Longterm Clinical and Immunological Effects of Anti-CD20 Treatment in Patients with Refractory Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To retrospectively evaluate longterm clinical and immunological effects of anti-CD20 treatment in patients with systemic lupus erythematosus (SLE) with active nephritis or autoantibody-mediated cytopenias refractory to conventional immunosuppressive treatment.

Methods. Anti-CD20 treatment (rituximab) was added to the ongoing immunosuppressive treatment in 31 SLE patients with active nephritis (n = 17), thrombocytopenia (n = 10), and hemolytic anemia (n = 4) refractory to conventional therapy. Disease activity was evaluated by the SLE Disease Activity Index. The median followup time after anti-CD20 treatment was 22 months (range 1–61 mo).

Results. Complete B cell depletion was obtained in all patients. In 11 of the 17 lupus nephritis patients complete or partial responses were achieved after 6–12 months. Eight of these patients increased their glomerular filtration rate (GFR) by > 25%. The responders were characterized by having shorter nephritis duration, a baseline GFR > 30 ml/min, and detectable circulating CD19+ B lymphocytes before B cell depletion. Anti-CD20 treatment was highly effective in patients with autoimmune thrombocytopenia, inducing a significant increase of platelet counts after 1 month ($p < 0.01$). Five of 10 patients achieved complete normalization of their platelet counts within 6 months. The anti-CD20 treatment was followed by a significant reduction of autoantibodies against dsDNA and platelets, in nephritic and in thrombocytopenic patients, respectively.

Conclusion. Addition of anti-CD20 treatment to conventional immunosuppressive therapy may be a beneficial strategy in refractory lupus nephritis and autoimmune cytopenias, possibly by reducing the levels of pathogenic autoantibodies. (First Release April 1 2008; J Rheumatol 2008;35:826–33)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS B LYMPHOCYTES NEPHRITIS
THROMBOCYTOPENIA ANEMIA

Systemic lupus erythematosus (SLE) is an autoimmune relapsing disease characterized by multiorgan involvement and loss of tolerance against self-antigens followed by autoantibody production. Nephritis is a severe manifestation of SLE associated with significant morbidity and mortality due to progressive loss of renal function. Other manifestations of SLE associated with high morbidity and mortality are autoimmune hemolytic anemia and severe thrombocytopenia. Current treatment of severe SLE flares consists of toxic immunosuppressive drugs, most commonly cyclophosphamide together with high doses of corticosteroids[1]. However, the therapeutic options in cases of SLE refractory to standard treatment are indeed limited, and new treatment strategies are needed.

Anti-CD20 treatment has emerged as a potential attractive agent for autoimmune rheumatic diseases and has been shown to be effective in rheumatoid arthritis[2,3]. Antibodies against CD20, a B cell-specific calcium channel, were originally developed for treatment of B cell lymphomas. The B cell-specific monoclonal antibody rituximab has been shown to selectively deplete B cells while sparing plasma cells without significantly increasing susceptibility to infections[4]. B cells are important in the pathogenesis of SLE, being the source of autoantibodies and immunomodulatory cytokines, and acting as antigen-presenting cells for autoreactive T cells[5].

Although no randomized, controlled studies of anti-

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CD20 treatment in SLE have been published, reports suggest some efficacy of anti-CD20 treatment in refractory SLE[6-11]. In addition, recent small open uncontrolled studies of anti-CD20 cell treatment in non–SLE autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura have shown that this treatment regimen might have promise in refractory cases[12-14]. However, the effect of anti-CD20 treatment in autoimmune cytopenias in patients with SLE has not been extensively studied.

We retrospectively evaluated the longterm clinical and immunological effects of anti-CD20 treatment in 31 patients with SLE with refractory lupus nephritis, autoantibody-mediated thrombocytopenia, hemolytic anemia, hypocomplementemic urticarial vasculitis syndrome (HUVS), central nervous system (CNS) and cutaneous SLE manifestations. In addition, the influence of anti-CD20 treatment on the levels of pathogenic autoantibodies was evaluated.

MATERIALS AND METHODS

Patients. Thirty-three patients (28 women, 5 men) were treated with monoclonal antibodies against CD20 (rituximab; Mabthera®, Roche) at the Rheumatology Clinic, Sahlgrenska University Hospital, during the period September 2001 to November 2006. Thirty-one patients had SLE, diagnosed according to the American College of Rheumatology criteria[15]. Disease activity at baseline in these patients was scored by the SLE Disease Activity Index (SLEDAI). The remaining 2 patients had an overlap syndrome (mixed connective tissue disease and SLE) and HUVS, respectively. Seventeen of the patients had lupus nephritis with a progressive loss of renal function and signs of active renal inflammation despite ongoing treatment with cyclophosphamide (n = 14) or mycophenolate mofetil (n = 3). Renal biopsies were obtained at nephritis onset from all patients but one; 3 patients had class II nephritis, 5 had class III nephritis, and 8 had class IV nephritis, according to the World Health Organization classification system. Two patients had CNS lupus with epileptic seizures and myelitis, respectively. The remaining patients had autoantibody-related conditions refractory to intensive immunosuppressive treatment; 10 patients had thrombocytopenia (2 of these patients also had nephritis), 4 patients had hemolytic anemia (one patient also had thrombocytopenia), and 2 patients had HUVS associated with presence of anti-C1q antibodies (one of these patients subsequently developed lupus nephritis). Finally, 2 patients had SLE with severe mucocutaneous ulcers in the scalp and oral mucosa. The median followup time after the anti-CD20 treatment was 22 months (range 1–61 mo). Table 1 shows the clinical and demographic characteristics of the patients. The majority of patients were Caucasian (n = 24), 3 were Asian, 3 African, and 3 Persian in origin. All patients were informed about the aim and potential complications of the anti-CD20 treatment, and all gave informed consent before treatment.

Anti-CD20 treatment. Rituximab was added to the ongoing immunosuppressive treatment, which was maintained until remission was achieved. Rituximab was given as 4 consecutive intravenous infusions once weekly at a dose of 375 mg/m^2. All patients were given paracetamol orally (Panodil®, 1 g) and antihistamine (klemastin; Tavegyl®, 2 mg) intravenously 1 hour before infusion of rituximab. Corticosteroids were not administered simultaneously with the rituximab infusions.

Evaluation of B cell numbers and function. The number of circulating B cells defined as CD19+ lymphocytes in peripheral blood was assessed at baseline and after 1, 3, 6, 12, and 24 months by flow cytometry. CD19+ B cells were chosen for determination of the number of circulating B cells before and after anti-CD20 treatment to avoid any possible interference of rituximab with the flow cytometric assay. Levels of CD19+ B cells < 1% of the total lymphocyte population were considered to indicate B cell depletion from the peripheral blood.

Serum levels of immunoglobulins (IgG, IgM, IgA) were determined by nephelometry, and the number of circulating immunoglobulin-producing cells was determined by an Elispot assay.

Assessment of clinical responses to anti-CD20 treatment. The clinical efficacy of anti-CD20 treatment in patients with lupus nephritis was evaluated by analyses of glomerular filtration rate (GFR) determined by Cr-EDTA clearance, proteinuria, hematuria, urine casts, and serum albumin concentrations. Renal responses were evaluated by the criteria used by Sfikakis, *et al*[16]. A complete renal response was defined as normal serum creatinine and serum albumin levels, inactive urinary sediment, and a 24-hour urinary albumin secretion < 0.5 g; a partial response was defined as ≥ 50% improvement of the renal measures being abnormal at baseline and absence of deterioration of any of them. For patients with thrombocytopenia the platelet counts were followed, and presence of specific antiplatelet antibodies was studied in the majority of these patients. The clinical effectiveness of anti-CD20 in treatment of autoimmune hemolytic anemia was evaluated by measurement of serum hemoglobin and haptoglobin levels and reticulocyte numbers. Additionally, the presence of autoantibodies against erythrocytes and C1q was analyzed. Direct and indirect agglutination tests and gammaglobulin levels were followed when required. All evaluations were performed at baseline and after 1, 3, 6, 12, and 24 months following the anti-CD20 treatment.

Evaluation of anti-DNA antibodies. Levels of antibodies against double-stranded DNA (dsDNA) were determined by a radioimmunoassay and also by indirect immunocytochemistry using *Crithidia lucillae* as substrate for analysis of anti-dsDNA of IgG and IgM isotypes.

Statistical evaluation. Clinical measures such as disease duration and followup time were presented as medians and ranges. All laboratory data were expressed as means ± SEM. Responses to anti-CD20 treatment at 1, 3, 6, and 12 months were compared with baseline values. Paired Student's t-test was used for comparison of different variables at baseline and followup. P values < 0.05 were considered to indicate statistically significant differences. All analyses were performed using Prism 4.0 (GraphPad Software Inc.).

RESULTS

Safety aspects of anti-CD20 treatment. Rituximab was given to 33 patients, and all but 3 patients received 4 weekly infusions. Treatment was discontinued after the first infusion in one patient due to osteitis in the jaw (possibly present before the first anti-CD20 treatment), and in one patient after the third infusion due to serum sickness with fever, rash, and arthritis. One patient with hemolytic anemia died of respiratory and cardiac failure in the interval between the second and third infusion. These patients were excluded from further analyses. One patient was hospitalized 2 months after the last rituximab infusion due to neutropenia and pseudomonas sepsis, one patient treated for lupus nephritis died of pulmonary infection 23 months after the anti-CD20 treatment, and one patient with autoimmune thrombocytopenia died of dilated cardiomyopathy after 18 months. Finally, one patient underwent allogeneic stem-cell transplant due to severe life-threatening autoimmune hemolytic anemia resistant to all treatments, and died from generalized Candida infection in conjunction with this 35 months after anti-CD20 treatment.

The remaining 26 patients did not develop any severe allergic reactions or adverse events after rituximab infu-

Table 1. Clinical and demographic characteristics of patients before anti-CD20 treatment.

Patient	Age/ Sex	Ethnicity	Disease Duration, yrs	SLEDAI	Previous Therapies	Reason for Anti-CD20 Treatment	Concomitant Treatment	Followup, mo	Time to Flare, mo
1	53 F	C	21	18	AZA, CYC, MTX, PRED	Nephritis (III)	CYC	29	12
2*	19 F	C	1	14	CYC, PRED	Nephritis (IV)/ nephritis, thrombocytopenia	CYC/MMF	41	26/36
3	22 F	P	1	12	CYC, PRED	Nephritis (IV)	CYC	22	22
4*	53 F	C	15	18	AZA, CYC, PRED	Rash/nephritis (II)	AZA/ CYC	55	30/ no flare
5	37 F	C	9	16	AM, AZA, CYC, MTX, PRED	Nephritis (II)	CYC	24	20
6	39 F	C	13	16	AM, AZA, ATG, CYA, CYC, MTX, PRED	Nephritis (nd), rash	CYC	23	8
7	50 M	C	1	12	CYC	Nephritis (II)	CYC	26	No flare
8	29 F	C	1	20	CYC, PRED	Nephritis (III)	CYC	20	17
9	40 F	Af	4	16	AM, PRED	Nephritis (III)	CYC	14	No flare
10	48 F	C	12	12	AZA, CYC, PRED	Nephritis (IV)	MMF	55	No flare (ESRD)
11	20 M	C	4	16	AZA, CYA, CYC, PRED	Nephritis (IV)	CYC	58	No flare (ESRD)
12	47 F	C	10	17	AZA, CYA, CYC, PRED	Nephritis (IV)	CYC	29	18†
13	39 F	C	13	30	AM, AZA, MTX, PRED, Spl	Nephritis (III), rash	CYC	22	No flare
14	26 F	Af	5	20	CYC, MTX, PRED	Nephritis (IV)	CYC	8	No flare
15	39 F	C	4	22	AM, MMF, MTX, PRED	Nephritis (IV)	MMF	7	No flare
16	37 F	C	0.5	19	CYC, PRED	Nephritis (IV), alveolitis	CYC	12	No flare
17	31 F	Af	11	20	AM, CSA, CYC, PRED	Nephritis (III)	CYC	24	No flare (ESRD)
18	33 F	C	8	3	IVIG, MTX, PRED, Spl	Thrombocytopenia	AZA	61	14
19	17 F	As	1	13	CSA, IVIG, PRED	Thrombocytopenia, AIHA	PRED	2**	No flare
20*	37 F	As	2	7	AZA, PRED	Thrombocytopenia /thrombocytopenia	MTX	15	8/no flare
21	39 F	P	10	3	AM, AZA, PRED	Thrombocytopenia	AZA	15	No flare
22	84 F	C	13	3	MTX, PRED	Thrombocytopenia	MTX	15	No flare
23	34 F	C	10	13	AZA, CSA, CYC, IVIG, MMF, MTX, PRED	Thrombocytopenia	IVIG, PRED	18	No flare† (ESRD)
24	49 F	C	14	3	AZA, CSA, IVIG, PRED	Thrombocytopenia	PRED	7	No flare
25	30 F	C	11	MCTD	AZA, PRED	Thrombocytopenia	AZA	7	6
26	71 M	C	11	3	AM, CYC, IVIG, PRED	AIHA	CYC	1	†
27	60 M	C	11	5	CSA, CYC, PRED, Spl	AIHA	CSA	52	No flare
28*	21 F	C	5	10	ATG, CSA, CYC, PRED, SCT	AIHA	MTX, CSA	35	No effect†
29	49 F	C	5	HUVS	AZA, MTX	Rash	None	6	No flare
30	32 F	P	9	18	AM, AZA, CYC, MMF, MTX, PRED	Rash/vasculitis	MMF	29	No effect
31	60 F	C	32	15	AZA, CYC, MTX, PRED	Rash/vasculitis	CYC	9	No effect
32	24 F	As	1	25	Chl, CYC, PRED, plasmapheresis	CNS lupus, hypergamma-globulinemia	CYC	35	6
33	30 M	C	13	19	AZA, Chl, CYC, MTX, PRED	CNS lupus	CYC	10	No flare

Af: African; AIHA: autoimmune hemolytic anemia; AM: antimalarial drugs; As: Asian; ATG: antithymocyte globulin; AZA: azathioprine; C: Caucasian; Chl: chlorambucil; CSA: cyclosporine; CYC: cyclophosphamide; ESRD: endstage renal disease; HUVS: hypocomplementemic urticarial vasculitis syndrome; IVIG: intravenous immunoglobulin; MCTD: mixed connective tissue disease; MMF: mycophenolate mofetil; MTX: methotrexate; P: Persian; nd: not done; PRED: prednisolone; SCT: stem-cell transplant; Spl: splenectomy. *Patients re-treated with anti-CD20 antibodies, first flare/second flare. **Patient was lost during followup after 2 months. †Patients died during followup.

sions. The mean total dose of rituximab was 2483 ± 601 mg. Four patients were re-treated with rituximab. One patient was re-treated after 7 months due to a new episode of thrombocytopenia. One patient was re-treated twice (after 26 and 36 mo) due to refractory glomerulonephritis and thrombocytopenia. The third patient, initially successfully treated for severe anti-C1q-mediated urticarial rash, received a second round of rituximab treatment after 30 months due to refractory glomerulonephritis. The last patient, with severe hemolytic anemia not responding to any treatment, was re-treated twice, 5 and 6 months after the first anti-CD20 treatment.

Clinical responses in lupus nephritis. Seventeen patients were treated with anti-CD20 antibodies due to signs of active lupus nephritis, i.e., significant hematuria and decreasing GFR, despite ongoing immunosuppressive treatment. The majority of the patients had impaired kidney function before start of anti-CD20 treatment — mean GFR 55

± 25 ml/min. Six to 12 months after addition of anti-CD20 treatment 2 of 17 (12%) patients had reached a complete renal response and 9 (53%) had a partial response. The remaining 6 patients showed no signs of renal improvement and were nonresponders according to the response criteria (Sfikakis, *et al*[16]). During the first 12 months after addition of anti-CD20 treatment GFR increased by > 25% in 8 of the 17 patients and remained mainly unchanged in 5 patients (Figure 1). The GFR in the remaining 4 patients continued to decrease despite the addition of anti-CD20 treatment, and hemodialysis was started in these patients due to endstage renal disease. These 4 patients all had severely impaired renal function, with GFR < 30 ml/min and highly increased serum creatinine levels, before the addition of anti-CD20 treatment.

The 24-hour urine albumin loss decreased significantly from 3.8 ± 2.8 g to 1.0 ± 1.3 g after 12 months in patients with complete or partial renal responses (p = 0.043). In addition, the mean serum albumin concentration increased significantly from 26 ± 6.2 g/l to 35 ± 5.5 g/l (p = 0.001) and 36 ± 6.2 g/l (p = 0.017) in these patients after 6 and 12 months, respectively.

Ten of the patients with nephritis were able to discontinue cyclophosphamide within 8 months of starting anti-CD20 treatment due to improvement of renal function.

To determine if certain baseline measures were associated with a more favorable response to anti-CD20 treatment we compared different baseline indicators of responders and nonresponders. As shown in Table 2, nonresponders were characterized by having a longer duration of nephritis and a GFR < 30 ml/min (p = 0.002) and higher serum creatinine levels at baseline (p = 0.0066) than the patients with complete or partial renal responses.

In addition, patients with complete or partial renal responses after anti-CD20 treatment were more likely to have circulating CD19+ B lymphocytes at baseline than nonresponders (p = 0.046). In contrast, 3 of 4 patients who developed endstage renal disease had no detectable CD19+ B lymphocytes in the circulation at baseline. However, presence of detectable CD19+ B lymphocytes in the circulation before anti-CD20 treatment was not a prerequisite for treatment efficacy, since 2 patients without detectable CD19+ B lymphocytes at baseline had a complete and a partial response, respectively. In addition, 2 of the patients having circulating CD19+ B cells before B cell depletion did not respond to anti-CD20 treatment.

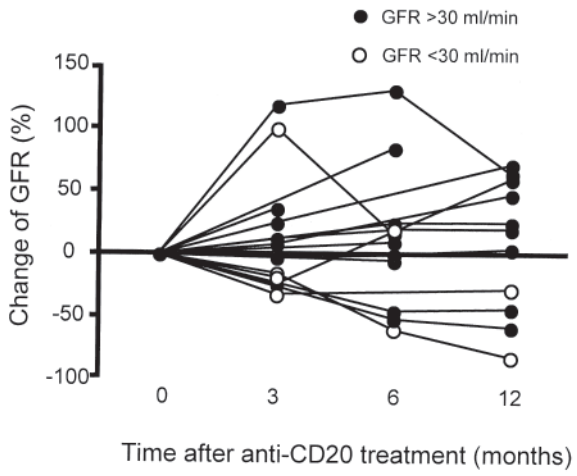

Figure 1. Relative change of glomerular filtration rate (GFR), as determined by Cr-EDTA, following anti-CD20 treatment in patients with lupus nephritis. Baseline GFR was 100%.

Table 2. Comparison of baseline clinical characteristics of lupus nephritis patients with complete/partial responses and no responses to anti-CD20 treatment.

Feature	Complete/Partial Responders n = 11	Nonresponders n = 6	p
Duration of nephritis, mo, median (range)	9 (2–31)	19 (10–72)	NS
Patients with GFR< 30 ml/min	0/11	4/6	0.002*
Serum creatinine, µmoles/l	86.1 ± 30.9	207.2 ± 86.6	0.0066**
Urine albumin loss, g/24 h	3.4 ± 2.1	5.0 ± 1.6	NS
Anti-dsDNA, U/ml	38 ± 4.9	37.5 ± 8.0	NS
C3 level, g/l	1.0 ± 0.1	1.1 ± 0.3	NS
Patients with detectable CD20+ lymphocytes at baseline	9/11	2/6	0.0456*

Values are mean ± SEM. *Chi-square test. ** Students t-test. NS: nonsignificant.

Four patients had relapses of glomerulonephritis after 20, 20, 22, and 26 months, respectively, and were re-treated with cyclophosphamide (n = 3) or mycophenolate mofetil (n = 1). One of these patients was also re-treated with anti-CD20 antibodies twice, after 26 and 36 months, due to active nephritis despite continuing cyclophosphamide treatment.

Anti-CD20 treatment in refractory autoimmune thrombocytopenia and hemolytic anemia. Ten patients with autoantibody-mediated thrombocytopenia resistant to high doses of corticosteroids and intravenous immunoglobulin (IVIG) treatment were treated with anti-CD20 antibodies. The treatment was stopped in one patient who developed serum sickness after the third infusion; this patient was excluded from further analyses. The remaining 9 patients all responded with a rapid increase of platelet counts, the mean platelet count increasing from 41 ± 9.7 × 10^9/l to 93 ± 18 × 10^9/l after 1 month (p = 0.0095; Figure 2). Within 6 months of followup, platelet counts had normalized completely in 5 patients (56%) and additionally remained at stable levels > 100 × 10^9/l in 2 patients despite tapering of oral corticosteroids. The remaining 2 patients increased their platelet counts to stable levels of roughly 50 × 10^9/l. One of these patients died of dilated cardiomyopathy after 18 months. The mean daily oral prednisolone dose decreased from 21.4 ± 18.1 mg at baseline to 8.9 ± 5.2 mg and 8.6 ± 5.4 mg after 3 and 6 months, respectively (not significant).

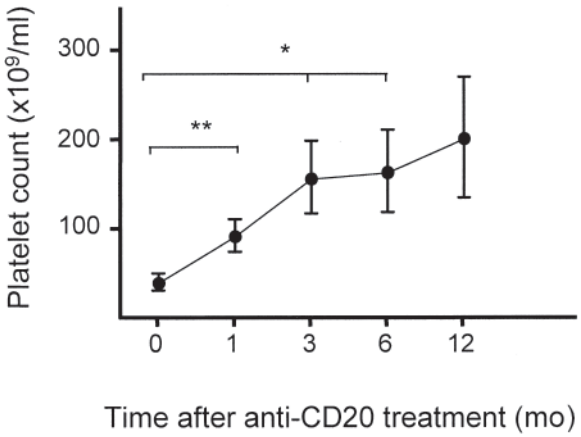

Figure 2. Platelet counts in peripheral blood after anti-CD20 treatment. *p < 0.05, **p < 0.01, paired Student t-test.

Antithrombocyte antibodies of IgG and IgM isotypes bound to the surface of platelets were detected in 8 of the 10 patients by flow cytometry. Followup measurements were available in 4 patients, and the levels of IgG and IgM antibodies decreased after anti-CD20 treatment in all these patients; indeed, IgM antithrombocyte antibodies disappeared after 1 and 6 months, respectively, in 2 patients. Relapses of thrombocytopenia were seen in 2 patients after 6 and 8 months, respectively, and one of these patients was successfully retreated with anti-CD20 antibodies.

Four patients with refractory autoimmune hemolytic anemia received anti-CD20 treatment. One patient died of respiratory and cardiac failure in the interval between the second and third infusions. Two patients responded with a rapid increase in hemoglobin levels and normalization of haptoglobin and reticulocyte levels. One of these responding patients continued to have normal hemoglobin levels at 52 months of followup. The second responding patient moved to another region of Sweden and was lost after 2 months of followup. The remaining patient, with severe SLE, who earlier had been successfully treated for severe CNS lupus with autologous stem-cell transplant[17], did not respond to anti-CD20 treatment or any other therapy tested, and due to severe life-threatening hemolytic anemia she underwent allogeneic stem-cell transplantation, and died from a generalized Candida infection in conjunction with this 35 months after anti-CD20 treatment.

In addition, 2 SLE patients with refractory cutaneous ulcers and 2 patients with HUVS received anti-CD20 treatment. The cutaneous manifestations disappeared in the HUVS patients and were followed by a dramatic reduction of anti-C1q antibodies in both cases (data not shown), whereas the effect against cutaneous ulcers was modest. Anti-CD20 treatment was also given to 2 patients with CNS lupus; at present, after 10 months of followup, one patient presenting with epilepsy remains free of seizures on concomitant antiepileptic therapy. The other patient, with myelitis and severe hypergammaglobulinemia, showed some improvement of CNS symptoms, i.e., paraplegia and impaired cognitive function, and the serum IgG levels decreased from 70 g/l to 40 g/l and 37 g/l after 3 and 12 months, respectively, following anti-B cell treatment.

Effects of anti-CD20 treatment on number of circulating B cells and immunoglobulin production. Circulating B cells, defined as CD19+ lymphocytes by flow cytometric analysis, were detected in 26 of the 33 patients before anti-CD20 treatment, the mean level being 6.5% (range 0–21%). Depletion of B cells from the circulation was observed in all patients 1 month after anti-CD20 treatment (Figure 3A). B cells returned to the circulation in 17% of the patients after only 3 months, in 42% after 6 months, and in 80% of patients after 12 months following anti-CD20 treatment (Figure 3A).

We also analyzed the changes of anti-dsDNA antibodies following anti-CD20 treatment. The majority of the SLE patients (84%) had detectable levels of anti-dsDNA antibodies at baseline (Farr assay), being most frequently found in lupus nephritis patients (in all patients but one). After anti-CD20 treatment the levels of anti-dsDNA decreased significantly in the lupus nephritis patients, from 34.5 ± 4.9 U/ml to 27.3 ± 5.3 U/ml after 6 months ($p = 0.0384$; Figure 4), and to 16.3 ± 3.9 U/ml after 12 months ($p = 0.0049$; Figure 4). In the lupus nephritis patients with detectable anti-dsDNA antibodies of the IgG isotype decreased significantly from a mean reciprocal titer of 188 (range 0–320) to 17 (range 0–40) after 12 months ($p = 0.0235$). Eight of the 17 nephritis patients had detectable anti-dsDNA of IgM isotype at baseline, and anti-dsDNA IgM was undetectable in all but one of them at 12 months. In contrast, the levels of total, IgG, or IgM anti-dsDNA antibodies remained unchanged in non-nephritis SLE patients.

The presence of anti-dsDNA antibodies at baseline was not related to increased risk of flare.

The anti-CD20 treatment had no significant effect on the levels of circulating immunoglobulins of IgG, IgA, or IgM isotypes (data not shown).

The presence of circulating immunoglobulin-producing cells was analyzed in 27 of the 33 patients, and all these patients had detectable levels of immunoglobulin-producing cells before anti-CD20 treatment. The levels of IgG, IgM, and IgA-producing cells decreased after anti-CD20-treatment, but the decrease reached only statistical significance for IgA-producing cells at 3 months after treatment ($p = 0.0216$; Figure 3B). Decreases of circulating immunoglobulin-secreting cells corresponded to the depletion of B cells from the circulation, while the decreases of anti-dsDNA antibodies occurred at later timepoints.

DISCUSSION

We report the longterm clinical and immunological outcome after anti-CD20 treatment in patients with refractory SLE

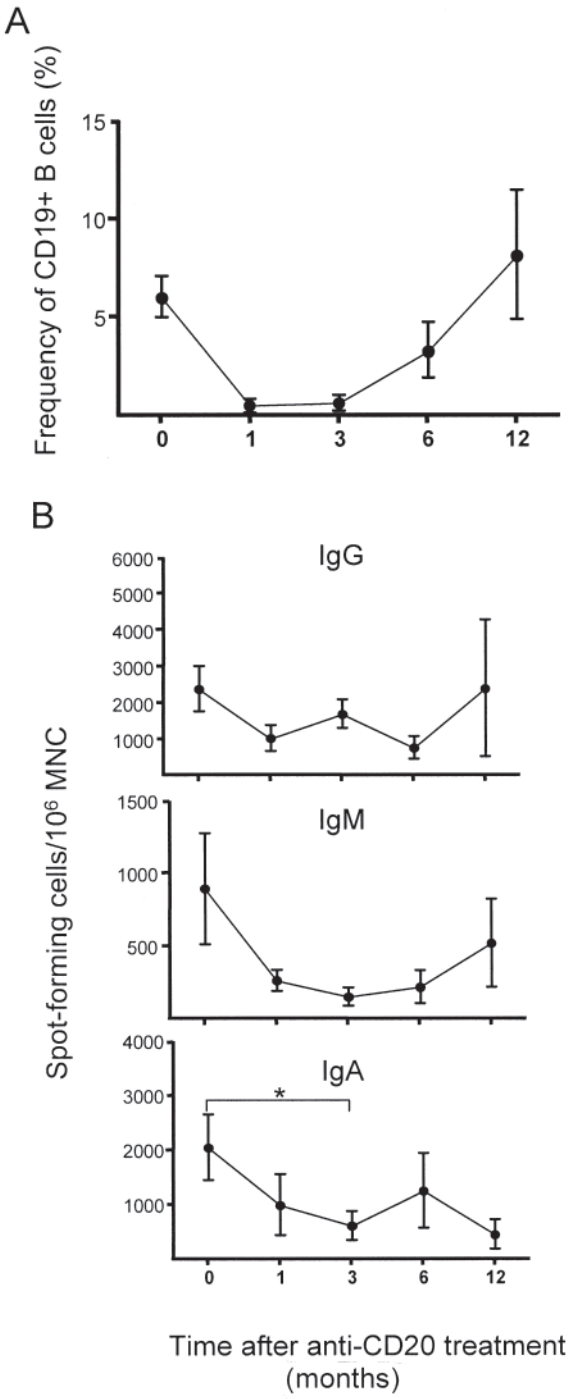

Figure 3. A. Frequency of detectable CD19+ lymphocytes in peripheral blood before and after anti-CD20 treatment. Amount of CD19+ cells is expressed as the proportion of CD19+ cells in relation to numbers of lymphocytes determined by flow cytometry. B. Number of circulating immunoglobulin-secreting cells as determined by Elispot. Results are expressed as numbers of spot-forming units/10^6 peripheral blood mononuclear cells (MNC). *p < 0.05, paired Student t-test.

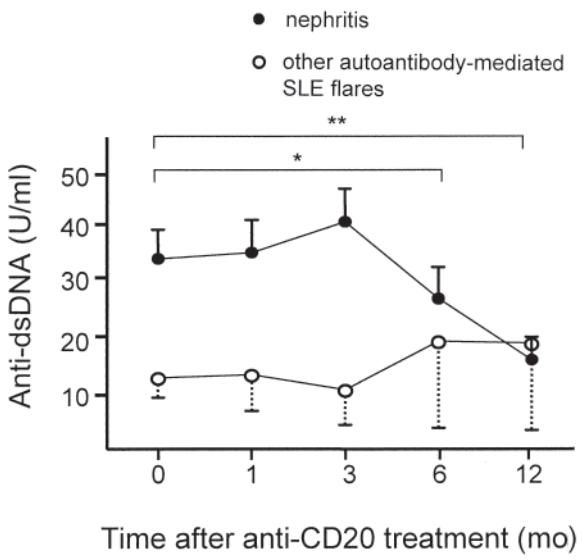

Figure 4. Levels of anti-dsDNA antibodies following anti-CD20 treatment in SLE patients with nephritis flares and other autoantibody-mediated flares. Values are given as means ± SEM. *p < 0.05, **p < 0.01.

manifestations. Our data support the previously reported beneficial effects of anti-CD20 treatment in lupus nephritis and autoimmune cytopenias. We found that the effect of anti-CD20 treatment in lupus nephritis was seen more than 6 months after the treatment, while in autoantibody-mediated cytopenias the effect was rapid and longstanding in the majority of the treated patients. Our data also show that anti-CD20 treatment decreases the levels of disease-specific autoantibodies.

Eleven of the 17 patients with refractory lupus nephritis described here had a complete or partial response to addition of anti-CD20 to conventional immunosuppressive treatment with cyclophosphamide or mycophenolate mofetil and high-dose corticosteroids. Of these 11 patients with favorable renal responses, 7 remain in remission after followup for up to 26 months.

The patients with refractory lupus nephritis treated with anti-CD20 antibodies generally had more severely impaired renal function, as indicated by decreased GFR, than patients with lupus nephritis reported previously[10,16,18]. Indeed, the nonresponders in our cohort were characterized by severely impaired renal function; 4 of them had GFR < 30 ml/min before the anti-CD20 treatment and also had longer duration of their renal involvement. Thus, anti-CD20 treatment may be less efficacious in lupus nephritis patients with severely impaired kidney function.

We observed that SLE patients with detectable circulating B lymphocytes before anti-CD20 treatment were significantly more likely to achieve a complete or partial renal response to anti-CD20 treatment. However, patients with lupus nephritis without detectable circulating B lymphocytes also responded to anti-CD20 treatment. This observation supports a report indicating a lack of correlation between circulating and renal populations of CD20+ cells in the majority of patients with lupus nephritis[19].

We found that anti-CD20 treatment was highly effective in refractory autoantibody-mediated thrombocytopenia with a rapid, significant, and stable increase of platelet counts in 7 of the 9 patients who completed the treatment. Our retrospectively evaluated experience of addition of anti-CD20 treatment in patients with autoimmune thrombocytopenia associated with systemic rheumatic disease corresponds well to the recently reported efficacy of anti-CD20 treatment in thrombotic thrombocytopenic purpura, where anti-CD20 treatment was found to increase the progression-free survival compared to standard treatment consisting of corticosteroids and IVIG[12]. Indeed, the complete responses with normalization of the platelet counts seen in 5 of 9 patients in our study exceeds the response rate reported in a recent systemic review of the clinical efficacy of rituximab in idiopathic thrombocytopenic purpura[14].

A recent study indicated that the presence of antibodies against extractable nuclear antigens before anti-CD20 treatment might predict flares after the treatment, and that patients with low C3 levels at baseline had a shorter time to flare following anti-CD20 treatment[20]. However, we could not detect any association between the presence of anti-dsDNA antibodies or low C3 levels at baseline (data not shown) and the risk of flare or time to flare post-anti-CD20 treatment.

CD19+ B lymphocytes could be detected in the circulation of 24 of the 33 patients before anti-CD20 treatment, and a complete depletion of B lymphocytes was achieved in all patients receiving 4 weekly infusions of rituximab. The finding that 9 of 33 patients had no detectable CD19+ or CD20+ B cells in the blood at baseline could be explained by longterm intensive immunosuppressive treatment prior the anti-CD20 treatment. Another possible explanation for this finding is that the disturbance of the peripheral B lymphocyte pool is related to SLE disease itself. The early return (starting after only 3 months) of B cells to the circulation observed in a substantial proportion of the SLE patients in our study is in good agreement with previous reports, and may be associated with an increased maturation rate of B cells in these patients. The CD20-binding monoclonal antibody rituximab selectively depletes B lymphocytes, whereas antibody-producing plasma cells are spared. Accordingly, serum immunoglobulin levels have been reported to be largely unchanged after rituximab treatment[4,21]. We observed that antibody-producing cells in the circulation are significantly decreased after anti-CD20 treatment, and that their reoccurrence paralleled the return of CD20-positive B lymphocytes in peripheral blood. Even though the total immunoglobulin levels in serum were not substantially changed, we detected a significant decrease of specific anti-dsDNA antibodies in the nephritis patients and also a substantial decrease of antithrombocyte, antierythrocyte, and anti-C1q antibodies in patients with autoimmune thrombocytopenia, autoimmune hemolytic anemia, and HUVS, respectively. Together these findings indicate that disease-specific autoantibodies are at least partly secreted by circulating CD20-positive B lymphoblasts. Another explanation could be that short-lived anti-dsDNA-producing plasma cells are deleted by anti-CD20 treatment. Yet another explanation for the finding of decreased levels of autoantibodies could be that autoreactive CD4+ T cells are affected by the anti-B cell treatment. A reduction of T-helper activation and an increase in $CD25_{high}$ FOXP3+ regulatory T cells after rituximab treatment of SLE have recently been reported[22]. However, the source of autoantibodies of pathogenic value in SLE remains to be determined.

Anti-CD20 therapy may represent a promising new treatment in refractory SLE nephritis and autoantibody-mediated conditions; a possible mechanism might be reduction of levels of pathogenic autoantibodies.

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