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*J Immunol* 2007; 178:6624-6633; doi: 10.4049/jimmunol.178.10.6624
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A New Population of Cells Lacking Expression of CD27 Represents a Notable Component of the B Cell Memory Compartment in Systemic Lupus Erythematosus

Chungwen Wei,* Jennifer Anolik,* Amedeo Cappione,2* Bo Zheng,* Aimee Pugh-Bernard,3* James Brooks,4* Eun-Hyung Lee,† Eric C. B. Milner,* and Iñaki Sanz5*

Human memory B cells comprise isotype-switched and nonswitched cells with both subsets displaying somatic hypermutation. In addition to somatic hypermutation, CD27 expression has also been considered a universal memory B cell marker. We describe a new population of memory B cells containing isotype-switched (IgG and IgA) and IgM-only cells and lacking expression of CD27 and IgD. These cells are present in peripheral blood and tonsils of healthy subjects and display a degree of hypermutation comparable to CD27+ nonswitched memory cells. As conventional memory cells, they proliferate in response to CpG DNA and fail to extrude rhodamine. In contrast to other recently described CD27-negative (CD27neg) memory B cells, they lack expression of FcRHa4 and recirculate in the peripheral blood. Although CD27neg memory cells are relatively scarce in healthy subjects, they are substantially increased in systemic lupus erythematosus (SLE) patients in whom they frequently represent a large fraction of all memory B cells. Yet, their frequency is normal in patients with rheumatoid arthritis or chronic hepatitis C. In SLE, an increased frequency of CD27neg memory cells is significantly associated with higher disease activity index, a history of nephritis, and disease-specific autoantibodies (anti-dsDNA, anti-Smith (Sm), anti-ribonucleoprotein (RNP), and 9G4). These findings enhance our understanding of the B cell diversification pathways and provide mechanistic insight into the immunopathogenesis of SLE. The Journal of Immunology, 2007, 178: 6624–6633.


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cell hyperactivity. Similar to conventional CD27+ memory B cells, DN cells can be divided into isotype-switched and nonswitched subsets and express somatically mutated VH genes. In contrast to another subset of CD27-negative (CD27neg) memory cells described in healthy subjects during the preparation of this manuscript, these cells are found in the peripheral circulation and lack expression of the FcRH4 (13).

Materials and Methods

Clinical samples

Samples were obtained with informed consent using protocols approved by the University of Rochester Medical Center Institutional Review Board. PBMCs from SLE patients, hepatitis C patients, rheumatoid arthritis (RA) patients, and healthy controls were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia Biotech). Eligible SLE and RA

Table I. Distribution of B cell subsets among SLE patients

<table>
<thead>
<tr>
<th>SLE</th>
<th>Naive CD27+IgD-</th>
<th>CD27+IgD- (DN)</th>
<th>CD27-IgD-</th>
<th>CD27-IgD- (DN)</th>
<th>DN% of memory cells</th>
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<tbody>
<tr>
<td>SLE1</td>
<td>73</td>
<td>2</td>
<td>16</td>
<td>9</td>
<td>33</td>
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<td>15</td>
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<tr>
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<td>2</td>
<td>11</td>
<td>6</td>
<td>31</td>
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<td>94</td>
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<td>3</td>
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<td>81</td>
<td>8</td>
<td>10</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

*S Numbers represent the percentage of all PBL B cells contributed by naive, CD27+ nonswitched (IgD-), CD27+ switched (IgD+), and DN B cells (CD27-/IgD-). Also shown is the percentage contributed by DN cells to the memory compartment. For comparison, naive and DN B cells represented 63.2 ± 9.9% and 4.6 ± 1.8% (mean ± SD) of all B cells, respectively, in healthy control subjects (n = 29). Naive lymphopenia was defined as having a naive population that is 2 SD lower than the mean (<43%), and the cutoff between low and high percentage DN was arbitrarily established at 3 SD above the mean (≥10%).
subjects were male or female, ages >18 years, who met the American College of Rheumatology revised criteria for the classification of disease. Disease activity was assessed by the Systemic Lupus Activity Measure. Tonsil samples were obtained from normal subjects undergoing routine tonsillectomy.

**Serological analyses**

Autoantibodies in the sera against dsDNA were detected by ELISA using the Kallestad anti-dsDNA kit (Bio-Rad). Detection of Abs in the sera against RNP, Sm, Ro, and La were conducted using the QUANTA Lite Microwell ELISA kits (INOVA Diagnostics). C3 levels in the sera were quantitatively determined by rate nephelometry using the IMMAGE immunochemistry systems (Beckman Coulter). Assays for autoantibodies and C3 were conducted following the manufacturers’ recommendations, respectively, in the Department of Pathology and Laboratory Medicine Immunology Laboratory and Protein Laboratory at the University of Rochester.

**Flow cytometry analysis**

Experiments were performed as previously described in our laboratory (12, 14, 15). PBMCs purified through Ficoll-Hypaque density gradient centrifugation were stained in PBS/2 mM EDTA/0.5% BSA/5% normal mouse serum with fluorescein-conjugated mouse monoclonal anti-CD19 (SJ25C1; BD Biosciences), anti-IgD (IA6-2; BD Biosciences), anti-CD27 (O323; eBioscience), and one of the following Abs: anti-CD38 (HIT2; BD Biosciences), anti-IgM (G20-127; BD Biosciences), anti-IgG (G18-145; BD Biosciences), anti-CD10 (HI10a; BD Biosciences), or anti-CD10 (H1/10a; BD Biosciences). FcγRIII was detected either with biotinylated goat anti-human FcγRIII (R&D Systems)
followed by fluorochrome-conjugated streptavidin or with a mAb provided by Dr. M. D. Cooper (University of Alabama at Birmingham). Tonsillar B cells were first isolated by SRBC rosetting, then stained with anti-IgD, anti-CD27, anti-CD38, and one of the following Abs: anti-CD10, anti-IgG, anti-IgM, and anti-CD45R/B220. To analyze FcRH4 expression, tonsil cells were stained with anti-CD19, anti-IgD, anti-FcRH4, and anti-CD27 or anti-CD38. After washing, cells were fixed in PBS/0.5% formaldehyde and analyzed on a FACSCalibur (BD Biosciences). To analyze the extrusion of Rhodamine 123 (R123) by different subsets of B cells, PBMCs or tonsillar mononuclear cells were pulsed with 6 μM R123 (Molecular Probes) in the culture medium at 37°C for 10 min, then chased for 3 h. After washing, cells were stained with anti-CD19, anti-IgD, and anti-CD27 at 4°C for 30 min as described above.

To carry out nine-color flow cytometry, PBMCs or tonsillar mononuclear cells were stained with the following fluorochrome-conjugated mouse anti-human mAbs: FITC-anti-IgD (IAE-2; BD Biosciences), PE-anti-CD38 (HIT2; BD Biosciences), PE-Alexa 610-anti-CD24 (SN3; Caltag Laboratories), PE-Cy5-anti-IgM (G20-127; BD Biosciences), PE-Cy5-anti-IgD (IA6-2; BD Biosciences), and PE-Cy7-anti-CD19 (B94; BD Biosciences). Anti-IgG, anti-IgM, and anti-CD45R/B220 were obtained from BD Biosciences. PE-Cy5-anti-IgM (G20-127; BD Biosciences), PE-Cy5-anti-IgD (IA6-2; BD Biosciences), PE-Cy7-anti-CD19 (B94; BD Biosciences). For our studies, memory cells were either identified on the basis of CD27 expression or according to their Bm5 phenotype within the Bm1–Bm5 fraction that represents GC cells as well as a CD38bright/dull fraction that lacks CD10 expression. In contrast, Bm5 cells are devoid of CD10+ GC cells due to the exclusion of cells expressing higher levels of CD38 but can be divided into CD27+ and CD27− fractions. Thus, the CD27− IgD−CD10+ fraction represents the tonsil equivalent of PBL DN cells. Further analysis of CD27− switched memory and DN cells reveals a virtually identical profile regarding the expression of CD24 and IgM. This identity was found whether DN were identified within CD27+ IgD− or Bm5 fractions.

V gene sequence analysis

PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation and stained with FITC-IgD, PE-IgG, PerCP-Cy205, and allophycocyanin-Cy7-Cy5, and allophycocyanin-Cy7-Cy5, and allophycocyanin-Cy7-Cy5. Total RNA was extracted from the sorted cells and reverse transcribed into cDNA using oligo(dT) priming, which was then amplified by PCR with a V_{H} family-specific primer (5'-GAGGTTGCG GTGKTGAGTGC-3') and a Cγ primer (5'-CGTTCGCGAAA GTAGCTTGACC-3'). The PCR products were then cloned into the pCR4Blunt-TOPO vector (Invitrogen Life Technologies) and used for the transformation of competent DH5α Escherichia coli cells. After transformation, plasmid DNA was isolated for sequencing from randomly picked colonies. Sequencing was conducted with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) using the M13F primer (5'-GTAAAACGAC GTGCCAGT-3') and analyzed in an automated Applied Biosystems sequencer.

Statistical analysis

Statistical significance was assessed by the two-tailed Student’s t test assuming unequal variances. The frequencies of clinical manifestations and autoantibodies between groups were compared by a χ² test of independence.
Results

A population of peripheral blood B cells lacking expression of CD27 and IgD is expanded in patients with SLE

When analyzed for their expression of surface IgD and CD27, human PBL B cells typically segregate into two dominant populations as follows: 1) an IgD−/CD27− fraction, which largely includes mature naïve B cells, and which generally represents the main population in healthy subjects and 2) a fraction containing CD27+ memory B cells, which can be further separated into IgD− and IgD+ subsets (Fig. 1A). As described by multiple groups, patients with SLE frequently have a relative expansion of CD27+ memory cells. In addition, Davidson’s laboratory and our own group have reported that some SLE patients also display an expansion of CD19+ IgD−/CD27− (DN) B cells (Fig. 1A) (11, 12). As shown in Fig. 1A and in Table I, in some patients, this fraction constitutes the largest PBL B cell population.

We have now analyzed this DN population in 36 patients with SLE, as well as in normal subjects and diseased controls. As a group, the frequency of DN cells among total PBL B cells is significantly increased in SLE patients as compared with healthy controls (p = 0.0001). In healthy subjects, DN cells are always lower than 10% of all CD19+ B cells, with a mean frequency of 4.5% (Table I and Fig. 1B). In contrast, DN cells represent >10% of CD19+ B cells in 50% (18 of 36) of SLE patients, with a mean frequency for this subgroup of 19.4%. In 14% (5 of 36) of patients, DN cells reached levels higher than 20% of all PBL B cells. Moreover, DN cells were present at frequencies higher than CD27+ memory cells in 25% of all SLE patients analyzed and in 40% of the SLE subset classified as having DN expansions (Table I). As illustrated in Table I, the relative increase observed in the frequency of DN cells was not solely due to the naïve lymphopenia that can be observed in SLE patients (18). Indeed, the frequency of DN cells was increased in 72% (13 of 18) of patients in whom the frequency of naïve B cells fell within the normal range for healthy controls.

To assess whether DN cells are also expanded in other conditions characterized by autoimmunity and/or B cell hyperactivity, we studied patients with RA and patients with chronic hepatitis C infection. As shown in Fig. 1B, the frequency of DN cells in these diseases was no different from the one observed in healthy subjects.

Surface phenotype of CD27/IgD DN B cells in human PBL

The surface phenotype of PBL DN B cells resembles that of conventional CD27+ memory cells, both in SLE patients and healthy controls (Figs. 2 and 3). Thus, DN cells contain similar frequencies of IgG+ isotype-switched cells (44.4 ± 13.0% for healthy controls and 53.7 ± 20.5% for SLE) as CD27+ cells (42.8 ± 10.8% and 43.2 ± 10.1%, respectively). The frequency of IgM+ cells was also similar between DN cells (17.5 ± 9.1% vs 20.6 ± 11.8%, respectively). It should be noted, however, that in contrast to DN cells, the vast majority of CD27+ memory cells that express IgM also express IgD (data not shown) (7, 19).

DN cells are also similar to CD27+ memory cells in terms of their level of expression of CD38 (Figs. 2 and 3). Indeed, DN cells...
include CD38dull and CD38− populations whose levels of CD38 parallel those expressed by early Bm5 and Bm5 memory cells, respectively, and below those of B cells that typically stain clearly positive for this marker in the PBL (pre-GC, transitional, and plasmablasts) (17). Interestingly, although Bm5, CD27+, and DN cells are almost equally split into CD38− and CD38dull in healthy subjects, in SLE patients these populations are largely formed by the CD38dull subset (early Bm5). Also, as previously described by Bleesing and Fleisher (20) and our own group (21) and in contrast to naive B cells, which universally express B220, conventional memory cells can be divided into B220-positive and -negative subsets. This heterogeneity is also found in DN cells.

It is worth bearing in mind that classification schemes of human B cells based on the expression of IgD, CD27, and/or CD38, although useful and widely used, have significant limitations. Thus, so-called naive Bm1 and Bm2 cells also contain non-switched CD27− memory cells; Bm2+ pre-GC cells also contain transitional B cells, which can also be found within the IgD−CD27− naive populations (22–24). We therefore used multichromatic flow cytometry to evaluate the expression of other useful differentiation markers including CD24 and CD10 (Figs. 2A and 3). This approach confirms the similarity in surface phenotype between DN and conventional CD27+ memory cells. Furthermore, it shows that DN cells are devoid of surface CD10, a feature that adds further to the distinction between DN cells and other B cell subsets that can be found in the PBL, including transitional cells (CD10+, CD38high, IgD+) and pregerminal center (CD10+, CD38high, IgD+/−); 15, 17, 23–26).

**Surface phenotype of CD27/IgD DN B cells in human tonsil**

Next, we sought to identify the tonsil counterpart of the DN population. As shown in Fig. 3, IgD−CD27− tonsil B cells can be further divided into CD38− and CD38dull−neg. The former fraction stains positive for CD10 and, as previously shown by multiple groups, it represents GC cells, whereas the latter subset lacks expression of CD10 and represents the tonsil counterpart of DN cells (15, 17, 23, 25, 26). As previously discussed for the PBL, the Bm1−Bm5 classification has significant limitations and, as shown in Fig. 3, the Bm5 subset contains not only conventional CD27− memory cells, but also a large fraction of CD27− cells. We therefore compared the extended surface phenotype between the global IgD+CD27−CD10− fraction, the Bm5 IgD+CD27+CD10− fraction, the Bm5 IgD−CD27−CD10− fraction, and the CD10− fraction of IgD+CD27+ memory cells. Remarkably, all four subsets display a virtually identical phenotype with regard to the expression of CD24 and IgM. Similarly, all fractions are comparable in terms of their expression of IgG and B220 (data not shown).

Another important difference between PBL and tonsil DN cells is determined by the expression of FcRH4, an interesting inhibitory FcR homolog that has been described during the preparation of this manuscript as being present in a subset of tonsil CD27neg memory cells, but absent in the PBL, bone marrow, and spleen (13, 27). As shown in Fig. 4, and consistent with the results of Ehrhardt et al. (13), FcRH4 expression is largely restricted to a fraction of CD27neg cells, whether IgD− or isotype switched. Tonsil CD27neg cells, however, include a significant component of GC cells. Therefore, we assessed the expression of FcRH4 in the tonsil

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### Table II. Mutational analysis of IgH genes

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls</th>
<th>SLE</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CD27−IgG+/− (n = 28)</td>
<td>CD27−IgG+ (n = 28)</td>
</tr>
<tr>
<td>No. of Mutations</td>
<td>252</td>
<td>431</td>
</tr>
<tr>
<td>No. of nucleotides sequenced</td>
<td>7983</td>
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<tr>
<td>% Mutated</td>
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**R:S ratio**

<table>
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<tr>
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<th>FR2</th>
<th>FR3</th>
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<td>FR3</td>
<td>3.8</td>
<td>3.8</td>
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</tr>
</tbody>
</table>

**CD27− and CD27+ memory B cells isolated from the peripheral blood of a healthy donor and a SLE patient were analyzed for the presence and distribution of somatic mutation. The percentage of mutated nucleotides is shown for individual framework (FR) and hypervariable regions (CDR).**

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**FIGURE 5.** V_{H} genes from CD27−IgG+ memory B cells exhibit fewer somatic mutations than those from CD27−IgG+ memory B cells. CD27−IgG− (□) and CD27−IgG+ (■) memory B cells were sorted from the periphery of a healthy donor (A) and a SLE patient (B), and their rearranged V_{H} gene sequences were analyzed. The percentages of the total V gene sequences exhibiting certain numbers of mutations per V gene sequence are plotted.
DN counterpart by analyzing the Bm5 CD27neg population. This approach demonstrates that tonsil DN cells contain a majority of FcRH4+ cells as well as a fraction that lacks expression of this marker. In contrast, virtually no expression of FcRH4 was detected in PBL DN cells, whether in healthy subjects or in SLE patients.

CD27/IgD DN B cells have phenotypic, genetic, and functional characteristics of memory cells

As previously discussed, with the critical exception of CD27, the surface phenotype of DN cells strikingly resembles that of conventional memory cells, whether they are identified through the expression of CD27 or by the Bm5 classification. Several additional features permit such characterization. Thus, as shown in Table II and Fig. 5, DN cells display a significant level of somatic hypermutation in their rearranged VH Ab genes. Interestingly, this level was not significantly different between healthy controls and SLE patients (means of 3.2 vs 2.6%, respectively). However, the mutation level displayed by CD27neg cells is lower than the mutation rate observed in their CD27+/H11001 counterparts (5.4% in normal subjects and 5.1% in SLE). Overall, the distribution of mutations and the replacement:silent mutation ratio suggests that DN cells are Ag experienced and may have been selected by an Ag-driven process.

As recently described by others, memory B cells are also characterized by their inability to extrude rhodamine or similar dyes owing to the absence of the ATP-binding cassette B1 transporter (28). As shown in Fig. 6, this property is shared by DN cells and conventional CD27+ memory cells, with both subsets showing a significant departure with the behavior of naive B cells. Finally, we determined whether DN cells behave like conventional memory cells with regard to their proliferative behavior in response to stimulation with CpG DNA. As shown in Fig. 7, both populations show a similar positive response in the absence of BCR costimulation. This type of response further differentiates DN cells from naive B cells, the latter of which require BCR costimulation for effective proliferation (29). Of note, CD27 gets up-regulated by the majority of DN cells that proliferate in response to CpG DNA stimulation.

Clinical correlations

To identify correlations between increased frequency of DN cells and clinical manifestations of SLE, we studied 46 patients that were classified as either DN-low (DN <10% of all PBL B cells;
significantly higher scores were found in the DN-high group (whom Systemic Lupus Activity Measure values were available, anti-Ro/La Abs, and serum C3 levels. In a subset of 23 patients for high and DN-low groups in terms of hematological manifestations, no significant differences were observed between the DN-
high and DN-low patients, DN-high patients had a significantly higher frequency of nephritis (15 of 26 vs 4 of 20; p = 0.025). Histology was available in three of four patients with nephritis in the DN-low group and in 12 of 15 patients in the DN-high group. Classes III and V (30) were predominant in this group, as they were present in six and five patients, respectively (of these patients, one had classes III plus V and another classes IV plus V). One additional patient had type II nephritis. In the DN-low group, classes III, IV, and V were diagnosed in one patient each. DN-high patients were also characterized by an increased titer of anti-dsDNA Abs (p = 0.001) and anti-RNP/Sm Abs (p = 0.009). No significant differences were observed between the DN-high and DN-low groups in terms of hematological manifestations, anti-Ro/La Abs, and serum C3 levels. In a subset of 23 patients for whom Systemic Lupus Activity Measure values were available, significantly higher scores were found in the DN-high group (p = 0.02). Interestingly, DN-high patients also had significantly higher levels of serum 9G4 Abs, an autoantibody species with high specificity for SLE, which has been shown to correlate with disease activity and lupus nephritis (21, 31, 32). For patients whose DN cells reached levels ≥20% of all PBL B cells, neither clinical nor serological profiles were significantly different from those of patients with DN levels between 10 and 20%.

Discussion
We define in this manuscript the expansion in SLE patients of a novel population of peripheral blood memory B cells lacking expression of CD27 and IgD. In healthy subjects, these cells represent on average <5% of all PBL B cells, a low frequency that may explain why DN cells had been previously neglected. Both in healthy subjects and SLE patients, DN cells contain sizable populations expressing either IgM or isotype-switched Abs (either IgG or IgA; Fig. 8).

Our contention that DN cells should be classified as memory cells is based on the following: 1) an extended surface phenotype that resembles that of conventional memory cells, whether defined as CD27+ or IgD−/CD38−/int Bm5 cells that distinguishes them from other PBL subsets such as naive, transitional, and pre-GC cells. This phenotype includes the expression in approximately two-thirds of these cells of isotype-switched Abs; 2) their inability to extrude R123; 3) their proliferative response to stimulation by CpG DNA in the absence of BCR cross-linking; and 4) the presence of somatic hypermutation in a pattern consistent with Ag-driven selection.

One of the central implications of our study is that in SLE CD27neg cells represent a major (sometimes dominant) component of the B cell memory compartment. Indeed, CD27 has been considered a universal marker of human memory B cells, although the existence of a population of CD27neg memory B cells in SLE had been initially suggested independently by Davidson and colleagues (11) and our group (12). Subsequently, during the preparation of this manuscript, two groups have reported on the existence of CD27neg memory cells in healthy subjects. Thus, Wirths and Lanzavecchia (28) used the differential ability of naive, memory, and transitional B cells to extrude R123 to identify a minor population of CD27neg PBL B cells (<1%) that behave like conventional CD27+ memory cells. Their contention was based on the inability of these cells to extrude R123 as well as on the expression and production in culture of isotype-switched Abs as the vast majority of these cells expressed IgG. However, no genetic analysis assessing the presence of somatic hypermutation was performed. In contrast to our findings, both in SLE and healthy subjects, the population identified by these investigators did not contain CD27neg IgM− memory cells and contained only a very small fraction of IgA cells. Also, in contrast to another population of CD27neg memory cells recently described in the tonsil, PBL CD27neg memory cells are devoid of FcRIH4, an ITIM-containing FcR homolog that can exert powerful inhibition of BCR signaling (13, 33). Along these lines, it is reasonable to speculate that the absence of this inhibitory receptor could make these cells more responsive to activation than their positive counterparts and contribute to their expansion in SLE, as it has been postulated for the inhibitory FcγRIIb (34, 35). However, our preliminary analysis indicates that CD27neg memory cells express levels of inhibitory FcγRIIb similar to CD27+ memory cells and higher than naive B cells.
cells (data not shown). The increased level of FcRγIIB in memory cells as compared with naive cells is consistent with previously published observations (36).

The origin of CD27neg memory B cells remains to be elucidated. Nonetheless, two models could be invoked. On the one hand, these cells could represent a distinct lineage of memory cells following a developmental pathway distinct from CD27+ memory cells, whether isotype-switched cells (which develop in GC reactions in a T-dependent fashion) or IgD+IgM- cells (which may also develop through GC-independent, CD154-independent pathways) (6). Alternatively, these cells could represent either a progenitor or the progeny of CD27+ memory cells. We submit that the totality of the evidence available suggests that CD27neg memory B cells represent cells that fail to go through a productive GC reaction. Indeed, CD27 is acquired by activated B cells after a GC reaction is initiated by cognate B-T cell interactions mediated by CD40–CD154 (3, 37–39). It is therefore tempting to postulate that CD27neg memory cells might develop outside the GC, perhaps in extrafollicular reactions capable of supporting somatic hypermutation as recently demonstrated in the mouse (40). This work suggested that the survival and differentiation of autoreactive B cells outside the GC could be mediated by unique signals provided by CD11c+ DC (40, 41). Interestingly, DC have recently been shown to activate extrafollicular B cells and are also known to induce isotype switching in an CD40-independent fashion through BlyS-BAFF-R interaction (42, 43). Whether these functions could induce a separate differentiation pathway for human memory cells is, at this time, only a tantalizing possibility.

Alternatively, CD27neg memory cells might represent activated follicular cells that initiate GC reactions after receiving early CD154-mediated T cell help, but fail to progress through this pathway, thereby explaining their failure to acquire CD27 and their lower rate of somatic hypermutation as compared with CD27+ memory cells. Given that CD27 interacts with CD70 on activated T cells, it is plausible that the very absence of CD27 might impair the ability of these cells to receive the full and sustained degree of T cell help required to complete a GC reaction (39, 44–46). Previous studies demonstrating a low level of somatic hypermutation in cells generated in abortive GC induced in the course of T cell-independent responses are also supportive of the notion that CD27neg memory cells could represent the progeny of formé fruste GC (47). Interestingly, somatic hypermutation in the absence of CD27 is present in two B cell tumors (Waldenstrom’s macroglobulinemia and hairy cell leukemia), for which an extra-GC origin has been proposed (48, 49). Finally, this model is also supported by the observation that DN cells did not decrease in SLE patients in whom productive T cell-dependent GC reactions were disrupted, presumably at very early stages, with anti-CD154 Abs (11, 50, 51). Also in keeping with this model is our previous observation that the expansion of DN cells in SLE is reversed by Rituximab, even in patients in whom a failure to fully interrupt GC reactions was suggested by rapid reaccumulation of CD27+ memory cells (12).

It is also plausible that CD27neg memory cells could represent intermediates in the life cycle of CD27+ cells. Thus, by analogy with the consequences of ICOS-ICOS ligand interaction, CD27 could be lost on memory B cells upon engagement of CD70 on activated T cells. Of note, similar to our observation with CD27neg cells, ICOSL-negative B cells have been shown to be increased in SLE patients (52). However, if CD27neg memory cells should merely represent the progeny of CD27+ cells one would expect to find the same rate of somatic hypermutation in both subsets. Given that differentiation of CD27+ memory cells into the plasma cell lineage is associated with up-regulation of CD38 and increased level of CD27 expression, it is unlikely that CD27neg memory cells represent an intermediate between these two compartments (44, 53). In contrast, whether or not they initially derive from CD27+ cells, CD27neg memory cells might be able to give rise to CD27+ memory cells as suggested by their acquisition of CD27 upon stimulation with CpG DNA, an important polyclonal stimulator that may be critical for the Ag-independent long-term replenishment of human CD27+ B cell memory (29).

The origin of the expansion in SLE of a heterogeneous population of CD27neg memory cells that includes previously unrecognized subsets should provide the impetus for a better understanding of the contribution of different memory subsets to protective vs autoimmune responses and to the autoimmune responses in different diseases. The actual participation of CD27neg memory cells in the autoimmune process remains to be formally established. However, initial evidence for this concept is provided by the observation that, in patients with expansions of 9G4 B cells (15, 21), the frequency of this autoreactive population was similar within the DN and CD27+ subsets (Fig. 9). Additional support for this notion is provided by the correlation observed between the expansion of these cells with global disease activity, lupus nephritis, and the presence of some disease-specific autoantibodies including 9G4 autoantibodies. Current studies in our laboratory are aimed at elucidating the contribution of CD27neg memory cells to the generation of anti-dsDNA and other SLE-associated autoantibodies. Longitudinal follow-up of SLE patients, currently underway, should also help establish the role of these cells as well as their prognostic and diagnostic value.

Acknowledgments
We thank Drs. G. R. Ehrhardt and M. D. Cooper (University of Alabama at Birmingham) for providing anti-FcγRIIB Abs. We also thank Dr. R. P. Kimberly (University of Alabama at Birmingham) for his gift of the 4F5 anti-FcγRIIB mAb.

Disclosures
The authors have no financial conflict of interest.

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12: 117–139.