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J Immunol 2001; 167:2361-2369; doi: 10.4049/jimmunol.167.4.2361
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Increased Frequency of Pre-germinal Center B Cells and Plasma Cell Precursors in the Blood of Children with Systemic Lupus Erythematosus

Edsel Arce,*† Deborah G. Jackson,*† Michelle A. Gill,*† Lynda B. Bennett,*† Jacques Banchereau,* and Virginia Pascual**†

We have analyzed the blood B cell subpopulations of children with systemic lupus erythematosus (SLE) and healthy controls. We found that the normal recirculating mature B cell pool is composed of four subsets: conventional naive and memory B cells, a novel B cell subset with pregerminal center phenotype (IgD+CD38+centerin+), and a plasma cell precursor subset (CD20+CD19+CD27+/++CD38++). In SLE patients, naive and memory B cells (CD20+CD38−) are ~90% reduced, whereas oligoclonal plasma cell precursors are 3-fold expanded, independently of disease activity and modality of therapy. Pre-germinal center cells in SLE are decreased to a lesser extent than conventional B cells, and therefore represent the predominant blood B cell subset in a number of patients. Thus, SLE is associated with major blood B cell subset alterations. The Journal of Immunology, 2001, 167: 2361–2369.

Lymphocyte counts are known to be significantly decreased in systemic lupus erythematosus (SLE) and lymphopenia of <1500 cells/μl is the most prevalent initial laboratory abnormality in this disease (3). Despite the low circulating lymphocyte levels, B cells play a major role in the pathogenesis of SLE in both humans and murine SLE models, as they are responsible for the hypergammaglobulinemia and autoantibody production that characterize this disease (4, 5). Most studies on lupus B cells have been performed on mice with lupus-like syndromes (6–9) rather than human SLE (10–14). Interestingly, MRL/lpr mice expressing surface Ig but lacking secreted Ig develop nephritis, suggesting that B cells may play a role in the pathogenesis of SLE nephritis that is independent from serum autoantibodies (15). With regard to humans, SLE B cells exhibit, upon signaling through the Ag receptor, increased Ca2+ flux and early protein tyrosine phosphorylation (12). SLE B cells express high levels of costimulatory molecules CD80 and CD86 (13) as well as CD40 ligand (CD40L)/CD154 (14). High levels of soluble CD40L are also found in the serum of active SLE patients (16, 17).

In recent years our laboratory has developed methods to isolate and characterize mature peripheral B cells. Using anti-IgD and anti-CD38 Abs, four mutually exclusive peripheral B cell populations can be isolated (reviewed in Refs. 18 and 19). Single-positive IgD+ cells correspond to follicular mantle cells (Bm1 + Bm2), whereas single-positive CD38 cells correspond to germinal center (GC) cells (Bm3 + Bm4). Double-negative B cells correspond to the memory population (Bm5), whereas double-positive cells represent a combination of cells at a transitional stage between follicular mantle and GC (Bm2′ and single-isotype IgD+ GC cells (20). More recently, CD27 has been reported as marker of memory B cells within both the slgD+ and slgD− peripheral B cell compartments (21, 22). The phenotypic summary of these populations is depicted in Table I.

These studies and those by others (23–30) have led to the proposal of a model of T cell-dependent, Ag-dependent mature B cell differentiation: naive B cells (Bm1 and Bm2) are activated in association with Ag-specific T cells and interdigitating cells within the extrafollicular areas. The activated B cell blasts either undergo terminal differentiation toward plasma cells (extrafollicular reaction) or become GC founder cells (Bm2′). In GCs, Bm2′ differentiate into centroblasts (Bm3) that proliferate and accumulate point mutations into the Ig variable region genes, yielding three types of mutants: high affinity, low affinity, and autoreactive mutants. These mutants will be selected while they differentiate into centrocytes (Bm4), their survival depending on their affinity for the Ag trapped within immune complexes bound to follicular dendritic cells. The high affinity mutants will pick up the Ag, process it, and present it to GC T cells, which are induced to express CD40L and secrete cytokines (i.e., IL-4 and IL-10), key elements for survival, proliferation, and isotype switching. These high affinity centrocytes differentiate into either memory B cells (Bm5) or plasma cells. Low affinity mutants that do not bind FDC-bound Ag will die by apoptosis, whereas autoreactive mutants are eventually deleted because they do not receive T cell help. During secondary humoral immune responses, recirculating memory B cells can be activated in extrafollicular areas, giving rise to plasma cells and GC founder cells.

Although extensive information has accumulated on the mature B cells that populate peripheral lymphoid organs such as human tonsils, little is known about blood B cell subsets. We have thus
analyzed the peripheral blood B cell compartment of healthy adults, healthy children, and children suffering from rheumatic diseases including juvenile dermatomyositis (JDM) and, most particularly, SLE. These studies have permitted us to identify a novel blood B cell population expressing a partial GC phenotype and an oligoclonal plasmablast population. Although these populations are not restricted to SLE patients, the disproportionate depletion of conventional naive and memory B cells in SLE make pre-GC cells and plasmablasts predominate in SLE blood.

**Materials and Methods**

**Samples and patient populations**

Blood samples from 35 healthy children, 68 children with SLE, 10 with JDM, and 17 healthy adults were drawn after informed consent in accordance with our institutional internal review board was obtained. All pediatric SLE patients included in this study fulfilled the established American College of Rheumatology criteria for SLE (31). The patients’ clinical and serological data were gathered during clinic visits, and the corresponding SLE disease activity index (SLEDAI) was recorded in the chart (32). The average ± SD age and the sex ratio for each of the groups were: 1) healthy children group, 12.15 ± 3.15 years, 3:1 female/male; 2) pediatric SLE group with SLEDAI >10 (n = 36), 14 ± 2.67 years, 5:1 female/male; 3) pediatric SLE group with SLEDAI <10 (n = 32), 13 ± 3.15 years, 6:1 female/male; 4) JDM group, 9.2 ± 3.8 years, 4:1 female/male; and 5) adult group, 36.8 ± 6.21 years, 3:2 female/male. SLE patients belong to different ethnic backgrounds, including Caucasian (32.3%), African-American (25.3%), Hispanic (23.9%), and Oriental (4.2%). The healthy children consisted of Caucasian (32.3%), Hispanic (23.9%), and Oriental (4.2%). The patients included in this study were receiving i.v. cyclophosphamide (20% of patients) and/or patients were being treated with oral prednisone and hydroxychloroquine.

**Flow cytometric analysis of blood B cells**

Two methods have been used to assess blood B cells. The first analyzes purified B cells, whereas the second analyzes total blood and has the considerable advantage of necessitating only 0.5 ml (rather than 10–20 ml) of blood. Samples from 44 SLE patients, 22 healthy children, 10 JDM, and 17 healthy adults were analyzed using enriched B cells, whereas samples from 24 SLE patients and 13 healthy children were assessed using whole blood.

**Isolation of peripheral blood B cells**

Mononuclear cells were isolated using gradient centrifugation over a Hypaque cushion. The resulting population was enriched for B cells using negative depletions with magnetic beads coupled to anti-CD2, CD3, CD4, CD14, CD16, CD56, and glycoporphin A (stem cell). The enriched B cells were stained with fluorochrome-labeled Abs (FITC, PE, Tricolor, PerCP, and allophycocyanin). The following were used: anti-human CD3-FITC, CD7-FITC, CD14-PE, CD19-allophycocyanin, CD20-PerCP (BD Biosciences, Mountain View, CA); CD10-FITC, CD40-PE, CD71-PE, CD79a-FITC (Immunotech Research, Quebec, Canada); CD23-PE, CD56-FITC (Caltag, South San Francisco, CA); CD38-PE, CD5-E, CD138-FITC, κ and λ light chain-PE (Serotec, Oxford, U.K.); CD154-FITC (Ancell, Bayport, MN); and anti-human IgD-FITC, IgM-PE, IgG-PE, IgE-FITC, IgA-FITC (Southern Biotechnology Associates, Birmingham, AL). Stained cells were analyzed using flow cytometry (FACSCalibur, BD Biosciences).

**Labeling of cell surface Ags from whole blood samples**

Whole blood was collected into tubes containing heparin or ACD and stained with the following Abs: IgG-FITC, CD38-PE, CD20-PerCP, and CD19-allophycocyanin and corresponding isotype controls. We used 50 µl blood and 3 µl of each Ab per tube for each staining. After staining, the blood was lysed with FACS Lysing Solution (BD Biosciences), rinsed with PBS, centrifuged at 1200 rpm for 10 min, and resuspended in 1% paraformaldehyde. Samples were then analyzed on a BD Biosciences flow cytometer (FACSCalibur).

**Amplification of the centerin gene**

Real-time PCR was performed using an ABI Prism 7700 sequence detector (PE Biosystems, Foster City, CA). The RT-PCR conditions were 30 min at 48°C and 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C. The Taqman PCR core kit reagents (PE Biosystems), Multiscribe reverse transcriptase (PE Biosystems), and RNase inhibitor (PE Biosystems) were used according to the manufacturer’s suggested concentrations for a multiplex reaction. The 18S ribosomal RNA and Centerin standard curves were generated using a serial dilution of a known quantity of Raji total RNA. Ribosomal RNA analysis was performed using the ribosomal RNA control reagent kit (PE Biosystems). The centerin probe (5′-FAM-tccacagcaacctgctgtaag-tamra) was used at a concentration of 250 nM, and the forward and reverse centerin primers (forward aagggaggtgtagacaattca; reverse gcttctcccacttggctttaaa) were used at a concentration of 900 nM.

**Sequencing of Ig VH genes**

Total RNA from between 1,000 and 100,000 sorted B cells was prepared using the mini-RNEASY kit (Qiagen, Valencia, CA) following the manufacturer’s protocol. RT-PCR was performed on 10% of the total RNA generated from the sorted cells using the Titan RT-PCR kit (Roche, Indianapolis, IN). The VH region of IgM transcripts was amplified using either a V H 4 - or a V H 5 - leader primer in combination with a μ-constant region reverse primer, as previously described (33, 34). The VH region of IgG was amplified using identical forward primers with a γ-specific constant region reverse primer. The VH fragments were excised from a low melt agarose gel and reamplified using heminested reverse primers and the high fidelity PFU polymerase (Stratagene, La Jolla, CA). The PCR fragments were either tailed with Taq polymerase (Promega, Madison, WI) and subsequently cloned into the pCRII-TOPO vector or directly cloned into the pCR-blunt-II-TOPO vector (Invitrogen, Carlsbad, CA) and sequenced in both directions using an automated DNA sequencer (ABI-377; Advanced Biotechnologies, Columbia, MD).
Analyses of DNA sequences
Sequences were edited and analyzed using the DNASTar software package (DNASTar, Madison, WI). Cloned products were searched against the IMGT (the international ImMunoGeneTics database, http://imgt.cines.fr; DNAstar, Madison, WI). Cloned products were searched against the IMGT database. Sequences were edited and analyzed using the DNAstar software package.

Statistical analysis
The data obtained in this study were evaluated using a two-tailed t test and multivariable statistical analysis, as well as the Pearson correlation ratio.

Results
T and B cells are profoundly decreased in SLE blood
Although lymphopenia has been described in SLE (3), the extent of T and B cell decrease remains uncharacterized. Therefore, we measured the absolute numbers of CD3+, CD14+, and CD20+/19+ cells in the blood of 1) 68 children suffering from SLE, 2) 35 age-matched healthy controls, 3) 10 children with JDM to control for the effect of steroid treatment, and 4) 17 healthy adults. SLE patients were divided into two groups according to their disease activity index (SLEDAI over or under 10) measured at the time of blood sampling. The ages (mean and SD) of the SLE patients and healthy controls were comparable (see Materials and Methods). As previously reported (36, 37), when compared with adults healthy children display significantly more blood CD3+ T cells (1687 ± 1139 vs 881 ± 202 cells/µl; p = 0.002) and CD19+ B cells (394 ± 196 vs 129 ± 67 cells/µl; p < 0.0001; Fig. 1). Children with JDM, treated with steroid regimens similar to those of SLE patients, display numbers of CD19+ cells comparable to those in healthy controls (Table II and Fig. 2). The slight difference (not statistically significant) may reflect the lower average age of the JDM group (9.2 ± 3.8 vs 12.1 ± 3.5 years in JDM and healthy controls, respectively).

Children with SLE showed significantly fewer circulating T cells than healthy children (450 ± 300 vs 1700 ± 380 cells/µl; p < 0.0001). Although patients with the highest disease activity (SLEDAI >10) had lower numbers of T cells than patients with lower disease activity (SLEDAI, <10; 310 ± 167 vs 510 ± 467 cells/µl), this difference was not statistically significant. SLE patients had fewer circulating monocytes than healthy children (144 ± 149 vs 313 ± 326 cells/µl), but this difference did not reach statistical significance (p = 0.06; Fig. 1).

Blood CD19+ B cells in SLE patients were reduced by 81% compared with those in age-matched healthy controls (82.6 ± 77.5 vs 394 ± 196 cells/µl; p < 0.0001). There was no difference in the number of circulating B cells between the two patient groups (Table II), suggesting that B cell lymphopenia in SLE is independent of disease activity. Although most of our patients had been treated for weeks to years with steroids at the time of study, the T and B cell lymphopenia is not a consequence of this therapy, as newly diagnosed patients (3 of 68) were also found to have similarly decreased numbers of T and B cells before they had entered into remission.

Table II. Mean and SD numbers of cells per microliter in each of the studied populations of healthy donors and patients

<table>
<thead>
<tr>
<th>Sample (n)</th>
<th>Total CD19+ Mean ± SD</th>
<th>CD19+CD20+CD38− Mean ± SD</th>
<th>CD19+CD20+CD38+ Mean ± SD</th>
<th>CD19+CD20−CD38+++ Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy adults (17)</td>
<td>129.6 ± 67.5a</td>
<td>97.7 ± 49.7a</td>
<td>18.1 ± 18.7c</td>
<td>1.4 ± 1.7c</td>
</tr>
<tr>
<td>Healthy children (35)</td>
<td>394.0 ± 196.1b</td>
<td>270.9 ± 157.9c</td>
<td>57.8 ± 59.3c</td>
<td>6.3 ± 9.2c</td>
</tr>
<tr>
<td>JDM (10)</td>
<td>470.7 ± 298.4d</td>
<td>428.4 ± 294.6d</td>
<td>37.4 ± 31.2d</td>
<td>4.2 ± 5.5d</td>
</tr>
<tr>
<td>Total SLE (68)</td>
<td>82.6 ± 77.5</td>
<td>28.0 ± 40.3</td>
<td>19.9 ± 24.5</td>
<td>18.7 ± 22.2</td>
</tr>
<tr>
<td>SLEDAI &gt;10 (36)</td>
<td>75.2 ± 81.1</td>
<td>28.6 ± 40.2</td>
<td>21.4 ± 27.7</td>
<td>19.5 ± 12.8</td>
</tr>
<tr>
<td>SLEDAI &lt;10 (32)</td>
<td>90.5 ± 74.0</td>
<td>27.1 ± 41.6</td>
<td>18.2 ± 20.6</td>
<td>18.0 ± 19.9</td>
</tr>
</tbody>
</table>

a Superscript letters indicate the statistical significance between control and SLE groups.
b p = 0.006.
c p < 0.001.
d NS.
e p = 0.001.
any therapy (64.2 ± 72.1 B cells/μl; n = 3). Additionally, nine of the patients treated with i.v. solumedrol and cyclophosphamide who were included in this study have been followed after discontinuation of these drugs for periods between 6 mo and 2 years without finding statistically significant differences in the number of B cells (data not shown).

Circulating naive and memory B cells are considerably reduced in SLE

Our earlier studies on tonsillar B cells showed that CD38 expression permits us to distinguish plasmablasts/plasma cells and GC B cells from naive and memory B cells (reviewed in Refs. 18 and 19). Thus, CD19⁺CD20⁺CD38⁻ blood cells include both naive and memory B cells. As shown in Table II, healthy children displayed significantly more conventional mature (CD19⁺CD20⁺CD38⁻) B cells than adults (270 ± 157 vs 97 ± 49 cells/μl; p < 0.0001). In contrast, SLE patients showed a marked reduction (~90%) in these cells compared with age-matched controls (28.0 ± 40.3 cells/μl; p < 0.0001). This reduction does not appear to be related to disease activity (27.1 ± 41.6 cells/μl for SLEDAI <10; 28.6 ± 40.2 cells/μl for SLEDAI >10; Table II).

The blood memory B cell population is best identified as CD20⁺CD27⁺ cells. We calculated the ratio of memory/naive B cells in healthy children and children with SLE and found no difference between the two groups (0.46 ± 0.30 and 0.49 ± 0.35 in healthy and SLE children, respectively).

B cells with pre-GC phenotype recirculate in blood of healthy and SLE children

Our initial studies on SLE total blood and enriched blood B cells revealed a strikingly high percentage of circulating CD20⁺IgD⁺CD38⁻ cells. A closer analysis of samples from non-SLE patients revealed that cells with similar phenotype were also present in the blood of healthy children, adults, and children with autoimmune diseases other than SLE, prompting us to report their characterization (Fig. 3). In absolute numbers healthy children have the highest numbers of IgD⁺CD38⁻ cells (57.6 ± 53.3 cells/μl), followed by patients with JDM (37.4 ± 31.2 cells/μl). The number of IgD⁺CD38⁻ cells in SLE patients (21.4 ± 27.7 cells/μl SLEDAI >10, 18.2 ± 20.6 cells/μl SLEDAI <10) is comparable to that in adults (18.1 ± 18.7 cells/μl; Table II). Due to the more drastic reduction in conventional CD20⁺CD38⁻ cells in SLE patients, this population overall represents 29 ± 17.7% of SLE blood B cells (range, 6–77%), whereas it represents 13.2 ± 8 and 18.5 ± 14.9% of the total blood B cells in healthy adults and children, respectively (Fig. 4).

In both patients and controls these cells express high CD20, a characteristic of GC B cells (data not shown). When sorted and analyzed with Giemsa staining, IgD⁺CD38⁻ cells appear very

![FIGURE 3](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org)
similar to the tonsilar Bm2 (IgD⁺CD38⁻CD23⁺) cell subset: they are larger than naive B cells and display a full cytoplasmic rim (Fig. 5, a and b). Using real-time PCR, these cells were found to transcribe centerin (Fig. 6), a GC-specific serpin not expressed in conventional naive and memory blood/tonsil B cells (36). Yet, the blood IgD⁺CD38⁺ cells seem less committed toward GC differentiation than the GC founder cells (Bm2⁺) that were previously identified within tonsils (37), as they mostly lack expression of CD10 and CD77, and only about one-fifth of these cells (21.5 ± 16.7% of 17 samples analyzed) express CD71.

One of the characteristics of tonsilar IgD⁺CD38⁺ cells is the initiation of somatic mutation within Ig V_H genes (38). Therefore, blood IgD⁺CD38⁺ cells were sorted from eight different SLE patients, and their V_H Ig RNA was amplified using primers specific to the small V_H 4 and V_H 5 family leader peptide and μ constant region. Fifty-six independent clones were sequenced and aligned to their closest germ-line counterparts, revealing the presence of low grade somatic mutation within 66% of the transcripts (1–7 bp substitutions/mutated V_H region; Table III). The same population in healthy adults showed a higher rate of mutation (80% transcripts), with a range of 1–13 bp

FIGURE 4. Percentage of conventional B cells (CD19⁺CD20⁺CD38⁻), pre-GC B cells (CD20⁺CD38⁻), and plasma cell precursors (CD20⁺CD19low CD27⁺CD38⁺⁺) in healthy adults, healthy children, and SLE patients.

FIGURE 5. a, Wright-Giemsa staining of cytopsin, magnetic bead-purified blood B cells; arrows show two resting naive B lymphocytes with scant cytoplasm next to three larger cells with more abundant cytoplasm corresponding to IgD⁺CD38⁻ B cells. b, Sorted blood IgD⁺CD38⁺ B cells. c and d, Sorted blood CD19⁺⁺⁺CD20⁺⁺CD27⁺⁺CD38⁺⁺ plasmablasts at ×40 and ×100 magnifications, respectively.
substitutions/mutated V_H region (data not shown). Thus, blood IgD^+CD38^- cells have initiated the process of somatic mutation. Taken together, our data indicate the presence in blood of a subset of B cells that may represent the link between naive and GC cells.

Increase in SLE blood of CD20^-CD19^-CD38++ clonally expanded plasma cell precursors that can be further subdivided into CD27^+ and CD27^-.

Most SLE patients display a distinct population of CD20^-CD19^-lowCD38++ blood cells (Fig. 7, A and B). Upon staining with CD27, these cells can be further subdivided into a CD27^+ and a CD27^- population. Although the ratio of CD27^+/CD27^- varies, the predominant population expresses CD27 with intensity comparable to that of memory (CD27^+) B cells (Fig. 7B). After sorting and Wright Giemsa staining, the majority of these cells do not look like mature plasma cells but like plasmablasts/early plasma cells (39, 40), as they have larger, less peripheral nuclei and less abundant cytoplasms (Fig. 5, c and d). The majority of these cells express both surface and intracytoplasmic Ig, with a kA ratio close to 1 (43.5 ± 17.9% λ), whereas only a small percentage (15.5 ± 8.8%) of them expresses the mature plasma cell marker CD138 or syndecan.

As shown in Table II, SLE patients have a 3-fold expansion of this population compared with healthy controls. This expansion does not correlate with disease activity as measured by the SLEDAI (18.0 ± 19.9 cells/µl for SLEDAI <10; 24.1 ± 33.1 cells/µl for SLEDAI >10).

We sorted these cells and analyzed 38 IgG V_H gene transcripts from four different SLE patients. All but two transcripts showed a high frequency of somatic mutations (mean, 16 ± 8.5 mutations/mutated transcript). However, a striking finding was the identification in three of four patients of clonally related transcripts. An example of the V_H sequences corresponding to an expanded clone (seven related transcripts), with unique and shared mutations, is displayed in Fig. 8. The pattern of nucleotide mutation within this clone strongly suggests that it is the product of an Ag-driven response, as there is a high ratio of replacement vs silent substitution, especially concentrated within the second hypervariable region and the third framework. Clonally related, somatically mutated transcripts were also found in the blood plasma cell precursors isolated from two healthy adults (data not shown), suggesting that these cells in health and disease are the product of oligoclonal expansions.

SLE serum does not alter the survival of normal blood B cells

To determine whether the consistently low numbers of blood B cells and/or the activated B cell phenotype that we observed in our SLE patients were due to soluble serum factors, we purified naive blood and tonsil B cells from healthy donors and cultured them in the presence of autologous sera, sera from four lymphopenic SLE patients with different SLEDAI, and sera from two patients with JDM. The percentage and absolute numbers of viable cells were calculated at 24, 48, 72, and 96 h using a hemocytometer after trypan blue staining. Apoptotic cells were also analyzed by flow cytometry using forward side scatter/side light scatter and annexin V binding/propiodium iodine staining. No consistent differences were observed (data not shown), thus suggesting that a soluble factor(s) is not responsible for mature B cell death and subsequent lymphopenia in all SLE patients.

Discussion
B cell subsets in the blood of healthy children

Our study shows that blood B cells in all age groups include at least four subsets: 1) naive (CD19^-CD20^-IgD^-CD38^-CD27^-) B cells, 2) pre-GC (CD19^-CD20^-IgD^-CD38^+CD27^-) B cells, 3) memory (CD19^-CD20^-CD38^-CD27^+) B cells, and 4) plasma cell precursors (CD19^-CD20^-CD27^-/CD38^+). When comparing children to adults, naive and memory B cells are 2.4-fold more abundant, whereas pre-GC B cells and plasma cell precursors are 3- and 4-fold expanded, respectively, in children.

A puzzling observation is the detection in blood of slgM^-slgD^+ B cells bearing a phenotype similar to that of tonsil GC B cell founders. As GC B cells, these cells express CD38 and centerin, but, unlike GC founders (Bm2^-) and centroblasts (Bm3), they lack the expression of CD10 and CD77. Furthermore, they are smaller than centroblasts, hence their denomination as pre-GC cells. Importantly, these cells have initiated the process of somatic mutation, which is another hallmark of GC reactions; sequencing the V_H transcripts from sorted slgD^-slgM^-CD38^- blood B cells from healthy adults revealed a mutation frequency similar to that described for tonsil GC B cell founders (1–12 bp mutations/V_H region in 80% transcripts) and higher than that of naive B cells (1–2 bp mutations/V_H region in 50% transcripts) (33, 39). Thus, IgM^-IgD^-CD38^- blood B cells may represent the link between naive (Bm1 and Bm2) and GC founders (Bm2^-). It remains to be established whether these cells result from 1) activation in lymphoid sites and recirculation in the blood, or 2) activation in nonlymphoid sites followed by recirculation in the blood and later homing to peripheral lymphoid organs.

<table>
<thead>
<tr>
<th>B Cell Subpopulation</th>
<th>No. Clones</th>
<th>Isotype</th>
<th>No. Mutated Clones</th>
<th>No. Mutations (Average/ST dev)</th>
<th>Mutation Range</th>
<th>Clonal Relatedness</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgD^-CD38^-</td>
<td>10</td>
<td>µδ</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>No</td>
</tr>
<tr>
<td>IgD^-CD38^+</td>
<td>56</td>
<td>µδ</td>
<td>37</td>
<td>1.4 ± 1.6</td>
<td>0–7</td>
<td>No</td>
</tr>
<tr>
<td>CD19^-CD20^-CD38^-++</td>
<td>30</td>
<td>γ</td>
<td>28</td>
<td>15.6 ± 8.6</td>
<td>0–31</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Plasma cell precursors constitute another underestimated circulating cell population. We show herein that they represent 1.4% of the total B cell compartment in healthy adults and 3.3% in healthy children. In the context of certain infections and malignancies, higher numbers of plasmablasts have been described in the blood (reactive plasmacytosis) (40, 41). These cells have been reported to characteristically lack the plasma cell marker CD138, but they acquire it in vitro upon exposure to IL-6 (41). Additionally, these cells express variable levels of CD27 (Refs. 42 and 43, and our own observation), suggesting caution when using CD27 to enumerate memory cells, especially in clinical situations where plasmacytosis may be expected.

Blood B cell subsets in children with SLE

Our studies reveal that children with SLE suffer profound B cell lymphopenia due to a dramatic reduction in all mature B cell subsets. SLE B cell lymphopenia does not correlate with any modality of therapy, SLEDAI, or anti-dsDNA or complement titers. SLE B cell lymphopenia could be due to 1) a reduction in the number of bone marrow B cell precursors, 2) shortened mature B cell life span, or 3) accelerated activation/differentiation of naive cells into downstream phenotypes including GC, memory, or plasma cells that would subsequently home into lymphoid tissues.

FIGURE 7. A, Enriched (>95% pure) blood B cells from two SLE patients (A and B). Squares depict the CD19<sup>+</sup>/CD38<sup>+</sup> plasma cell precursor population. Patient A was recently diagnosed and untreated at the time the sample was obtained, whereas the sample from patient B was obtained 1 mo after cytoxan and solumedrol pulses. B, Enriched B cells from a SLE patient stained with anti-CD20-PerCP, anti-CD19-allophycocyanin, anti-CD38-PE, and anti CD27-FITC. CD19<sup>+</sup>/CD38<sup>+</sup> cells gated in B are represented within a rectangle in D and divided by a dotted line according to the intensity of CD27 staining. The same CD19<sup>+</sup>/CD38<sup>+</sup> population is enclosed by a dotted circle (A) and a dotted rectangle (C). This experiment is representative of 12 individual experiments.

VH5-251 MGSTAILALLAVLQGCTCA EVQLVQGSQAELKYGDSKICSRRSYSH
SLE-1 N
SLE-2 N
SLE-3 A
SLE-4 T
SLE-5 T
SLE-6 D
SLE-7 P

CDR1

TVSADKSISTAYLQWSSLFASDTAYYCAR

CDR2

VH5-251 OKQIKELW M31IYPDGSDTTEYSSFPQQ
SLE-1 N
SLE-2 H
SLE-3 I
SLE-4 P
SLE-5 P
SLE-6 P
SLE-7 P

CDR3

SPQQ

FIGURE 8. Amino acid translation of seven clonally related V<sub>H</sub>6/7<sub>γ</sub> transcripts isolated from sorted plasmablasts from the blood of a SLE patient. The transcripts display unique and common mutations while sharing the same V-D and D-J junctions (only the V-D junction is shown). There is a high ratio of R/S nucleotide substitutions especially within CDR2 (R/S = 2 and 5 in transcripts SLE 7 and SLE 3, the least and most mutated V<sub>H</sub> regions from this clone, respectively) and FW3 (R/S = 3 and 8 in SLE 7 and SLE 3, respectively). The nucleotide sequences corresponding to these transcripts have been submitted to GenBank under accession numbers 384526, 384527, 384534, 384543, 384551, and 384565.
Killing of B cells by soluble factors (i.e., anti-lymphocytic Abs) has been implicated as a cause of SLE lymphopenia (44, 45). Although this mechanism may operate in some SLE patients, our results suggest that it is unlikely to explain the universal lymphopenia observed in this disease, as incubation of blood naive B cells from healthy donors with serum from active SLE patients failed to disclose any significant reduction in the number of viable cells. Additionally, the B and T lymphocyte propensity to undergo spontaneous and induced apoptosis has been recently described to be grossly intact in SLE (46).

The lymphopenia that we describe cannot be explained by bone marrow aplasia, as the neutrophil and platelet counts were within normal limits in the population that we studied. Furthermore, bone marrow aspirates from SLE patients, usually obtained in the context of severe blood cytopenias, have rarely revealed aplasias (47–49). Therefore, only a selective lymphoid cell precursor defect could explain the reduced numbers of T and B cells that we observed in the blood of our SLE patients. The increased proportion of CD38\(^+\) B cells in SLE blood may provide us with some clues regarding the lymphopenia and perhaps some ethiopathogenic factors in this disease. In trying to induce naive B cells to become GC B cells in vitro, we identified IFN-α as one of the most efficient signals to up-regulate CD38 expression on naive B cells (50). Interestingly, high levels of IFN-α have been described in the serum of SLE patients (51), and the PBMCs of patients without circulating IFN-α display high levels of oligoadenylate synthetase and Mx protein, a signature of exposure to IFN-α (52, 53). The potential role of this cytokine in SLE development is further suggested by the large proportion of patients receiving IFN-α therapy who develop autoimmune, including SLE-like, syndromes (reviewed in Ref. 54). Finally, and perhaps best explaining the generalized lymphopenia of SLE patients, administration of IFN-α to newborn mice induces T and B cell development in the bone marrow, thymus, and spleen by 80% (55). Therefore, all these findings make it tempting to speculate that SLE may be associated with a deregulation of IFN-α production. Consistent with this hypothesis, the blood pre-GC (IgD\(^+\)CD38\(^+\)) cell subpopulation is reduced to a lesser extent in SLE patients compared with controls and represents the predominant blood B cell population in many SLE patients.

In contrast to the reduction in all mature B cell subsets, children with SLE present a 3-fold expansion of blood plasma cell precursors that make up 8.7% of their blood B cell compartment. Plasma cells expressing CD138 and high levels of CD27 have been recently reported in the blood of 13 adult SLE patients (43). In our study only a small proportion of the CD20\(^+\)CD19\(^{low}\)CD38\(^++\) cells in the 68 patients analyzed display this more mature phenotype, whereas the majority lack CD138, express two levels of CD27 (comparable and higher than memory B cells), and upon sorting and Giemsa staining do not show a mature plasma cell morphology. Blood plasma cell precursors are post-GC cells, as they express highly mutated and isotype-switched Ig transcripts. Additionally, there is a high degree of clonal relatedness within this subset, as numerous transcripts share the same VDJ rearrangement while displaying common and unique nucleotide substitutions. This suggests that they are the products of a recent clonal expansion that probably occurred in a GC, given the presence of unique mutations. This expansion may be explained by increased IL-10, a major plasma cell differentiation factor (56). Indeed, high levels of IL-10 are found in the serum of SLE patients, and treatment of these patients with anti-IL-10 Abs has shown beneficial effects (57–59). Alternatively, the recently identified B lymphocyte stimulator (BLyS/BAFF/TALL-1), a TNF family cytokine (60–63), may contribute to the disease, as it seems to prominently enhance humoral responses. BLyS transgenic mice show hypergammaglobulinemia and an autoimmune lupus-like disease (61). Furthermore, the survival of lupus-prone mice is increased by treatment with a BLyS antagonist (63). Although altered expression of BLyS and/or its receptors may play a role in human SLE, significant differences between the B cell phenotype found in BLyS transgenic mice and human SLE exist, as these transgenic mice display B cell expansion in the blood rather than the profound B cell lymphopenia that we describe in our patients. SLE may thus be best explained by the combined ectopic expression of cytokines such as α-IFN, IL-10, and BLyS. The etiology of this disease may be explained at the level of cells that produce these cytokines, which include APC such as dendritic cells.

Acknowledgments
We thank Dr. Karolina Palucka for very helpful discussions. Steven Scholl and Elizabeth Kraus provided excellent technical assistance.

References