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Yoshiya Tanaka · Kazuhiko Yamamoto
Tsutomu Takeuchi · Norihiro Nishimoto
Nobuyuki Miyasaka · Takayuki Sumida · Yoshihito Shima
Kazuki Takada · Isao Matsumoto · Kazuyoshi Saito
Takao Koike

A multicenter phase I/II trial of rituximab for refractory systemic lupus erythematosus

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Abstract Although corticosteroids and immunosuppressants are widely used for the treatments of systemic lupus erythematosus (SLE), safer and more effective therapies are prerequisite. We and others have reported that anti-CD20 antibody rituximab targeting B cells are effective for refractory SLE and, therefore, safety and clinical efficacy of rituximab in SLE was evaluated by a multicenter phase I/II clinical trial. An open-label, multicenter study of 15 patients with active and refractory SLE (total British Isles Lupus Assessment Group [BILAG] score 8 to 17) was conducted. Rituximab was administered to 5 SLE patients as 4 infusions of 500 mg/body every week and to 10 SLE patients as 2 infusions of 1000 mg/body every other week. Assessment of safety, infusion reactions and adverse effects was used as the primary outcome for clinical tolerability and was evaluated by 28 weeks. Rituximab was well tolerated, with most experiencing no significant adverse effects. B cells rapidly reduced in all patients and remained low until 6 months post-treatment. Four patients developed human antichimeric antibodies without affecting efficacy of rituximab. Changes in routine safety laboratory tests clearly related to rituximab were not observed. Nine among 14 evaluable patients achieved the major or partial clinical response of BILAG score and prednisolone dose significantly decreased at the 28 weeks. Rituximab therapy appears to be safe for the treatment of active SLE patients and holds significant therapeutic promise, at least for the majority of patients experiencing profound B-cell depletion.

Key words B cells · CD20 · Monoclonal antibody · Rituximab · Systemic lupus erythematosus (SLE)

Y. Tanaka (✉) · K. Saito
First Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Japan, 1-1 Iseigaoka, Yahata-nishi, Kitakyushu 807-8555, Japan
Tel. +81-93-603-1611; Fax +81-93-691-9334
e-mail: tanaka@med.uoeh-u.ac.jp

K. Yamamoto
Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

T. Takeuchi
Division of Rheumatology and Clinical Immunology, Saitama Medical Center, Saitama Medical University, Kawagoe, Japan

N. Nishimoto · Y. Shima
Laboratory of Immune Regulation, Graduate School of Frontier Biosciences, Osaka University, Suita, Japan

N. Miyasaka · K. Takada
Department of Medicine and Rheumatology, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan

T. Sumida · I. Matsumoto
Department of Clinical Immunology, University of Tsukuba, Ibaraki, Japan

T. Koike
Department of Medicine II, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease in which immune complexes consisting of antigens and autoantibodies secreted from activated B cells cause severe inflammation on various tissues and organs. Although high-dose corticosteroids (CS) and immunosuppressants are widely used for the treatments, we often experience patients with SLE who are refractory to these conventional treatments, and innovative approaches to newer therapeutic agents aimed at more specific targets in the immune cascade need to be developed.

Three major critical pathways by which B cells initiate and perpetuate the disease processes of SLE have been emerging, which imply the significance of B-cell as a target to control SLE.[1–4] Initially, the polyclonal activation of autoreactive B cells produce autoantibodies, activating the complement system and immune responses. B cells also contribute to T-cell activation as highly efficient antigen-presenting cells (APC), which lead to production of proinflammatory signals from T cells. Furthermore, activated B cells produce cytokines such as tumor necrosis factor (TNF)-

α and interleukin (IL)-6, which promote inflammation and tissue damage in SLE.

CD20 is a surface molecule specific for B cells, and is expressed in most stages of B cells. Rituximab (Rituxan) is a chimeric monoclonal antibody specific for human CD20, consisting of human immunoglobulin (Ig) G1 constant regions and mouse variable regions. Rituximab is known to deplete B cells by complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity. This antibody has already been used and has demonstrated high effectiveness in the treatment of B-cell lymphomas. Recently, the potential efficacy of B-cell depletion therapy with rituximab has been reported in several autoimmune diseases such as rheumatoid arthritis (RA) and rituximab was, thereby, approved for the treatment of RA refractory to tumor necrosis factor (TNF) inhibitors in the United States.[1–8]

Rituximab has also been used for the treatment of SLE.[9–13] An open label study by Leandro et al.[10] in the UK initially showed improvement within 1 month in disease activity measured by British Isles Lupus Assessment Group (BILAG) scores in six female patients with active lupus nephritis. Improvements in fatigue, arthralgia/arthritis, and serositis were particularly impressive and reductions in urinary protein-creatinine ratio were noticed. Looney et al.[11] also confirmed the efficacy of rituximab for SLE in the United States. Ten among 16 SLE patients achieved complete B-cell depletion and disease activity improved, and sustained improvements were seen for up to 12 months with some patients requiring little or no CS, even though B-cell counts normalized between 3 and 12 months after the treatment. We have also reported that 10 patients with life-threatening SLE complicated with lupus nephritis and neuropsychiatric complications (NPSLE) improved, sometimes very rapidly, by rituximab in a series pilot study.[14–16] These results prompted further investigation into the efficacy and safety of rituximab in SLE. Herein, we describe a multicenter phase I/II clinical trial of rituximab in patients with moderately to highly active SLE, and report its safety profile and clinical efficacy in SLE.

Patients and methods

Study design

An open-label, multicenter study in 15 patients with refractory SLE who met the American College of Rheumatology (ACR) criteria for the classification of SLE was conducted.[17] The protocol was approved by the local ethics committees in each of seven universities (facilities of all the listed co-authors), and all patients enrolled were required to provide signed, detailed, informed consent. The study population consisted of patients ≥20 years of age and who had moderate to severe flare, although they were treated with ≥30.4 mg/kg per day of prednisolone. Clinical disease activity was defined by a BILAG scores and patients having one or more category A or two or more category B BILAG scores were enrolled.[18,19]

Primary outcome

Assessment of safety, infusion reactions, and adverse effects was used as the primary outcome for clinical tolerability. Any adverse events were graded according to toxicity criteria of National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 2.0 on a grade 1 to 4 scale for toxicity (1, mild; 2, moderate; 3, severe; 4, life threatening). Patients were assessed for adverse events and disease activity at baseline and at 2, 4, 8, 12, 16, 20, 24, and 28 weeks after starting rituximab. Evaluations by 28 weeks of follow-up included vital signs, physical examination, adverse events, routine safety laboratory tests (hematology, serum chemistry), urinalysis, and serum immunoglobulins (Igs). Serum levels of rituximab and human antichimeric antibodies (HACA) were measured using an enzyme-linked immunosorbent assay (ELISA). The percentage of peripheral CD19-positive and CD20-positive B lymphocytes was determined at each time point using standard direct-staining on whole blood using a flow cytometer (Becton Dickinson, San Jose, CA, USA).

Secondary outcome

Secondary objectives were to ascertain the clinical efficacy of rituximab on serologic activity and SLE activity determined by the BILAG scores. The BILAG system was used to categorize the severity level of disease activity in each patient at 2, 4, 8, 12, 16, 20, 24, and 28 weeks after starting rituximab. The BILAG system organizes clinical signs and symptoms according to eight different systems: general/constitutional, mucocutaneous, neurological, musculoskeletal, cardiovascular/respiratory, vasculitis, renal, and hematological domains.[18,19] At each evaluation, the presence and change of any signs and symptoms were recorded and the level of any disease activity within each body system determined on a treatment-intent basis, according to BILAG rules as: A (severely active), B (moderately active), C (stable mild disease), D, and E. Clinical responses were grouped into major clinical response (MCR), partial clinical response (PCR), and no clinical response (NCR), according to achievement of BILAG scores. Specifically, MCR was defined as subjects who achieved BILAG C scores or better at 28 weeks, PCR as subjects who achieved a maximum of one domain with a BILAG B score at 28 weeks and NCR as subjects who failed to meet the definition of an MCR or PCR. Other evaluations at these times included an SLE panel (anti-ds-DNA antibody, complements, and other laboratory tests).

Treatment schedule

Rituximab was provided by Zenyaku-Kogyo (Tokyo, Japan) and was administered to the initial five SLE patients as 4 infusions of 500 mg/body every week (as used for the treatment of lymphoma). After the assessment of safety, infusion reactions, and adverse effects during the infusion, the subsequent 10 SLE patients were treated with 2 infu-

sions of 1000 mg/body every other week (as used for the United States trial of rituximab for autoimmune diseases). Concomitant immunosuppressive medications and bolus CS were not allowed during the 28-week follow-up and one patient was excluded because intravenous cyclophosphamide pulse therapy was used.

Results

Patient characteristics at study entry

Rituximab was administered to 15 subjects (14 females and 1 male). Their age was 20–55 years (33.3 ± 11.1 years, mean ± SD) and their disease duration was 5 months to 22 years (6.7 ± 7.2 years) (Table 1). All patients were treated with ≥30.4 mg/kg per day of prednisolone and continued to use it without dose increase during the study period, but concomitant immunosuppressive agents and bolus CS were not allowed during the 28-week follow-up period. However, one patient withdrew at 2 months because of the newly additional use of intravenous cyclophosphamide pulse therapy, because biphasic disorder was flared and IL-6 levels in cerebrospinal fluid was elevated (41.6 pg/ml). Six patients among the evaluable 14 suffered from marked renal manifestation and five patients had NPSLE, as shown in Table 1. Patients had moderately to highly active SLE, which were categorized by total BILAG scores ranging from 8 to 17 (12.5 ± 3.0 points) at study entry (Table 2). Eight patients out of 14 had A-level disease activity in any body system

Table 1. Characteristics of the SLE patients at baseline

Age (years)/sex	Duration	Medication	Main clinical manifestations of SLE
40/F	14Y	PSL 0.4 mg/kg,CS-pulse, CsA, IV-CY, MZ	Nephritis (proteinuria 1.5 g/day), fatigue, nausea, erythema, Raynaud phenomena
30/F	22Y	PSL 0.5 mg/kg, MZ	NPSLE (acute confusional state, mood disorder), fatigue, nausea, erythema, myopathy
55/F	2Y	PSL 0.4 mg/kg,CS-pulse, IV-CY, CsA, MTX	Lymphoadenopathy/splenomegaly, erythema, diffuse eruption, severe anemia
21/F	6Y	PSL 0.4 mg/kg,CS-pulse, CY, MZ, MTX	NPSLE (mood disorder, myelopathy), fatigue, nausea, erythema, Raynaud phenomena
34/F	1Y	PSL 0.4 mg/kg, CS-pulse	Nephritis (proteinuria 2.2 g/day), fatigue, arthritis
27/F	3Y	PSL 0.4 mg/kg, CS-pulse, IV-CY, AZ, CsA, MZ	Appetite loss, diffuse eruption, aseptic necrosis, leukocytopenia
21/F	6Y	PSL 0.8 mg/kg, CS-pulse, IV-CY, CsA, MZ, AZ, TL	Severe thrombocytopenia, arthritis, erythema
20/F	6M	PSL 0.5 mg/kg, IV-CY, AZ	NPSLE (acute confusional state), arthritis, arrhythmia, Raynaud phenomena
20/F	2Y	PSL 0.4 mg/kg, CS-pulse	Nephritis (proteinuria 1.5 g/day), erythema, diffuse eruption
38/F	11Y	PSL 0.4 mg/kg, IV-CY, CsA, AZ	Nephritis (proteinuria 0.6 g/day), NPSLE (depression), serositis, arthritis, fatigue and others
46/F	9M	PSL 0.6 mg/kg, CsA	Nephritis (0.5 g/day), pleuritis, serositis, dyspnea, thrombosis
41/F[a]	21Y	PSL 0.9 mg/kg,CS-pulse, IV-CY, IA, PE	Nephritis (proteinuria 4.0 g/day), NPSLE (acute confusional state), TTP, fatigue and others
29/F	5M	PSL 0.6 mg/kg, IV-CY	High fever, fatigue, nausea, lymphadenopathy, erythema, arthritis
29/F	5Y	PSL 0.5 mg/kg, CsA	Nephritis (proteinuria 2.5 g/day), NPSLE (acute confusional state), erythema, fatigue
48/M	4Y	PSL 0.4 mg/kg,CS-pulse, IV-CY, AZ	Cutaneous vasculitis, arthritis, aseptic necrosis, fatigue, erythema, livedo

SLE, systemic lupus erythematosus; Y, years; M, months; PSL, prednisolone; CS, corticosteroid; CsA, cyclosporin A; IV-CY, intravenous pulse therapy of cyclophosphamide; MZ, mizoribine; NPSLE, neuropsychiatric SLE; MTX, methotrexate; AZ, azathioprine; IA, immunoabsorption; PE, plasma exchange; TTP, thrombotic thrombocytopenic purpura; TL, tacrolimus
[a]This patient withdrew from the study at 2 months

Table 2. Summary of British Isles Lupus Assessment Group (BILAG) global index before and after the treatment with rituximab

Years/sex	40/F	34/F	30/F	55/F	21F	21/F	27/F	20/F	20/F	38/F	46/F	29/F	29/F	48/M
General	B–D	C–D	B–D	C–D	B–B	D–D	B–C	D–D	D–D	C–C	D–D	A–A	C–D	C–C
Mucocutaneous	B–C	D–D	B–B	B–D	C–C	B–B	B–D	C–D	B–D	B–B	C–D	B–B	B–B	B–B
Nervous system	E–E	C–C	A–C	E–E	A–C	E–E	E–E	A–C	D–D	B–C	E–E	E–E	B–C	D–D
Musculoskeletal	D–D	C–C	C–D	E–E	D–D	C–D	C–C	C–D	E–E	B–D	E–E	B–B	D–B	C–C
Cardiovascular/ respiratory	E–E	E–E	E–E	C–C	E–E	E–E	E–E	C–D	E–C	D–D	A–A	E–E	E–E	E–E
Vasculitis	C–D	D–D	D–D	E–E	C–C	E–E	E–E	C–D	E–E	D–D	B–B	E–E	E–E	B–B
Renal	B–D	A–C	E–E	E–E	D–D	D–D	E–E	E–E	A–B	B–B	B–B	E–E	B–B	C–C
Hematological	D–D	C–C	C–C	B–B	D–D	A–C	C–B	D–B	D–D	B–C	E–C	D–B	D–D	C–D
TOTAL	10–1	13–4	17–5	8–4	14–6	13–4	8–5	13–4	12–4	16–9	16–16	15–18	10–10	10–9
Estimation	MCR	MCR	PCR	PCR	PCR	PCR	PCR	PCR	PCR	NCR	NCR	NCR	NCR	NCR

BILAG scores were determined before and at 28 weeks after the rituximab treatment in each patient
MCR, major clinical response; PCR, partial clinical response; NCR, nonclinical response

and all patients had B-level disease activity in at least two body systems. A-level disease occurred in the nervous (3), renal (2), general (1), cardiovascular/respiratory (1), and hematological (1) body system.

Pharmacokinetics and laboratory data

Serum samples for analysis of pharmacokinetics of rituximab and HACA by ELISA assay were collected from all 15 patients. Rituximab serum levels were variable but the range of serum levels attained approximately 200–500 ng/ml and 350–650 ng/ml, by 4 infusions of 500 mg/body every week and 2 infusions of 1000 mg every other week, respectively (Fig. 1). Rituximab levels were detected in 11 of 15 patients through at least 16 weeks after the first administration and were still detectable in 4 of 15 patients evaluated at 28 weeks. Figure 1 shows the individual measurements over time. Four of 15 patients developed detected levels of serum HACA during the follow-up period, just after serum rituximab levels became undetectable (limit of quantification 500 ng/ml). The titers of HACA were low (72 ng/ml at day 168, 287 ng/ml at day 56, 173 ng/ml at day 196, and 114 ng/ml at day 112), but they were gradually decreased as follow-up continued and HACA gave no evidence of clinical immunogenicity. Peripheral CD19- and CD20-positive B cells were rapidly reduced and were depleted within 28 days and 14 days, respectively, in all 15 patients with a similar tendency, which was discrepant from the American study reported by Looney et al.[11] By 28 weeks, B-cell counts recovered to less than 50% of baseline (Fig. 1). In contrast, serum levels of IgG, IgA (data not shown), and IgM did not decrease significantly following treatment (Fig. 2). Also, some patients had decreased values of anti-dsDNA IgG or IgM after the treatment, but any consistent pattern of decrease/increase of their levels was not observed. The levels of serum C3 or C4 and complement activity measured by CH50 had a tendency of increment after the rituximab treatment, but there were no significant differences (Fig. 2).

Safety

During or following treatment, two patients had post-treatment increases from grade 0 to grade 3 in NCI-CTC version 2.0 toxicity grades (Table 3). One was manifest as pneumonia and the other was enteritis infection, which were easily treated with intravenous application of antibiotics. Pneumonia occurred with low-grade fever and consolidation in left lung by X-ray at 28 weeks, and was cured by the intravenous administration of pazufloxacin mesilate for

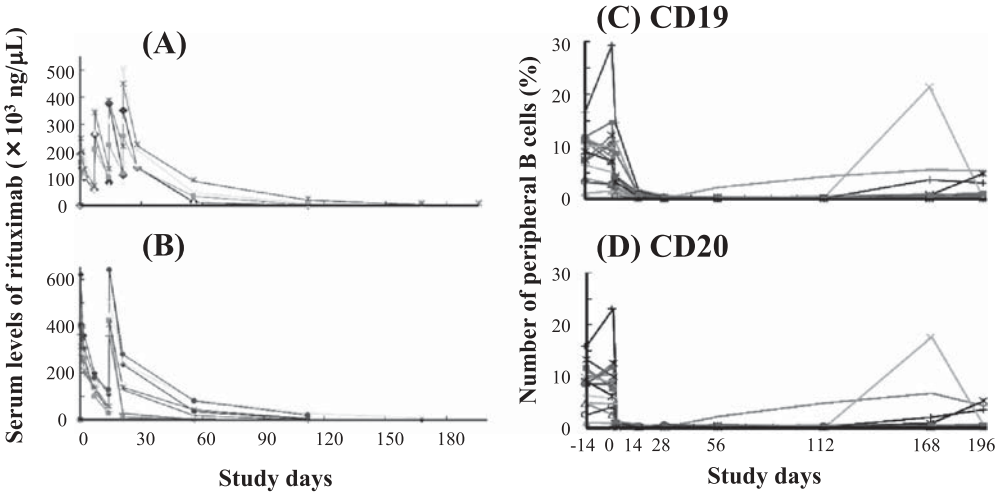

Fig. 1A–D. Serum levels of rituximab and peripheral B-cell levels after the initial administration in individual patients. Serum concentration of rituximab was detected by enzyme-linked immunosorbent assay in five systemic lupus erythematosus (SLE) patients who obtained 4 infusions of 500 mg/body every week (**A**) and 10 SLE patients who obtained two infusions of 1000 mg/body every other week (**B**) during the study. The percentage of peripheral CD19-positive B cells (**C**) and CD20-positive B cells (**D**) in individual patients was assessed at each time point using a flow cytometer

Table 3. Adverse events of the treatment with rituximab

Grade	Adverse events
Grade 1 ($n = 16$)	Infection (5), redness of foot pad (1), flush (1), muscle weakness of hands (1), tremor (1), tachycardia (1), edema of feet (1), subcutaneous nodule (1), hypertension (1), headache (1), sore throat (1), paresthesia (1)
Grade 2 ($n = 27$)	Infections (21)[a], diarrhea (1), skin eruption (1), muscular pain of lower limbs (1), abdominal pain (1), keratosis of foot pad (1), sleeping disturbance (1)
Grade 3 ($n = 3$)	Infections (3)[b]

Shown are adverse events observed during or following treatment graded by NCI-CTC version 2.0 toxicity grades. Parentheses contain number of events

[a] Oral antibiotics and intravenous [b] infusion of antibiotics were used for the treatment of infections

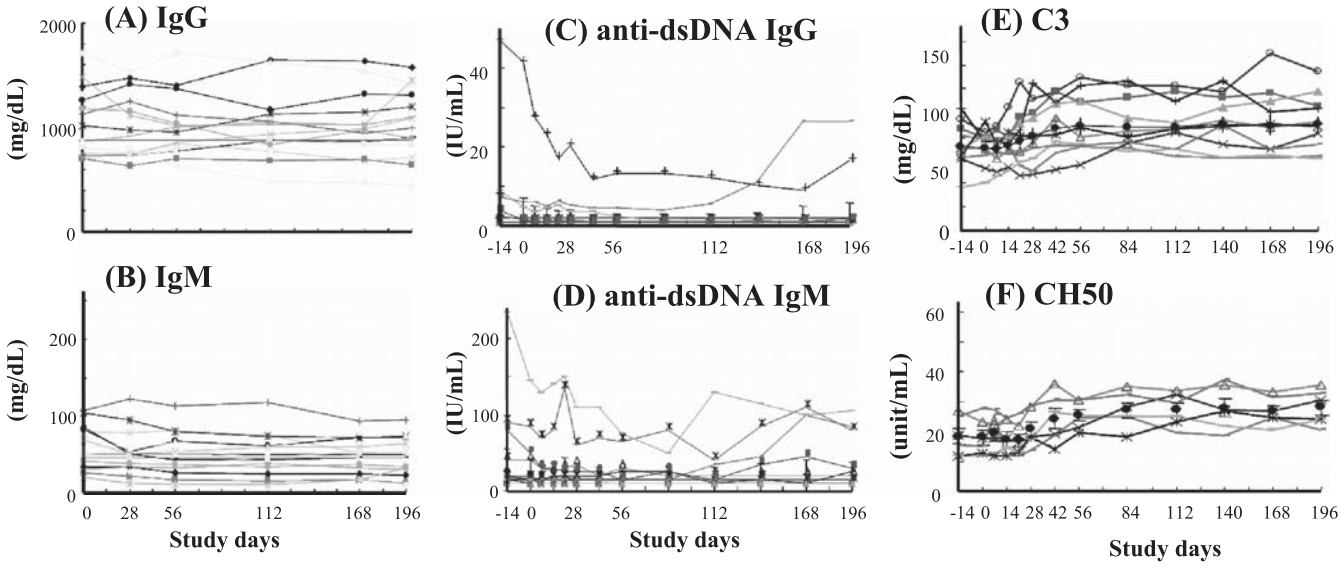

Fig. 2A–F. Changes of serum Igs and complements in individual patients. Serum levels of IgG (**A**), IgM (**B**), anti-double-stranded (ds) DNA IgG (**C**), anti-dsDNA IgM (**D**), and C3 (**E**) and CH50 activity (**F**) were assessed in individual patients during each time point

Table 4. Changes of BILAG score before and after the treatment with rituximab

	Number of patients with BILAG A and B level activities			
	Before	4 weeks	16 weeks	28 weeks
General	5	3	2	2
Mucocutaneous	10	9	8	6
Nervous	5	0	0	0
Musculoskeletal	2	2	1	2
Cardiovascular/respiratory	1	1	1	1
Vasculitis	2	2	2	2
Renal	6	5	4	4
Hematological	3	6	5	4

Shown are number of patients with BILAG A and/or B activities before and at 4, 16 and 28 weeks after the rituximab treatment

about 1 week. Enteritis was observed at 8 weeks and was improved by the use of pazufloxacin mesilate for 10 days. Causative bacteria were not identified in these two patients. Among 27 adverse events at a grade 2 level, 21 infections were reported in 6 patients, including upper respiratory infection (4), cystitis (3), bronchitis (2), urinary tract infection (2), and others that ware easily treated with oral antibiotics. Only one patient had mild and transient edema as infusion reaction, which was repeatedly observed after the treatment. Standard safety laboratory tests showed no consistent pattern of change from baseline. Also, significant difference in terms of safety profiles, depletion of peripheral B cells, the appearance of HACA, and changes of serum levels of Igs was not observed among patients treated with 4 infusions of 500 mg and those with 2 infusions of 1000 mg.

Clinical efficacy

The effect of rituximab on clinical manifestations was evaluated during the study period using numerical total BILAG scores and categorical scores (Table 4). In terms of changes in the total BILAG score, a statistically significant improvement was observed at 4, 16, and 28 weeks, compared to baseline score in 14 patients who accomplished the study (12.5 ± 3.0 points at study entry, 7.1 ± 4.9 at 28 weeks, $P < 0.001$ by Student's *t*-test) (Table 2). Among 14 patients, 2 satisfied MCR and 7 satisfied PCR criteria, according to achievement of BILAG scores defined as above and, therefore, 9 of 14 patients (64.3%) were categorized as MCR or PCR at week 28. However, 5 of 14 patients (35.7%) were grouped as NCR (Table 2). The compositions of A- and/or B-level activities improved after treatment, primarily in the general, mucocutaneous, nervous, and renal systems (Tables 2–4). It is noteworthy that all five patients who had A- or B-level disease in the nervous system rapidly improved to C-level activity within 4 weeks after the initiation of rituximab treatment. As described, serum titers of anti-dsDNA antibodies did not change significantly during the study. An additional indirect measure of clinical efficacy is the ability to taper the CS dosage. At study entry, all 14 patients received ≥30.4 mg/kg per day of CS (0.50 ± 0.14 mg/

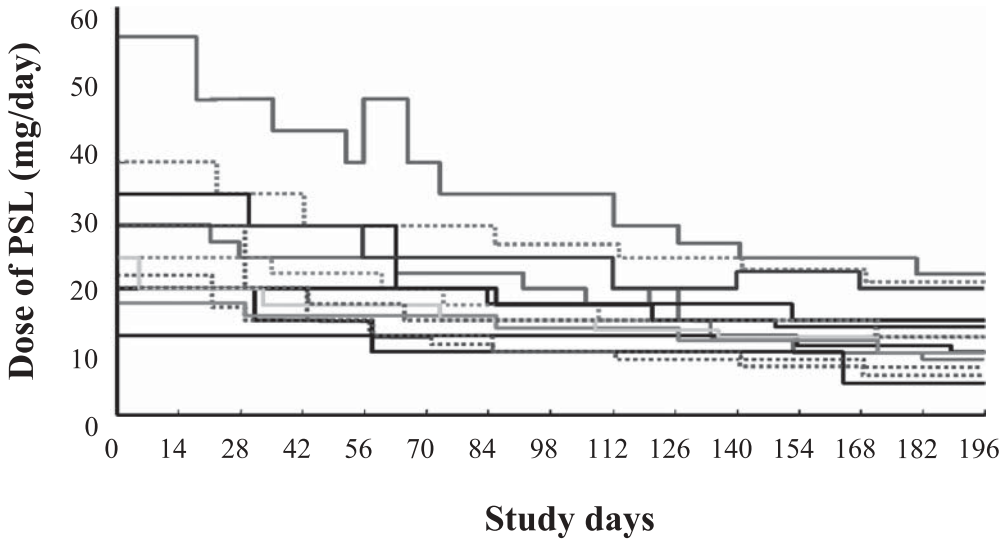

Fig. 3. Changes of required corticosteroid in each patients after the treatment with rituximab. Dose of corticosteroid, converted in oral prednisolone (mg/day), required for individual patients were plotted during each time point after the treatment with rituximab

kg per day), but after the rituximab treatment the dosage of CS was gradually decreased in individual patients (0.24 ± 0.10 mg/kg per day at 28 weeks, $P < 0.001$ compared to that at the study entry) and none of them had dose escalation at 28 weeks (Fig. 3).

Discussion

This multicenter phase I/II trial of rituximab in patients with moderately to highly active SLE showed profound B-cell depletion and holds significant therapeutic promise. Serum levels of rituximab were variable but rapidly attained high levels in all 15 patients. Peripheral CD19- and CD20-positive B cells were rapidly reduced and were depleted within 28 days and 14 days, respectively, in all 15 patients with a similar tendency. These results were discrepant from the American study reported by Looney et al. that B-cell depletion failed in 6 of 17 patients and the failure to deplete B cells was significantly more common in African-Americans.[11] The appearance of HACA has been a major concern of the treatment, but HACA were detected in 4 of 15 patients with low levels (72–287 ng/ml) and gradually decreased during further follow-up. HACA were not associated with infusion reactions, but HACA appeared just after serum rituximab became undetectable, indicating that the disappearance of rituximab resulted in HACA. Also, there was no evidence that HACA changed efficacy of rituximab and increased disease activity in their observed levels. Overall, abnormal changes clearly related to rituximab in routine safety laboratory tests were not observed. Although grade 3 adverse events in NCI-CTC version 2.0 toxicity grades were noted in two patients (pneumonia and enteritis in one patient each), these may have been related to the underlying disease and background treatments. No opportunistic infection, which is often observed in patients treated with biologic agents, was noted, probably because serum levels of IgG, IgA, and IgM remained steady following treatment.

Rituximab was effective for moderately to highly active refractory SLE patients whose total BILAG score ranged from 8 to 17. Nine of 14 patients achieved the major or partial clinical response according to BILAG score and BILAG score decreased by ≥350% in 8 patients at 28 weeks. Actually, the CS dose significantly fell; all patients received ≥30.4 mg/kg per day at entry and in none of them was the dose increased at 28 weeks (0.50 ± 0.14 mg/kg/day at entry, 0.24 ± 0.10 mg/kg/day at 28 weeks; $P < 0.001$ compared to entry). The compositions of A- and/or B-level activities improved after treatment, primarily in the general, mucocutaneous, nervous, and renal systems, but it is noteworthy that all five patients who had A- or B-level disease in the nervous system rapidly improved to C-level activity within 4 weeks after the treatment. In our pilot study, we reported that 10 patients with NPSLE improved, sometimes very rapidly, from acute confusional state and that improvement was also documented by MRI imaging.[14–16] Although the precise mechanism remains unclear, it can be emphasized that efficacy for NPSLE is a probable benefit of rituximab treatment.

The underlying mechanism of the efficacy of rituximab for long-term remission remains unknown. Although it takes about 2–4 weeks for rituximab to completely deplete B cells from the periphery, NPSLE was rapidly improved within 1 week in some patients. We found that the expression of the costimulatory molecules CD40 and CD80 on B cells was rapidly reduced after rituximab treatment in most of our SLE patients.[15,16] Furthermore, the expression of CD40L, a ligand for CD40, was also down-regulated on $CD4^+$ T cells, suggesting that effects on costimulatory molecules might also play a role.[16,20] On the other hand, Anolik et al. reported that rituximab improved abnormalities in B-cell homeostasis, with a decreased proportion of autoreactive memory B cells after treatment.[21,22] Together with several reports, reconstitution of autoreactive B-cell lineage could be induced following B-cell depletion by rituximab.[20–25] Therefore, rituximab-induced depletion of memory B cells could also prevent the activation of autoreactive T cells through interactions with B cells, resulting in

the down-regulation of CD40L on $CD4^+$ T cells. Thus, rituximab may improve the disease course of SLE by partially resetting the autoimmune responses.

Taken together, anti-CD20 rituximab therapy appears to be safe for the treatment of active and refractory SLE patients and holds significant therapeutic promise. Thus, therapeutic B-cell depletion has not only brought a promising treatment a step closer to the clinic but has also provided an opportunity to learn more about the biology of B cells and their roles in the pathogenesis of SLE and other autoimmune diseases. Based on these results, the next phase of a double-blind controlled trial of rituximab would be warranted.

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