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Disturbed Peripheral B Lymphocyte Homeostasis in Systemic Lupus Erythematosus

Marcus Odendahl,* Annett Jacobi,† Arne Hansen,‡ Eugen Feist,† Falk Hiepe,* Gerd R. Burmester,† Peter E. Lipsky,§ Andreas Radbruch,‡# and Thomas Dörner* ‡,3†

In patients with active systemic lupus erythematosus (SLE), a marked B lymphocytopenia was identified that affected CD19+/CD27− naive B cells more than CD19+/CD27+ memory B cells, leading to a relative predominance of CD27-expressing peripheral B cells. CD27high/CD38+/CD19dim/surface IgDlow/CD20+/CD138− plasma cells were found at high frequencies in active but not inactive SLE patients. Upon immunosuppressive therapy, CD27high plasma cells and naive CD27− B cells were markedly decreased in the peripheral blood. Mutational analysis of V gene rearrangements of individual B cells confirmed that CD27+ B cells coexpressing IgD were memory B cells preferentially using VH3 family members with multiple somatic mutations. CD27high plasma cells showed a similar degree of somatic hypermutation, but preferentially employed VH4 family members. These results indicate that there are profound abnormalities in the various B cell compartments in SLE that respond differently to immunosuppressive therapy. The Journal of Immunology, 2000, 165: 5970–5979.

Systemic lupus erythematosus (SLE) is characterized by the production of multiple autoantibodies. Although the pathogenesis of SLE remains enigmatic, autoantibodies against dsDNA and ribonucleoproteins, deposition of immune complexes, complement activation, and leukocyte infiltration are thought to represent a consequence of immune dysregulation in this entity (1–5). Whereas B cell hyperreactivity and spontaneous Ig production by PBLs have been documented in SLE, distinct abnormalities of B cells have not been elucidated in detail. However, it is known that peripheral B cells from SLE patients contain populations that spontaneously produce Ig and also cells that can mature into Ab-secreting cells when cultured in vitro in the absence of obvious activators of B cell differentiation (6–11).

In vivo, Ag-specific activation and differentiation of B cells occur in germinal centers (12–16). Within germinal centers, naive B cells undergo activation, proliferation, somatic hypermutation of rearranged V region genes, Ig isotype switching, and subsequent positive and/or negative selection by Ag (13, 15, 17–19). Within germinal centers, activated B cells mature into Ab-producing plasma cells or, alternatively, become memory B cells. This developmental dichotomy of B cells is reflected by differential expression of a variety of B cell surface Ags, such as surface Ig, CD38, CD20, and CD138 (syndecan-1; see Refs. 14 and 20).

Peripheral blood as well as in the bone marrow, memory B cells have been identified in populations of B cells expressing either class-switched Ig isotypes: IgM and IgD, or IgM only (21–25). More recently, IgD+/CD27+ B cells have been identified as having somatically mutated Ig genes and, therefore, being memory B cells (25). In normal persons, IgM+/IgD+/CD27− naive B cells represent about 60% of the peripheral blood B cell population (25, 26).

In this study, we demonstrate that the frequencies of CD27-expressing B cells were significantly enhanced as a result of a relative and absolute reduction of the total number of naive B cells and a less prominent reduction of memory B cells in the periphery of patients with SLE. A significant population of CD27high plasma cells was identified in the periphery of patients with active SLE. Upon immunosuppressive therapy, the CD27+ B cell population in SLE patients remained stable, whereas the frequencies of naive B cells and CD27high plasma blasts decreased significantly.

Materials and Methods

Patients' material and preparation of samples

Heparinized whole blood (10–20 ml) from patients with various autoimmune diseases (Table I) were obtained from the Department of Rheumatic Diseases, University Hospitals Charite (Berlin, Germany). In detail, we analyzed 13 patients with SLE, fulfilling the criteria revised in 1982 (27), and a group of 9 patients with other autoimmune diseases (2 patients with primary Sjögren’s syndrome, 2 patients with polymyositis, 2 patients with progressive systemic sclerosis, 1 patient with polymyalgia rheumatica, 1 patient with polychondritis, and 1 patient with acquired factor VIII resistance) (Table I). Six patients with SLE exhibited a flare at the time of analysis and subsequently underwent immunosuppressive therapy. Two patients had not been diagnosed before (patients 11 and 13), one patient discontinued taking prednisolone 3 wk before the analysis (patient 6), and three patients were taking <10 mg of prednisolone/day (patients 1, 5, and 10) at the time of disease flare. The remaining patients with SLE were being treated with azathioprine (100–150 mg daily) and/or methylprednisolone (12 mg daily) or prednisolone, respectively (<20 mg daily). As a control, fresh blood from 14 apparently normal healthy blood donors (NHS) were also analyzed. PBMC were prepared as reported previously (24).

Cytometric analysis

Immunofluorescence staining for flow cytometric analysis was performed by incubating PBMC with biotinylated anti-CD19 (S125-C1; Southern Bio-technology Associates, Birmingham, AL), anti-CD27 Cy5 (clone 2E4), and either anti-CD38 FITC (clone HIT-2; Pharmingen, San Diego, CA), anti-
HLA-DR FITC (clone R30), anti-CD95 FITC (clone CH-11; Immunotech, Marseille, France), anti-CD20 FITC (clone B-Ly1; Southern Biotechnology Associates), anti-human CD138 biotinylated (clone B-B4; Diaclone, Sunnyvale, CA.), anti-human Igκ FITC (G20–193; PharMingen) and light chain FITC (JDC-12; PharMingen), anti-human IgG FITC (rabbit anti-human IgG; Dako, Hamburg, Germany), anti-human IgM FITC (rabbit anti-human IgM; Dako), or anti-human IgD FITC (clone IA6-2, mouse anti-human IgD; PharMingen). Incubation with Abs was performed in PBS/0.5%BSA/5 mM EDTA at 4°C for 10 min. Propidium iodide (1 mg/ml; Sigma, Munich, Germany) was added immediately before cytometric analysis to exclude dead cells. Before incubation with streptavidin-PE (0.5 mg/ml; PharMingen), cells were washed twice. For intracellular staining, the cells were fixed in 2% (w/v) formaldehyde (Merck, Darmstadt, Germany) for 20 min at room temperature, washed, and stored at 6 – 8°C in PBS/0.5%BSA. The cells were then incubated in PBS/0.5%BSA, with or without 0.5% saponin (saponin buffer; Sigma), and fluoresceinated Ab for 10 min at 4°C and then washed in saponin buffer and PBS. For intracellular analyses, anti-IgE FITC (rabbit anti-human IgE; Dako) was used. In addition, anti-CD5 FITC was added immediately before cytometric analysis to exclude dead cells. Before incubation with streptavidin-PE (0.5 μg/ml; PharMingen), cells were washed twice. For intracellular staining, the cells were fixed in 2% (w/v) formaldehyde (Merck, Darmstadt, Germany) for 20 min at room temperature, washed, and stored at 6 – 8°C in PBS/0.5%BSA. The cells were then incubated in PBS/0.5%BSA, with or without 0.5% saponin (saponin buffer; Sigma), and fluoresceinated Ab for 10 min at 4°C and then washed in saponin buffer and PBS. For intracellular analyses, anti-IgE FITC (rabbit anti-human IgE; Dako) was used. In addition, anti-CD5 FITC was added immediately before cytometric analysis to exclude dead cells. 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Enhanced frequencies of CD27+/CD19− cells were calculated according to statistical threshold sets in reference to control stainings, as shown in Fig. 1. Patients with inactive SLE treated with azathioprine and/or glucocorticoids had significantly lower total numbers of peripheral B cells regardless of their treatment regimen (Fig. 2) compared with both control groups. When the overall frequency of peripheral B cells expressing CD27 was examined, SLE patients with active and inactive disease had significantly higher frequencies of CD27-expressing B cells than both control groups (Fig. 3). The two control groups did not differ in the overall frequency of CD27-expressing B cells (p = 0.4310).

The increased frequency of CD19+/CD27− peripheral B cells in patients with SLE results from a reduction of the CD19+/CD27− naive peripheral B cell pool and a lesser decline in CD19+/CD27+ memory B cells

The increase in the frequency of CD19+/CD27+ peripheral B cells in SLE patients was not caused by an expansion of the CD19+/CD27+ subpopulation. Rather, it was a consequence of a significantly reduced total number of naive CD19+/CD27− peripheral B cells of SLE patients with active (85 ± 54 × 10^6 cells/l) and inactive disease (74 ± 70 × 10^6 cells/l) compared with other patients (546 ± 941 × 10^6 cells/l, p = 0.012 and p = 0.0052, respectively) and normal controls (418 ± 204 × 10^6 cells/l, p = 0.0006 and p = 0.0004, respectively) (Fig. 4). Patients with active disease did not differ in their frequency of naive B cells compared with those without disease flares (p = 0.7308, Fig. 4). In contrast, the total number of CD27+ B cells was not significantly lower in patients with other autoimmune diseases than in normal controls (p = 0.0833). As shown in Fig. 4, the reduction in these absolute numbers of naive B cells coincided with a reduced frequency of CD27− B cells in SLE patients compared with both control groups, whereas these frequencies did not differ between the two groups of SLE patients (p = 0.9452) or between the control groups (p = 0.8749).

Further analysis led to the discrimination of two distinct CD27-expressing B cell populations in peripheral blood: one CD27 at high levels (CD27hi/b) and one expressing CD27 less brightly (CD27+). The frequency of CD27+ B cells in patients with other autoimmune diseases did not exceed those found in NHS (p = 0.5496, Fig. 5). Moreover, patients with active lupus and those

**FIGURE 2.** Absolute numbers of peripheral B cells were compared between SLE patients with active (n = 6) and inactive disease (n = 7) as well as in patients of the control groups (AID, patients with other autoimmune diseases (n = 9) and NHS (n = 14)).
with inactive treated disease did not differ in regard to the frequency of CD27⁺ peripheral B cells (p = 0.2949). In most patients with SLE (10/13), CD27⁺ B cells comprised 39.2% or more of the peripheral B cells, with a mean frequency of 43 ± 17% in active and 55 ± 22% in inactive SLE patients. One female patient (donor 4) was exceptional in that only 15.9% of her B cells expressed CD27, a frequency in the range of NHS controls. Interestingly, this patient had delivered a healthy child 1 wk before the analysis and did not manifest any disease activity at that time. Her white blood cell count and levels of complement factors C3 and C4 were normal. Anti-dsDNA Abs were not detectable, although previously she had had a 1:8 titer of anti-dsDNA and 1:80 titer of anti-nuclear Abs (fine speckled pattern).

As opposed to SLE patients, CD27 was expressed on B cells from patients with other autoimmune diseases (mean, 29 ± 14%; highest value, 48.4%) at a similar frequency as found in NHS controls (mean, 34 ± 9%; highest value, 48.9%). Two patients with other autoimmune diseases exhibited frequencies of peripheral CD27⁺ B cells higher than 39%, a similar frequency as that found in the patients with SLE. This included one patient with Sjögren’s syndrome (44.7%, patient 15) and another one with acquired factor VIII resistance and hemophilia A (48.4%, patient 22).

Careful analysis of the clinical characteristics of these two patients in comparison to the other patients with autoimmune diseases did not document any significant differences. Most notably, these patients lacked an enhanced frequency of CD27high B cells in the periphery as detected in patients with SLE (see below).

Although the frequency of CD27⁺ B cells among CD19⁺ cells was significantly increased in inactive SLE patients compared with patients with other autoimmune diseases (p < 0.017) and NHS
only, the absolute number of these cells was significantly higher in NHS compared with the other groups analyzed (Fig. 5). Moreover, the absolute number of CD27+ cells did not differ between any of the patient groups. Although the absolute number of CD27+ B cells was diminished in all patient groups, the magnitude of the decrease noted in the SLE patients was markedly less than the decrease in the number of circulating CD19+CD27− naive B cells.

An increase in the frequency and the number of the peripheral CD27high/CD19+ B cell subpopulation is characteristic of active SLE

Patients with active and inactive SLE showed an increased frequency of peripheral B cells expressing high levels of CD27 (CD27high, Figs. 1 and 6) in contrast to NHS (p < 0.0006 and p < 0.001, respectively, Fig. 6) and to controls with other autoimmune diseases (p < 0.004 and p < 0.0007, respectively). Among the SLE patients with active disease, the mean frequency of these cells was 26 ± 15%, ranging between 7.4 and 43.1% of peripheral B cells, significantly higher than in SLE patients with inactive disease (mean, 6 ± 4%; minimum, 1.9%; maximum, 11.2%; p < 0.022). The CD27high B cell subpopulation was uncommon in the blood of NHS (1.4 ± 0.8% of peripheral B cells). In patients with autoimmune diseases other than SLE, such cells were found at frequencies of 0.9 ± 0.9%. The frequencies of CD27high B cells in the seven SLE patients without disease activity were higher than those in patients with other autoimmune diseases (p < 0.0007) as well as in NHS (p < 0.001). However, the absolute numbers of CD27high B cells were only significantly increased in active SLE patients compared with inactive SLE patients (p < 0.008), patients with other autoimmune diseases (p < 0.0004), and NHS (p < 0.002). It should be pointed out that among the non-SLE autoimmune controls, one of the patients with Sjögren’s syndrome was the only one with a significantly increased population of CD27high B cells (3.2%).

CD19+/CD27high cells express CD38, CD95, HLA-DR, CD138, and intracellular Ig

As shown in Fig. 7, and representative for the SLE patients analyzed, CD19+/CD27high cells express CD38, CD95, HLA-DR, CD138, and intracellular Ig. As shown in Fig. 7, and representative for the SLE patients analyzed, CD19+/CD27high cells express CD38, CD95, HLA-DR, CD138, and intracellular Ig. As shown in Fig. 7, and representative for the SLE patients analyzed, CD19+/CD27high cells express CD38, CD95, HLA-DR, CD138, and intracellular Ig. As shown in Fig. 7, and representative for the SLE patients analyzed, CD19+/CD27high cells express CD38, CD95, HLA-DR, CD138, and intracellular Ig. As shown in Fig. 7, and representative for the SLE patients analyzed, CD19+/CD27high cells express CD38, CD95, HLA-DR, CD138, and intracellular Ig. As shown in Fig. 7, and representative for the SLE patients analyzed, CD19+/CD27high cells express CD38, CD95, HLA-DR, CD138, and intracellular Ig. 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CD19<sup>+</sup>/CD27<sup>high</sup> and CD19<sup>+</sup>/CD27<sup>−</sup> cells are decreased by immunosuppressive treatment of SLE patients

To determine whether the presence of CD27<sup>high</sup> B cells in peripheral blood was related to disease activity and/or treatment, we performed a follow-up analysis on two of the patients who initially had a prominent population of these cells in the periphery at the time of flare symptoms and consequently had been treated with immunosuppressive therapy. As seen in Fig. 10, the administration of i.v. methylprednisolone, 1000-mg bolus for 2 days and 500 mg for the successive 3 days, led to a marked reduction of the peripheral CD27<sup>high</sup> plasma cell subpopulation. Subsequently, the patient received i.v. cyclophosphamide (800 mg bolus) once. Afterward, the patient’s condition improved and the CD27<sup>high</sup> B cells had almost completely disappeared from the periphery and the number of naive B cells was reduced significantly, with CD27<sup>+</sup> memory B cells not detectably affected. The phenotype shown in Fig. 10c was characteristic of three SLE patients treated with immunosuppressive therapy and without apparent disease activity. Another SLE patient (Fig. 1c), treated with hemodialysis, still exhibited smoldering activity including lowered complement factors. This patient still showed an increased frequency of peripheral plasma cells (10.7%) but a low frequency of naive B cells (5.3%).

**V<sub>H</sub>** gene usage and hypermutation in CD27<sup>+</sup> and CD27<sup>high</sup> B cells in SLE

Individual IgD<sup>−</sup>/CD27<sup>−</sup>, CD27<sup>−</sup>/IgD<sup>+</sup>, and CD27<sup>high</sup>/IgD<sup>−</sup> B cells were sorted by FACS using single-cell deposition. The mutational frequencies of <i>V<sub>H</sub></i> gene segments were determined for the three cell types (Table II). Among productively rearranged <i>V<sub>H</sub></i> genes, there was a significant difference in the mean frequency of mutations between the CD27<sup>−</sup> (mutational frequency, 0.4%) and the CD27<sup>+</sup>-expressing B cell populations (mutational frequency, 6.46%,<i>p < 0.0001</i>), which is consistent with the classification of CD27<sup>−</sup> cells as memory B cells. CD27<sup>+</sup> (mutational frequency,
cells/l; inactive SLE, 90,000 were still present in significantly lower numbers in all patients were the predominant peripheral blood population in SLE, yet they were the last cytometric analysis was performed. The populations of CD27⁺, CD27⁻, and CD27high cells were gated and their frequencies among CD19⁺ B cells are indicated.

In the B cells of this patient, the ratio of productively rearranged V₄D3 and V₄H⁺ genes representing the most frequently used V₄H families was remarkably different between CD27⁻ and CD27⁺ cells (Table II). Whereas V₄D3 genes were preferentially used by B cells expressing IgD but not CD27 (13/14) and no IgD⁺CD27⁺ B cells used V₄D4 gene segments, the latter gene segments were used by some IgD⁻/CD27⁻ memory B cells (2/15) and >50% of IgD⁻/CD27high plasma cells (8/15, if the genes used by all cells of a clone were considered as one). With regard to individual genes, the V₁₄–23 gene was found most often in CD27⁻/IgDⁿ naïve (6/14) and CD27⁻/IgD⁺ memory B cells (4/15), whereas V₁₄–34 was amplified from 3 of 15 and V₁₄–59 from 3 of 15 CD27high/IgD⁻ cells. The increase in mutational frequencies coincided with an increase in frequencies of B cells using V₄H in the productive repertoire. Notably, V₄H1 family members were found to be rearranged in all B cell subpopulations. However, productively rearranged V₄H1 family members occurred in CD27⁺ B cells (2/15) and CD27high plasma cells (1/15) only.

In addition, a clonally expanded population within the CD27high plasma cells employing V₄H−61/D3–09/I₄D4 could be identified with an almost identical CDR3 of 66 bp and a common insertion of 6 inserted bp in CDR1 (Fig. 11). The three rearranged V₄H−61 genes of this clone carried 25 mutations (gene D12 IV V₄H4 A1), 44 mutations (gene D12 IV V₄H4 A1), and 54 mutations (gene D12 IV V₄H4 H3).

**Discussion**

The current study provides evidence that in patients with SLE there is significant B lymphocytopenia associated with major disturbances in the homeostasis of all three major B cell types, naive and memory B cells and plasma cells. CD27⁻ memory B cells were the predominant peripheral blood population in SLE, yet they were still present in significantly lower numbers in all patients compared with normal subjects, active SLE, 122 ± 130 × 10⁹ cells/l; inactive SLE, 90 ± 59 × 10⁹ cells/l; other autoimmune diseases, 136 ± 89 × 10⁹ cells/l; NHS, 205 ± 64 × 10⁹ cells/l; all p < 0.05 compared with NHS. A distinct population of CD19⁻ B cells (CD27high) expressing CD27 highly, CD38, CD138, and intracellular but not slg was identified as an expanded population in SLE patients with a lupus flare. It remains to be shown whether these abnormalities of B cell homeostasis are interdependent. Upon immunosuppressive therapy, both the populations of naive CD27⁻ B cells and CD27high plasma cells were reduced in the SLE patients, whereas the CD27⁻ memory cell population was apparently not affected.

Extensive work has been devoted to analysis of autoantibody-producing cells and perturbation of T lymphocyte homeostasis in patients with SLE (32; reviewed in Ref. 5). With regard to B lymphocytes, spontaneously activated B cells and polyclonal production of Ig, including autoantibodies, have been repeatedly demonstrated in the peripheral blood and in the bone marrow of SLE patients (reviewed in Refs. 5, 6, and 8–11). The current data are consistent with these findings, indicating that there are expanded numbers of phenotypically defined plasma cells in the blood of patients with active SLE. For the first time, we show here that these plasma cells express high levels of CD27.

CD27 belongs to the TNF receptor family and is expressed preferentially by T cells but also by B cells. CD27 signals after interaction with its ligand, CD70, which is expressed on T cells. CD27/CD70 signaling appears to act at late stages of B cell differentiation, providing a key signal for the maturation of memory B cells into Ig-secreting cells in the germinal center reaction (33–35). Expression of CD27 on B cells is apparently induced in the context of germinal center reactions and is maintained on memory B cells (25). CD27⁺ B cells in human peripheral blood show extensive somatic hypermutation of their V genes, irrespective of the isotype they express, marking them as descendants of cells activated previously in vivo (25, 36). Here, we confirm this observation and extend it to peripheral CD27⁺/IgD⁺ B cells and CD27high B cells from a patient with a lupus flare.

Of the V₄H gene rearrangements from the CD27⁺/IgD⁺ B cells analyzed, 14 of 15 showed mutation rates of 2–15% (overall mutational frequency, 6.1%). In comparison, 10 productively rearranged V₄H segments from CD27⁻/IgD⁻ B cells showed an overall mutational frequency of only 0.4%. In peripheral B cells from normal subjects, Klein et al. (25) had observed similar frequencies for CD27⁻ and for CD27⁺ B cells. Eleven of 14 V₄H regions obtained from CD27high plasma cells were highly mutated (3.4–10.5%; mean, 6.9%). In addition, three cells of a heavily mutated CD27high plasma cell clone expressed the V₄H4–61 gene segment with 25–54 mutations. CD27⁺ and CD27high cells both showed a high degree of somatic hypermutation, but they differed...
in their VH gene preference. Thirteen of 14 IgH loci of CD27- IgD+ B cells and 11 of 15 IgH loci of CD27+/IgD+ cells used VH3 genes in VDJ recombination. In CD27+ B cells, however, only 5 of 15 IgH loci used VH3 but 9 genes of the VH4 family. In addition, the three clonally related cells used the VH4-61 segment. Preferential usage of VH4 genes by postswitch cells has been reported by other groups for patients with rheumatoid arthritis (37, 38), whereas VH3 was most frequently found in naive B cells or in unfractionated peripheral B cells from normal subjects (24, 28, 39). Moreover, the gene VH4-34 frequently used in the clonally unrelated CD27high cells analyzed here has been reported previously to be involved in the formation of anti-dsDNA Abs in SLE patients (40–42) and to be expanded in patients with disease activity (41). This VH4 gene encodes cold agglutinins (43–45). It was also frequently used in immune responses of infectious mononucleosis (41, 46). In normal subjects, this particular gene occurred at frequencies of 3.5% among peripheral CD5+ and 3.9% among CD5- B cells (24) or 3–10.8% among peripheral B cells (44, 45, 47, 48). The high frequency of VH4-34 usage in peripheral CD27 high B cells, the high frequency of such cells in untreated SLE patients, and their disappearance upon successful immunosuppressive treatment imply that CD27 high plasma cells expressing this VH gene rearrangement may be involved in the etiopathogenesis of SLE.

### Table II. Analysis of productively rearranged VH gene sequence obtained from individual peripheral B cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>VH Segment</th>
<th>Reading length (bp)</th>
<th>Mutations (n)</th>
<th>Sequence Homologya (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD27+/IgD+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D12 II VH3 E1</td>
<td>VH3-09/DP-31</td>
<td>222</td>
<td>4</td>
<td>98.2</td>
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<tr>
<td>D12 II VH3 G2</td>
<td>VH3-23/DP-47</td>
<td>223</td>
<td>1</td>
<td>99.6</td>
</tr>
<tr>
<td>D12 II VH3 E3</td>
<td>VH3-74/DP-53</td>
<td>226</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>D12 II VH3 F4</td>
<td>VH3-23/DP-47</td>
<td>225</td>
<td>1</td>
<td>99.6</td>
</tr>
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<td>D12 II VH3 G4</td>
<td>VH3-23/DP-47</td>
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<td>100.0</td>
</tr>
<tr>
<td>D12 II VH3 D5</td>
<td>VH3-64/DP-61</td>
<td>226</td>
<td>2</td>
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</tr>
<tr>
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<td>VH3-23/DP-47</td>
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<td>100.0</td>
</tr>
<tr>
<td>D12 II VH3 C8</td>
<td>LSG 6.1</td>
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</tr>
<tr>
<td>D12 II VH3 E8</td>
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</tr>
<tr>
<td>D12 II VH3 A1b</td>
<td>VH3-09/DP-31</td>
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<tr>
<td>D12 II VH3 C2b</td>
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<tr>
<td>D12 II VH3 E12b</td>
<td>VH3-73</td>
<td>222</td>
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</tr>
<tr>
<td>D12 II VH D9b</td>
<td>VH1-08/DP-15</td>
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<td>0</td>
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<tr>
<td>CD27+/IgD+</td>
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<td></td>
<td></td>
<td></td>
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<td>100.0</td>
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<tr>
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<td>VH3-21/DP-77</td>
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<td>15</td>
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<td>218</td>
<td>14</td>
<td>93.6</td>
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<td>VH3-07/DP-54</td>
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<td>9</td>
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<td>VH3-23/DP-47</td>
<td>225</td>
<td>11</td>
<td>95.1</td>
</tr>
<tr>
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<td>VH3-23/DP-47</td>
<td>224</td>
<td>14</td>
<td>93.8</td>
</tr>
<tr>
<td>D12 III VH3 C7</td>
<td>VH4-30-4/DP-78</td>
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<td>5</td>
<td>97.6</td>
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<tr>
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<td>VH4-59/DP-71</td>
<td>210</td>
<td>21</td>
<td>90.0</td>
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<tr>
<td>D12 III VH3 A3</td>
<td>VH1-69/DP-10</td>
<td>218</td>
<td>32</td>
<td>85.3</td>
</tr>
<tr>
<td>D12 III VH3 B1</td>
<td>VH1-46/Hg+1</td>
<td>219</td>
<td>13</td>
<td>94.1</td>
</tr>
<tr>
<td>D12 III VH3 C8b</td>
<td>VH3-33/DP-50</td>
<td>222</td>
<td>10</td>
<td>95.5</td>
</tr>
</tbody>
</table>

a Sequence homology and the underlying VH segments were determined as described in Materials and Methods.

b Nonproductive VH4 rearrangement.

c Clonally related VH4 gene rearrangements.
CD19. All cells expressing intracellular Ig are CD19dim (data not shown). The expression of CD19 on peripheral plasma cells has been shown before (49, 50) and contrasts with the apparent absence of CD19 on myeloma cells (50, 51). Only two-thirds of them also express CD1381 (syndecan-1). As for CD5, it has been shown that both CD51 as well as CD52 B cells (52) obtained from SLE patients can produce anti-DNA Abs. The current data indicate that CD271 and CD27 high B cells are almost exclusively members of the CD52 B cell population, also in patients with an SLE flare. The expression of CD38, CD138, CD95, and intracellular Ig, down-modulation of CD20 and CD19, and hypermutated rearranged V\textsubscript{H} genes identifies these cells as plasma cells (30, 53).

For CD27 memory B cells, Agematsu et al. (34, 35) have shown that these cells can be induced in vitro to differentiate into Ig-secreting plasma blasts upon stimulation with CD70, IL-2, and IL-10. CD38\textsuperscript{high} peripheral cells have been shown by Lakew et al. (54) to secrete Ig in vitro spontaneously. Since expression of CD38 and CD27\textsuperscript{high} on peripheral B cells is perfectly correlated, it can be inferred that CD27\textsuperscript{high}/CD19\textsuperscript{dim} cells are Ab-secreting plasma cells.

In summary, the current study provides clear evidence that the expression of CD27 identifies marked disturbances of B cell homeostasis with respect to naive and memory B cells and plasma cells. Notably, a striking lymphocytopenia and a marked reduction of CD27\textsuperscript{+} B cells appear to be characteristic of SLE and not only the result of therapeutic interventions. In addition, active SLE is characterized by a marked increase in circulating plasma cells that is dramatically reduced by immunosuppressive therapy. The pool of CD27\textsuperscript{+} peripheral B cells is less susceptible to immunosuppressive therapy in contrast to the pools of naive B cells and CD27\textsuperscript{high} plasma cells. These results have clear implications for diagnosis and therapy of SLE. Cytometric monitoring of the various B cell populations using CD27 in conjunction with CD19 may provide an important diagnostic parameter for monitoring disease activity in SLE patients.

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References


