Analyses of CD27^{++} Plasma Cells in Peripheral Blood from Patients with Bacterial Infections and Patients with Serum Antinuclear Antibodies

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The number of CD27^{++} plasma cells (PCs) in peripheral blood may be a valuable biomarker for systemic lupus erythematosus (SLE) disease management. More insights into the behavior of the PC population are, however, required to validate CD27 as a reliable biomarker. In the current study, we have monitored the PC compartment of patients with acute bacterial infections and patients with SLE and, in addition, examined the relationship between the presence of serum antinuclear antibodies (ANAs) and the number of peripheral PCs. Kinetic analyses in patients with bacterial infection revealed a 10–60-fold expansion of the CD27^{++} PC compartment that peaked at day 2–5 and returned toward normal values at day 7–9 after hospital admission. The transient expansion of the PC population appeared to be a late phenomenon in the process of recovering from a bacterial infection. SLE subjects had significantly increased frequencies of PCs compared with patients suspected of a connective tissue disease and healthy controls. In patients suspected of a connective tissue disease, no relationship was found between the presence of serum ANAs and the number of CD27^{++} PCs. Additionally, the presence of serum ANAs was not associated with abnormalities in other peripheral B-cell subsets. It remains to be established at which stage of SLE development the expansion of the PC compartment is initiated.

KEY WORDS: Antinuclear antibodies; CD27; Infection; Plasma cells; SLE.

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INTRODUCTION

B lymphocytes continuously recirculate through peripheral blood and secondary lymphoid organs. B lymphocytes that encounter antigens become activated and differentiate into antibody-producing plasma cells or, alternatively, become memory B cells. This process of antigen-specific B-cell differentiation occurs within germinal centers in secondary lymphoid organs, where activated naïve B cells undergo vigorous proliferation, somatic hypermutation of immunoglobulin (Ig) variable-region genes, Ig isotype switching, and antigen-driven selection (1, 2).

The CD27 molecule belongs to the tumor necrosis factor receptor family and is involved in the late-stage differentiation of B cells by providing a key signal for the maturation of B cells into plasma cells (PCs) (3–5). On the basis of CD27 cell-surface expression peripheral blood B cells can be divided into three different subpopulations. CD27^{+} B cells carry somatically mutated Ig variable genes and have been identified as memory B cells. Naïve B cells that do not display such mutations are defined as CD27^{-} (IgM^{+} IgD^{+}) B cells (6–9). Peripheral blood B lymphocytes with high levels of CD27 (CD27^{++}) were characterized as PCs, which express low levels of CD19 and surface Ig, high amounts of CD38 and CD138, and are CD20^{-} (10–13). Disturbances in homeostasis of specific peripheral blood B-cell subsets have been described in patients with systemic autoimmune disorders. Patients with systemic lupus erythematosus (SLE) exhibit an expanded population of CD27^{++} PCs, while the number of CD27^{-} naïve B cells is reduced (10, 12, 14, 15). Analysis of peripheral blood from patients with primary Sjogren's syndrome and systemic sclerosis showed a predominance of CD27^{-} naïve B cells and reduced frequencies of CD27^{+} memory B cells compared with normal

donors (15–17). Recently, an immature B-cell subpopulation, so-called transitional B cells, has been identified in peripheral blood (18). Increased frequencies of transitional B cells, which are IgD$^+$ CD38^{++}, were observed in patients with SLE and other autoimmune disorders such as primary Sjogren's syndrome (18–20). Connective tissue diseases (CTD) are autoimmune diseases characterized by various organ manifestations and the production of a broad variety of autoantibodies that are frequently targeted against intracellular antigens of the cell nucleus (i.e., antinuclear antibodies, ANAs) (21). Although B-cell hyperreactivity and ANA production are characteristic hallmarks of CTD, especially SLE, serum ANAs are often found in subjects who do not fulfil the classification criteria for a CTD (22). Recently, it has been demonstrated that ANAs are typically present many years before the diagnosis of SLE, while patients are still asymptomatic (23). It is unknown whether the presence of serum ANAs coincide with elevated frequencies of CD27^{++} PCs. Accurate diagnosis and assessment of disease activity of SLE is important to reduce morbidity and mortality. However, in view of its heterogeneous presentation and unpredictable course, clinical management of SLE is difficult (24). The frequency of CD27^{++} PCs has been shown to significantly correlate with SLE-disease activity: patients with active SLE had higher frequencies of PCs than patients with inactive SLE (12, 25). Hence, CD27 appears a promising biomarker for monitoring SLE-disease activity. Additionally, analyses of the CD27^{++} PC compartment may be of diagnostic value in SLE. Insights into the behavior of the PC compartment are essential to validate CD27 as a reliable biomarker for SLE. Indeed, polyclonal B-cell activation due to infection could be considered as a possible cause of CD27^{++} PC expansion. There is, however, a paucity of data on the behavior of the CD27^{++} PC compartment in a well-controlled immune response. In the current study, the appearance and disappearance of PCs in patients suffering from acute bacterial infection were studied. Further, we have investigated whether the presence of serum ANAs in subjects who do not fulfil the classification criteria for a CTD is associated with abnormalities in B-lymphocyte subsets.

MATERIALS AND METHODS

Subjects and Blood Sampling

Blood samples of 120 consecutive patients suspected of CTD (82 female, 38 male; mean age 48 years, range 18–66 years) and 30 clinically quiescent SLE patients (28 female, 2 male; mean age 50 years, range 24–75 years)

sent to the clinical laboratory for ANA assessment were included. The control group consisted of 16 healthy blood donors (14 female, 2 male; mean age 48 years, range 25–57 years). The patients were referred by inhouse specialists, including rheumatologists, internists, dermatologists, neurologists, and nephrologists. Clinical data were obtained from the patient records. SLE was diagnosed according to the American College of Rheumatology classification criteria (21, 26). Patients known with other types of autoimmune diseases were excluded. Within the group of patients suspected of CTD, six patients were treated with immunosuppressive drugs at the time of analysis (two patients were receiving hydrochloroquine (200 mg/day), three patients were receiving low doses of prednisone (one patient: 10 mg weekly; two patients 2.5–5.0 mg daily), and one patient was being treated with 7.5 mg methorexate weekly). The remaining CTD-suspected patients were not receiving therapy. All six patients receiving immunosuppressive therapy had arthritis-like symptoms. Within the patient group of SLE, 21 patients received low doses of prednisone (between 5 and 10 mg daily) and nine patients were not receiving therapy. During the study, two newly diagnosed SLE patients admitted to our hospital due to an elevated disease activity were examined. One patient was admitted due to abdominal pain and deep venous thrombosis. Subsequently, an antiphospholipid antibody syndrome and active nephritis were established. Furthermore, shortly (day 4) after hospital admission, the patient developed a malar rash and leucocytopenia. In serum high titres of anti-ds DNA antibodies, antinuclear antibodies and low levels of complement were detected. Immunosuppressive therapy including treatment with methylprednisolone (1000 mg for 3 days, thereafter 20 mg/day) and mycophenolate mofetil (1000 mg twice daily) was started 4 weeks after admission. The second SLE patient was admitted due to excessive polyarthritis. Furthermore, a leucocytopenia, thrombocytopenia, elevated titres of anti-ds DNA antibodies, serum antinuclear antibodies, circulating immune complexes, and low levels of complement were established. Upon admission, the patient was receiving low dosages of prednisone (5 mg/day). The patient's condition improved without increasing the dose of prednisone or starting therapy with other immunosuppressive agents.

A total of six patients with an acute bacterial infection admitted to the Emergency Department were monitored for their plasma cell number in peripheral blood and serum C-reactive protein (CRP) during their hospital stay. All patients were treated with antibiotics upon admission. Blood was sampled from an antecubital vein by venipuncture (Becton Dickinson Vacutainer, Meylan Cedex, France). The study was approved by the ethical com-

mittee of Sint Lucas Andreas Hospital, Amsterdam, The Netherlands.

Immunofluorescence Staining and Flow Cytometric Analysis

Total white blood cell and lymphocyte count were performed on an automated Bayer ADVIA 120 blood cell counter (Bayer HealthCare, Tarrytown, NY, USA). Cell-surface expression of CD markers on leukocytes was analyzed by three-color immunofluorescence cytometry using a whole blood staining procedure as previously described (27). Briefly, 100 μL of EDTA anticoagulated whole blood was incubated with peridinin chlorophyll protein (PERC)-labeled anti-CD19 and fluorescein isothiocyanate (FITC)-labeled CD27 in combination with phycoerythrin (PE)-labeled CD20, or CD138 monoclonal antibodies (Becton Dickinson, San Jose, CA). Erythrocytes were lysed with FACS-lysing solution (Becton Dickinson) according to the manufacturer's instructions. Samples were washed with PBS/0.5% bovine serum albumin and analyzed on a FACS-track cytometer (Becton Dickinson). Lymphocytes were gated on the basis of forward- and right-angle scatter characteristics. For the detection of CD27, CD20, and CD138 expressing B cells, a live gate was set on the CD19$^+$ cell population. Frequencies of CD27^{++}/CD20$^-$ PCs, CD27$^-$ naïve B cells, and IgD$^+$ CD38^{++} transitional B cells of total CD19$^+$ cells were calculated using CellQuest software (Becton Dickinson).

Determination of ANA, Anti-ENA, and Anti-ds DNA Antibodies

Assays for serum antinuclear antibodies were performed by indirect immunofluorescence with HEp-2000 cells according to the recommendations of the manufacturer (ImmunoConcepts, Sacramento, CA). Detection of ANAs at a dilution of 1:40 was considered a positive result. Enzyme-linked immunosorbent assays were used to evaluate the presence of serum antibodies to SS-A, SS-B, RNP, Sm, Scl70, Jo-1, and histones (EliA, Pharmacia Diagnostics, Freiburg, Germany). Antidouble-stranded DNA antibodies were determined by FARR assay (Diagnostic Products Corporation, LA, USA).

Statistical Analysis

Statistical analyses were performed by using SPSS version 15.0 software. Nonparametric Mann–Whitney U test was used to establish the level of significance of differences between two groups of subjects. A P value of < 0.05 was considered statistically significant.

RESULTS

Appearance and Disappearance of PCs During Bacterial Infections and SLE

Sizes of the CD27^{++}/CD20$^-$ PC compartment were established in peripheral blood from patients with bacterial infection, SLE, and healthy controls. Furthermore, the behavior of the PC compartment was monitored in the group of patients with bacterial infection. In the healthy control group, the mean frequency of CD27^{++}/CD20$^-$ PCs was $0.8 \pm 0.1\%$ (SD $= 0.5\%$) and absolute number was 2.9 ± 0.8 10^6/L (SD $= 3.3$ 10^6/L). Increased mean frequencies of CD27^{++}/CD20$^-$ PCs were found in SLE patients (mean: $6.3 \pm 1.1\%$) compared with the control group ($P < 0.001$) (Fig. 1). The absolute number of CD27^{++}/CD20$^-$ PCs did not differ between SLE and controls ($P = 0.6$). Since immunosuppressive therapy may influence the presence of CD27^{++}/CD20$^-$ PCs, the relationship between immunosuppressive therapy and the number of PCs was analyzed (12, 25). No differences in either the frequency ($P = 0.4$) or absolute number ($P = 0.6$) of PCs between SLE patients ($N = 21$) receiving immunosuppressive therapy and those who received no therapy ($N = 9$) were found. Similarly, no relationship between immunosuppressive therapy and the number of PCs was detected within the group of patients suspected of CTD ($P = 0.8$). During the study, two newly diagnosed SLE patients exhibiting lupus flare were examined. On admission (day 0), both SLE patients had elevated frequencies and absolute numbers of CD27^{++}/CD20$^-$ PCs (i.e., > 2 SD above the mean of controls) that persisted on day 3 and 7 of hospitalization (Table I). One SLE patient (patient #1) showed an increase in the number of PCs at day 7 compared with day 3 that coincided with worsening of the patient's clinical condition (see the section on *Materials and Methods*). After receiving intensive immunosuppressive therapy, a complete clinical remission of SLE was reached. Analysis of PCs in blood from this patient several months later showed a normal number of CD27^{++}/CD20$^-$ PCs (Table I and Fig. 2).

The clinical characteristics of the patients with acute bacterial infection and the results of the PC analyses are summarized in Tables II and III, respectively. Upon hospital admission (day 0), patients with bacterial infection showed PC frequencies ranging from 0.5 to 5.6%. At day 0, two out of six patients had an increased frequency of PCs, i.e., CD27^{++} PCs $> 1.8\%$ (> 2 SD above the mean of controls). The absolute number of PCs at day 0 was moderately higher than controls ($P = 0.03$) (Fig. 1). More interestingly, a marked increase in PC number was detected during the first days of hospital-
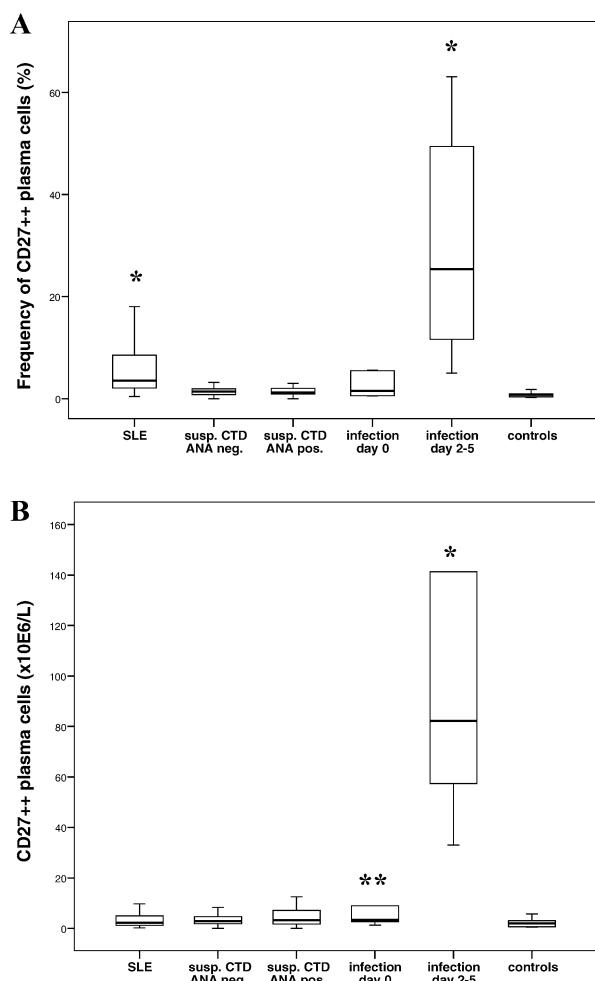
A

B

Fig. 1. *Box-and-whisker* plot of frequencies (A) and absolute number (B) of CD27^{++} plasma cells in peripheral blood from patients with SLE ($N = 30$), patients suspected of connective tissue disease (ANA negative, $N = 75$; ANA positive, $N = 45$), patients with bacterial infection ($N = 6$) on day 0 and day 2–5 after hospital admission, and healthy donors (controls, $N = 16$). The lower and upper limits of each *box* represent the 25th and 75th percentile, respectively. The *horizontal bar* within each *box* indicates the median. The *whiskers* emerging from the *boxes* extend to the upper and lower adjacent values and are defined as values < 1.5 times the box height. Significant differences from controls are shown (*$P < 0.001$,**$P = 0.03$).

Table I. Follow-Up Analysis of CD27^{++} PCs in Peripheral Blood from Two SLE Patients Admitted with Increased Disease Activity

Patient	Day(s) after admission	PC^a (%)	PC ($\times 10^6$/L)
1.	0	11.3	21.7
	3	15.4	24.3
	7	39.1	58.0
	122	1.1	1.9
2.	0	9.4	15.2
	3	8.0	14.5
	7	7.1	13.8

^{a}PC frequency of total CD19$^+$ B lymphocytes.

conditions early on (day 0) after antimicrobiotic treatment was initiated and recovered completely during their hospital stay. In support, decreasing CRP levels were found at the stage of expansion of the PC compartment (Table III).

Relationship Between the Presence of Serum ANAs and Sizes of B-Cell Compartments in CTD-Suspected Patients

The clinical characteristics of the group of CTD-suspected patients are summarized in Table IV. Sizes of IgD$^+$ CD38^{++} transitional B cell, CD27$^-$ naïve B cell, and CD27^{++}/CD20$^-$ PC subpopulations were established in peripheral blood of the patient groups and control group. The mean frequency of CD27^{++}/CD20$^-$ PCs, CD27$^-$ naïve B cells, and IgD$^+$ CD38^{++} transitional B cells in healthy controls was 0.8 ± 0.1%, 74 ± 2%, and 1.5 ± 0.2%, respectively. The observed frequencies are consistent with prior reports (12, 15, 17, 18, 28). SLE patients showed increased frequencies of CD27^{++}/CD20$^-$ PCs ($P < 0.001$) and IgD$^+$ CD38^{++} transitional B cells (mean: 5.5 ± 1.1%, $P = 0.01$) compared with controls. No differences in absolute numbers of CD27^{++}/CD20$^-$ PCs ($P = 0.6$) and IgD$^+$ CD38^{++} transitional B cells ($P = 0.1$) between SLE and controls were found.

In the group of patients suspected of CTD, the frequency of CD27^{++}/CD20$^-$ PCs was slightly increased compared with controls ($P = 0.03$). The absolute number of CD27^{++}/CD20$^-$ PCs did not differ from controls ($P = 0.06$). Within the group of CTD-suspected patients, patients with serum ANAs had similar frequencies of CD27$^-$ naïve B cells (mean: 76 ± 2% vs. 73 ± 2%, $P = 0.4$), IgD$^+$ CD38^{++} transitional B cells (mean: 2.3 ± 0.3% vs. 2.1 ± 0.2%, $P = 0.9$), and CD27^{++} PCs (mean: 1.6 ± 0.2% vs. 2.0 ± 0.4%, $P = 0.9$) as patients without serum ANAs (Fig. 1). These results indicate that the presence of serum ANAs does not coincide with aberrant B-cell-subset homeostasis.

ization. The peak of expansion, which resulted in a 10–60-fold increased PC-compartment size, was found between day 2 and 5 of hospitalization (Fig. 1). Between day 7 and 9 of hospital admission, the frequency of PCs returned toward normal values. As a representative, the kinetic of the PC compartment from one septic patient is shown in Fig. 3. Notably, the increase in PC number was not related to the worsening of the clinical condition of the patients. All patients showed improved clinical
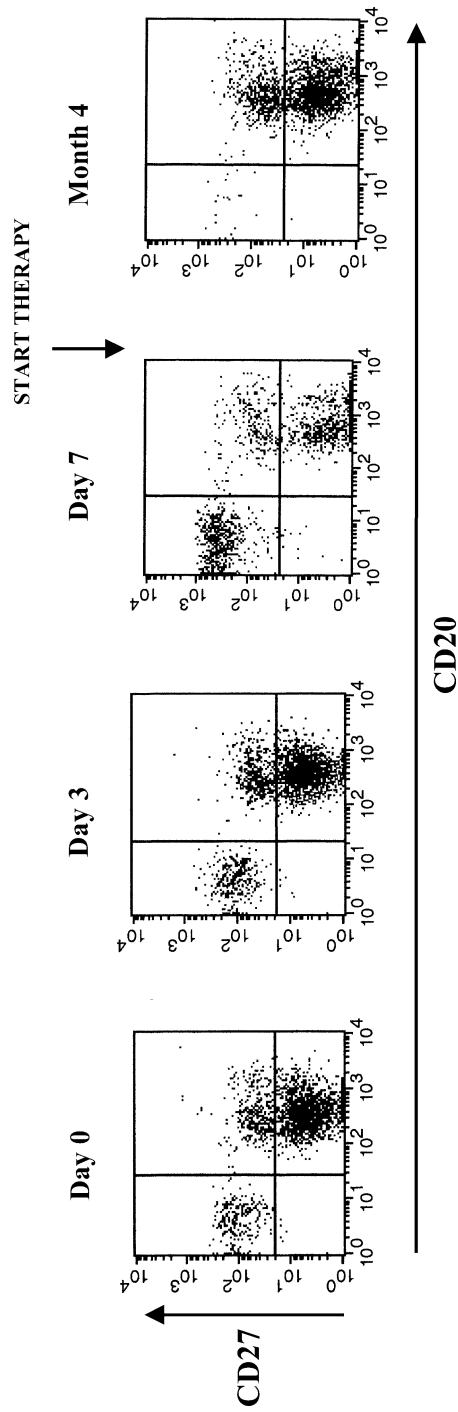
Fig. 2. Kinetics of the plasma cell compartment in peripheral blood from a SLE patient with a lupus flare. Days after hospital admission are indicated. Peripheral blood CD19^{+} lymphocytes were gated and stained for expression of CD27 in combination with CD20. Plasma cells were identified as CD27^{++}/CD20^{−} cells. Frequencies of CD27^{++} plasma cells were established at day 0, 3, 7, and 4 months after hospital admission. After 4 weeks of admission, the patient received intensive immunosuppressive therapy resulting in a clinical remission of SLE.
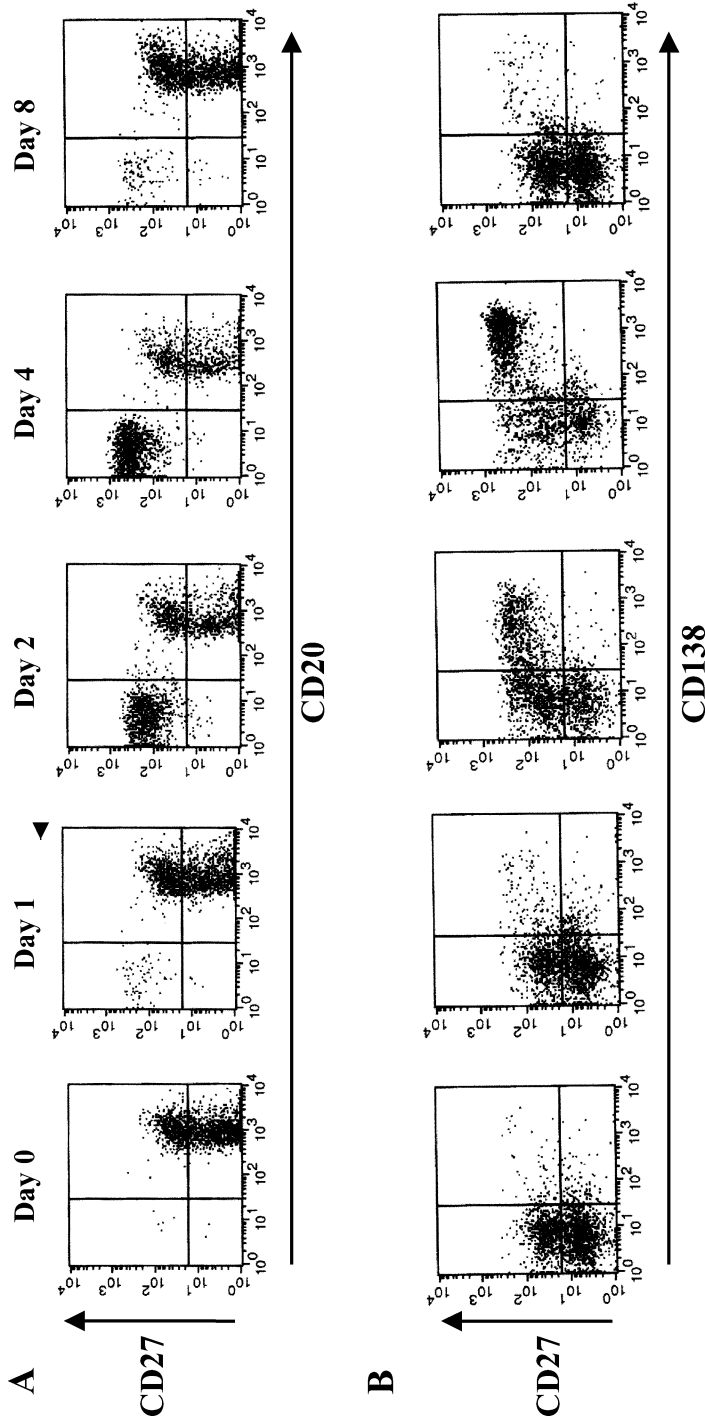
Fig. 3. Kinetics of the plasma cell compartment in peripheral blood from a patient with a bacterial infection. Days after hospital admission are indicated. Peripheral blood CD19^{+} lymphocytes were gated and stained for expression of CD27 in combination with CD20 or CD138 expression. Plasma cells were identified as (A) CD27^{++}/CD20^{−} cells and (B) CD27^{++}/CD138^{+} cells. Frequencies of CD27^{++} plasma cells were established at day 0, 1, 2, 4, and 8 after hospital admission. At day 4 of hospitalization, an expansion peak of plasma cells is seen.

Table II. Clinical Characteristics of the Studied Patients with Bacterial Infection

Patient	Sex	Age (years)	Results of culture	Diagnosis
1.	Female	55	*Blood*: Streptococcus pneumoniae	Acute pneumonia
2.	Female	26	*Blood*: Negative	Acute pyelonephritis
			Urine: Escherichia coli	
3.	Male	54	*Respiratory tract*: Streptococcus pneumoniae	Acute pneumonia
4.	Male	71	*Blood*: Streptococcus pyogenes	Acute renal failure
5.	Female	85	*Joint fluid*: Streptococcus pyogenes	Septic arthritis
			Blood: Streptococcus pyogenes	
6.	Female	62	*Respiratory tract*: Streptococcus pyogenes	Acute pneumonia
			Blood: Negative	

Table III. Percentage of CD27^{++} PCs in Peripheral Blood and Serum CRP Levels of Patients with Bacterial Infection During Hospitalization

Patient	Day(s) after admission	PC^a (%)	CRP (mg/L)
1	0	0.5	426
	1	1.2	450
	3	5.0	192
	7	2.1	27
	9	1.1	35
2	0	2.1	389
	1	4.0	219
	2	5.5	198
	4	12.6	88
	5	15.6	50
	11	2.8	6
3	0	1.0	616
	1	4.3	523
	2	45.4	324
	4	63.1	162
	8	5.0	72
4	0	5.5	449
	1	6.9	413
	2	14.8	327
	3	49.4	216
	5	20.5	93
	7	3.1	38
5	0	5.6	339
	2	35.1	273
	5	16.8	45
	8	5.0	67
	9	3.0	58
6	0	0.5	13
	1	0.6	67
	2	0.5	325
	4	11.6	68
	7	2.5	17

^{a}PC frequency of total CD19$^+$ B lymphocytes.

Relationship Between the Presence of Serum Anti-ENA Antibodies and Frequency of CD27^{++} Plasma Cells

ANA-positive serum samples were further analyzed for identification of the fine-antigen specificity by anti-ENA- and anti-ds DNA antibody testing. Within the group of SLE patients, the presence of serum anti-RNP antibodies ($N = 13$) was found to be associated with a higher frequency of CD27^{++}/CD20$^-$ PCs (mean:

Table IV. Prevalence of Clinical Manifestations of CTD-Suspected Patients ($N = 120$)

Clinical manifestation	%
Mucocutanous	14
Musculoskeletal	16
Serositis	5
Cardiovascular	2
Pulmonary	6
Gastrointestinal	19
Renal	9
Central nervous system	9
Hematologic	34
Others	6

8.5 $\pm$ 1.8%) compared with patients without these antibodies (mean: 4.2 $\pm$ 0.8%, $P = 0.02$). Anti-dsDNA ($N = 9$), anti-SS-A/B ($N = 10$), anti-SM ($N = 4$), and antihistones ($N = 14$) antibodies were not found to be related to a higher frequency of CD27^{++}/CD20$^-$ PCs (data not shown). In the group of CTD-suspected patients, anti-ENA positivity was found in five cases. No correlations between CD27-expressing B-cell subsets and the occurrence of anti-ENA antibodies were found. However, the small number of anti-ENA antibody-positive patients did not allow conclusions of statistical significance.

DISCUSSION

The current study identifies a characteristic pattern of appearance and disappearance of PCs in peripheral blood during bacterial infection. Patients showed a transient, 10–60-fold expansion of the PC compartment, that peaked at day 2–5 after hospital admission. A wave of PCs in blood has also been found in humans after secondary vaccination with tetanus toxin. Large numbers of PCs were detectable in blood on day 6 and 7 after immunization and disappeared from the blood after day 8 (29). There have been no data reported on monitoring of the PC compartment

during bacterial infections in humans. Earlier studies on PCs have examined selected group of patients with reactive plasmacytoses or in patients upon active secondary immunizations (11, 29–32). The prior-examined patients with reactive plasmacytoses had massive amounts of PCs that are rarely seen during infection and inflammation (11, 30, 33). One study established the number of PCs in three patients with bacterial septicaemia and reported, in agreement with our results, high amounts of PCs in all patients (33). Additionally, we show that the PC expansion is transient and is a rather late phenomenon as patients already showed clear clinical improvements with decreasing CRP levels.

There is strong evidence that PCs generated in germinal centers during immune responses either remain within the tissues of their origin or leave the secondary lymphoid organs and migrate via blood to the bone marrow, mucosal-associated lymphoid tissue, or sites of inflammation (34, 35). Hence, it is conceivable that the PCs transiently present in blood during bacterial infections are on their way to the bone marrow or other organs. Plasma cell homing via blood to different tissues appears to have a crucial role in regulating strength and duration of humoral immune responses. PCs predominantly home to the bone marrow where they contribute to long-term antibody production. In fact, the vast majority of serum immunoglobulins produced are derived from bone marrow PCs (36). Recent experiments have established a key role for chemokines and their receptors expressed on PCs in guiding the release of PCs from secondary lymphoid organs and migration to their final destination (34, 35).

In SLE patients, we found disturbances of peripheral B-cell homeostasis with accumulation of IgD^+ $CD38^{++}$ transitional B cells and $CD27^{++}$ PCs. Our data, therefore, confirm results reported by others (10, 12, 18, 20, 25). Elevated frequencies of $CD27^{++}$ PCs, but no differences in absolute numbers between the clinically quiescent SLE patients and healthy controls were detected. In support, others described that patients with inactive SLE had increased frequencies of $CD27^{++}$ PCs but normal absolute numbers of PCs, whereas patients with active SLE had elevated frequencies and absolute numbers of PCs (12, 14). We found that patients with bacterial infections reached similar frequencies and absolute numbers of $CD27^{++}$ PCs as previously reported in active SLE patients (12, 14). Patients with SLE are known to be more susceptible to common and opportunistic infections due to immunologic defects and immunosuppressive therapy (37). Therefore, in case CD27 is chosen as a biomarker for monitoring SLE-disease activity, infections should be considered as a possible cause of $CD27^{++}$ PC increase.

However, the kinetic of PC expansion between infection and active SLE may differ. In support, we found that two newly diagnosed SLE patients admitted to our hospital due to an elevated disease activity had strongly elevated $CD27^{++}$ PC numbers that persisted during their hospital stay. One patient reached a clinically quiescent status of SLE after intensive immunosuppressive therapy that coincided with a marked reduction of PC number. Accordingly, others have reported a decline in frequency of PCs after effective immunosuppressive therapy of an SLE patient (12).

Since virtually all SLE patients have ANAs in their serum, this triggered us to investigate the association between the presence of ANAs and B-cell subset homeostasis. The presence of serum ANAs appeared not to be associated with abnormalities in the distribution of specific B-cell subpopulations within the group of CTD-suspected patients. Thus, the presence of serum ANAs itself does not coincide with increased numbers of IgD^+ $CD38^{++}$ transitional B cells and $CD27^{++}$ PCs as characteristically seen in SLE patients. These findings may indicate that autoantibody production is not preceded by accumulation of $CD27^{++}$ PCs. Our preliminary results suggest that the presence of antibodies against RNP is related with increased frequencies of $CD27^{++}$ PCs in SLE. A weak relationship between the number of $CD27^{++}$ PCs and the occurrence of anti-ENA antibodies was also observed in a prior study (25). In contrast to our data, in the prior study, anti-SS-A/B and anti-dsDNA antibodies were found to be associated with increased $CD27^{++}$ PC frequencies (25). The discrepancy may be due to differences in level of disease activity of the studied patients or differences in assays used for detection of these type of antibodies.

The majority of included CTD-suspected patients had clinical and laboratory features suggestive for SLE but, however, did not fulfil the classification criteria for SLE. These patients had significantly lower frequencies of $CD27^{++}$ PCs compared with SLE patients suggesting a possible diagnostic value of PC analyses in SLE. Autoantibodies are present many years before the onset of SLE, while patients are still asymptomatic, and are believed to contribute directly to the pathogenesis of SLE (23). Our results may indicate that disturbances in homeostasis in B-cell subsets is a phenomenon that appears in more advanced, pathogenic stages of development of SLE and is not yet present at the stage of antinuclear antibody production. Additional studies are needed to further validate the suitability of PC analysis as a diagnostic tool for SLE. For instance, it remains to be established at which developmental stage of SLE PCs start to accumulate in peripheral blood.

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