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Correlation Between Circulating CD27^{high} Plasma Cells and Disease Activity in Patients With Systemic Lupus Erythematosus

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Objective. **Disease activity in systemic lupus erythematosus (SLE) is usually assessed with complex disease activity scores comprising a variety of different parameters. In order to determine whether SLE disease activity correlates with abnormal B lymphocyte activity, B cell subsets were analyzed, and their relationship to clinical measures of disease activity was assessed.**

Methods. **The distribution of B cell subsets was determined by fluorescence-activated cell sorting analysis and assessed in relation to the autoantibody profile, disease activity measured by the SLE Disease Activity Index (SLEDAI) and the European Consensus Lupus Activity Measure (ECLAM) scores, disease duration, and therapy.**

Results. **The number and frequency of CD27^{high} plasma cells were significantly correlated with the SLE disease activity indices and with the titer of anti–double-stranded DNA autoantibodies. Circulating B cell subsets were not influenced by age or sex, but appeared to relate to the duration of disease and the therapeutic regimen, with the number and frequency of CD27^{high} plasma cells increasing and those of CD27− naive B cells decreasing over time. Patients were divided into those with a SLEDAI score of 0–8 (low disease activity) and those with a SLEDAI score >8 (high disease activity). Patients with high disease activity had an increased frequency of both CD19+ B cells and CD27^{high} plasma cells. By using a nonparametric data sieving algorithm, we observed that these B cell abnormalities provided predictive values for nonactive and active disease of 78.0% and 78.9%, respectively. The predictive value of the B cell abnormalities (77.8%) was greater than that of the humoral/clinical data pattern (70.0%), including anti-DNA antibody levels, circulating immune complexes, increased erythrocyte sedimentation rate, mucocutaneous involvement, and acute renal involvement.**

Conclusion. **Flow cytometric monitoring of B cell subsets in the peripheral blood provides new insights into abnormalities of B cell function in SLE and may also be a diagnostically valuable option for monitoring the activity of this autoimmune disease.**

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by various organ manifestations and the production of a broad variety of autoantibodies (1). In addition to immunologic disturbances such as abnormalities of the complement system (2,3), T cell–B cell interaction (4–6), or phagocytosis (7–9), B cell hyperactivity represents a central feature of SLE (10). Therefore, characterization of the abnormalities of B cell biology is essential to gain further insights into the pathogenesis of this disease. Spontaneous immunoglobulin production by peripheral blood lymphocytes (PBLs) ex vivo has been previously documented in SLE (11,12); distinct abnormalities of peripheral B cell homeostasis in patients with SLE have also been reported (13). In several studies, aberrant expression of various receptors or activation of signaling pathways controlling B cell

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proliferation and differentiation, antibody production, and apoptosis (13–18) was reported. Moreover, abnormal expression of factors such as BAFF (BLyS), interleukin-10 (IL-10), IL-6, and soluble CD154 that are involved in B cell activation and differentiation has been reported in patients with SLE or in animal models of lupus (19–26).

Recently, disturbances of specific B cell subsets in the peripheral blood have been described in a small number of patients with SLE compared with normal healthy controls and patients with other autoimmune diseases (13). A reduction of naive CD20+/CD27− B cells was observed, which accounted for the known B lymphocytopenia in SLE. In addition, expansion of a unique population of immunoglobulin-secreting plasma cells was observed in patients with active disease. These cells expressed CD19 and high levels of CD27 and contained cytoplasmic immunoglobulin, but lacked expression of CD20. Analysis of the V_H gene rearrangements of these plasma cells revealed clonally related, extraordinarily highly mutated V_H genes and a predominant usage of V_H4 family members. Another study in children with SLE also identified enhanced numbers of peripheral plasma cell precursors in patients with active disease (27), but reduced frequencies of naive CD27− B cells were not characteristic of juvenile SLE.

In SLE, disease activity is usually assessed by composite scores (28,29), e.g., the SLE Disease Activity Index (SLEDAI) or the European Consensus Lupus Activity Measure (ECLAM) (30), integrating clinical parameters and serologic findings, such as anti–double-stranded DNA (anti-dsDNA) antibody titer and complement levels. The heterogeneity of the disease requires a complete recording of all parameters for the assessment of disease activity. No single parameter, not even the anti-dsDNA antibody titer, allows a reliable assessment of disease status, because not all patients produce antibodies against dsDNA. Use of assessment tools such as the SLEDAI or ECLAM has permitted a description of disease activity in patients with SLE, regardless of the affected organ system.

Because B cells are centrally involved in the pathogenesis of SLE, we tested the hypothesis that the degree of abnormalities of circulating B cell subsets would correlate with disease activity. Therefore, results of flow cytometric evaluation of peripheral B lymphocyte subsets were compared with clinical and humoral data included in the SLEDAI or ECLAM as well as with individual parameters of disease activity, such as autoantibody profile, certain organ manifestations, and the influence of therapy.

MATERIALS AND METHODS

Patients and preparation of blood samples. Heparinized whole blood (5 ml) from 60 patients with SLE was obtained from the Department of Rheumatic Diseases, University Hospital Charité. Between 1999 and 2001, we analyzed 60 consecutive patients (53 women and 7 men) with SLE fulfilling the 1982 revised criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (1), after informed consent was obtained. Disease activity was assessed by the SLEDAI (28) and the ECLAM (30). Additional demographic, clinical, and serologic features, as well as the therapeutic regimen, were recorded at the time of analysis (Table 1). Patients who received intravenous cyclo- T1

Table 1. Demographic characteristics, clinical features, and serologic parameters of the 60 patients with systemic lupus erythematosus* AQ: A

Characteristic	
Age, mean ± SD years	38.7 ± 12.7
Disease duration, mean ± SD years	8.7 ± 7.7
Mean SLEDAI at the time of analysis	9.1
Female	53 (88.3)
Male	7 (11.7)
Autoantibody profile	
Anti-Sm+	12 (20.7)†
Anti-Ro+	31 (53.4)†
Anti-La+	15 (25.9)†
Antihistone+	13 (22.8)†
Anti-dsDNA+ by immunofluorescence	11 (18.3)
Anti-dsDNA+ by ELISA (cutoff 10 units/ml)	22 (36.7)
Disease activity	
SLEDAI ≤8	36 (60)
SLEDAI >8	24 (40)
Therapeutic regimen	
Less intensive or short-lasting therapy‡	23 (38.3)
More intensive therapy	33 (55)
Azathioprine (50–150 mg/day) + prednisone (median 7,5 mg/day, range 5–75 mg/day)	17 (28.3)
Cyclophosphamide bolus + prednisone (median 8.25 mg/day, range 5–50 mg/day	12 (20)
Cyclosporine (100–200 mg/day) + prednisone (5–20 mg/day)	4 (6.7)
Other therapeutic regimen§	4 (6.7)
Disease manifestations	
Arthritis	7 (11.7)
Rash	28 (46.7)
Serositis	4 (6.7)
CNS involvement	7 (11.7)
Renal involvement	28 (46.7)
Active lupus nephritis	4 (6.7)
Hematologic	23 (38.3)
Vasculitis	9 (15.3)†
Reduced complement levels	35 (78.7)†

* Except where indicated otherwise, values are the number (%). SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; anti-dsDNA = anti–double-stranded DNA; ELISA = enzyme-linked immunosorbent assay; CNS = central nervous system.
† Because of the lack of information from patients 57 and 59, n <60.
‡ Six patients were receiving hydroxychloroquine (200–400 mg/day) ± 5 mg prednisone/day, 13 patients were receiving 5–312.5 mg prednisone equivalent/day [median 30 mg/day]), and 4 patients were not receiving therapy.
§ Three patients were receiving experimental therapy, and 1 patient was receiving mycophenolate (1 gm twice daily) and 7.5 mg prednisone/day.

Table 2. Correlation of the frequency and absolute number of CD27^{high} plasma cells with SLEDAI and ECLAM scores*

	Frequency		Absolute no.	
	r_p	*P*	r_s	*P*
SLEDAI	0.4959	<0.0001	0.2618	0.0433
ECLAM	0.4205	0.0008	0.4890	<0.0001

* SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; ECLAM = European Consensus Lupus Activity Measure.

phosphamide bolus therapy were examined at least 4 weeks after the last bolus had been administered. Peripheral blood counts were determined by routine laboratory tests. Peripheral blood mononuclear cells (PBMCs) were prepared as previously described (31). In order to detect production of IgG, IgA, and IgM by various B cell subsets, an ELISpot assay (53) was performed using PBLs from a patient with active SLE. This study was approved by the local ethics committee at the University Hospital Charité.

Measurement of autoantibodies and circulating immune complexes. IgG anti-dsDNA antibodies were detected by *Crithidia luciliae* immunofluorescence and enzyme-linked immunosorbent assay (ELISA). Anticardiolipin (aCL) antibodies, circulating immune complexes, and anti-Ro, anti-La, anti-Sm, and antihistone antibodies were measured by commercially available ELISA kits (Imtec, Berlin, Germany).

Cytometric analysis. Immunofluorescence labeling for flow cytometry was performed by incubating PBMCs with phycoerythrin-labeled anti-CD19 (SJ25-C1; SBA, Birmingham, AL), Cy5-labeled anti-CD27 (clone 2E4), and fluorescein isothiocyanate–labeled anti-CD20 antibodies (clone B-Ly1; SBA). Incubation with antibodies was performed in phosphate buffered saline/0.5% bovine serum albumin/5 m*M* EDTA at 4°C for 10 minutes. Propidium iodide (1 μg/ml; Sigma, Munich, Germany) was added immediately before cytometric analysis to exclude dead cells. Intracytoplasmic IgA, IgG, and IgM were analyzed as described previously (13). Flow cytometry was performed using a FACSCalibur and CellQuest software (Becton Dickinson, San Jose, CA). A total of 80,000 events was collected for each analysis.

Statistical analysis. Two disease activity indices (SLEDAI and ECLAM), anti-dsDNA autoantibodies, complement levels and erythrocyte sedimentation rate (ESR), the therapeutic regimen, and 12 clinical parameters were recorded and compared with 11 cellular parameters (leukocyte and lymphocyte count, total B cell number, number of CD27−/CD20+ naive B or CD27+/CD20+ memory B cells, number of CD27^{high}/CD20− plasma cells, as well as the respective frequencies). Additional humoral parameters such as autoantibodies (aCL antibodies and anti-Ro, anti-Sm, anti-La, and antihistone antibodies) and circulating immune complexes were also analyzed. In 36 patients, we retrospectively analyzed serum IgG, IgM, and IgA levels by routine nephelometry.

Frequencies of B cell populations were calculated using CellQuest software (Becton Dickinson, Franklin Lakes, NJ). Differences between median values of defined patient groups were compared using the nonparametric Mann-Whitney U test. Correlation was examined by Spearman's rank test or using Pearson's coefficient if the data followed a Gaussian distribution. The absolute number (per microliter of blood) of B cells of various phenotypes was calculated, based on the frequencies of those cells among PBMCs and the absolute numbers of PBMCs. AQ: 6

The CLASSIF1 nonparametric data sieving algorithm (32,33) was used in separate and combined analyses to determine the most discriminatory data pattern of disease activity among the 11 cellular and 29 humoral/clinical parameters of the study. For these analyses, patients were divided into those with low disease activity (SLEDAI score 0–8) and those with active disease (SLEDAI score >8). The algorithm transforms numerical parameter values into the triple matrix characters decreased (−), unchanged (0), or increased (+), depending on their position relative to the lower and upper percentile threshold of the value distribution of reference patients (SLEDAI = 0). Parameters are then temporarily removed from the resulting triple matrix data pattern in an iterative manner, the degree of discrimination between low disease activity (SLEDAI score 0–8) and active disease (SLEDAI score >8) of the remaining parameters is recorded, and the parameter is reinserted into the data pattern. Only the most discriminatory parameters are retained in the optimal data pattern. All nondiscriminatory parameters are eliminated from further consideration. *P* values less than 0.05 were considered significant. The software automatically determines the optimal discriminatory data pattern for the most discriminatory percentile pair. AQ: 7

RESULTS

Relationship of B cell subsets, humoral/clinical parameters, and disease activity. The initial analysis documented a correlation between the number of circulating CD27^{high} plasma cells and disease activity in SLE. As shown in Table 2, the frequency and absolute number T2

Table 3. Classification of patients according to the SLEDAI score*

	SLEDAI 0–8 (n = 36)		SLEDAI >8 (n = 24)		
Parameter	Specificity	Negative predictive value	Sensitivity	Positive predictive value	Classifiable patients
Humoral/clinical	82.9	78.4	65.2	71.4	96.7
B lymphocyte	88.9	78.0	62.5	78.9	100.0
Combined humoral/clinical/ B lymphocyte	80.0	77.8	66.7	70.0	98.3

* Values are the percent. The CLASSIF1 program was used (32,33). SLEDAI = Systemic Lupus Erythematosus Disease Activity Index.

AQ: B

Table 4. Discriminatory parameter patterns according to the SLEDAI score*

Mask parameter	Disease classification masks: SLEDAI score 0–8	SLEDAI score >8
Humoral/clinical		
Anti-DNA by ELISA	0−	+
Circulating immune complexes	0−	+
Mucocutaneous manifestations	0−	+
Acute renal manifestations	0−	+
Increased ESR	0−	+
B lymphocyte		
Frequency of CD19+	0−	+
Frequency of CD27−/CD20+	0+	−
Absolute no. of CD27−/CD20+	0+	−
Frequency of $CD27^{high}$/CD20−	0−	+
AQ: 8 Absolute no. of $CD27^{high}$/CD20−	0−	+
Combined humoral/clinical/B lymphocyte		
Anti-DNA by ELISA	0−	+
Mucocutaneous manifestations	0−	+
T3 Frequency of $CD27^{high}$/CD20−	0−	+

* By applying the CLASSIF1 program, discriminatory mask parameters were identified that allowed distinction between patients with systemic lupus erythematosus (SLE) who had less active disease and those who had active disease. The optimally discriminatory lower and upper percentiles for the triple matrix transformation of the parameter distributions of patients with an SLEDAI score of 0–8 were 20–80% AQ: 9 for humoral/clinical, 30–70% for B lymphocyte, and 25–75% for combined parameters. The software automatically determines the optimal discriminatory data pattern for the most discriminatory percentile pair. 0 = unchanged, − = decreased, + = increased (see Table T4 5 for other definitions).

of $CD27^{high}$ plasma cells were correlated significantly with both the SLEDAI ($r_P = 0.4959$, $P < 0.0001$ and $r_s = 0.2618$, $P = 0.0433$, respectively) and the ECLAM ($r_P = 0.4205$, $P = 0.0008$ and $r_s = 0.4890$, $P < 0.0001$, respectively).

To confirm this correlation and to assess the features of disease activity that were most predictive of disease activity, a nonparametric data sieving algorithm (CLASSIF1) was used. Patients were divided into those with low disease activity (SLEDAI score 0–8) and those with active disease (SLEDAI score >8). The humoral/clinical parameters and the B lymphocyte parameters were then examined for their ability to discriminate between the two groups.

Humoral/clinical parameters alone provided predictive values of 78.4% and 71.4% for the discrimination of less active (SLEDAI score 0–8) and active disease (SLEDAI score >8) (Table 3), with 96.7% of patients considered classifiable. Patients who were not classifiable were given transitional classifications, which means that they were equally distributed between patients with low-activity disease and those with active disease. When only humoral/clinical parameters were considered, increased anti-DNA antibodies, circulating immune complexes, mucocutaneous and acute renal manifestations, as well as increased ESR were indicative of patients with active disease (SLEDAI score >8) (Table 4A).

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Table 5. Quantitation of discriminatory parameters*

Parameter	SLEDAI score 0–8 (n = 36)	Lower/upper percentiles for SLEDAI scores 0–8†	SLEDAI score >8 (n = 24)	*P*
Humoral/clinical				
Anti-DNA by ELISA	7.25 ± 1.74	0.77/10.7	30.5 ± 8.0	<0.0005
Circulating immune complexes	36.6 ± 6.2	3.59/54.2	53.4 ± 11.5	NS
Mucocutaneous manifestations	1.25 ± 0.07	0.078/1.20	1.58 ± 0.10	<0.005
Acute renal manifestations	1.43 ± 0.08	0.166/1.52	1.52 ± 0.11	NS
Increased ESR	1.42 ± 0.08	0.167/1.53	1.54 ± 0.10	<0.01
B lymphocytes				
Frequency (%) of CD19+ cells	9.27 ± 1.40	3.92/10.5	10.3 ± 2.0	NS
Frequency (%) of CD27−/CD20+ cells	61.1 ± 3.7	49.5/74.7	50.9 ± 5.2	NS
Absolute no. of CD27−/CD20+ cells/μl	84.6 ± 24.4	10.0/64.7	53.9 ± 17.0	NS
Frequency (%) of $CD27^{high}$/CD20− cells	8.20 ± 1.60	2.62/9.12	16.7 ± 2.6	<0.0025
Absolute no. of $CD27^{high}$/CD20− cells/μl	4.38 ± 0.69	1.52/5.92	8.62 ± 2.04	<0.0125
Combined humoral/clinical/B lymphocytes				
Anti-DNA by ELISA	7.25 ± 1.74	1.28/8.39	30.5 ± 8.0	<0.0005
Mucocutaneous manifestations	1.25 ± 0.07	0.13/1.00	1.58 ± 0.10	<0.005
Frequency (%) of $CD27^{high}$/CD20− cells	8.20 ± 1.60	2.47/10.3	16.7 ± 2.6	<0.0025

* Except where indicated otherwise, values are the mean ± SEM. *P* values were determined by Student's *t*-test. SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; ELISA = enzyme-linked immunosorbent assay; ESR = erythrocyte sedimentation rate; NS = not significant.

† The optimally discriminatory lower and upper percentiles for the triple matrix transformation of the parameter distributions of patients with an SLEDAI score of 0–8 were 20/80% for humoral/clinical, 30/70% for B lymphocytes, and 25/75% for combined parameters, as determined automatically using the CLASSIF1 program.

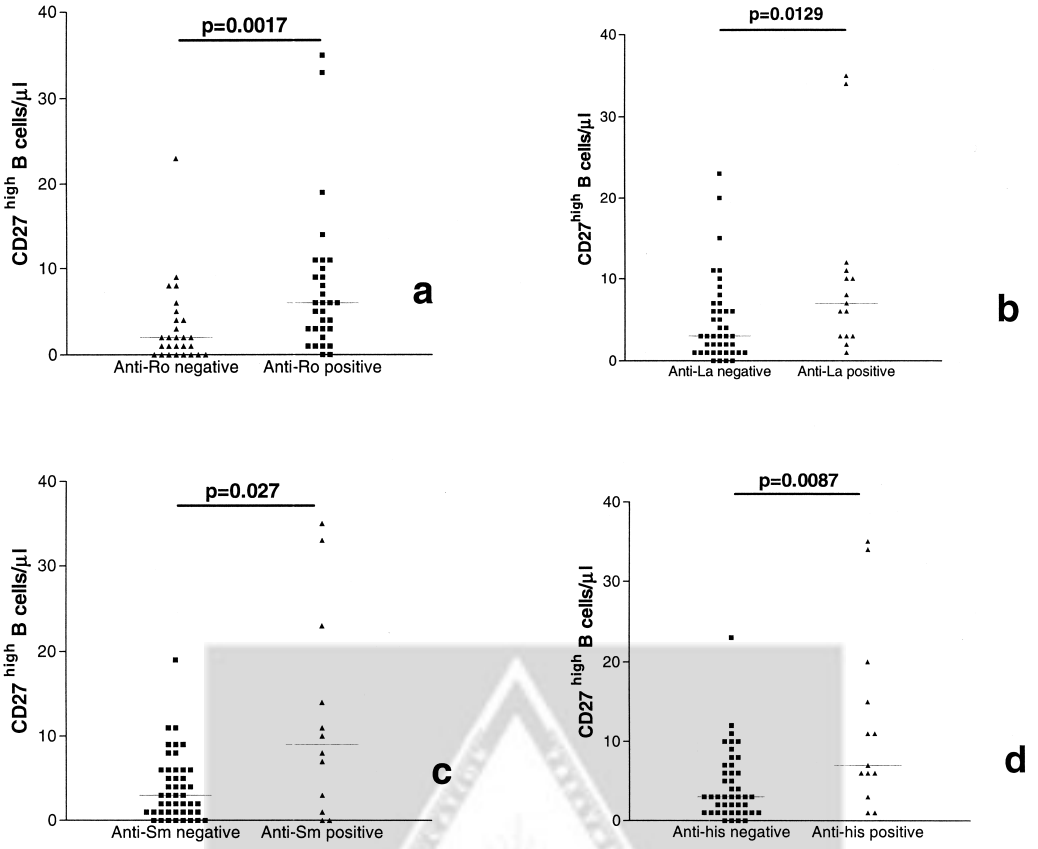


Figure 1. Comparison of absolute numbers of CD27^{high} plasma cells in systemic lupus erythematosus (SLE) patients with and those without anti-Ro (**a**), anti-La (**b**), anti-Sm (**c**), and antihistone (anti-his) (**d**) antibodies. Values are the medians, by Mann-Whitney U test.

Quantitative comparison identified anti-DNA antibodies ($P < 0.0005$), mucocutaneous manifestations ($P < 0.005$), and increased ESR ($P < 0.01$) as being significantly different between the two patient groups (Table 5). T5

B cell parameters provided higher predictive values for low-activity disease and active disease (78.0% and 78.9%, respectively) (Table 3), and all patients were classifiable. A pattern of increased frequencies of CD19+ B cells and CD27^{high}/CD20− plasma cells, enhanced absolute numbers of CD27^{high}/CD20− B cells, as well as decreased frequencies and absolute numbers of CD27−/CD20+ naive B cells, was identified as indicative of patients with a SLEDAI score >8 (Table 4B). The frequency and absolute number of CD27^{high} plasma cells were significantly increased in patients with active SLE ($P < 0.0025$ and $P < 0.0125$, respectively) when the discriminatory parameters were compared quantitatively between the patient groups (Table 5).

Notably, combining humoral/clinical and B cell parameter classification did not provide a better result than did classification based on B cell parameters alone (Table 3). The discriminatory data pattern identified by CLASSIF1 contained 3 parameters (frequency of CD27^{high} plasma cells, anti-DNA antibodies, and mucocutaneous manifestations) (Table 4C). Anti-DNA antibodies ($P < 0.0005$), mucocutaneous manifestations ($P < 0.005$), and an enhanced frequency of CD27^{high} plasma cells ($P < 0.0025$) were all significantly associated with active disease (SLEDAI score > 8) (Table 5). AQ: 10 AQ: 11

B cell subsets and age. Previous data have shown that the frequency of CD27+ memory B cells reflects the accumulation of antigen-experienced B cells in healthy individuals and appears to be related to age (34–36). Therefore, we tested this hypothesis for the patients examined in the current study (mean [± SD] age 38.7 ± 12.7 years) but found no significant correlation between the age of patients with SLE and the frequency or absolute number of CD27− naive B cells, CD27+ memory B cells, and CD27^{high} plasma cells.

B cell subsets and disease duration. Further analysis addressed the relationship between the frequency of CD27− naive B cells, CD27+ memory B cells, or $CD27^{high}$ plasma cells and the duration of SLE. Interestingly, disease duration was correlated inversely with the frequency as well as the absolute number of CD27− naive B cells ($r_P = -0.326$, $P = 0.0161$ and $r_s = -0.4330$, $P = 0.0011$) (results not shown). In contrast, the frequency of $CD27^{high}$ plasma cells was significantly correlated with the duration of disease ($r_P = 0.2902$, $P = 0.0333$) (results not shown). The frequency and absolute number of CD27+ memory B cells were found to be independent of disease duration in the patients examined.

Relationship between B cell subsets and the presence of autoantibodies. The occurrence of anti-Ro, anti-La, antihistone, and anti-Sm antibodies, the titer of anti-dsDNA, and the titer of aCL (IgG and IgM) antibodies were analyzed to investigate whether the number or frequency of $CD27^{high}$ plasma cells is related to the individual autoantibody profile of patients with SLE. Indeed, patients who were positive for anti-Ro, anti-La, anti-Sm, or antihistone autoantibodies (Table 1) showed significantly higher absolute numbers of peripheral $CD27^{high}$ plasma cells compared with patients lacking these antibodies ($P = 0.0017$, $P = 0.0129$, $P = 0.027$, $P = 0.0087$, respectively) (Figures 1a–d). The absolute number and the frequency of plasma cells were significantly higher in patients producing anti-dsDNA autoantibodies compared with patients lacking these antibodies ($P = 0.0011$ and $P = 0.0059$, respectively) (Figures 2A and B). In addition, there was a significant correlation between the frequency of $CD27^{high}$ plasma cells and the anti-dsDNA antibody titer ($r_s = 0.4049$, $P = 0.0015$). The correlation between the absolute number of $CD27^{high}$ plasma cells and the anti-dsDNA antibody titer was also significant, but of a lesser magnitude ($r_s = 0.3893$, $P = 0.0023$). Neither the occurrence nor the titer of aCL antibodies or circulating immune complexes was found to be correlated with the number or frequency of $CD27^{high}$ plasma cells in patients with SLE (data not shown).

To rule out the possibility that the correlation between the SLEDAI and the frequency of $CD27^{high}$ plasma cells was limited to a subgroup of anti-dsDNA–positive patients, and to investigate the relationship of these cells and disease activity, a further analysis was performed. Patients were subdivided into those who were positive or negative for anti-dsDNA antibodies by either ELISA or *C luciliae* immunofluorescence. In patients producing large amounts of anti-dsDNA antibodies and showing positivity of the *C luciliae* immunofluorescence test (n = 11), the correlation between the frequency of $CD27^{high}$ plasma cells and the SLEDAI ($r_s = 0.6713$, $P = 0.0237$) was greater than that in patients lacking anti-dsDNA antibodies detectable by immunofluorescence (n = 49) ($r_s = 0.4359$, $P = 0.0017$). The correlation coefficients of both subgroups differed only moderately.

Several patients had anti-dsDNA–specific antibodies detectable by ELISA but not by *C luciliae* immu-

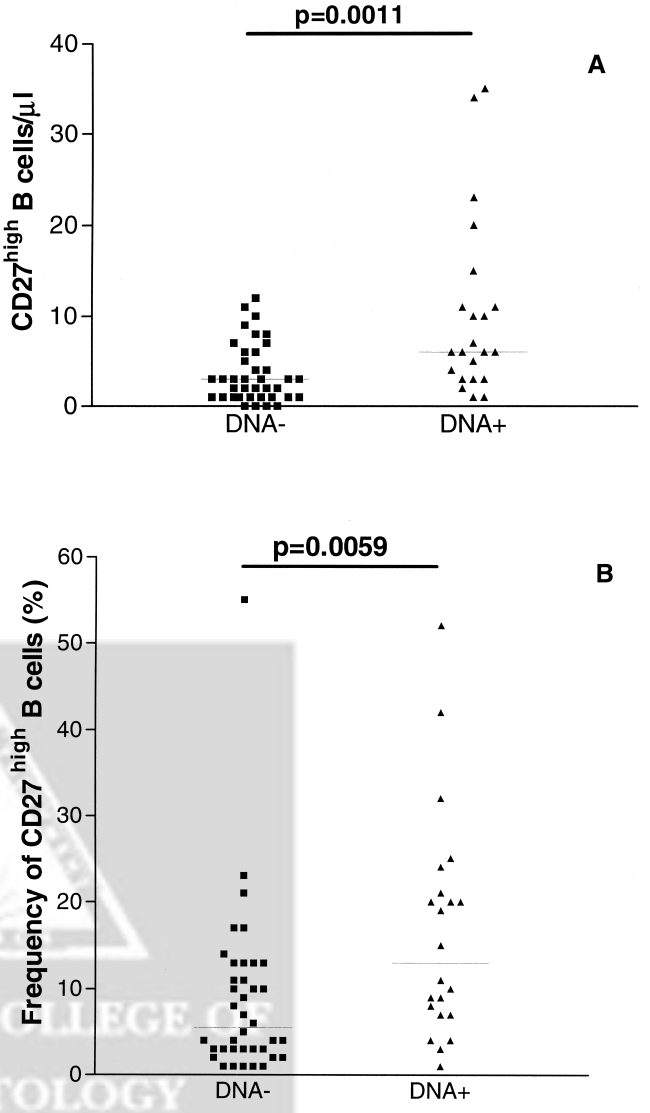
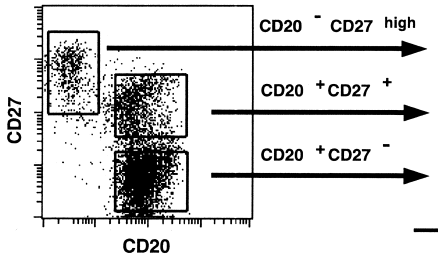


Figure 2. Comparison of the absolute number (**A**) and frequency (**B**) of $CD27^{high}$ plasma cells in anti–double-stranded DNA (anti-dsDNA)–positive patients with systemic lupus erythematosus (SLE) versus anti-dsDNA–negative patients with SLE. Values are the medians, by Mann-Whitney U test.



Total number of sorted cells/well	Total number of spots/well					
	IgA		IgG		IgM	
100	29	33	12	10	0	1
20	3	6	2	0	0	0
500	0	0	2	0	0	0
100	0	0	0	1	1	0
500	0	0	0	0	0	0
100	0	0	0	0	0	0

Figure 3. Detection of spontaneous immunoglobulin production by peripheral B cells in a patient with systemic lupus erythematosus. CD27^{high} plasma cells as well as CD27− naive and CD27+ memory B cells were separated by fluorescence-activated cell sorting. Different total numbers of cells were then subjected to an ELISpot assay. Values are the numbers of IgG-, IgA-, and IgM-producing cells in these populations.

nofluorescence (Table 1). Therefore, a second subanalysis examined patients who had anti-dsDNA antibodies detectable only by ELISA (cutoff 10 units/ml). A group of 22 patients with anti-dsDNA antibody titers >10 units/ml was compared with a group of 38 patients who had negative anti-dsDNA tests by ELISA. For both patient groups, a significant correlation between the SLEDAI and the frequency of CD27^{high} plasma cells was found (for anti-dsDNA–positive patients, $r_s = 0.4425$, $P = 0.0349$; for anti-dsDNA–negative patients, $r_s = 0.3465$, $P = 0.0331$).

B cell subsets and immunoglobulin levels. Spontaneous production of immunoglobulins by PBLs ex vivo in SLE has been documented previously (11,12). To confirm that CD27^{high} plasma cells were actually secreting immunoglobulin, B cell subsets were sorted, and an ELISpot assay was performed (Figure 3). The production of IgG, IgA, and IgM by CD27− naive B cells, CD27+ memory B cells, and CD27^{high} plasma cells in a patient with SLE with 8.54% CD27^{high} plasma cells (9 cells/μl blood), 21.31% CD27+ memory B cells (23 cells/μl blood), and 70.20% CD27− naive B cells (74 cells/μl blood) was determined. The results of this assay revealed that 10–12% of CD27^{high} plasma cells produced IgG and that 29–33% produced IgA, whereas IgM was produced by only very few CD27^{high} plasma cells (1%). Therefore, >50% of CD27^{high} plasma cells were actively secreting immunoglobulin, which was confirmed in other patients with SLE. Few immunoglobulin-secreting cells were found in the other B cell subsets.

In a subgroup of patients (n = 5), flow cytometric analysis documented that a large percentage of CD27^{high} plasma cells contained cytoplasmic IgA or IgG (median 54.72% for IgA [range 44.00–86.00%] and 40.20% for IgG [range 22.30–44.00%]), which is typical of plasma cells. Analysis of the serum IgA, IgG, and IgM levels of 36 of the analyzed patients with SLE, however, did not reveal any significant correlation between levels of immunoglobulins and the number or frequency of CD27^{high} plasma cells. Furthermore, no significant correlation was found between the serum levels of IgG or IgA and disease activity assessed by SLEDAI.

B cell subsets and therapy. SLE itself (37) as well as immunosuppressive therapy are known to influence the overall number of lymphocytes in the peripheral blood (38–41). Therefore, the influence of immunosuppressive therapy on the presence of B lymphocytes, CD27− naive B cells, CD27+ memory B cells, and CD27^{high} plasma cells in the blood was analyzed in more detail. To minimize variations related to the influence of disease activity, only patients with an SLEDAI score ≥8 who were receiving conservative therapy were included (n = 31). Within this group, patients receiving less intensive therapy (n = 13) were compared with patients receiving more intensive therapy (n = 18) (Table 1). The mean (± SD) SLEDAI score was comparably high in both patient groups (12.86 ± 7.37 versus 13.95 ± 5.86). In patients receiving more intensive therapy, the absolute number of all B cell subsets was diminished compared with that in patients receiving less intensive therapy (Figures 4a–d). This difference was statistically significant for CD27− naive B cells ($P = 0.0008$) and CD27+ memory B cells ($P = 0.0209$) but was not significant for CD27^{high} plasma cells. As a result of the

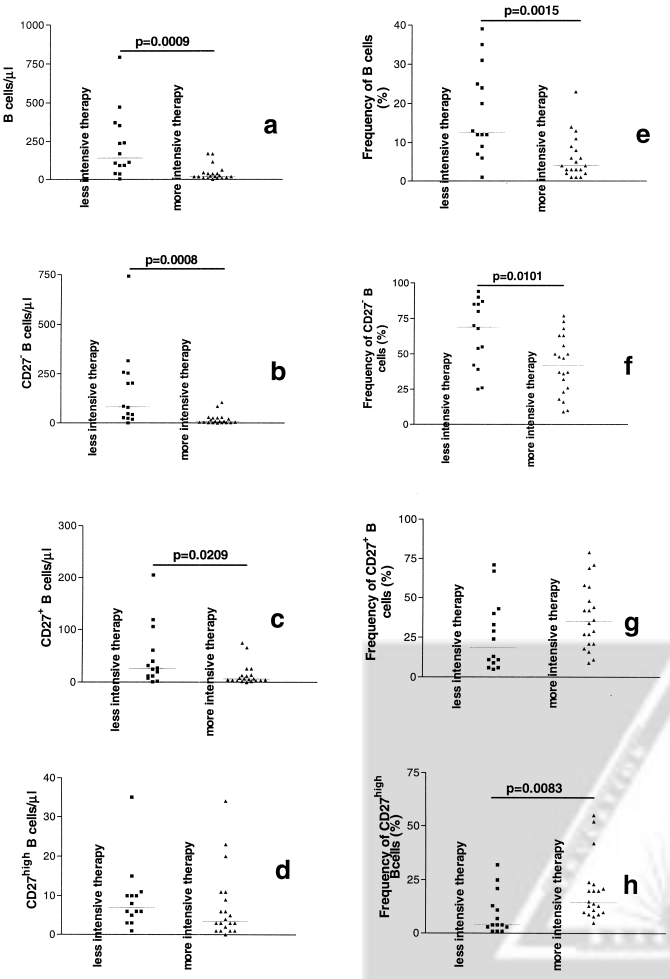


Figure 4. Comparison of the influence of the intensity of immunosuppressive therapy on the absolute number (**a**–**d**) and frequency (**e**—**h**) of total B cells, CD27− naive B cells, CD27+ memory B cells, and CD27^{high} plasma cells in patients with a Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score ≥8. The mean (± SD) SLEDAI score was comparably high in both patient groups. Values are the medians, by Mann-Whitney U test.

predominant influence of SLE therapy on CD27− naive B cells, the frequency of CD27^{high} plasma cells was relatively increased in patients receiving more intensive therapy compared with those who received less intensive treatment ($P = 0.0083$), although absolute numbers of CD27^{high} plasma cells were found to be reduced (Figure 4).

AQ: 12

DISCUSSION

Flow cytometric monitoring of B cells made it possible to identify characteristic changes in peripheral B cell homeostasis that are significantly correlated with disease activity in patients with SLE. By applying the CLASSIF1 program (32,33), which allows identification of discriminatory patterns between patient subsets, we observed that anti-DNA antibodies, mucocutaneous manifestations, and B cell abnormalities (especially increased frequencies of CD27^{high} plasma cells) discerned SLE patients with low-activity disease (SLEDAI score 0–8) from those with active disease (SLEDAI score >8). Moreover, an expansion of CD27^{high} plasma cells was found to be associated with the serologic presence of particular autoantibodies (anti-dsDNA, anti-Ro, anti-Sm, anti-La, and antihistone antibodies) and the duration of the disease.

The absolute number as well as the frequency of CD27^{high} plasma cells correlated with increased disease activity measured by both SLEDAI and ECLAM, but also after patients received very intensive immunosuppressive therapy cyclophosphamide, cyclosporine, azathioprine plus prednisone). The latter effect appeared to be related to the major reduction of naive CD27− B cells caused by intensive therapy. This indicates that disease activity as well as immunosuppressive therapy can influence the number and frequency of circulating B cell subsets and suggests that careful monitoring of B cell subsets (in particular the frequency of CD27^{high} plasma cells) in the blood is a useful tool to assess not only disease activity but also the efficacy of therapy in SLE. However, analysis of B cell subsets is not only diagnostically valuable in SLE but also allows differentiation of patients with primary Sjögren's syndrome. In primary Sjögren's syndrome, an enhanced naive B cell pool and reduced numbers of memory B cells and plasma cells were observed (42,43).

AQ: 13

The positive correlation between the absolute number and frequency of CD27^{high} plasma cells and disease activity is consistent with an important role for these cells in the pathogenesis of SLE. This correlation seemed to be independent of the presence of anti-dsDNA antibodies. Thus, flow cytometric analysis of peripheral CD27^{high} plasma cells allows direct monitoring of those cells critically involved in disease pathogenesis and is a reliable assessment of disease activity, regardless of the presence of anti-dsDNA antibodies. In contrast to the current study, a study by Arce et al, who also described an expansion of plasma cell precursors (CD19+,CD20−,CD38++) in children with SLE, could not demonstrate a correlation of these cells with either disease activity or therapy (14). Children with highly active disease (SLEDAI score of 10), however, showed a greater mean absolute number of CD19+,CD20−,

CD38++ B cells and a larger standard deviation than those with less disease activity. The apparent discrepancy between the studies may be explained by differences in therapy, because those children with lupus had been extensively treated. In addition, CD27^{high} plasma cells were not directly analyzed in the previous study. Finally, differences in the baseline distribution of B cell subsets in children (27), especially normal CD27− naive B cells, may have also contributed to the differences.

Besides an activity-related increase in the number of CD27^{high} plasma cells, a reduction of naive B cells possibly caused by autoantibodies against these cells in patients with active disease can be considered. Bhat et al (44) characterized V_H4–34–encoded anti-i/anti-B cell antibodies in SLE patients as having both a complement-independent cytotoxic effect for human B lymphocytes and the capacity to bind erythrocytes. In addition, Cappione et al (45) described antibodies against a CD45 glycoform ("i" determinant; B220) that is preferentially expressed on the cell surface of naive B cells. These antibodies were also encoded by a V_H4–34 heavy chain gene. Interestingly, absorption experiments revealed that >75% of the lymphocytotoxic activity in sera obtained from patients with SLE is related to these V_H4–34–encoded antibodies. Furthermore, elevated titers of V_H4–34–encoded antibodies have been detected in sera of patients with SLE, showing a fluctuation with disease activity in some patients (46–48). In this regard, we previously reported an expansion of V_H4–34–expressing cells in the CD27^{high} plasma cell population (13), and patients with B lymphopenia had a significantly higher frequency of CD27^{high} plasma cells compared with the other patients.

Previous studies in adult and juvenile patients with SLE (13,27) demonstrated an increase of CD27^{high} plasma cells in the peripheral blood, whereas these cells were found at a mean ± SD frequency of 1.4 ± 0.8% in normal individuals (13). Although disease activity and the frequency of CD27^{high} plasma cells were correlated positively in both anti-dsDNA–positive and anti-dsDNA–negative patients, significantly higher absolute numbers of CD27^{high} plasma cells were also observed in patients who were positive for other autoantibodies (anti-Sm, anti-La, anti-Ro, antihistone, or anti-dsDNA).

These findings again emphasize a central role of these cells in the pathogenesis of SLE, likely based on their capacity to produce immunoglobulin, particularly pathogenically relevant autoantibodies such as anti-dsDNA antibodies, which usually result from an autoantigen-driven process involving the induction of somatic hypermutation, immunoglobulin heavy chain switching, and affinity maturation. Notably, the majority of CD27^{high} plasma cells expressed intracytoplasmic immunoglobulin. Moreover, previous studies of V_H gene analysis (13,14) demonstrated extraordinary highly mutated and clonally related V_H gene rearrangements within the CD27^{high} plasma cell population. These results indicate that CD27^{high} plasma cells were the products of a B cell response that involved induction of somatic hypermutation, immunoglobulin heavy chain isotype switching, and affinity maturation as well as clonal expansion. However, polyclonal B cell activation must also be considered as a possible cause of CD27^{high} plasma cell expansion. To determine the specificity of these cells, potential autoantigens and foreign antigens are currently being tested using immunoglobulin secretion assays and intracellular cell staining. Notably, however, the serum immunoglobulin levels (IgG and IgA) of a subgroup of patients correlated with neither the frequency of CD27^{high} plasma cells nor disease activity.

The percentage of plasma cells and their absolute number in the peripheral blood of patients with SLE might increase during active disease, as a result of either rapid generation or prolonged survival of these cells. In addition, disturbances in homing behavior or B cell trafficking can be considered as possible causes for the observed expansion of this B cell population in the peripheral blood of patients with active SLE. Increased blood levels of IL-10 and IL-6 (22–25), BLyS (19), and soluble CD154 (26), all of which are capable of perpetuating B cell maturation and causing the generation of plasma cells and subsequent antibody production (23,49), have been reported in patients with SLE. Disturbed expression of costimulatory molecules might also contribute to the enhanced B lymphocyte activation in SLE. In this context, enhanced or prolonged expression of CD154 on SLE T cells and B cells in vitro and ex vivo as well as overexpression of CD86 in SLE B cells (ex vivo) have been described recently (6,15,50). However, less is known about the in vivo expression and function of these costimulatory molecules on B cell subpopulations in patients with SLE. In addition, altered homing behavior of differentiated B cells must be considered as a contributing reason for disturbances in the B cell homeostasis in active SLE, and the expression of adhesion molecules and chemokine receptors needs further study.

Patients with additional infectious disease or vaccination were excluded from the current study. Similar to lupus flares, vaccination or infectious disease has been shown to cause reactive plasmacytosis (51,52). Further examinations are currently underway to attempt

to discriminate between plasmacytosis caused by infectious disease and expansion of plasma cells caused by increased SLE disease activity, based on further characterization of the B cell phenotype by flow cytometry.

Immunosuppressive therapy is known to reduce lymphocyte counts (38–41). When only those patients in the current study who had a SLEDAI score ≥8 were analyzed, the percentage and absolute number of $CD27^{high}$ plasma cells were increased, with a predominant reduction of CD27− naive B cells. This suggests that naive B cells are more susceptible to immunosuppressive drugs (azathioprine, cyclophosphamide, cyclosporine). However, in the followup analyses of these patients, which are currently underway, monitoring of B cell subsets is required to allow an exact comparison of the different therapeutic effects of the most frequently used immunosuppressive drugs (azathioprine, cyclophosphamide, cyclosporine, hydroxychloroquine, prednisone, mycophenolate).

AQ: 16

The duration of disease was found to be positively correlated with the percentage and absolute number of the $CD27^{high}$ plasma cells but was negatively associated with the absolute number of CD27− naive B cells. Based on the observations mentioned above, this is probably related to both the impact of disease activity and the influence of long-term immunosuppressive therapy, and apparently reflects the chronicity of this autoimmune disease. Whether the changing pattern of enhanced numbers of $CD27^{high}$ plasma cells and declining numbers of naive CD27− B cells is also characteristic of individual courses of the disease needs to be addressed.

In conclusion, therapy as well as disease activity seem to influence the pattern of peripheral B cell subsets interdependently. Nevertheless, flow cytometric monitoring of circulating B cell subsets, especially monitoring the frequency of $CD27^{high}$ plasma cells, provides new insights and diagnostically valuable options for monitoring disease activity in patients with SLE.

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