Phenotypic Changes of Lymphocytes in Patients with Systemic Lupus Erythematosus Who Are in Longterm Remission After B Cell Depletion Therapy with Rituximab

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ABSTRACT. ***Objective.*** Rituximab has recently emerged as a novel treatment strategy for systemic lupus erythematosus (SLE). We investigated longitudinally the differentiation and phenotypic changes of peripheral B cells and T cells in patients with SLE after rituximab treatment.

Methods. Phenotypic changes on B cells and T cells in 10 patients with SLE treated with rituximab were analyzed before, 28 days after, and 2 years after rituximab treatment, and at relapse.

Results. Rituximab rapidly depleted naive and memory B cells from the peripheral blood. In the patients with prolonged remission, the memory B cells remained depleted while naive B cells recovered within 3–9 months, and the expression levels of CD40 and CD80 remained downregulated for 2 years. There was also a decrease of memory T cells relative to naive T cells, and the expression of CD40L and inducible costimulator (ICOS) on CD4-positive T cells rapidly decreased and remained downregulated for 2 years. In 1 patient, an increase in the number of memory B cells with upregulation of CD40 and CD80 expression was noted just before relapse. In another patient with relapse, however, recovery of CD4-positive memory T cells with upregulation of ICOS expression was noted, with no change in the number of memory B cells.

Conclusion. Our results suggest that the phenotypic changes of peripheral B cells result in inhibition of T cell differentiation and activation mediated by B cells and thereby bring about longterm remission of SLE. Activated memory B cells or ICOS-positive CD4-positive memory T cells reappeared in association with relapse, probably reflecting the heterogeneity of SLE. (First Release Dec 15 2010; J Rheumatol 2011;38:633–41; doi:10.3899/jrheum.100729)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS RITUXIMAB B CELL DEPLETION THERAPY

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease induced by activation of autoreactive T cells and overproduction of autoantibodies by B cells. Rituximab is a human-mouse chimeric monoclonal antibody that targets the CD20 antigen, a B cell-specific antigen, and causes depletion of CD20-expressing pre-B to mature B cells. Rituximab has recently been reported to show a rapid onset of effect and prolonged efficacy in patients with refractory SLE[1,2,3,4,5,6,7,8], emerging as a promising new agent for the treatment of this disease. In regard to the mechanism underlying the longterm remission of SLE induced by rituximab, Anolik, *et al*[9] reported that the drug caused peripheral blood depletion of memory B cells[10,11] and plasma cells[12] that play important roles in the pathogenesis of SLE, as well as a depletion of memory B cells from the secondary lymphoid tissue[13].

It has been reported that not only activated B cells, but also T cells and dendrocytes are involved in the pathogenesis of SLE in humans[14,15,16,17]. Treatment with rituximab has been demonstrated to produce an increase in the number of CD4+CD25^{bright}Foxp3+ regulatory T cells in the peripheral blood during the recovery phase from SLE or an increase in the messenger RNA expression of Foxp3 at 1 to

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3 months after treatment[18,19]. We have also shown that rituximab may downregulate the expression of CD40L, a costimulatory molecule expressed on T cells, in patients with SLE[7]. To our knowledge, however, there are no reported comprehensive studies that have investigated the effects of rituximab on the differentiation of T and B cells or on the expression of costimulatory molecules on these cells. The precise mechanisms underlying the longterm remission of SLE induced by rituximab and the lymphocyte subsets involved in the pathogenesis of SLE remain unknown.

We investigated longitudinally the pattern of B cell and T cell differentiation and the changes in the expression levels of costimulatory molecules on these cells in rituximab-treated patients with SLE showing longterm remission and relapse, in order to determine the mechanisms underlying both the longterm remission of SLE induced by rituximab and the lymphocyte subsets involved in the pathogenesis of SLE.

MATERIALS AND METHODS

Patients. Our cohort study involved 10 patients diagnosed with SLE based on their fulfilling at least 4 of the 11 modified American College of Rheumatology (ACR) criteria for the diagnosis of SLE[20]. Table 1 shows the background variables of the patients before they started rituximab treatment. The subjects were 9 women and 1 man, with a mean age of 27.4 ± 8.8 years (range 16–41 yrs). The mean duration of illness from SLE diagnosis to administration of rituximab was 96.7 ± 113.3 months (range 3–324 mo). The mean steroid dose prior to the start of rituximab treatment was 35.0 ± 16.8 mg/day. Despite receiving conventional treatments such as pulse steroid therapy, intermittent intravenous cyclophosphamide pulse therapy (IVCY), cyclosporine A, mizoribine, azathioprine, mycophenolate, plasma exchange, and immunoadsorption therapy, all the patients had highly active disease, with a mean SLE Disease Activity Index (SLEDAI) of 16.2 ± 9.6 and a British Isles Lupus Assessment Group (BILAG) activity index of 19.8 ± 8.2 (all patients falling in the BILAG score category) at the start of rituximab treatment. The organ involvement was as follows: lupus nephritis in 7 patients [World Health Organization (WHO) type I in 1 patient and type IV in 6 patients], neuropsychiatric SLE in 5 patients, and thrombotic thrombocytopenic purpura in 1 patient. All patients were treated with rituximab at our facility between 2004 and 2009, and all completed the course of the anti-CD20 antibody treatment protocol formulated for our study. Written informed consent was obtained from each patient in accord with the requirement of the study protocol approved by the ethics committee of our university.

Treatment schedule. Rituximab was administered twice, with a 1-week interval between administrations, at a dose of 375 mg/m^2 in all patients.

Assessment. Laboratory measurements included the serum levels of complements, and the titers of antinuclear antibody, antiribonucleoprotein antibody, anti-SSA antibody, anti-SSB antibody, anti-Sm antibody, and anti-dsDNA antibody. To assess the activity of SLE, the BILAG index[21] and SLEDAI[22] were calculated. Disease activity was scored on a 5-category scale by the BILAG index: A (severely active), B (moderately active), C (stable mild disease), D, and E. Responses to rituximab were categorized according to the improvement of the BILAG index, as major clinical response (MCR), partial clinical response (PCR), and no clinical response (NCR). More specifically, MCR was defined as improvement of the BILAG index to C or better at 2 years, PCR as improvement of the BILAG index to B in at least 1 domain at 2 years, and NCR as failure to meet the definition of either MCR or PCR[5].

Flow cytometry. Analysis of the B cell and T cell phenotypes and expression of the surface molecules on these cells was carried out by flow cytometry before, 28 days after, and 2 years after rituximab treatment, as well as at the time of relapse. Mononucleated cells were isolated from the peripheral blood, and were treated with the following antibodies:

Table 1. Characteristics of the study participants with systemic lupus erythematosus.

Patient	Age, yrs/ Sex	Disease Duration, mo	CS Dose, mg	Treatments Prior to RTX	Organ Involvement	Anti-dsDNA antibody, IU/ml	ENA	C3/C4/CH50	ANA	CS Dose at 2 yrs	SLEDAI Day 0 to 2 yrs	BILAG Day 0 to 2 yrs	Clinical Response at 2 yrs
1	39 F	144	22.5	IVCY, CsA	nephritis (II)	123.2	Ro, Sm, RNP	52/< 5/26	2560	5	10→0	15→0	MCR
2	21 F	84	12.5	IVCY, MTX	CNS	1.4	Ro, RNP	105/19/49	320	6	3→0	15→1	MCR
3	20 F	10	25	IVCY	CNS	< 1.0	—	119/24/53	< 40	10	10→0	13→0	MCR
4	41 F	324	60	IVCY, PE	CNS, TTP nephritis (IV)	500.6	Sm, RNP	28/< 5/13	10240	9.5	20→0	28→0	MCR
5	32 F	252	60	IVCY	CNS nephritis (IV)	8.4	—	56/12/27	< 40	2	28→2	25→0	MCR
6	29 F	6	30	IVCY	lymphadenopathy	5.4	—	114/18/31	320	7.5	9→17*	12→21*	NCR*
7	17 M	3	30	MMF	nephritis (IV)	6.3	—	103/26/55	< 40	5	18→2	13→0	MCR
8	32 F	108	50	IVCY, CsA, AZA, PE	nephritis (IV)	52.3	Ro, Sm, RNP	90/15/39	640	7	16→0	17→0	MCR
9	16 F	30	20	ICVY, MZ, CsA	nephritis (IV) CNS	610.7	Ro	54/8/25	320	12.5	13→10*	23→13*	NCR*
10	27 F	6	40	IVCY, PE IA	nephritis (IV)	242.1	—	35/< 5/9	1280	2.5	35→4	37→3	MCR

* At relapse. RTX: rituximab; CNS: central nervous system; ANA: antinuclear antibody; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; BILAG: British Isles Lupus Assessment Group Activity Index; CS: prednisolone (or equivalent); CY: cyclophosphamide; IVCY: intravenous cyclophosphamide pulse therapy; CsA: cyclosporin; MZ: mizoribine; PE: plasma exchange; IA: immune adsorption; AZA: azathioprine; MMF: mycophenolate mofetil; TTP: thrombotic thrombocytopenic purpura; ENA: extractable nuclear antigen; AIHA: autoimmune hemolytic anemia; MCR: major clinical response; NCR: no clinical response.

FITC-labeled mouse $IgG_1\kappa$, FITC-conjugated anti-CD40, FITC-conjugated anti-CD80, FITC-conjugated anti-CD69, FITC-conjugated anti-CD45RA, phycoerythrin (PE)-conjugated anti-IgD, PE-conjugated anti-CD45RO, PE-conjugated anti-inducible costimulator (ICOS), PE-Cy5-conjugated anti-CD4, PE-Cy7-conjugated anti-CD19 (Pharmingen, San Diego, CA, USA), FITC-conjugated anti-CD40L (Ancell, Bayport, MN, USA), and allophycocyanin-conjugated anti-CD27 (BioLegend, San Diego, CA, USA). They were then incubated 30 minutes at 4°C, and the cells were washed 3 times with FACS solution and analyzed using the FACSCalibur (Becton-Dickinson, San Jose, CA, USA) and FlowJo software (Digital Biology, Tokyo, Japan). The numbers of CD40 and CD80 molecules expressed per CD19-positive cell were counted using QIFIKIT Beads (Dako Japan, Kyoto, Japan).

Statistical analysis. The results were analyzed using SPSS version 16. The statistical significance of differences between the pretreatment and posttreatment values was tested by Wilcoxon's test. P values < 0.05 were considered statistically significant.

RESULTS

Changes in levels of B cell and T cell surface antigens following rituximab treatment. We analyzed longitudinally the pattern of B cell and T cell differentiation and the changes in the expression levels of the costimulatory molecules on these cells in the longterm responders to rituximab therapy. Figures 1A and 1B show a representative patient with longterm remission after the treatment (Patient 7). Rituximab treatment resulted in a disappearance of CD19+IgD+CD27– naive B cells, CD19+IgD–CD27– memory B cells, and CD19+IgD–CD27+ class-switched memory cells from the peripheral blood within 28 days after treatment. On the other hand, $CD19^{low}CD27^{high}$ or IgD–CD38+ plasma cells persisted in the peripheral blood of these patients until Day 28, although these cells also disappeared completely from the peripheral blood by 6 months after rituximab treatment. The naive B cells recovered within 3 to 9 months after treatment, while the memory B cells and plasma cells remained depleted for 2 years. The similar changes of B cell phenotype were observed in all of the 8 patients with longterm remission of SLE (Figure 1C, 1D). The ritux-

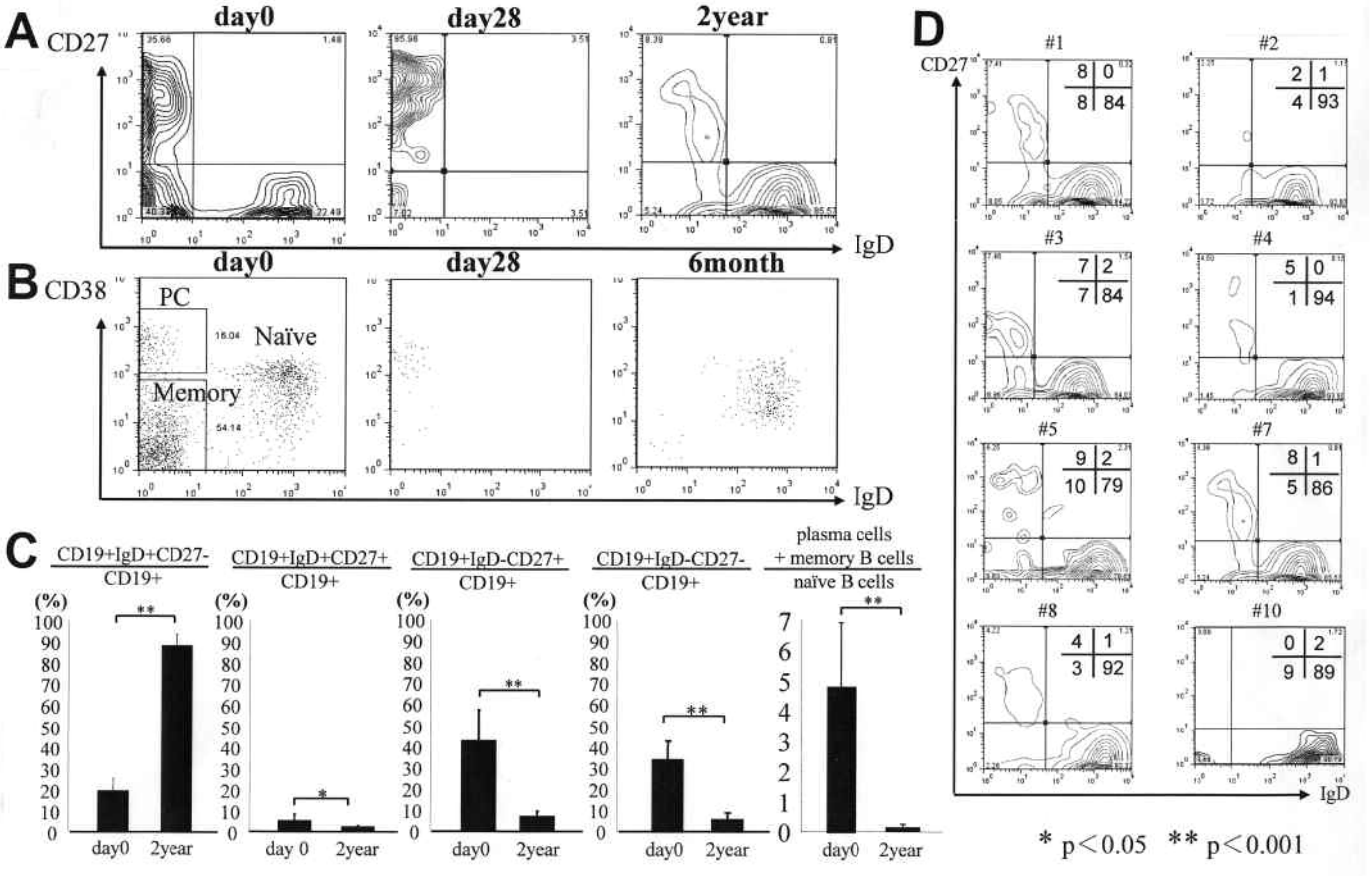

Figure 1. Changes in CD19-positive cell subsets in cases of SLE showing prolonged remission and major clinical response following rituximab therapy. (A) B cell subsets immediately before, 28 days after, and 2 years after rituximab treatment in Patient 7 (a representative case following a typical course). Left upper quadrant identified plasma cells (CD27++) and class-switched memory B cells (CD27+). Right upper quadrant identified IgM memory B cells. Left lower quadrant identified double-negative memory B cells. Right lower quadrant identified naive B cells. (B) B cell subsets and plasma cells immediately before, 28 days after, and 6 months after rituximab treatment in Patient 7 (a case following a typical course). IgD–CD38+ cells show plasma cells. IgD–CD38– cells show memory B cells. IgD+CD38+ cells show naive B cells. (C) Changes in percentages of CD19+IgD+CD27– naive B cells, CD19+IgD+CD27+ IgM memory B cells, CD19+IgD–CD27+ class-switched memory B cells, CD19+IgD–CD27– (double-negative) memory B cells among the CD19-positive cells and the ratio of plasma cells plus memory B cells to naive B cells immediately before and 2 years after rituximab treatment in 8 patients with prolonged remission. (D) CD19-positive cell subset in 8 patients at 2 years.

imab therapy resulted, by 2 years after treatment, in a significant fall in the percentage of CD19+IgD+CD27+ memory B cells (3.0 ± 1.8% to 1.2 ± 0.7%, p < 0.05; 1.0 ± 0.8 cells/μl to 0.9 ± 0.9 cells/μl, p = 0.8203), CD19+IgD–CD27+ class-switched memory B cells (43.5 ± 10.0% to 5.4 ± 3.2%, p < 0.001; 22.8 ± 24.4 cells/μl to 6.7 ± 5.9 cells/μl, p = 0.1187), and CD19+IgD– CD27– memory B cells (34.7 ± 8.8% to 5.9 ± 3.2%, p < 0.001; 14.7 ± 11.0 cells/μl to 5.8 ± 5.9 cells/μl, p = 0.1096); and a significant reduction of the plasma cells and memory B cells/naive B cells ratio (4.80 ± 2.12 to 0.15 ± 0.07, p < 0.001; 4.8 ± 2.1 to 0.1 ± 0.2, p < 0.001).

Next, we assessed the effects of rituximab treatment on the expression levels of costimulatory molecules on CD19-positive cells. Figure 2A shows the changes of phenotypes in a representative case and similar trends were noted in all of the 8 patients with SLE who showed longterm remission following rituximab therapy (Figure 2B). At 2 years after the initial infusion, no significant change from the baseline level of the percentage and the number of CD40-expressing cells among the CD19-positive cells was observed (88.8 ± 10.6% to 76.4 ± 16.0%, p = 0.0742; 78.5 ± 69.1 cells/μl to 80.1 ± 54.5 cells/μl, p = 0.5212), even though the number of CD40 molecules per CD19-positive cell had fallen significantly within 28 days after the treatment (from 1957.8 ± 769.6 to 1200.9 ± 120.2 molecules/cell, p = 0.0357). In contrast to CD40, a significant reduction from baseline levels remained at 2 years in the percentage and number of CD80-expressing cells among the CD19-positive cells (55.8 ± 27.3% to 10.0 ± 5.4%, p = 0.0008; 46.8 ± 29.0 cells/μl to 8.9 ± 9.3 cells/μl, p = 0.0042) and in the number of CD80 molecules per CD19-positive cell (1657.2 ± 1936.1 to 158.4 ± 88.4 molecules/cell, p = 0.0016), indicating the reduction of memory B cells, since CD80 is expressed only on memory B cells.

On the other hand, the percentage of CD4+CD45RO^{bright} memory T cells in the peripheral blood was high before the start of rituximab therapy, and a similar trend was observed until Day 28 in a representative case of Patient 4 (Figure 3A), and similar trends were observed among 8 patients

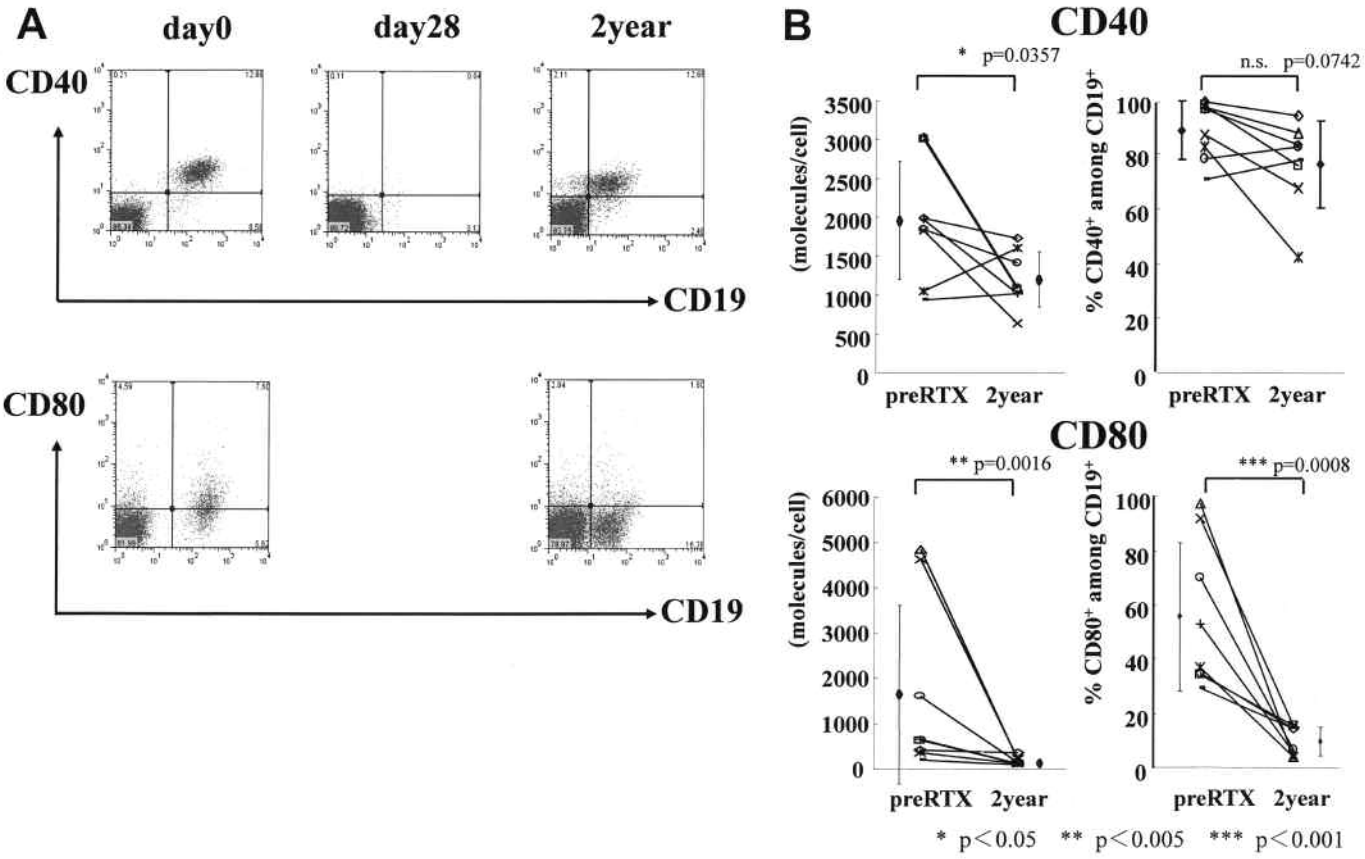

Figure 2. Sequential changes in levels of CD40 and CD80, costimulatory molecules expressed on CD19-positive cells, in patients with SLE who have prolonged remission and show major clinical response following rituximab treatment. (A) Course from immediately before rituximab treatment to 28 days after and 2 years after treatment in representative Patient 7, who followed a typical course (x axis, CD19; y axis, CD40 in the upper panel, CD80 in the lower panel). (B) Changes in the number of CD40 and CD80 molecules per CD19-positive cell (left, using QIFIKIT Beads) and the percentage of CD40-positive and CD80-positive cells among the CD19-positive cells (right) before and 2 years after rituximab treatment in the 8 patients with prolonged remission. RTX: rituximab; n.s.: nonsignificant.

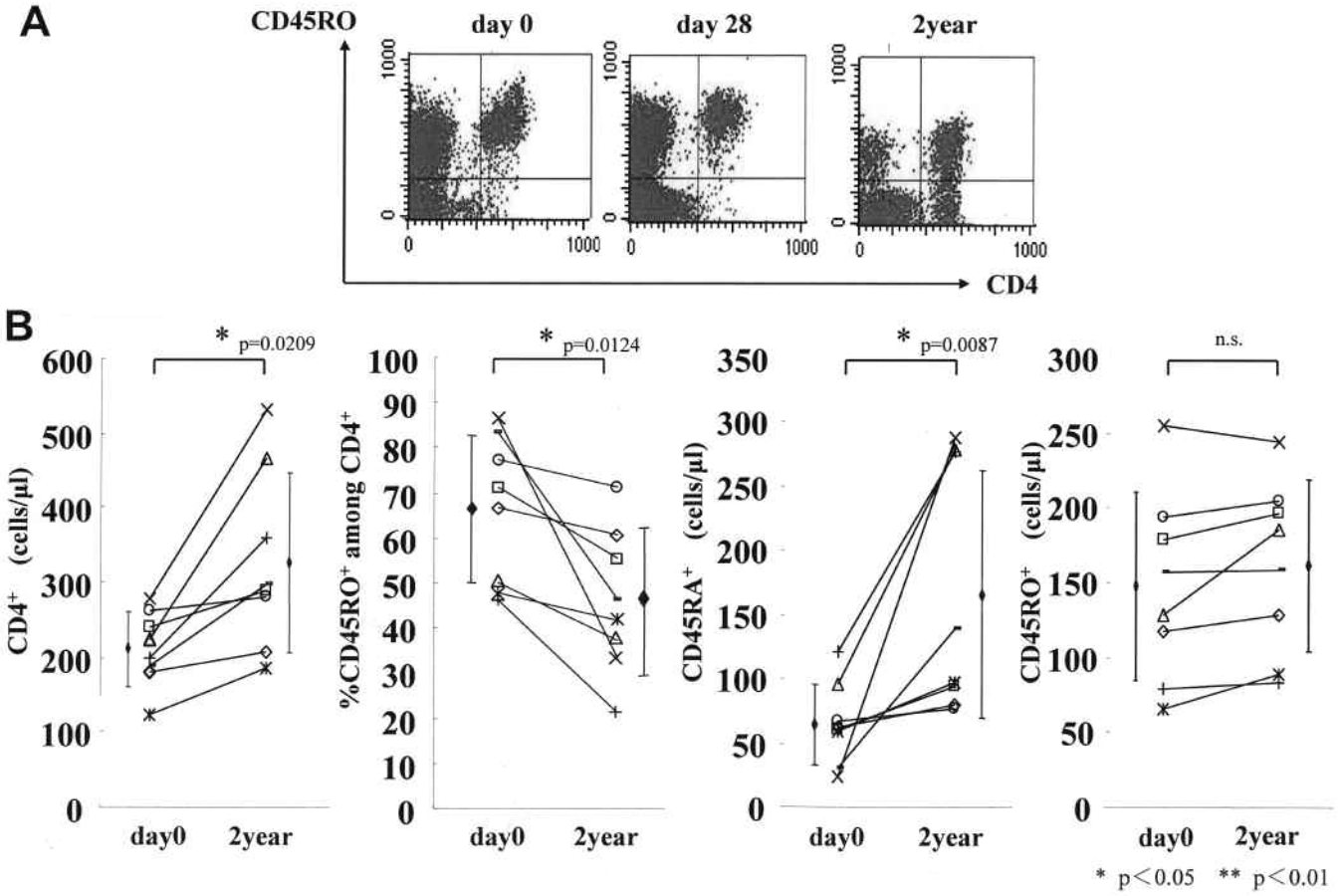

Figure 3. Changes in T cell subsets in patients with SLE with long-sustained remission and major clinical response following rituximab treatment. (A) Course of a representative Patient 4 (x axis, CD4; y axis, CD45RO). The percentage of CD4+CD45RO^{bright} memory cells was high before rituximab treatment and remained high until Day 28 after treatment. After 2 years, however, the level of this costimulatory molecule decreased (CD45RO^{bright} to CD45RO^{intermediate}), along with an increase in the number of CD45RO-negative naive T cells. (B) Changes from baseline to 2 years after treatment in the 8 patients with prolonged remission. From left to right, changes in the actual number of CD4-positive cells, percentage of CD45RO-positive cells among the CD4-positive cells, actual number of CD4+CD45RA+ cells, and the actual number of CD4+CD45RO+ cells are shown.

treated with rituximab (Figure 3B). At 2 years after initial infusion, a significant reduction was observed in the percentage of memory T cells among the CD4-positive T cells (66.2 ± 16.2% to 45.9 ± 16.2%, p = 0.0124) and a significant increase in the number of naive T cells (64.6 ± 31.6 to 165.8 ± 97.2 cells/μl, p = 0.0087). Further, the expression of CD69, an activation marker expressed on CD4-positive cells, and of the costimulatory molecules CD40L and inducible costimulator decreased rapidly by Day 28 in a representative patient (Figure 4A). Expression of these molecules remained reduced for 2 years in 8 patients treated with rituximab (CD69, 17.9 ± 18.7% to 3.8 ± 5.7%, p = 0.0117; 30.5 ± 34.3 cells/μl to 12.1 ± 17.3 cells/μl, p = 0.032; CD40L, 10.0 ± 6.6% to 2.3 ± 1.0%, p = 0.0008; 23.6 ± 21.9 cells/μl to 6.9 ± 2.7 cells/μl, p = 0.0502; and ICOS, 8.7 ± 5.0% to 2.3 ± 1.7%, p = 0.0063; 21.1 ± 10.6 cells/μl to 8.4 ± 7.3 cells/μl, p = 0.0311; Figure 4B).

Changes in expression of lymphocyte surface antigens in patients showing relapse after prolonged remission of SLE. We observed 1 case with B cell-dominant relapse and another with T cell-dominant relapse. The patient with B cell-dominant relapse was a 16-year-old girl (Patient 9) with lupus nephritis (WHO type IV). In this patient, despite intense immunosuppressive therapy, disease activity remained high (SLEDAI 13 and BILAG 23). She achieved remission of SLE after rituximab treatment, with rapid disappearance of the CD19+IgD–CD27– memory B cells and CD19+IgD–CD27+ class-switched memory B cells from the peripheral blood by Day 28, along with rapid reduction in the expression of the costimulatory molecules CD40L and ICOS on the T cells. However, the disease relapsed at 1.5 years after rituximab treatment, and simultaneously the butterfly rash reappeared, the anti-dsDNA antibody titer increased again, and proteinuria recurred. Just before relapse, the percentages of CD19+IgD–CD27– memory B cells and CD19+IgD–CD27+ class-switched memory B

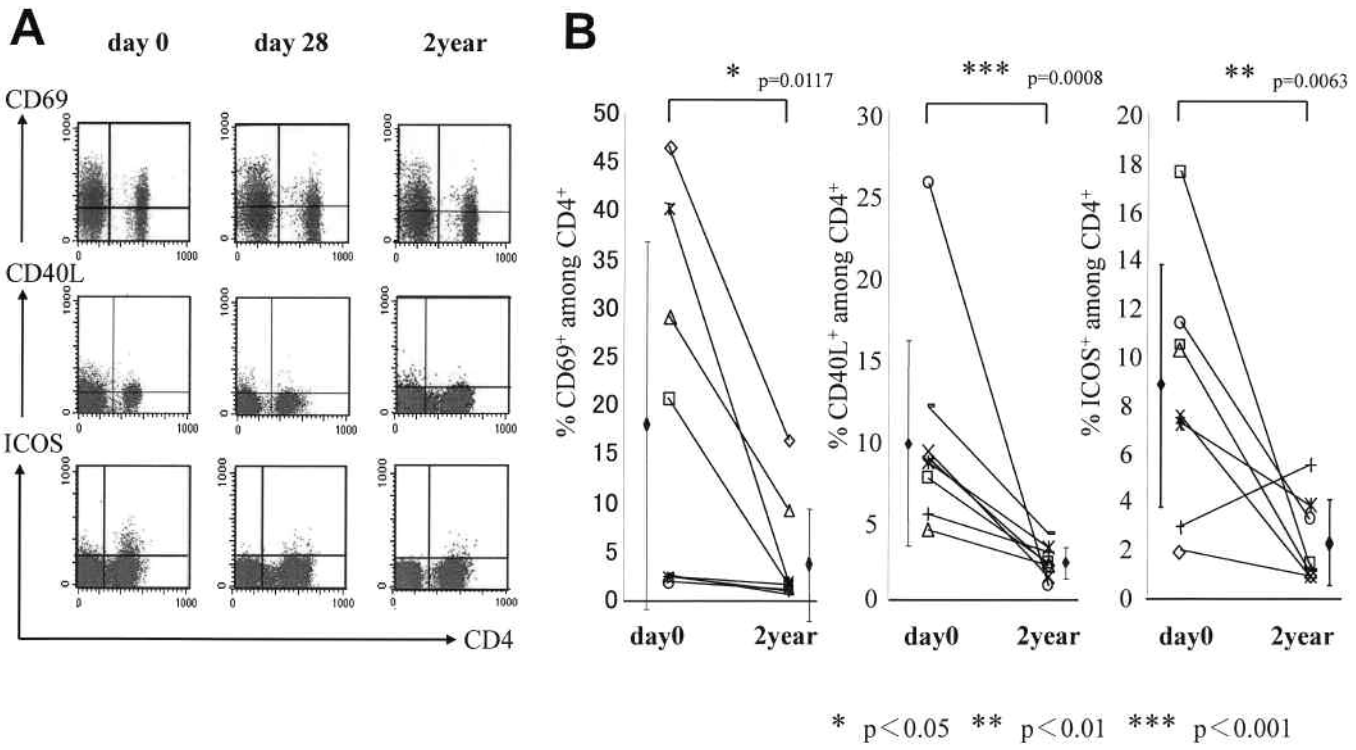

Figure 4. Changes in levels of the T cell activation marker CD69, and of the costimulatory molecules CD40L and inducible costimulator (ICOS) in patients with SLE who have prolonged remission and show major clinical response following rituximab therapy. (A) Cases showing typical courses (x axis, CD4). From top to bottom, changes from baseline to 28 days and 2 years after rituximab treatment in Patient 3 (y axis, CD69), Patient 4 (y axis, CD40L), and Patient 1 (y axis, ICOS). (B) Changes in percentages of CD69-positive cells, CD40L-positive cells, and ICOS-positive cells in the CD4-positive cell population before and 2 years after rituximab treatment in the 8 patients with prolonged remission.

cells, as well as the levels of CD40 and CD80 on the CD19-positive cells, increased, without any significant change in the number of T cells (Figure 5A). This patient was treated again with rituximab. The retreatment resulted in the disappearance again of memory B cells from peripheral blood and a decrease in disease activity. The butterfly rash disappeared, the anti-dsDNA antibody test was negative, and urinary protein excretion and occult blood disappeared.

The patient with T cell-dominant relapse was a 29-year-old woman (Patient 6). Despite intense immunosuppressive therapy, she continued to have central nervous system (CNS) symptoms and a high disease activity level (SLEDAI 9, BILAG 12). The results of tests for anti-dsDNA antibody and anti-Sm antibody were negative. Treatment with rituximab induced remission of SLE along with rapid disappearance of both naive and memory B cells from the peripheral blood. Two years later, however, the disease relapsed and the patient presented with CNS disease manifestations. While no changes in B cells were seen either before or after the relapse, an increase in the population of memory T cells was noted, along with markedly elevated levels of ICOS on CD4-positive cells (Figure 5B). In this patient, disease activity was found to be worse and to involve predominantly T cell abnormalities. Therefore, she was retreated with IVCY, because this drug is considered to be effective against T cells as well. Systemic symptoms, such as fever, malaise, and lymph node swelling, and also the psychiatric symptoms improved with IVCY treatment. In addition, brain perfusion scintigraphy showed an improvement of blood flow, and the level of ICOS on CD4-positive T cells decreased (data not shown).

DISCUSSION

Rituximab has recently been demonstrated to be effective in the treatment of SLE[1,2,3,4,5,6,7,8] and we undertook our study to determine the mechanisms of the longterm remission of SLE induced by rituximab and the relapse after remission. When patients with highly active SLE were treated with rituximab, rapid depletion of CD19+IgD+CD27– naive B cells, CD19+IgD–CD27– memory B cells, and CD19+IgD–CD27+ memory B cells from the peripheral blood was observed, while $CD19^{low}CD27^{high}$ or IgD–CD38+ plasma cells persisted in the blood until Day 28. For patients with clinical remission for about 2 years after rituximab treatment, the plasma cells as well as memory B cells remained depleted or in markedly reduced numbers, although the naive B cells recovered. Analysis of the

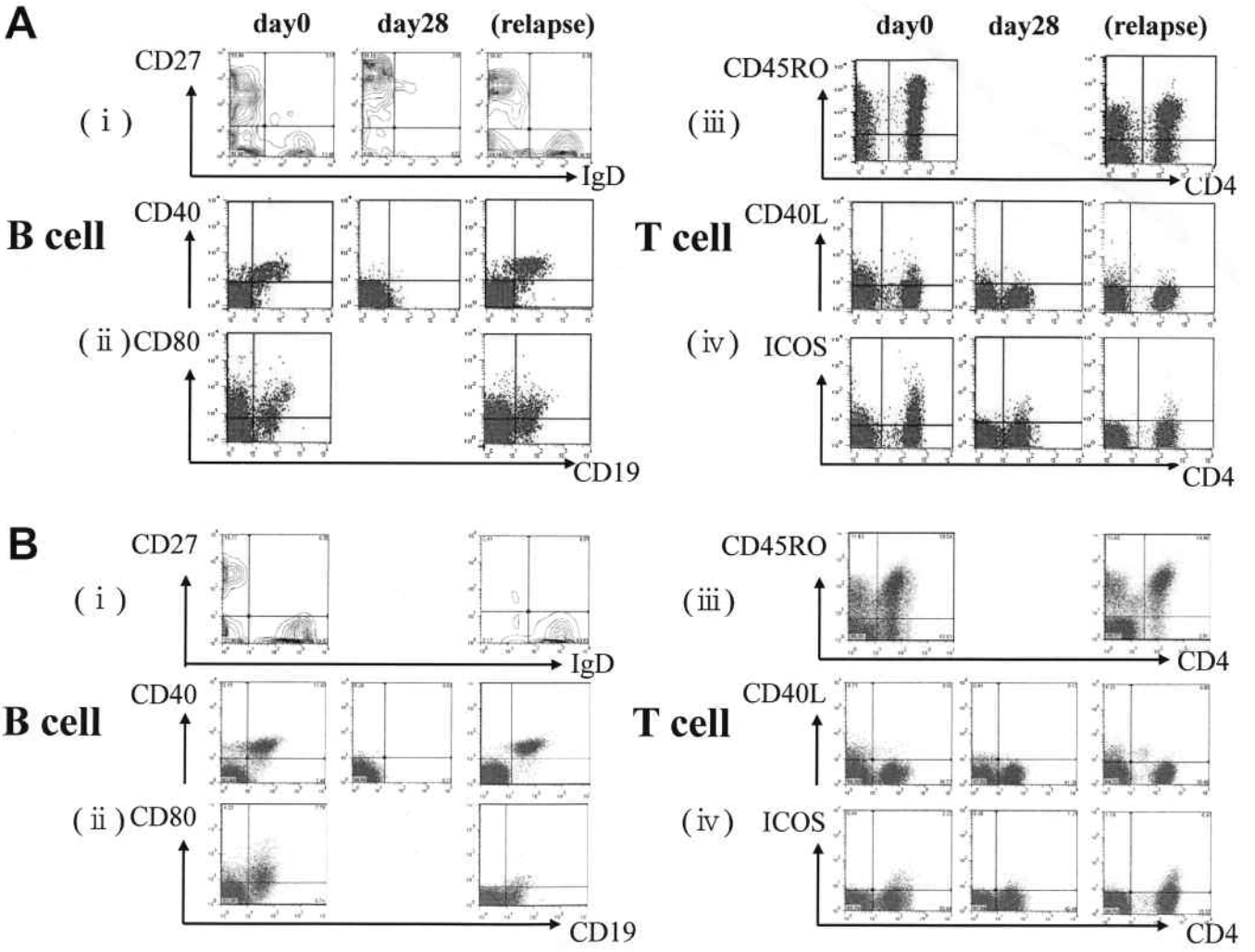

Figure 5. (A) Changes in levels of B cell and T cell surface antigens before and 28 days after treatment, and at the time of SLE relapse, 1.5 years after treatment, in Patient 9, who showed B cell-dominant relapse. (i) Changes in CD19-positive cell subsets (x axis, IgD; y axis, CD27; changes in the numbers of naive B cells, memory B cells, and plasma cells). (ii) Changes in levels of CD40 and CD80, costimulatory molecules expressed on CD19-positive cells. (iii) Changes in the CD4-positive cell subsets (x axis, CD4; y axis, CD45RO; changes in the numbers of naive T cells and memory T cells). (iv) Changes in levels of CD40L and ICOS, costimulatory molecules expressed on CD4-positive cells. (B) Changes in levels of B cell and T cell surface antigens before and 28 days after rituximab treatment, and at the time of relapse, 2 years after treatment, in Patient 6, who showed T cell-dominant relapse. (i) Changes in the CD19-positive cell subsets (x axis, IgD; y axis, CD27; changes in the number of naive B cells, memory B cells, and plasma cells). (ii) Changes in levels of CD40 and CD80. (iii) Changes in the CD4-positive cell subset (x axis, CD4; y axis, CD45RO; changes in numbers of the naive T cells and memory T cells). (iv) Changes in levels of CD40L and ICOS.

changes in the levels of the costimulatory molecules on the B cells revealed that levels of both CD40 and CD80 remained suppressed until 2 years after rituximab therapy.

However, in Patient 9, who showed relapse after prolonged remission, an increase in the percentage of CD19+IgD–CD27– memory B cells and CD19+IgD–CD27+ memory B cells, as well as levels of CD40 and CD80 on these cells, was noted just before the relapse of SLE. Further, in these patients who showed relapse, the anti-dsDNA antibody titers increased, along with development of lupus nephritis as organ involvement, suggesting the correlation between changes in the B cells and the pathophysiology of SLE. Thus, in the patients in whom B cells were successfully depleted by rituximab therapy, the numbers of memory B cells and plasma cells, which express costimulatory molecules, remained suppressed for prolonged periods of time, even though the naive B cells recovered. These findings suggest that reconstitution of the peripheral B cell compartment is crucial for sustaining longterm SLE remission and that recovery of memory B cells expressing costimulatory molecules precedes the SLE relapse.

A significant finding was that rituximab used to produce B cell depletion also affected T cell differentiation and activation. In cases with highly active SLE complicated by

lupus nephritis or CNS disorders, findings suggestive of T cell subset involvement in the pathophysiology of SLE have been reported, such as reduction in the population of naive T cells and an inverse correlation with the antibody-forming potential[23,24,25,26,27]. In patients with sustained SLE remission for 2 years after rituximab treatment, however, there were significant increases in the peripheral blood CD4-positive and CD4+CD45RA+ naive T cells. Further, although no changes were seen in the number of CD4+CD45RO+ memory T cells, the expression of CD45RO decreased ($CD45RO^{bright}$ to $CD45RO^{intermediate}$), suggesting the reduced activation potential of the cells. In fact, reduction or disappearance of the expression of CD69 and the costimulatory molecules CD40L and ICOS was noted. As described, activated B cells in patients with SLE showed enhanced expression of MHC class II antigens and costimulatory molecules and an antigen-presenting potential close to that of dendritic cells, suggesting T cell activation. However, the costimulatory molecule-expressing B cells disappeared, thereby reducing the costimulatory molecule-expressing memory T cells, a change that probably contributes to longterm remission in patients with SLE.

The case of Patient 6 in this study is interesting because the SLE relapse was associated with predominant T cell abnormalities. With regard to the clinical presentation of this patient, there were marked systemic symptoms such as fever (over 38˚C), polyarthritis, and lymphadenopathy, along with CNS involvement. However, this patient cannot be viewed as a specific or extraordinary case of SLE. The fact that an increase in the memory T cells and an increase in the levels of ICOS on the CD4-positive cells preceded the relapse of SLE, without any changes in the number of B cells, B cell subsets, or surface antigen expression, indicated that T cell activation was predominantly involved in the SLE relapse.

Many clinical studies revealed that some patients do not benefit at all from peripheral B cell depletion therapy with rituximab[1,2,7,13]. When those findings are considered with our findings, it would appear that the existence of T cell-dependent/B cell-independent abnormalities may be involved in the pathogenesis of SLE. This may reflect the heterogeneity in the pathophysiology of SLE. Indeed, the patient with B cell-dominant relapse in our study responded well to retreatment with rituximab, and a favorable outcome of the patient with T cell-dominant relapse was obtained following treatment with IVCY. Thus, a higher efficacy of B cell-targeted therapy may be obtained in patients with B cell-dominant SLE, while T cell-targeted therapy may be needed for patients with T cell-dominant SLE.

Our findings support the notion that activated T cells, in addition to activated B cells, may be potentially involved in the pathogenesis of SLE, and that interaction between activated B cells and T cells may worsen the pathophysiology of SLE. Depletion of B cells by rituximab may result in the reconstitution of B cells in the peripheral compartment. That could cause inhibition of T cell activation and differentiation mediated by memory B cells, which in turn might lead to longterm remission of SLE.

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