.

Anti-tocilizumab antibodies were detected in 4 patients (2.5%). Only one patient showed a skin eruption at the third injection, while the other three were asymptomatic. They were all withdrawn according to the study protocol.

DISCUSSION

This 52-week, *x* ray reader-blinded, randomised, controlled trial demonstrated that tocilizumab monotherapy in patients with active RA significantly inhibited the progression of structural joint damage compared with conventional DMARDs therapy. Note that even monotherapy with tocilizumab significantly retarded the radiological progression. It is of interest whether tocilizumab in combination with MTX would provide greater benefit; this is being investigated in the European studies.

The results of this study confirmed that IL-6 plays a pathological role in the joint destruction in RA. IL-6 blockade can inhibit the osteoclast activation in RA. Additionally, tocilizumab therapy reduced MMP-3 levels (data not shown), which could also contribute to the radiographic benefit.

In addition to the radiographic benefits, tocilizumab monotherapy improved signs and symptoms as well as functional evaluation with MHAQ. Although this was an open-label study for clinical efficacy endpoints, the results of previous phase II studies[21 22] were confirmed. Moreover, significant improvement in MHAQ scores indicates that tocilizumab improves patients' daily living activity.

There was no decrease in the efficacy of tocilizumab during the 1-year treatment. The benefit of a humanised antibody was again emphasised in the repetitive treatment, as anti-tocilizumab antibodies were detected in only 4 patients (2.5%) without requiring the use of immunosuppressive agents such as MTX. It could also be that tocilizumab that blocks IL-6 action to induce B cell differentiation into antibody producing cells.

Tocilizumab monotherapy was generally well tolerated. There was no specific infection related to tocilizumab therapy, including tuberculosis, which is often a problem in anti-TNF therapy,[30] although patients had neither prophylactic medication nor screening in this study.

Three malignancies occurred in patients treated with tocilizumab in this study. There are many reports describing the relationship between IL-6 and malignant diseases, where IL-6 is often a causative factor.[31] Further analysis to compare the incidence with an epidemiology surveillance data base regarding malignancies in Japanese patients with RA will be required to ascertain the carcinogenesis risk or the benefit of IL-6 inhibition.

Table 3 Adverse events observed in at least 5% of patients*

	Conventional DMARDs (n = 145)	8 mg/kg Tocilizumab (n = 157)
Nasopharyngitis	47 (32.4)	56 (35.7)
Rash	6 (4.1)	17 (10.8)
Diarrhoea	13 (9.0)	13 (8.3)
Headache	3 (2.1)	11 (7.0)
Stomatitis	13 (9.0)	9 (5.7)
Eczema	6 (4.1)	9 (5.7)
Nausea	2 (1.4)	9 (5.7)
Pruritus	2 (1.4)	9 (5.7)
Paronychia	1 (0.7)	9 (5.7)
Vomiting	5 (3.4)	8 (5.1)
Vertebral compression fracture	8 (5.5)	3 (1.9)

*Values are the number (%) of patients.
DMARDs, disease-modifying antirheumatic drugs; Tocilizumab, humanised anti-interleukin-6 receptor antibody.

The increase in TC is also observed in anti-TNF therapy[32] and therefore it could be secondary to the improvement in inflammation. We have reported that tocilizumab treatment improves wasting in Castleman's disease, an atypical lymphoproliferative disease with overproduction of IL-6 but not TNFα, where tocilizumab monotherapy normalises hypocholesterolemia but seldom causes hypercholesterolemia.[33] Therefore, IL-6 plays a role in regulating serum cholesterol levels.

As an increase in serum IL-6 levels has been reported as a cardiovascular risk[34 35] and as IL-6 could contribute to the atherosclerosis,[35] it is of interest whether tocilizumab therapy reduces the incidence of cardiovascular events. This issue will be also proven in a future epidemiological surveillance of patients treated long-term with tocilizumab.

In conclusion, this study clearly demonstrates the superiority of tocilizumab monotherapy in preventing joint damage to conventional DMARDs in Japanese RA patients. However the results need to be confirmed in the trials in western RA patients.

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REFERENCES

1 **Harris ED Jr.** Rheumatoid arthritis: Pathophysiology and implications for therapy. *N Engl J Med* 1990;**322**:1277–89.
2 **Nishimoto N.** Interleukin-6 in rheumatoid arthritis. *Curr Opin Rheumatol* 2006;**18**:277–81.
3 **Nakahara H,** Song J, Sugimoto M, Hagihara K, Kishimoto T, Yoshizaki K, *et al.* Anti-interleukin-6 receptor antibody therapy reduces vascular endothelial growth factor production in rheumatoid arthritis. *Arthritis Rheum* 2003;**48**:1521–9.
4 **Tamura T,** Udagawa N, Takahashi N, Miyaura C, Tanaka S, Yamada Y, *et al.* Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. *Proc Natl Acad Sci USA* 1993;**90**:11924–8.
5 **Kotake S,** Sato K, Kim KJ, Takahashi N, Udagawa N, Nakamura I, *et al.* Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. *J Bone Mineral Res* 1996;**11**:88–95.
6 **Hirano T,** Matsuda T, Turner M, Miyasaka N, Buchan G, Tang B, *et al.* Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. *Eur J Immunol* 1988;**18**:1797–801.
7 **Houssiau FA,** Devogelaer JP, Van Damme J, de Deuxchaisnes CN, Van Snick J. Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides. *Arthritis Rheum* 1988;**31**:784–8.
8 **Guerne PA,** Zuraw BL, Vaughan JH, Carson DA, Lotz M. Synovium as a source of interleukin 6 in vitro. Contribution to local and systemic manifestations of arthritis. *J Clin Invest* 1989;**83**:585–92.
9 **Miyasaka N,** Sato K, Hashimoto J, Kohsaka H, Yamamoto K, Goto M, *et al.* Constitutive production of interleukin 6/B cell stimulatory factor-2 from inflammatory synovium. *Clin Immunol Immunopathol* 1989;**52**:238–47.
10 **Bhardwaj N,** Santhanam U, Lau LL, Tatter SB, Ghrayeb J, Rivelis M, *et al.* IL-6/IFN-beta 2 in synovial effusions of patients with rheumatoid arthritis and other arthritides. *J Immunol* 1989;**143**:2153–9.

11 **Dasgupta B**, Corkill M, Kirkham B, Gibson T, Panayi G. Serial estimation of interleukin 6 as a measure of systemic disease in rheumatoid arthritis. *J Rheumatol* 1992;**19**:22–5.
12 **Madhok R**, Crilly A, Watson J, Capell HA. Serum interleukin 6 levels in rheumatoid arthritis: correlations with clinical and laboratory indices of disease activity. *Ann Rheum Dis* 1993;**52**:232–4.
13 **Sack U**, Kinne RW, Marx T, Heppt P, Bender S, Emmrich F. Interleukin-6 in synovial fluid is closely associated with chronic synovitis in rheumatoid arthritis. *Rheumatol Int* 1993;**13**:45–51.
14 **Uson J**, Balsa A, Pascual-Salcedo D, Cabezas JA, Gonzalez-Tarrio JM, Martin-Mola E, *et al.* Soluble interleukin 6 (IL-6) receptor and IL-6 levels in serum and synovial fluid of patients with different arthropathies. *J Rheumatol* 1997;**24**:2069–75.
15 **Roux-Lombard P**, Eberhardt K, Saxne T, Dayer JM, Wollheim FA. Cytokines, metalloproteinases, their inhibitors and cartilage oligomeric matrix protein: relationship to radiological progression and inflammation in early rheumatoid arthritis. A prospective 5-year study. *Rheumatology* 2001;**40**:544–51.
16 **Yamanaka H**, Matsuda Y, Tanaka M, Sendo W, Nakajima H, Taniguchi A, *et al.* Serum matrix metalloproteinase 3 as a predictor of the degree of joint destruction during the six months after measurement, in patients with early rheumatoid arthritis. *Arthritis Rheum* 2000;**43**:852–8.
17 **Ribbens C**, Andre B, Jaspar JM, Kaye O, Kaiser MJ, De Groote D, *et al.* Matrix metalloproteinase-3 serum levels are correlated with disease activity and predict clinical response in rheumatoid arthritis. *J Rheumatol* 2000;**27**:888–93.
18 **Sato K**, Tsuchiya M, Saldanha J, Koishihara Y, Ohsugi Y, Kishimoto T, *et al.* Reshaping a human antibody to inhibit the interleukin 6-dependent tumor cell growth. *Cancer Res* 1993;**53**:851–6.
19 **Choy EHS**, Isenberg DA, Garrood T, Farrow S, Ioannou Y, Bird H, *et al.* Therapeutic benefit after blocking interleukin-6 activity in rheumatoid arthritis with an anti-interleukin-6 receptor monoclonal antibody. *Arthritis Rheum* 2002;**46**:3143–50.
20 **Nishimoto N**, Yoshizaki K, Maeda K, Kuritani T, Deguchi H, Sato B, *et al.* Toxicity, pharmacokinetics, and dose-finding study of repetitive treatment with the humanized anti-interleukin 6 receptor antibody MRA in rheumatoid arthritis. Phase I/II clinical study. *J Rheumatol* 2003;**30**:1426–35.
21 **Nishimoto N**, Yoshizaki K, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, *et al.* Treatment of rheumatoid arthritis with humanized anti-interleukin-6 receptor antibody: a multicenter, double-blind, placebo-controlled trial. *Arthritis Rheum* 2004;**50**:1761–9.
22 **Maini RN**, Taylor PC, Szechinski J, Pavelka K, Broll J, Balint G, *et al.* Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. *Arthritis Rheum* 2006;**54**:2817–29.
23 **Arnett FC**, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315–24.
24 **van der Heijde D**. How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol* 2000;**27**:261–3.
25 **van der Heijde D**, Simon L, Smolen J, Strand V, Sharp J, Boers M, *et al.* How to report radiographic data in randomized clinical trials in rheumatoid arthritis: guidelines from a roundtable discussion. *Arthritis Rheum* 2002;**47**:215–8.
26 **Kashiwazaki S**, Ichikawa Y, Sugawara S, Nagaya I, Kawai S, Hakota M, *et al.* Determination of the clinical optimal dose of L-377 (methotrexate capsule) for the treatment of rheumatoid arthritis. *Ensyo* 1996;**16**:437–58.
27 **Ito S**, Gross WL, Reinhold-Keller E, Gause A, Aries P, Ruther W, *et al.* Rheumatology in Japan, Germany, and Egypt: a comparison of medical practices. *Acta Medica et Biologica* 2006;**54**:51–58.
28 **Fransen J**, Creemers MC, Van Riel PL. Remission in rheumatoid arthritis: agreement of the Disease Activity Score (DAS28) with the ARA preliminary remission criteria. *Rheumatology* 2004;**43**:1252–5.
29 **Kosinski M**, Zhao SZ, Dedhiya S, Osterhaus JT, Ware JE Jr. Determining minimally important changes in generic and disease-specific health-related quality of life questionnaires in clinical trials of rheumatoid arthritis. *Arthritis Rheum* 2000;**43**:1478–87.
30 **Keane J**, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, *et al.* Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001;**345**:1098–104.
31 **Akira S**, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. *Adv Immunol* 1993;**54**:1–78.
32 **Seriolo B**, Paolino S, Sulli A, Fasciolo D, Cutolo M. Effects of Anti-TNF-α Treatment on Lipid Profile in Patients with Active Rheumatoid Arthritis. *Ann N Y Acad Sci* 2006;**1069**:414–9.
33 **Nishimoto N**, Kanakura Y, Aozasa K, Johkoh T, Nakamura M, Nakano S, *et al.* Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. *Blood* 2005;**106**:2627–32.
34 **Fisman EZ**, Benderly M, Esper RJ, Behar S, Boyko V, Adler Y, *et al.* Interleukin-6 and the risk of future cardiovascular events in patients with angina pectoris and/or healed myocardial infarction. *Am J Cardiol* 2006;**98**:14–8.
35 **Rattazzi M**, Puato M, Faggin E, Bertipaglia B, Zambon A, Pauletto P. C-reactive protein and interleukin-6 in vascular disease: culprits or passive bystanders? *J Hypertens* 2003;**21**:1787–803.

EXTENDED REPORT

Efficacy of rituximab (anti-CD20) for refractory systemic lupus erythaematosus involving the central nervous system

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Aim: Neuropsychiatric systemic lupus erythaematosus (NPSLE) is a serious treatment-resistant phenotype of systemic lupus erythaematosus. A standard treatment for NPSLE is not available. This report describes the clinical and laboratory tests of 10 patients with NPSLE before and after rituximab treatment, including changes in lymphocyte phenotypes.

Methods: Rituximab was administered at different doses in 10 patients with refractory NPSLE, despite intensive treatment.

Results: Treatment with rituximab resulted in rapid improvement of central nervous system-related manifestations, particularly acute confusional state. Rituximab also improved cognitive dysfunction, psychosis and seizure, and reduced the SLE Disease Activity Index Score at day 28 in all 10 patients. These effects lasted for >1 year in five patients. Flow cytometric analysis showed that rituximab down regulated CD40 and CD80 on B cells and CD40L, CD69 and inducible costimulator on CD4+ T cells.

Conclusions: Rituximab rapidly improved refractory NPSLE, as evident by resolution of various clinical signs and symptoms and improvement of radiographic findings. The down regulation of functional molecules on B and T cells suggests that rituximab modulates the interaction of activated B and T cells through costimulatory molecules. These results warrant further analysis of rituximab as treatment for NPSLE.

Systemic lupus erythaematosus (SLE) is an autoimmune disease characterised by multiple lesions induced by activation of autoreactive T cells and overproduction of autoantibodies by B cells. The involvement of the central nervous system (CNS) in SLE is often intractable, complicating the course of the disease in about 12–75% of patients with SLE. The involvement of the CNS has a negative clinical impact with a 5-year survival of 55–85% and is associated with poor prognosis.[1 2] Neuropsychiatric systemic lupus erythaematosus (NPSLE) exhibits a wide range of symptoms unrelated to SLE activation, which include organic and mental disorders, often associated with impairment of consciousness and/or convulsions. These organic disorders may become permanent, eventually leading to long-term or irreversible decline in higher mental functions.

CNS immune abnormalities have an important role in such disease states. Therefore, a trial of intensive treatment, including the combination of potent immunosuppressive treatment and plasma exchange (PE), depending on the disease type and its severity, may be advisable in an effort to control autoreactive lymphocytes.[3–10] Although the severity of NPSLE correlates with prognosis, there is no established treatment protocol and many cases are resistant to treatment making this condition difficult to control.

This study describes the results of treatment of patients with NPSLE who had previously failed to respond to various immunosuppressants. Our approach was based mainly on the use of anti-CD20 antibody (rituximab), a chimeric antibody that directly targets B cells.[11 12] Rituximab is a biological preparation that eliminates B cells through a variety of mechanisms such as antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity and apoptosis. Rituximab has recently been used for the treatment of a variety of SLE disease conditions and good therapeutic response has been reported.[13–16] We investigated the short-term and long-term responses to rituximab treatment in 10 patients with NPSLE, and report that some showed marked improvement following rituximab treatment. Moreover, the results showed that rituximab modulated the functional molecules of activated lymphocytes, implying the efficacy of anti-CD20 antibody treatment for CNS lesions in patients with SLE, otherwise resistant to other treatments.

MATERIALS AND METHODS

Patients

The study subjects were 10 patients who had been previously diagnosed with SLE based on the American College of Rheumatology criteria.[17] The inclusion criteria were (1) the presence of a highly active disease and (2) CNS lesions resistant to conventional treatment. None of the patients showed improvement in CNS-related symptoms in response to conventional immunosuppressive treatment such as intravenous cyclophosphamide pulse treatment (IV-CY), cyclosporine A (CsA), PE and immunoadsorption therapy. All patients completed the course of anti-CD20 antibody treatment described in this study. Patients 1–8, and patients 9 and 10 were treated at the University of Occupational and Environmental Health Hospital and Kyoto University Hospital, respectively, from 2000 to 2005. Informed consent was obtained from all patients in accordance with the regulations of the aforementioned two hospitals, and rituximab was administered in accordance with the study protocol approved by the ethics committee of each hospital.

Abbreviations: CNS, central nervous system; FACS, fluorescence-activated cell sorter; NPSLE, neuropsychiatric systemic lupus erythaematosus; PBS, phosphate-buffered saline; PE, plasma exchange; SLE, systemic lupus erythaematosus; SLEDAI, SLE Disease Activity Index; SPECT, single-photon-emission computed tomography

Treatment protocol

Patients 1–5 and 10 were treated with 375 mg/m^2 rituximab once a week for 2 weeks, and patient 9 received a single administration of the same dose. Patients 6 and 7 received 500 mg rituximab once a week for 4 weeks, while patient 8 was treated with 1000 mg once a week for 2 weeks. Blood pressure and ECG were monitored within the first 3.5 h of the administration to check for any reaction to the drug infusion.

Assessment

Clinical symptoms and treatment-induced adverse reactions were assessed before treatment, every week during treatment, every week within 1 month after treatment and once monthly thereafter. Laboratory tests included blood count, erythrocyte sedimentation rate, liver and renal function tests, urinary protein, serum complement titre and autoantibody level (such as anti-ds-DNA antibody). To evaluate the impact of rituximab on CNS lesions, we measured the immunoglobulin (Ig)G index and interleukin (IL)6 level in the cerebrospinal fluid, MRI, cerebral flood flow scintillator (single-photon-emission computed tomography (SPECT), and ^{18}FTG-positron emission tomography. To assess SLE activity, the SLE Disease Activity Index (SLEDAI) was determined before and after treatment. The level of expression of functional molecules on the lymphocyte cell surface was assessed by flow cytometry.

Flow cytometry

Mononuclear cells were isolated from peripheral blood using lymphocyte separation medium (ICN/Cappel Pharmaceuticals, Aurora, Ohio, USA). After washing twice with phosphate-buffered saline (PBS), the cells were incubated in blocking buffer (0.25% human globulin, 0.5% human albumin (Yoshitomi, Osaka, Japan), and 0.1% NaN_3 (Sigma Aldrich, St Louis, Missouri, USA) in PBS) and left to stand in a 96-well plate at 4℃ for 15 min. In the next step, the cells were incubated in 100 μl of fluorescence-activated cell sorter (FACS) solution (0.5% human albumin and 0.1% NaN_3 in PBS) and then treated with fluorescein isothiocyanate-labelled mouse IgG_1 and antihuman CD40, CD69, inducible costimulator (ICOS), CD19, CD4 (Pharmingen, San Diego, California, USA), CD80 (Chemicon Europe, Chandlers Ford, UK), or CD40L (Ancell, Bayport, USA) antibody, and left to react for 30 min at 4℃. The cells were washed three times with FACS solution and analysed using FACScalibar (Becton–Dickinson, San Jose, California, USA).

Statistical analysis

All data were expressed as mean (SD). Differences between data collected before and after treatment were examined for statistical significance using the Student's t test. $p<0.05$ denoted the presence of a significant difference.

RESULTS

Characteristics of patients

Table 1 summarises the NPSLE classification and laboratory data of the 10 patients. All patients were females with a mean (range) age of 31 (20–55) years. The mean (range) duration of illness from the onset of SLE to administration of rituximab was 9.6 years (3 months to 25 years). Immunosuppressants used for treatment before enrollment in the rituximab protocol included CsA, cyclophosphamide, mizoribine, and azathioprine. In addition, five patients with intractable disease did not respond to the combination treatment, and thus received PE as well.

With regard to CNS-related symptoms, acute confusional state was noted in 5, psychosis in 4, seizures in 2, mood disorders in 2, and one patient each had headache, demyelinating syndrome, myelopathy, anxiety disorder and cognitive dysfunction, based on the NPSLE classification of the American College of Rheumatology.[18 19] MRI findings included abnormal signals in the cerebral white matter in six patients. SPECT showed reduced cerebral blood flow in eight patients. Although a high IgG index[20] was noted in five patients (>0.66), an increase in IL6 was confirmed in only one patient.

Serious haemolytic anaemia, cardiomyopathy-associated decreased cardiac function, muscle pain, mucocutaneous disorders, peripheral neural deficits such as abnormal sensation and neurogenic bladder were also seen in these patients, in addition to the CNS-related changes (tables 1 and 2). In all participants, conventional immunosuppressive therapy produced either no improvement of symptoms or only a poor response. The SLEDAI values (range, 2–49) reflected the presence or absence of organ system-specific activity, with large scores representing involvement of CNS and low scores reflecting haematological activity. In the present study, involvement of organs was limited to those that could be confirmed objectively, while subjective signs such as headache, fatigue and paresthesia were not recorded. Thus, using this approach, the SLEDAI scores of patients with objective signs reflecting multiple involvement of CNS were high whereas those of patients with subjective symptoms only were low. In our study, patients 1 and 3 had multiple CNS signs, patients 1 (49 points) and 3 (37 points) had seizures, psychosis and organic brain syndrome. On the other hand, patient 2 had MRI abnormality in the medulla oblongata but had only paresthesia as a subjective symptom (2 points), and patient 7 had MRI abnormality in the dorsal medulla spinalis and paralysis of the lower extremities, mood and anxiety disorders. However, the SLEDAI scores of both patients were based on subjective symptoms, and thus the scores were low (2 and 3, respectively).

Clinical outcome

At the start of rituximab treatment, patients were treated with low to moderate doses of corticosteroids (15–40 mg of prednisolone, 1–3 mg betamethasone), and continued to use this treatment during the rituximab arm of the study. However, immunosuppressants were stopped at entry to the study in all patients except for patient 8 who continued her treatment of 50 mg azathioprine. The postrituximab follow-up period was 7–45 months. Table 2 provides details of the clinical symptoms and laboratory tests before and 28 days after rituximab treatment (unless otherwise indicated in the table). Improvement in the skin and mucocutaneous lesions was fast, and the ejection fraction recovered from 44% to 72.1% in patient 4. All patients showed improvement in haematopenia and complement titre and marked falls in PE-resistant autoantibodies after treatment. Analysis of SLE activity before and after the treatment showed a significant decrease in SLEDAI from 19.9 (range, 49–2) before treatment to 6.2 (range, 15–0) after treatment ($p = 0.013$, fig 1). Moreover, SLEDAI decreased to 0 in 9 of the 10 patients at 1–6 months after rituximab treatment.

Rituximab treatment was also effective against CNS lesions in all patients. In particular, the consciousness state of all the five patients who were in acute confusional state before treatment, improved rapidly after the treatment. For example, the GCS score of patient 1 improved from 7–11 to 15 after 5 days of treatment, and that of patient 2 from 3 to 14 after 2 days of treatment. This rapid recovery was clinically significant. In addition, even in three patients who were in a dazed state and needed to be woken up before rituximab treatment, became alert the next day (patient 2) or after a few days of treatment (patients 9 and 10). Furthermore, rituximab also improved neuropsychiatric symptoms such as psychosis

Table 1 Characteristics of 10 female patients with neuropsychiatric systemic lupus erythaematosus at study entry

Patient	Age (years)	Duration of disease	Previous treatment	NP classification	MRI/SPECT	IgG index /IL6 (pg/ml)	Clinical manifestations	SLEDAI
1	35	19 years	CS (40 mg, pulse 14), IV-CY (22), VCR (10 mg), CsA (300 mg, 3 years), AZ (100 mg, 2 months), MTX (8 mg/w, 4 months), PE (11), IA (15)	Acute confusional state, seizure, psychosis	Normal/abnormal	Not done/not done	Fever, fatigue, nephritic syndrome, leukopenia, low Hb, high ESR, CH50, anti-ds DNA ↑	49
2	55	25 years	CS (40 mg, pulse 3), IV-CY (7), PE (2)	Acute confusional state	II, III/abnormal	0.73 ↑ / 1.8	Paresthesia of fingers, severe AIHA, anti-ds DNA ↑	2
3	46	3 months	CS (50 mg), IV-CY (1), PE (2), IA (3)	Acute confusional state, seizure	II, III/abnormal	0.46/33.8 ↑	Leukopenia, low Hb, thrombocytopenia, proteinuria, AIH, anti-ds DNA ↑	37
4	20	1 year	CS (50 mg), CsA (175 mg, 1 m)	Headache	Normal/not done	1.05 ↑ /3.1	Fever, fatigue, skin rash, alopecia, cardiomyopathy, polyneuropathy, leukopenia, C4 ↓, anti-ds DNA ↑	16
5	34	3 years	CS (60 mg), IV-CY (8), MZ (150 mg, 25 months)	Demyelinating syndrome	II, III/normal	0.85 ↑ /0.9	Sensory deficit, photosensitivity, mouth ulcer, lymphocytopenia, C4 ↓	16
6	30	22 years	CS (40 mg), MZ (150 mg, 22 years)	Mood disorder	Normal/abnormal	0.54/1.5	Polyneuropathy, muscular pain, skin rash, leukopenia, anti-ds DNA ↑	17
7	21	7 years	CS (60 mg, pulse 3), IV-CY (14), MTX (intrathecal 30 mg), MZ (300 mg, 2 years)	Myelopathy, mood disorder, anxiety disorder	II, III/abnormal	0.80 ↑ /4.7	Periungual erythaema, leukopenia	3
8	20	9 months	CS (45 mg), IV-CY (6), AZ (50 mg, 1 month)	Psychosis, cognitive dysfunction	III/abnormal	0.56/1.0	Lymphadenopathy, alopecia, malar rash, lymphocytopenia	18
9	20	8 months	CS, CY, DFPP	Acute confusional state, psychosis	III/abnormal	0.98 ↑ /4.2	Fever, lymphadenopathy, low Hb, lymphocytopenia, high ESR, anti-Sm ↑	28
10	29	17 years	CS, AZ, CsA, CY, PE	Acute confusional state, psychosis	Normal/abnormal	0.60/2.4	Severe AIHA, CH50 ↓	18

The disease activity was high in all patients and none had responded to conventional immunosuppressants.

AIHA, autoimmune haemolytic anaemia; AZ, azathioprine; CS, corticosteroid; CsA, cyclosporine; CY, cyclophosphamide; DFPP, double filtration plasmapheresis; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; IA, immunoadsorption; MTX, methotrexate; MZ, mizoribine; PE, plasma exchange; SLE-DAI, Systemic Lupus Erythaematosus Disease Activity Index; VCR, vincristine.

For IV-CY, PE and IA, numbers in parentheses represent the number of treatments. For CS, CsA, AZ and MZ, the doses in parentheses represent maximum dosage. For VCR in patient 1 and MTX in patient 7, the dose in parentheses expresses total dosage.

Table 2 Clinical outcomes of neuropsychiatric systemic lupus erythaematosus after anti-CD20 antibody treatment

Patient	Dose of rituximab	Other treatments at study entry (mg)	CNS manifestations		Objective NPSLE findings after treatment	Duration of remission (m)
			before	after		
1	375 mg/m^2 day 1, 8	Bet 1.0	Consciousness disorder, seizure, psychosis	Complete recovery (GCS 7–11→15/5 days)	Improvement of SPECT	22
2	375 mg/m^2 day 1, 15	Bet 1.5	Consciousness disorder	Improved consciousness	No follow-up data	18
3	375 mg/m^2 day 1, 8	Bet 1.0	Consciousness disorder, seizure	Complete recovery (GCS 3→14/2 days)	No improvement in MRI and SPECT	23
4	375 mg/m^2 day 1, 8	m-PSL 20	Headache	Resolution of headache	Improved IgG index (1.05 →0.84/4 w)	29
5	375 mg/m^2 day 1, 8	Bet 1.25	Paresthesia of fingers, toes and left precordial-back	Resolution of paresthesia	Improvement of neck MRI	7
6	500 mg day 1, 8, 15, 22	Bet 2.5	Depressive state, insomnia	Improvement of depressive state	Improvement of SPECT	7
7	500 mg day 1, 8, 15, 22	Bet 1.25	Paresis of both lower limbs, muscle weakness, depressive state	Reduction of paresis, improvement of depressive state (SDS, 58→50/2 w)	Improvement of SPECT, improvement of IgG index (0.80 →0.72/3 m)	14
8	1000 mg day 1, 15	Bet 1.25, AZ 50	Psychosis, cognitive dysfunction	Improvement of psychosis (BPRS 26→7/8 w)	Improvement of SPECT	11
9	375 mg/m^2 day 1	PSL 45	Consciousness disorder, psychosis, paresis of both lower limbs, neurological bladder	Complete recovery	Improvement of PET and MRI, improved IgG index (0.98 →0.61/2 w)	10
10	375 mg/m^2 day 1, 8	Bet 3	Consciousness disorder, hallucination, cataplexy	Complete recovery	No significant improvement in objective findings	4

Bet, betamethasone; BPRS, brief psychiatric rating scale; CNS, central nervous system; GCS, Glasgow Coma Scale; m-PSL, methylprednisolone; MRI, magnetic resonance imaging; NPSLE, neuropsychiatric systemic lupus erythaematosus; PET, 18FTG-positron emission tomography; PSL, prednisolone; SDS, self-rating depression scale; SPECT, single photon emission computed tomography. For other abbreviations, see table 1.

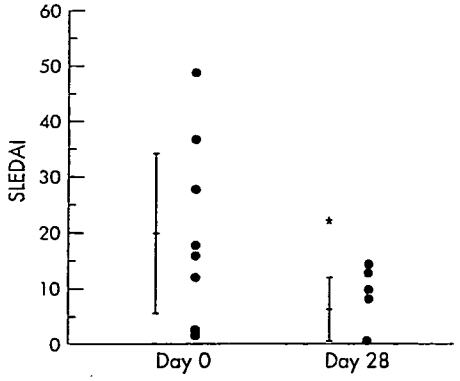

Figure 1 Systemic lupus erythaematosus disease activity index (SLEDAI) score before and 28 days after rituximab treatment. A decrease in SLEDAI score was detected in 9 of the 10 patients. Data are mean (SD). *p<0.05.

and mood disorder within a few weeks to a few months after treatment. For example, the Brief Psychiatric Rating Scale, which is used for the assessment of schizophrenia, markedly decreased in patient 8 from 26 to 7 points within 2 months, together with recovery of communication skills. In addition, patients 1 and 9 showed rehabilitation into society after rituximab treatment although they had serious neuropsychiatric symptoms before treatment. In addition to the improvement in SLE activity and clinical symptoms, rituximab also improved the quality of life of the patients.

We also assessed the effects of rituximab treatment by comparing the findings of MRI and SPECT before and after treatment. In four patients (patients 1, 6, 7 and 8), rituximab treatment improved cerebral blood flow as determined by SPECT; in patient 1, such improvement was noted at the early stage of treatment and paralleled the improvement in clinical symptoms. For patient 5, rituximab treatment resulted in improvement in the abnormal findings in T2-weighted images of the cervical cord on MRI, along with the improvement in sensory deficits due to inflammation at the same site. For patient 9, rituximab treatment resulted in reduction of the high-intensity lesion in the head MRI T2-weighted image.

Five of our patients had peripheral neuropathies in addition to CNS lesions. Treatment with rituximab resulted in remission or marked improvement of paresthesia in patient 2, radiculopathy in patient 4, ulnar neuropathy in patient 6, and neurological bladder in patient 9. Rituximab also improved quality of life based on improvement of peripheral neuropathy-related symptoms although such symptoms tended to persist after treatment.

While the overall therapeutic effect of rituximab was excellent, some patients developed relapse after long-term remission. Six of the 10 patients showed reactivation of SLE including reappearance of CNS-related symptoms. For patient 1, remission was maintained with low-dose steroid for 22 months after rituximab treatment. However, the patient showed recurrence associated with an increase in autoantibodies and proteinuria. Recurrence was also noted 18 months after treatment in patient 2, associated with haemolysis. Both patients 1 and 2 required retreatment with rituximab. At 23 months after completion of rituximab treatment, patient 3 showed worsening of the head MRI findings and cerebrospinal fluid abnormalities and developed witnessed seizure attacks. In patient 5, a reduction in the steroid dose was followed by recurrence of CNS-related symptoms after 7 months. Generalised skin rashes appeared in patient 9 after 10 months and patient 10 reported worsening of lupus headache after 4 months. Patients 3 and 5 received IV-CY treatment, and patient 9 and 10 required an increase in the steroid dose. However, four patients (patients 4, 6, 7 and 8) maintain a remission state at the time of writing this report (at 35 months in patient 4, at 7 months in patient 6, at 19 months in patient 7 and 16 months in patient 8) after the completion of rituximab treatment.

Adverse effects

Of the 10 patients, two developed pneumonia, one had herpes zoster, one developed chickenpox and one had intractable infection of decubitus ulceration. These infections were successfully controlled with antibiotics.

Phenotypic analysis of SLE lymphocytes

T cells and B cells are activated by antigen stimulation via T cell receptors and signals from costimulatory molecules. The responsible costimulatory molecules, such as CD40/40L, CD80, CD86/CD28 and ICOS/B7h, are known to be expressed in patients with active SLE.[21–26]

We performed serial analysis of the expression of functional molecules in eight patients with SLE before and after rituximab treatment by flow cytometry. Rituximab treatment resulted in rapid disappearance of CD20, a specific antigen to B cells, marked decrease in CD19-positive cells, within several days to 2 weeks after treatment. Rituximab also resulted in rapid falls in the percentages of CD40-expressing and CD80-expressing CD19 cells within 1 day and both were hardly detected after the second day (fig 2). The expression levels of these molecules were still low at 3 months after completion of rituximab treatment.

We also assessed the effects of treatment on the expression levels of CD40L (a costimulatory molecule on CD4-positive cells), ICOS and CD69 (an early activation antigen). While only three patients showed high expression of these molecules before treatment, rituximab treatment reduced the expression levels of these molecules in all three patients (fig 3), suggesting that rituximab does not only affect B cells but also T cells in patients with SLE.

DISCUSSION

To date, reports on rituximab treatment for autoimmune diseases have covered various conditions, including RA, SLE, dermatomyositis, Sjögren's syndrome and vasculitis.[27–30] Rituximab treatment resulted in improvement, manifested by a decrease in the British Disease Activity score and SLE DAI score, of arthropathy, nephropathy, thrombocytopenia and haemolytic anaemia.[11–16]

Although few reports described the efficacy of rituximab treatment in patients with SLE with CNS lesions,[11 14 31] to our knowledge, there are no published reports that provide detailed analysis of the effects of such treatment in a large group of patients. Rituximab has a large molecular weight of 146 kDa, and hence cannot readily cross the blood–brain barrier; therefore, it is unlikely to reach the cerebrospinal fluid following systemic administration. We measured rituximab concentration in the cerebrospinal fluid of patient 8 at 24 h after treatment. The value (0.3 μg/ml) was slightly higher than the lower detection limit of the assay, whereas the serum concentration was 279 μg/ml. Based on this finding, we assume that the central effects of rituximab are mediated through another mechanism, not through antibody-dependent cellular cytotoxicity and/or complement-dependent cytotoxicity.[32]

To assess autoreactive lymphocyte activity, we determined the expression of various functional molecules on the surface of peripheral blood lymphocytes before and after rituximab treatment by using flow cytometry. We previously proposed that rituximab could regulate SLE disease activity and correct

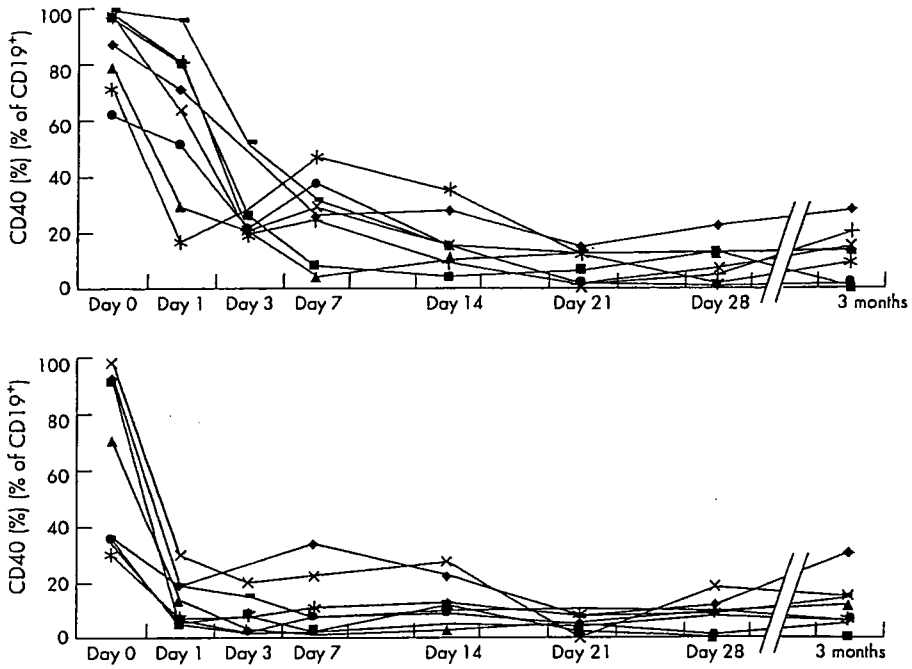

Figure 2 Serial changes in CD40 and CD80 expression on CD19-positive cells after rituximab treatment in eight patients with systemic lupus erythaematosus. CD40 and CD80 expression was measured before and 28 days after rituximab treatment.

autoimmune abnormalities.[12] The present results showed a rapid decrease in the expression of functional surface molecules and maintenance of long-term control following rituximab treatment (fig 2). Specifically, a marked decrease in the proportion of CD40-expressing and CD80-expressing cells was detected on the day after initiation of rituximab treatment. In this regard, Leng *et al*[33] found CD40 overexpression in CD19 cells in patients with rheumatoid arthritis compared with healthy controls. Others also reported that the percentage of CD80-positive cells among activated B cell subset was higher in SLE than the controls.[34] These results suggest that the target of rituximab treatment is activated B cells. Anolik *et al*[35] examined B cell phenotypes after rituximab treatment and reported that the proportion of autoreactive memory B cells was decreased after rituximab treatment. Considered together, the above results and those of the present study suggest that T cell activation is negatively influenced by a rapid decrease in B cell to T cell stimulation in parallel with the loss of B cells. Our

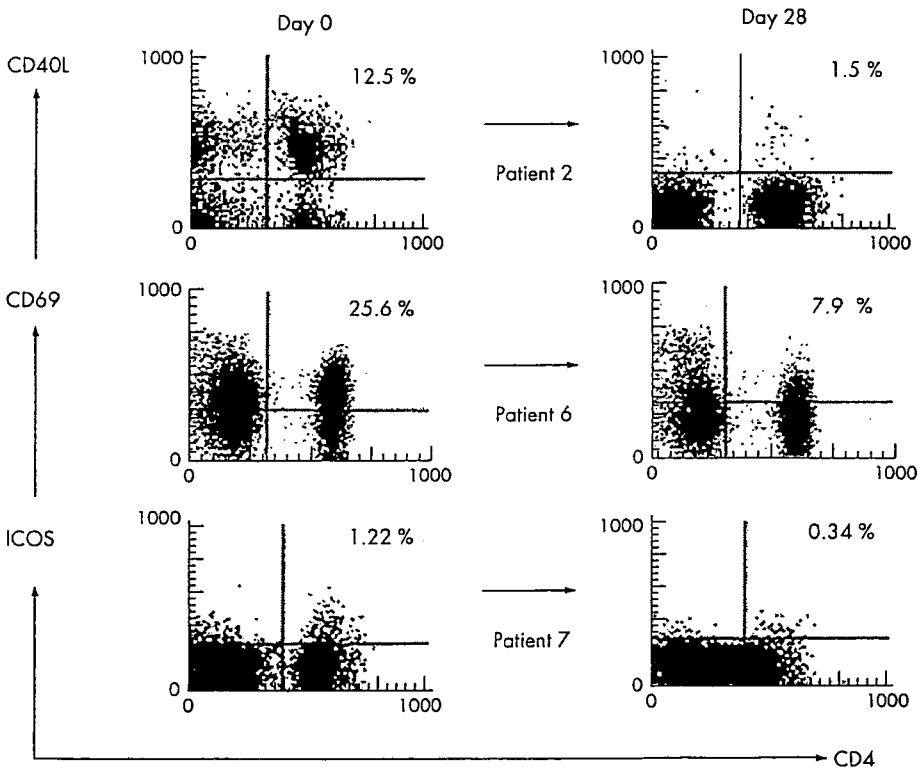

Figure 3 Changes in expression of functional molecules on CD4-positive cells induced by rituximab treatment. The expression of CD40L (patient 2), CD69 (patient 6) and ICOS (patient 7) on CD4-positive cells was measured before (day 0) and 28 days after rituximab treatment. Percentages represent the percentage of CD4-positive cells expressing the functional molecules.

results also showed that rituximab down regulated CD40L, ICOS and CD69 on CD4-positive cells in patients with active SLE (fig 3). Sfikakis *et al*[36] also reported that rituximab treatment decreased CD40L and CD69 expression in patients with SLE. These results imply that rituximab could eliminate B cells bearing functional molecules and inhibit the interaction between these B cells and activated T cells by down regulating costimulatory molecules, and also possibly by reducing the production of certain cytokines and complement activation, which could lead to rapid improvement of CNS manifestations of the disease.

At present, there is no treatment strategy for patients with NPSLE who fail to respond to conventional therapies. In such patients, large doses of steroids are provided on long-term basis, and IV-CY is administered continuously. Our study showed that rituximab is useful as a new treatment for such cases. However, recurrence after rituximab treatment was noted in our patients, as has been reported previously in patients with rheumatoid diseases.[28] Two of our patients who experienced recurrence received rituximab re-treatment. However, these patients experienced recurrence at 18 and 22 months after rituximab treatment, suggesting that remission could be maintained for a comparatively long period of time with rituximab treatment. Further studies are needed to develop strategies for the prevention of recurrence and counter measures for inhibiting the production of antichimeric antibodies.[37 38] There is also a need to investigate the long-term effects of rituximab treatment and its organ specificity.

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REFERENCES

1 **Blanco FJ,** Gomez-Reino JJ, de la Mata J, *et al.* Survival analysis of 306 European Spanish patients with systemic lupus erythematosus. *Lupus* 1998;7:159–63.
2 **Kasitanon N,** Louthrenoo W, Sukitawut W, *et al.* Causes of death and prognostic factors in Thai patients with systemic lupus erythematosus. *Asian Pac J Allergy Immunol* 2002;20:85–91.
3 **Hahn BH.** Systemic lupus erythematosus. In: Kasper DL, eds. *Origins of Harrison's principles of internal medicine.*16th edn. Columbus: McGraw-Hill, 2005:1960–7.
4 **Ad Hoc Working Group on Steroid-Sparing Criteria in Lupus.** Criteria for steroid-sparing ability of interventions in systemic lupus erythematosus: report of a consensus meeting. *Arthritis Rheum* 2004;50:3427–31.
5 **Goldblatt F,** Isenberg DA. New therapies for systemic lupus erythematosus. *Clin Exp Immunol* 2005;140:205–12.
6 **Petri M.** Cyclophosphamide: new approaches for systemic lupus erythematosus. *Lupus* 2004;13:366–71.
7 **Boumpas DT,** Yamada H, Patronas NJ, *et al.* Pulse cyclophosphamide for severe neuropsychiatric lupus. *Q J Med* 1991;81:975–84.
8 **Barile-Fabris L,** Ariza-Andraca R, Olguin-Ortega L, *et al.* Controlled clinical trial of IV cyclophosphamide versus IV methylprednisolone in severe neurological manifestations in systemic lupus erythematosus. *Ann Rheum Dis* 2005;64:620–5.
9 **Sherer Y,** Levy Y, Langevitz P, *et al.* Successful treatment of systemic lupus erythematosus cerebritis with intravenous immunoglobulin. *Clin Rheumatol* 1999;18:170–3.
10 **Valesini G,** Priori R, Francia A, *et al.* Central nervous system involvement in systemic lupus erythematosus: a new therapeutic approach with intrathecal dexamethasone and methotrexate. *Springer Semin Immunopathol* 1994;16:313–21.
11 **Saito K,** Nawata M, Nakayamada S, *et al.* Successful treatment with anti-CD20 monoclonal antibody (rituximab) of life-threatening refractory systemic lupus erythematosus with renal and central nervous system involvement. *Lupus* 2003;12:798–800.
12 **Tokunaga M,** Fujii K, Saito K, *et al.* Down-regulation of CD40 and CD80 on B cells in patients with life-threatening systemic lupus erythematosus after successful treatment with rituximab. *Rheumatology* 2005;44:176–82.
13 **Perrotta S,** Locatelli F, La Manna A, *et al.* Anti-CD20 monoclonal antibody (Rituximab) for life-threatening autoimmune haemolytic anaemia in a patient with systemic lupus erythematosus. *Br J Haematol* 2002;116:465–7.
14 **Leandro MJ,** Edwards JC, Cambridge G, *et al.* An open study of B lymphocyte depletion in systemic lupus erythematosus. *Arthritis Rheum* 2002;46:2673–7.
15 **Looney RJ,** Anolik JH, Campbell D, *et al.* B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. *Arthritis Rheum* 2004;50:2580–9.
16 **Leandro MJ,** Cambridge G, Edwards JC, *et al.* B-cell depletion in the treatment of patients with systemic lupus erythematosus: a longitudinal analysis of 24 patients. *Rheumatology* 2005;44:1542–5.
17 **Tan EM,** Cohen AS, Fries JF, *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–7.
18 **ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature.** The American College of Rheumatology nomenclature and classification and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum* 1999;42:599–608.
19 **Borchers AT,** Aoki CA, Naguwa SM, *et al.* Neuropsychiatric features of systemic lupus erythematosus. *Autoimmun Rev* 2005;4:329–44.
20 **French CA,** Tracy RP, Rudick RA, *et al.* Simultaneous determination of cerebrospinal fluid oligoclonal bands and the "gamma-protein index" by agarose electrophoresis and densitometry. *Clin Chem* 1986;32:84–7.
21 **Datta SK,** Kalled SL. CD40-CD40 ligand interaction in autoimmune disease. *Arthritis Rheum* 1997;40:1735–45.
22 **Koshy M,** Berger D, Crow MK. Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. *J Clin Invest* 1996;98:826–37.
23 **Yellin MJ,** Thienel U. T cells in the pathogenesis of systemic lupus erythematosus: potential roles of CD154-CD40 interactions and costimulatory molecules. *Curr Rheumatol Rep* 2000;2:24–31.
24 **Kovacs B,** Thomas DE, Tsokos GC. Elevated *in vivo* expression of the costimulatory molecule B7-BB1 (CD80) on antigen presenting cells from a patient with SLE. *Clin Exp Rheumatol* 1996;14:695–7.
25 **Bijl M,** Horst G, Limburg PC, *et al.* Expression of costimulatory molecules on peripheral blood lymphocytes of patients with systemic lupus erythematosus. *Ann Rheum Dis* 2001;60:523–6.
26 **Andreas H,** Kerstin B, Karin R, *et al.* Involvement of inducible costimulator in the exaggerated memory B cell and plasma cell generation in systemic lupus erythematosus. *Arthritis Rheum* 2004;50:3211–20.
27 **Leandro MJ,** Edwards JC W, Cambridge G. Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. *Ann Rheum Dis* 2002;61:883–8.
28 **Keogh KA,** Ytterberg SR, Fervenza FC, *et al.* Rituximab for refractory Wegener's granulomatosis: report of a prospective, open-label pilot trial. *Am J Respir Crit Care Med* 2006;173:180–7.
29 **Levine TD.** Rituximab in the treatment of dermatomyositis: an open-label pilot study. *Arthritis Rheum* 2005;52:601–7.
30 **Pijpe J,** van Imhoff GW, Spijkervet FK, *et al.* Rituximab treatment in patients with primary Sjogren's syndrome: an open-label phase II study. *Arthritis Rheum* 2005;52:2740–50.
31 **Armstrong DJ,** McCarron MT, Wright GD. SLE-associated transverse myelitis successfully treated with Rituximab (anti-CD20 monoclonal antibody). *Rheumatol Int* 2005;18:1–2.
32 **Reff ME,** Carner K, Chambers KS, *et al.* Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood* 1994;83:435–45.
33 **Leng JH,** Hu ZY, Zhuo GC, *et al.* The expression and significance of costimulatory molecule in peripheral blood B lymphocytes in rheumatoid arthritis. *Zhonghua Nei Ke Za Zhi* 2004;43:519–21.
34 **Folzenlogen D,** Hofer MF, Leung DY, *et al.* Analysis of CD80 and CD86 expression on peripheral blood B lymphocytes reveals increased expression of CD86 in lupus patients. *Clin Immunol Immunopathol* 1997;83:199–204.
35 **Anolik JH,** Barnard J, Cappione A, Pugh-Bernard AE, *et al.* Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus. *Arthritis Rheum* 2004;50:3580–90.
36 **Sfikakis PP,** Boletis JN, Lionaki S, *et al.* Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial. *Arthritis Rheum* 2005;52:501–13.
37 **Weide R,** Heymanns J, Pandorf A, *et al.* Successful long-term treatment of systemic lupus erythematosus with rituximab maintenance therapy. *Lupus* 2003;12:779–82.
38 **Edelbauer M,** Jungraithmayr T, Zimmerhackl LB. Rituximab in childhood systemic lupus erythematosus refractory to conventional immunosuppression: case report. *Pediatr Nephrol* 2005;20:811–13, .