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In Vivo \( \text{V}_L \)-Targeted Microbial Superantigen Induced Global Shifts in the B Cell Repertoire

Caroline Grönnwall,*,† Sergei L. Kosakovsky Pond,‡ Jason A. Young,‡ and Gregg J. Silverman*†

To subvert host defenses, some microbial pathogens produce proteins that interact with conserved motifs in \( \text{V} \) regions of B cell Ag receptor shared by large sets of lymphocytes, which define the properties of a superantigen. Because the clonal composition of the lymphocyte pool is a major determinant of immune responsiveness, this study was undertaken to examine the in vivo effect on the host immune system to exposure to a B cell superantigen, protein L (PpL), a product of the common commensal bacterial species, *Finegoldia magna*, which is one of the most common pathogenic species among Gram-positive anaerobic cocci. Libraries of \( \text{V}_L \) chain transcripts were generated from the spleens of control and PpL-exposed mice, and the expressed \( \text{V}_\kappa \) rearrangements were characterized by high-throughput sequencing. A total of 120,855 sequencing reads could be assigned to a germline \( \text{V}_\kappa \) gene, with all 20 known \( \text{V}_\kappa \) subgroups represented. In control mice, we found a recurrent and consistent hierarchy of \( \text{V}_\kappa \) gene usage, as well as patterns of preferential \( \text{V}_\kappa -\text{J}_\kappa \) pairing. PpL exposure induced significant targeted global shifts in repertoire with reduction of \( \text{V}_\kappa \) that contain the superantigen binding motif in all exposed mice. We found significant targeted reductions in the expression of clonotypes encoded by 14 specific \( \text{V}_\kappa \) genes with the predicted PpL binding motif. These rigorous surveys document the capacity of a microbial protein to modulate the composition of the expressed lymphocyte repertoire, which also has broad potential implications for host–microbiome and host–pathogen relationships. *The Journal of Immunology*, 2012, 189: 000–000.

Coevolution of microbial species with the host immune system has given rise to diverse mechanisms by which commensals and pathogens can evade and subvert, or at times reinforce, immunological defenses. The adaptive immune system provides special host advantages, with the capacity to select and expand Ag-specific lymphocyte clones and generate memory for rapid recall responses. However, bacteria and viruses, in turn, have developed high-avidity proteins with specificity for conserved and highly represented framework-associated motifs within the \( \text{V} \) regions of Ag receptors, and these are distinct from conventional Ag binding pockets. Although these proteins may not be critical for the microbe’s metabolic pathways for growth and survival, in several cases, they have been shown to act as virulence factors. The best characterized of the naturally occurring superantigens that target B cells are protein A (SpA) produced by *Staphylococcus aureus* and protein L (PpL) by *Finegoldia magna*, also termed *Peptostreptococcus magnus* (1) (reviewed in Ref. 2).

\( F. \) magna is a common component of commensal human flora that colonizes epithelial surfaces of the skin, mucosal surfaces, and gastrointestinal tract. It is also one of the most common pathogenic species among Gram-positive anaerobic coccoids and the cause of serious clinical infections of bone and joints, as well as wound infections and abscesses (3, 4). The bacterial virulence of *F. magna* isolates correlates with expression of PpL (5), a 76- to 106-kDa protein composed of four to five homologous Fab-binding domains that recognize a conserved framework-associated site (Table I) in the \( \text{V} \) regions of many Ig L chain (\( \text{V}_\kappa \)) gene products (6–9).

In vivo challenge studies have demonstrated that PpL can cause activation-induced apoptotic cell death resulting in the loss of >40% of splenic B cells (10). The greatest targeted depletion occurs among splenic marginal zone B cells and B-1 cells, which provide innate-like, Ag-specific B cell defenses against infectious pathogens (10, 11). To better understand the immunobiologic implications of interactions with a B cell-targeting superantigen, we performed high-throughput sequencing of Ig L chain gene rearrangements to investigate how a limited in vivo exposure of PpL can affect the expressed B cell repertoire.

Materials and Methods

Mice and immunogens

C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and bred under specific pathogen-free conditions under the supervision of the University of California San Diego (UCSD) Animal Subjects Program. All animal protocols were approved by the UCSD Institutional Animal Care and Use Committee. Adapting a well-studied regimen developed for the evaluation of responses to putative B cell superantigens (10–15), on day 0, one group of four 8- to 10-wk-old C57BL/6 mice received 0.5 mg endotoxin-free recombinant PpL (Biovision, Mountain View, CA) in 500 μl PBS by i.p. injection, which was repeated on day 4. Four sex- and age-matched control mice received injections of saline alone. On day 7 after the initial dose, mice were sacrificed, and the spleen from each mouse was harvested and divided, with 50% used for immediate ex vivo flow cytometry analysis and 50% for RNA extraction.

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Abbreviations used in this article: FR1, framework 1; IMGT, international immunogenetics (database); PpL, protein L; SpA, protein A; UCSD, University of California San Diego.

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Flow cytometry analysis

Spleens were dissociated into single-cell suspensions and RBCS lysed using ACK Lysing Buffer (Lonza, Walkersville, MD). Adapting previously reported methods (10), we identified subsets of splenocytes with fluorochrome-labeled Abs specific for B220, CD3, λ, and κ chains, using isotype control Abs, as appropriate. PpL-binding cells were detected with biotinylated recombinant PpL (Biovision) and fluorochrome-labeled streptavidin (BD Biosciences, San Diego, CA). Staining was performed in the presence of FC-block (BD Biosciences). Data were acquired using a FACS-Calibur (BD Biosciences) and analyzed with FlowJo software (Tree Star, Ashland, OR).

Preparation of Vκ amplicon libraries

Immediately after harvest, spleens were stored in RNAlater (Qiagen, Hilden, Germany) and RNA extracted using Qiagen RNA extraction kit following manufacturer’s instructions. RNA concentration was determined with an ND-1000 (NanoDrop, Thermo Fisher Scientific, Wilmington, DE). Isolated RNA (1 μg) was used for first-strand cDNA synthesis and amplification by Rapid Amplification of cDNA Ends (RACE) from the 5′ end (5′/3′ RACE second generation kit; Roche) using a specific reverse primer annealing in the constant part of the κ L chain gene. Purified first-strand cDNA was polya-Tailed, then amplified by PCR using a nested Ig κ C region-anneling reverse primer and an Oligo(dt) anchor forward primer that added a 3′ anchor sequence. For each library, we prepared a 50 μl PCR reaction, consisting of 0.25 mM forward and reverse primer mixes, 1 μl deoxyribonucleotide mix (10 mM), 5 μl 10× High Fidelity reaction buffer (Roche, Indianapolis, IN), 5 μl purified cDNA, 0.5 μl Fast start High Fidelity polymerase (Roche), and 36.5 μl nuclease-free water. The thermocycle program was: 94˚C for 2 min; then 72˚C for 10 min; and 4˚C storage. PCR products were gel purified (QiAex; Qiagen), then used as template (2–6 μl) for a second PCR step, using two PCRs per library, and following the same conditions as earlier, with the addition of library-specific multiplex identifier tags and sequencing primer key. Final amplicon libraries were then separately gel purified. No differences were found in the concentration or purity of PCR products obtained for each of the libraries. Sequences for oligonucleotides used in library generation are compiled in Supplemental Table I.

High-throughput sequencing

The sequences of transcripts in the eight Vκ gene cDNA libraries from four PpL-treated mice and four control-treated mice were determined by pyrosequencing with a 454 GS-FLX instrument (Roche) following the manufacturer’s protocol.

Bioinformatics analysis

For rapid processing of the sequence data, we developed a custom bioinformatics pipeline (Supplemental Fig. 1) based on two published approaches originally developed in the context of HIV-1 sequence analysis. In brief, all reads were examined for the presence of one of the eight library-specific multiplex identifiers, allowing up to one nucleotide mismatch. Reads were then filtered based on the presence of at least 100 consecutive nucleotides with q-scores ≥20 (corresponding to ≤1% base error rates), as previously described (16). Vκ–Jκ rearrangements were assigned to individual reads using a modification of the phylogenetic algorithm (SCUEAL) for classifying recombinant HIV-1 strains (17). We refer to this modification as Ig-SCUEAL. A collection of 100 germline Vκ genes, excluding pseudogenes and genes with large deletions or stop codons, and 5 Jκ genes was taken from the international immunogenetics (IMGT) database (http://www.imgt.org/textes/vquest/refseqh.html, September 9, 2011) (18), aligned in amino-acid space using MUSCLE (19), and mapped to corresponding nucleotide sequences to yield a codon-based alignment.

We next reconstructed maximum likelihood phylogenetic trees for the Vκ and Jκ germline sequences using the GTR+G+I model in Garli v2.0 (http://code.google.com/p/garli/) (20). Vκ and Jκ alignments (and the 318-bp sequence of the 5′ end of the C region) and corresponding trees were compared to the recombinant reference alignment for rearrangement mapping using Ig-SCUEAL. The most recent common ancestors (MRCA) of Vκ and Jκ genes were reconstructed using codon-based maximum likelihood models (17) and used for homology screening. This step served the dual purpose of removing reads that were insufficiently similar to germline genes and rapidly correcting for 454-instrument-specific errors in regions with homopolymers. We developed a computationally parsimonious approach to aligning a version of the Smith–Waterman algorithm that generates an alignment of two codon sequences and allows for penalized frameshifts. The advantage of this algorithm over nucleotide-based read mapping is that it permits for the alignment of divergent sequences that contain stretches of lower nucleotide homology, yet encode for amino acid sequences that are similar. Furthermore, when a read contains a frameshift because of a homopolymer length miscall (a commonly observed technical shortcoming of 454 instruments), the subsequent protein alignment can also be unreliable as only a part of the read may be in-frame. A codon-based alignment can explicitly correct for such frameshifts, whereas being cognizant of the amino-acid homology. To remove reads with poor homology even after correcting for possible frameshifts, we applied the requirement that each read have a per-codon alignment score that exceeded a factor of five that of a random sequence with the same average amino-acid composition, as previously validated (16). Sequences were also determined for the reverse complement, and we retained the direction with the highest homology to germline genes/alleles.

For each read that passed the homology screen, Ig-SCUEAL identified the most closely related Vκ and Jκ germline genes using a genetic algorithm, which provided phylogenetic assignments, with likelihood-based confidence estimates and alternative assignments. Unlike a purely homology-based approach (e.g., IgBLAST, http://www.ncbi.nlm.nih.gov/igblast/) or IMGT V-Quest (21), this approach takes phylogenetic relatedness of germline genes into account and uses an evolutionary model to correct for nucleotide substitution biases, unequal base frequencies, and site-to-site substitution rate variation.

Related transcript reads were assigned to a single clonotype that covered the Vκ-Jκ junction and mapped to a particular rearrangement with >70% confidence, if two conditions were met: 1) each had the exact same infragenus junctional sequence at the nucleotide level, and 2) each included no more than 0.05 substitution/base distant from any other read assigned to the same clonotype, that is, the maximal pairwise nucleotide distance did not exceed 0.05 (≈95% homology). Distances were estimated using pairwise codon-based alignments and the maximum likelihood fitting of a substitution model that allows for unequal base frequencies and transition/transversion rates (22).

All analyses were implemented in the HyPhy package (23) and executed in parallel on a message passing interface cluster. The current pipeline throughput is ~100,000 sequences/day on 200 CPUs, with the majority of time consumed by Ig-SCUEAL, a processor-intensive phylogenetic approach.

Statistical analysis

For each study, the specific statistical test is indicated, with significance based on a p value <0.05.

Results

PpL depleted a subset of Vκ-bearing B cells in vivo

To investigate the impact on the B cell repertoire, we modified a previously reported protocol (10–15), in which groups of four adult mice received injections of the Vκ-targeting superantigen, PpL, or control treatments with saline. Although PpL injections did not result in significant differences in spleen weights, the total number of mononuclear splenocytes was significantly lower in the PpL-exposed mice compared with control mice (mean 56 × 10⁶ compared with 73 × 10⁶ cells, p = 0.038). There was also a significant decrease in the ratio of B cells (B220⁺ cells) versus T cells (CD3⁺ cells) in the PpL-exposed mice (p = 0.0008; Fig. 1A).

In naive mice, there are both weak and strong PpL binders among κ-bearing B cells, whereas λ-bearing B cells do not bind PpL (10). After exposure to PpL, both the representation of PpL-high binding B cells, as well as the mean fluorescent intensity of overall PpL-binding by B cells, were significantly lower (p < 0.0001; Fig. 1B). In addition, PpL exposure resulted in a significant decrease in the overall representation of κ-bearing B cells and an increase in the proportion of λ-bearing B cells (Fig. 1C). Taken together, these findings demonstrate that in vivo exposure to PpL results in the significant depletion of B cells that bind PpL, which is likely mediated by a BCR-mediated intrinsic pathway of apoptotic death (10). However, the direct impact on the composition of the polyclonal B cell repertoire has not been previously investigated.

High-throughput sequencing shows a shift in the expressed Vκ repertoire after PpL exposure

We characterized the expressed Vκ L chain repertoire in control (i.e., saline)-treated animals compared with those exposed to PpL.
In mice (*Mus musculus*), the Vκ repertoire includes 177 Vκ germline genes, assigned by sequence similarity to 20 subgroups (18, 24) (http://www.imgt.org). Although 170,821 reads passed initial quality filtering (see Materials and Methods), a total of 120,885 reads were found to be homologous to one of the Vκ germline genes, of which 84,771 spanned a productive junction and contained sufficient phylogenetic signal to be reliably assigned to a unique rearrangement/clonotype.

From each of the libraries, 3,979 to 23,425 of the sequences derived from the control mice were successfully assigned as Vκ–Jκ rearrangements, and 649 to 15,146 sequences from libraries derived from the PpL-treated mice (Supplemental Table II). Although there were intragroup variations, the libraries from the mice in the control group shared similar expression patterns at a Vκ subgroup level, which was distinctly different from those generated from the PpL-treated mice. Specifically, in the control mice, the majority (59.5–68.4%, mean of 62.5%) of all transcripts were encoded by only five dominant Vκ subgroups: Vκ1, Vκ4, Vκ8, Vκ6, and Vκ12.

From comparisons of the expression levels of specific families within the libraries, we found that after PpL treatment, there were significant reductions of the families Vκ3, Vκ8, Vκ9, Vκ14, and Vκ15 (Supplemental Table II). In fact, we found PpL treatment was associated with significant increases only for the Vκ13 family, which was otherwise uncommonly expressed, representing 0.2 ± 0.2% in control-treated libraries (Supplemental Table II). Taken together, these findings documented nonrandom PpL-induced changes in the expressed Vκ repertoire.

The conserved PpL binding motif identifies Vκ gene usage susceptible to negative selection

The structural basis for PpL binding has been best explored in the human system, where PpL is bound by Ig of some, but not other, Vκ subgroups (9). The crystallographic solution of the structure of a PpL cocomplex with a Fab Ab demonstrated two binding interfaces, in which the primary interface had a binding interaction that was estimated to be the stronger by more than an order of magnitude (7). This primary interface is largely determined by 10 conserved residues located in framework 1 (FR1) of the Vκ region, with residues at positions 8 to 12 appearing to be the most important (Table I). The evolutionary conservation of this surface likely explains reports of PpL-binding interactions with Ig from other mammalian species (25), and in many cases, Vκ genes from mice may have common evolutionary ancestors with human analogs. We therefore assessed the homology of the deduced amino acid sequences of the murine Vκ subgroups with the amino acid sequence residues implicated in the human consensus PpL binding motif (7, 9) (Supplemental Table II). Our analysis predicted conservation of the PpL primary binding motif in products of the murine Vκ subgroups 3, 5, 8, 9, 12, 14, and 19, with impaired or absent binding predicted for the other Vκ subgroups or with λ VL (Vλ).

In light of the predicted PpL interactions, we next reconsidered Vκ expression within libraries and found that the representation of the sum of all seven Vκ subgroups containing the conserved PpL-binding motif in the control libraries was significantly reduced by PpL exposure (mean 44.5 versus 18.5%; p = 0.02; Fig. 2A, Sup-
Protein L Superantigen Modulates the B Cell Repertoire

Table I. Variations in amino-acid residue expression in murine VL at positions implicated in PpL binding

<table>
<thead>
<tr>
<th>Residue&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PpL Motif</th>
<th>Predicted PpL Binding Motif from Human κ L Chains</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL Subgroup&lt;sup&gt;b&lt;/sup&gt;</td>
<td>T</td>
<td>Q</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Vx1 (10)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx2 (4)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx3 (9)</td>
<td>+</td>
<td>T</td>
</tr>
<tr>
<td>Vx5 (4)</td>
<td>+</td>
<td>T</td>
</tr>
<tr>
<td>Vx7 (1)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx8 (11)</td>
<td>+</td>
<td>T, S</td>
</tr>
<tr>
<td>Vx9 (4)</td>
<td>+</td>
<td>T, I</td>
</tr>
<tr>
<td>Vx10 (3)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx11 (1)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx12 (7)</td>
<td>+</td>
<td>T</td>
</tr>
<tr>
<td>Vx13 (2)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx14 (3)</td>
<td>+</td>
<td>T</td>
</tr>
<tr>
<td>Vx15 (1)</td>
<td>−</td>
<td>N</td>
</tr>
<tr>
<td>Vx16 (1)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx17 (2)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx18 (1)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx19 (1)</td>
<td>+</td>
<td>T</td>
</tr>
<tr>
<td>Vx20 (1)</td>
<td>−</td>
<td>I</td>
</tr>
<tr>
<td>Vx1 (1)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx2 (1)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx3 (1)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx4 (5,7) (3)</td>
<td>−</td>
<td>T</td>
</tr>
<tr>
<td>Vx5 (2)</td>
<td>−</td>
<td>T</td>
</tr>
</tbody>
</table>

<sup>a</sup>The PpL binding motif has been previously described (7, 9). Based on deduced amino-acid residues implicated in the PpL–Fab cocryostal, sequences belonging to Vx and Vx groups highlighted in bold possess the conserved PpL primary binding motif as observed in the human Fab Ab PpL complex structure. Positions 6 and 7 in italics are not directly involved in the binding interface but contribute to important structural differences between κ and λ chains. The Fab–PpL binding interaction is dominated by the contribution of residues at positions 8–12. In the above prediction of PpL binders, proline at position 8 was deemed essential, whereas the leucine-to-methionine substitution at position 11 was deemed a conservative change.

<sup>b</sup>Underlining indicates nonconserved residues compared with the consensus human Vκ sequences of known PpL binders.

<sup>c</sup>Dashes indicate missing residues.

FIGURE 2. PpL treatment results in significant reductions in the representation of Vκ subgroups that include the PpL binding motif. Results are shown from 454 sequencing of Vκ transcripts in mice that received saline or the superantigen, PpL. The representation of Vκ subgroups is shown and is organized based on whether the Vκ subgroup is predicted to have the conserved PpL binding motif, with comparisons of representation between the two treatment groups (n = 4/group), using a one-sided Wilcoxon test. (A) Results from analysis of all transcripts. (B) Results for data sets after simplification that enables comparisons of unique Vκ clonotypes, which are here each counted only once.

The number of germ line sequences in each subgroup, as reported in the IMGT database, is indicated in parentheses. Nonfunctional open reading frames, pseudogenes, and vestigial genes are not included.

In the earlier described surveys, each transcript was individually counted, without regard to whether there were multiple reads of the same or a clonally-related transcript in the same library. In the generation of the libraries, we did not use cell sorting, which could have restricted our samplings to include only B cells of distinct subsets, maturation level, or activation state, but which could also have introduced other biases. Our surveys of relative Vκ expression could still be influenced by differences in transcript abundance among lymphocytes, and especially by the inclusion of end-differentiated plasma cells that may have 1000-fold more Ig transcripts per cell than a resting B cell. To attempt to correct for these influences, using rules outlined in Materials and Methods (Supplemental Fig. 1), we repeated the analysis after assigning reads to unique Vκ clonotypes (Fig. 2B, Table II, Supplemental Figs. 1, 2). In the libraries from the control mice, we found that the number of unique clonotypes ranged from 1158–4101, whereas in the PpL-treated mice, they ranged from 258–2684. Importantly, an analysis of the distribution of Vκ subgroup assignments in control versus PpL-exposed mice at the clonotype level identified similar
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Table II. Vk subgroup assignments for unique Vk clonotypes

<table>
<thead>
<tr>
<th>Vk Subgroup with conserved PpL motif</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mean ± SD</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Mean ± SD</th>
<th>p Value (Two-Tailed t Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vκ5</td>
<td>9.7</td>
<td>8.9</td>
<td>11.4</td>
<td>11.7</td>
<td>10.4 ± 1.3</td>
<td>0.8</td>
<td>3.8</td>
<td>5.5</td>
<td>1.3</td>
<td>2.9 ± 2.2</td>
<td>0.004</td>
</tr>
<tr>
<td>Vκ6</td>
<td>4.6</td>
<td>3.2</td>
<td>5.1</td>
<td>2.8</td>
<td>3.9 ± 1.1</td>
<td>0.8</td>
<td>4.2</td>
<td>1.2</td>
<td>1.9</td>
<td>2.0 ± 1.5</td>
<td>0.10</td>
</tr>
<tr>
<td>Vκ8</td>
<td>10.3</td>
<td>14.3</td>
<td>12.7</td>
<td>12.7</td>
<td>12.5 ± 1.6</td>
<td>4.3</td>
<td>4.7</td>
<td>7.5</td>
<td>5.9</td>
<td>5.6 ± 1.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Vκ9</td>
<td>3.8</td>
<td>4.4</td>
<td>3.2</td>
<td>3.6</td>
<td>3.8 ± 0.5</td>
<td>0.4</td>
<td>1.5</td>
<td>2.0</td>
<td>1.0</td>
<td>1.2 ± 0.7</td>
<td>0.002</td>
</tr>
<tr>
<td>Vκ12</td>
<td>9.3</td>
<td>8.0</td>
<td>4.3</td>
<td>7.3</td>
<td>7.2 ± 2.1</td>
<td>3.5</td>
<td>2.3</td>
<td>7.0</td>
<td>1.8</td>
<td>3.7 ± 2.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Vκ14</td>
<td>4.8</td>
<td>3.3</td>
<td>3.7</td>
<td>3.7</td>
<td>3.9 ± 0.6</td>
<td>2.7</td>
<td>1.2</td>
<td>1.4</td>
<td>1.8</td>
<td>1.8 ± 0.7</td>
<td>0.006</td>
</tr>
<tr>
<td>Vκ19</td>
<td>1.8</td>
<td>0.9</td>
<td>1.0</td>
<td>1.9</td>
<td>1.4 ± 0.5</td>
<td>1.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.2</td>
<td>0.6 ± 0.4</td>
<td>0.06</td>
</tr>
<tr>
<td>Total % with a PpL motif</td>
<td>44.3</td>
<td>43</td>
<td>41.4</td>
<td>43.7</td>
<td>43.1 ± 1.3</td>
<td>13.7</td>
<td>18</td>
<td>21.2</td>
<td>23.9</td>
<td>17.7 ± 5.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Vk Subgroup without conserved PpL motif</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vκ1</td>
<td>13.4</td>
<td>15.2</td>
<td>9.9</td>
<td>11.1</td>
<td>12.4 ± 2.4</td>
<td>39.5</td>
<td>18.8</td>
<td>19.6</td>
<td>22.7</td>
<td>25.2 ± 9.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Vκ2</td>
<td>2.8</td>
<td>3.5</td>
<td>5.3</td>
<td>3.4</td>
<td>3.8 ± 1.1</td>
<td>9.7</td>
<td>10.7</td>
<td>4.8</td>
<td>10.9</td>
<td>9.0 ± 2.9</td>
<td>0.04</td>
</tr>
<tr>
<td>Vκ4</td>
<td>19.9</td>
<td>17.6</td>
<td>17.1</td>
<td>15.5</td>
<td>17.5 ± 1.8</td>
<td>13.2</td>
<td>24.5</td>
<td>23.3</td>
<td>23.8</td>
<td>21.2 ± 5.4</td>
<td>0.28</td>
</tr>
<tr>
<td>Vκ6</td>
<td>9.7</td>
<td>12.6</td>
<td>18.8</td>
<td>17.1</td>
<td>14.6 ± 4.2</td>
<td>14.3</td>
<td>20.9</td>
<td>20.8</td>
<td>20.0</td>
<td>19.0 ± 3.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Vκ7</td>
<td>0.3</td>
<td>0.2</td>
<td>0.9</td>
<td>0.4</td>
<td>0.5 ± 0.3</td>
<td>0.4</td>
<td>0.1</td>
<td>0.0</td>
<td>0.2</td>
<td>0.2 ± 0.2</td>
<td>0.20</td>
</tr>
<tr>
<td>Vκ10</td>
<td>2.5</td>
<td>2.5</td>
<td>2.3</td>
<td>2.8</td>
<td>2.5 ± 0.2</td>
<td>6.2</td>
<td>3.0</td>
<td>2.9</td>
<td>4.5</td>
<td>4.2 ± 1.6</td>
<td>0.13</td>
</tr>
<tr>
<td>Vκ11</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.1 ± 0.1</td>
<td>0.8</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3 ± 0.3</td>
<td>0.33</td>
</tr>
<tr>
<td>Vκ17</td>
<td>0.7</td>
<td>0.4</td>
<td>0.6</td>
<td>0.3</td>
<td>0.5 ± 0.2</td>
<td>0.8</td>
<td>1.0</td>
<td>0.8</td>
<td>1.3</td>
<td>1.0 ± 0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Vκ18</td>
<td>1.3</td>
<td>2.0</td>
<td>1.4</td>
<td>2.4</td>
<td>1.8 ± 0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>1.2</td>
<td>0.2</td>
<td>0.5 ± 0.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Vκ19</td>
<td>0.4</td>
<td>0.6</td>
<td>0.5</td>
<td>1.5</td>
<td>0.8 ± 0.5</td>
<td>0.0</td>
<td>0.4</td>
<td>0.3</td>
<td>0.6</td>
<td>0.3 ± 0.3</td>
<td>0.21</td>
</tr>
<tr>
<td>Vκ20</td>
<td>3.8</td>
<td>1.9</td>
<td>1.0</td>
<td>0.8</td>
<td>1.9 ± 1.4</td>
<td>1.2</td>
<td>0.8</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8 ± 0.3</td>
<td>0.22</td>
</tr>
<tr>
<td>Vκ21</td>
<td>0.2</td>
<td>0.1</td>
<td>0.0</td>
<td>0.3</td>
<td>0.2 ± 0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0 ± 0.0</td>
<td>—</td>
</tr>
<tr>
<td>Vκ24</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1 ± 0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Total % without a PpL motif</td>
<td>55.3</td>
<td>56.7</td>
<td>57.9</td>
<td>55.6</td>
<td>56.4 ± 1.2</td>
<td>86.5</td>
<td>80.7</td>
<td>74.5</td>
<td>85.1</td>
<td>81.7 ± 5.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Total Vk unique clonotypes</td>
<td>2152</td>
<td>2809</td>
<td>4101</td>
<td>1158</td>
<td>258</td>
<td>2684</td>
<td>1449</td>
<td>1147</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bold text represents statistically significant values.

*Results from analysis of 454 sequencing of 4 libraries from 4 control-treated mice (libraries 1–4) and 4 libraries from 4 PpL-treated mice (libraries 5–8). Results are presented as percentage unique clonotypes into which all transcripts were grouped. Two transcripts were binned as the same clonotype if three conditions were met: both were identical except for a single nucleotide change or were identical amino acids at the same position.

*The two groups were compared by two-tailed Welch’s t test assuming unequal variances.

**Note:** Differences in Vk subgroup assignments for the conserved PpL-binding motif among clonotypes was still significantly reduced by PpL exposure compared with the control group (mean 17.7 compared with 43.1%, p = 0.003; Fig. 2B, Table II).

**Table III.** We therefore performed analyses of each of the individual Vk genes in subgroups with PpL-associated binding motifs (Table III). We found that compared with control-treated libraries, there were consistent and significant reductions of rearrangements of 14 of these 39 individual genes in the PpL-exposed mice, although many, but not all, of the other related genes also showed decreases that did not reach significance in our studies. However, sequence analyses did not find evidence of increased representation of FR1 replacement mutations after PpL exposure that might contribute to inefficient negative selection of otherwise susceptible Vk genes (data not shown).

Despite overall homology to human Vk families associated with PpL-binding activity, because of germline sequence variations, not all murine genes within the analyzed subgroups were predicted to bind PpL, and sequence analyses found no reductions in the representation of these genes in PpL-exposed animals. Our studies therefore identified several germline-associated amino acid variations that correlate with altered properties of the motif. For example, in the presence of threonine or serine at position 18, and arginine at position 22, there was no significant PpL-associated depletion, which suggested that these variations greatly reduced or abolished the capacity for PpL binding. In contrast, other common murine variations appeared to be permissive of PpL-associated depletion, such as methionine at residue 11, and lysine or valine at position 18, which did not appear to adversely affect the PpL binding interface (Table III). Based on evidence of the murine Vk genes that were significantly reduced by the PpL exposure, we formulated a primary sequence consensus motif for nonimmune binding of PpL for the murine immune system (Fig. 3).

We also characterized the diversity within our libraries with regard to specific Jκ usage for different Vk subgroups (Fig. 4). These analyses demonstrated a remarkable conservation of preferential Vk-Jκ association between the individual mice within each of the treatment groups. As previously well-documented, immunologically intact mice display a general bias in Jκ usage, with preferential usage that favors in favor of Vk-proximal Jκ elements, leading to a frequency hierarchy of Jκ1 > Jκ2 > Jκ4 > Jκ5, as the murine Jκ3 is a pseudogene (27). In our studies, clonotypes from the Vκ1, Vκ3, and Vκ4 subgroups showed a consistently higher level of Jκ1 rearrangements. Notably, for some of the other Vk subgroups, we found very different patterns, and the Vκ4 subgroup displayed the highest frequency of Jκ5 pairing in all eight libraries, which is consistent with previous reports (28–31). These patterns may reflect differences in Jκ preferences in primary rearrangements or result from secondary rearrangements caused by receptor editing (reviewed in Ref. 32).

We also looked for variations in the distribution of CDR3 length in the libraries. Overall, in comparisons of clonotypic sets in libraries from saline-treated and PpL-treated mice, we did not find significant differences in the distribution of CDR3 length.
| V_{L} Subgroup | V_{L} Gene | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 20 | 22 | 24 |
|---------------|------------|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|
| V_{c}3        | V_{c}3-1^{d} | T | Q | S | P | A | S | L | A | V | R | T | S | R | 0.97 | 0.25 | 0.44 | 0.52 | 0.55 |
|               | V_{c}3-2^{d} | T | Q | S | P | A | S | L | A | V | R | T | S | S | K | 3.06 | 1.88 | 3.00 | 2.85 | 2.70 |
|               | V_{c}3-3^{d} | T | Q | S | P | A | S | L | A | V | R | T | S | K | 0.60 | 0.75 | 1.46 | 1.47 | 1.07 |
|               | V_{c}3-5^{d} | T | Q | S | P | A | S | L | A | V | R | T | S | R | 0.60 | 0.53 | 0.73 | 1.90 | 0.94 |
|               | V_{c}3-7    | T | Q | S | P | A | S | L | A | V | R | T | S | R | 0.14 | 0.14 | 0.20 | 0.00 | 0.12 |
|               | V_{c}3-10   | T | Q | S | P | A | S | L | A | V | R | T | S | K | 0.46 | 0.60 | 0.37 | 0.78 | 0.55 |
| V_{c}3-12    | T | Q | S | P | A | S | L | A | V | R | T | S | S | K | 0.00 | 0.00 | 0.07 | 0.09 | 0.04 |
| V_{c}5        | V_{c}5-37   | T | Q | S | P | A | T | L | S | V | T | S | S | S | S | S | S | S | S |
|               | V_{c}5-39   | T | Q | S | P | A | T | L | S | V | S | T | S | S | S | S | S | S | S |
|               | V_{c}5-43   | T | Q | S | P | A | T | L | S | V | S | S | S | S | S | S | S | S | S |
|               | V_{c}5-45   | T | Q | S | P | A | T | L | S | V | S | S | S | S | S | S | S | S | S |
| V_{c}5-48^{d} | T | Q | S | P | A | T | L | S | V | S | S | S | S | S | S | S | S | S | S |
| V_{c}8        | V_{c}8-16   | T | Q | T | S | P | S | L | T | V | R | T | S | K | 0.28 | 0.07 | 0.12 | 0.00 | 0.12 |
|               | V_{c}8-19   | T | Q | S | P | S | L | A | V | T | S | S | R | R | R | R | R | R | R |
|               | V_{c}8-21^{d} | T | Q | S | P | S | L | A | V | T | S | S | R | R | R | R | R | R | R |
|               | V_{c}8-26^{d} | T | Q | S | P | S | L | A | V | T | S | S | R | R | R | R | R | R | R |
|               | V_{c}8-27^{d} | T | Q | S | P | S | L | A | V | T | S | S | R | R | R | R | R | R | R |
|               | V_{c}8-28^{d} | T | Q | S | P | S | L | A | V | T | S | S | R | R | R | R | R | R | R |
| V_{c}8-30^{d} | T | Q | S | P | S | L | A | V | T | S | S | R | R | R | R | R | R | R | R |
| V_{c}8-34^{d} | T | Q | S | P | S | L | A | V | T | S | S | R | R | R | R | R | R | R | R |
| V_{c}9        | V_{c}9-120^{d} | T | Q | S | P | S | L | A | V | R | T | S | R | 1.62 | 2.59 | 2.88 | 3.37 | 2.62 |
|               | V_{c}9-129  | T | Q | S | P | S | L | A | V | R | T | S | R | 0.05 | 0.32 | 0.24 | 0.17 | 0.09 |
|               | V_{c}9-124  | T | Q | S | P | S | L | A | V | R | T | S | R | 0.02 | 1.10 | 0.68 | 0.14 | 0.96 |
|               | V_{c}9-129  | T | Q | S | P | S | L | A | V | R | T | S | R | 0.32 | 0.43 | 0.07 | 0.00 | 0.21 |
| V_{c}12       | V_{c}12-38 | T | Q | S | P | S | L | A | V | R | T | S | R | 0.37 | 0.21 | 0.22 | 0.09 | 0.22 |
|               | V_{c}12-44 | T | Q | S | P | S | L | A | V | T | S | S | R | S | S | S | S | S | S |
|               | V_{c}12-44 | T | Q | S | P | S | L | A | V | T | S | S | R | S | S | S | S | S | S |
|               | V_{c}12-44 | T | Q | S | P | S | L | A | V | T | S | S | R | S | S | S | S | S | S |
|               | V_{c}12-89^{d} | T | Q | S | P | S | L | A | V | S | S | S | S | S | S | S | S | S | S |
|               | V_{c}12-98 | T | Q | S | P | S | L | A | V | T | S | S | S | S | S | S | S | S | S |
| V_{c}14       | V_{c}14-100 | T | Q | S | P | S | M | S | L | V | R | T | S | R | 1.16 | 0.37 | 0.54 | 1.22 | 1.10 |
|               | V_{c}14-111^{d} | T | Q | S | P | S | M | S | L | V | R | T | S | R | 0.71 | 0.73 | 0.78 | 0.85 | 0.73 |
|               | V_{c}14-130 | T | Q | S | P | S | M | S | L | V | R | T | S | Q | 0.05 | 0.07 | 0.05 | 0.09 | 0.06 |
| V_{c}19       | V_{c}19-93 | T | Q | S | P | S | M | S | L | V | R | T | S | S | N | N | N | N | N |

**Table III. Analysis of changes after PpL exposure in individual murine genes in subgroups predicted to bind PpL**

<table>
<thead>
<tr>
<th>% V_{L} Clonotypes Assigned to V_{L} Gene</th>
<th>Control-Treated Mice</th>
<th>PpL-Treated Mice</th>
<th>CDR3 Length, aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-Treated Mice</td>
<td>PpL-Treated Mice</td>
<td>CDR3 Length, aa</td>
<td></td>
</tr>
</tbody>
</table>

**Bolg text represents statistically significant values.**

1. Amino-acid residues implicated in the primary FR1 binding interface in the PpL-Fab co-crystal as described previously (7).
2. Individual functional V_{L} genes in subgroups with conserved PpL binding motif. In all cases, *01 alleles of indicated genes were used.
3. Down arrow (↓) indicates that the mean was significantly lower by two-tailed t test with Welch correction assuming unequal variances.
4. *Gene was found to be significantly reduced in the PpL-treated mice compared with controls.
5. Up arrow (↑) indicates that the mean was significantly higher by two-tailed t test with Welch correction in the PpL-treated mice compared with control mice.
6. Underlined residues were not consistent with the predicted human PpL-binding motif.
7. Residues are likely to considerably change the motif properties and the data suggest these are intolerant to PpL binding.
8. Reference sequence from IMGT was originally reported in C57BL/6 (18). The IMGT sequences for V_{c}5-48, V_{c}8-30, and V_{c}14-111 have also been reported in the literature in C57BL/6 as annotated in IMGT (18).
significant reduction in the V\textsubscript{\textalpha} superantigen exposure. Individual mice within the different groups, and the outcome of the remaining three (V\textsubscript{\textkappa} families (V\textsubscript{\textgamma} reductions, and it was statistically significant in four of these seven families predicted to encode for PpL binding showed consistent between members of the same group. Moreover, each of the a Fab–PpL cocomplex (7). These findings were remarkably consistent with the superantigen-binding motif in the V\textsubscript{\textalpha} framework (7). The consensus sequence is based on the relative representation of amino-acid variations at specific positions in the PpL binding motif (7) in the 14 germline V\textsubscript{\textalpha} genes that were significantly reduced in libraries from PpL-treated mice compared with control mice (see Table III). These data are presented as a WebLogo image (54). The image represents the alignment of each residue by a stack of letters, where the height of each letter is proportional to the observed frequency of the corresponding amino acid, and the overall height of each stack is proportional to the sequence conservation, at that position.

(data not shown). In addition, we examined the V\textsubscript{\textkappa} genes in subgroups associated with the PpL binding motif, but did not find consistent differences in CDR3 length in rearrangements identified by specific V\textsubscript{\textkappa} gene usage (Table III). Taken together these findings suggest that there was no relationship between the overall size of the CDR3, the somatically generated subdomain with the greatest structural diversity, and the nonimmune binding interactions with PpL.

Discussion
In these studies, we examined the in vivo outcome of exposure to a bacterial virulence factor, PpL, which has the functional properties of an Ig L chain-targeted superantigen. In control mice, among Ig\textsubscript{\textkappa}-bearing B cells, there was a continuous distribution of binding capacities to PpL, with some B cells displaying strong binding and others with none detectable. After PpL exposure, cellular analyses documented a decrease in the B cell/T cell ratio and in overall \kappa-bearing B cells, which was consistent with a specific targeted depletion of B cells with the strongest PpL-binding capacity. Because the effect of such a microbial toxim on the expressed BCR repertoire at a molecular level was unknown, we compared the representation of Ig\textsubscript{\textkappa} transcripts in libraries made from the spleens of control mice and PpL-exposed mice. In control mice raised under specific pathogen-free conditions, we made from the spleens of control mice and PpL-exposed mice. In addition, fine analysis of the Ig\textsubscript{\textkappa} rearrangements also demonstrated conserved patterns of preferential pairing of V\textsubscript{\textkappa}-J\textsubscript{\textkappa}. Therefore, using an approach technically similar to a recently reported characterization of libraries made from cells from pooled donors (33), our investigations provide novel insights into the conservation of overall expression patterns within the overall \kappa L chain repertoire, for V–J joining patterns in individual mice within the different groups, and the outcome of superantigen exposure.

The principal finding in our studies was striking evidence of induced global shifts in the repertoire of each of the four PpL-exposed mice, with significant reductions of the V\textsubscript{\textkappa} subgroups that include the superantigen-binding motif in the V\textsubscript{\textkappa} framework region that has been characterized in crystallographic studies of a Fab–PpL cocomplex (7). These findings were remarkably consistent between members of the same group. Moreover, each of the seven families predicted to encode for PpL binding showed reductions, and it was statistically significant in four of these families (V\textsubscript{k}3, V\textsubscript{k}8, V\textsubscript{k}9, and V\textsubscript{k}14), and with similar trends in the remaining three (V\textsubscript{k}5, V\textsubscript{k}12, and V\textsubscript{k}19). There was also a significant reduction in the V\textsubscript{k}15 family, which remains unexplained. Interestingly, the cellular analysis confirmed evidence from V\textsubscript{\textkappa} gene analyses that some PpL binding to a subset of B cells can still be detected in the PpL-exposed mice. This may suggest that some B cells, with weaker binding interactions with PpL, were not completely depleted. However, it is possible that this could be related to differences in in vivo PpL availability, the B cell surface density of BCR and other H chain isotypes, or even receptor dilution that may be prevalent in marginal zone B cells. In the future, more detailed studies using single cell sorting and BCR cloning of these remaining cells with lower PpL binding activity will be needed to further investigate this topic.

We interpret our findings as consistent with negative supraclonal selection of PpL-binding B cells, which results from strong B cell superantigen-mediated, BCR-mediated first signal in the absence of a balanced and sufficient second signal (12, 13). In other settings, if there is impaired BCR-mediated death signaling or in the presence of second signals (12, 13), which could occur during active infection, we predict that PpL-binding B cell clones could be greatly expanded to further dominate the immune repertoire. Notably, these superantigen-induced effects were completely different from the outcome commonly associated with immunization with a conventional Ag as there was no evidence of expansions of specific clonotypes in the PpL-exposed animals (see Supplemental Fig. 2). Our surveys therefore also demonstrate that even limited exposure to this bacterial virulence factor can globally shift the immune repertoire in a manner that is highly diagnostic of the influence of a superantigen–BCR interaction. We have earlier reported evidence that similar superantigen-induced changes can alter immune responsiveness to new microbial threats, as well as for recall immune responses (15).

In the murine and human immune systems, immune competence is, in part, dependent on the formation of a clonally diverse pre-immune repertoire. Murine BCRs are formed and molded by stochastic mechanisms that can access a large number of germline V\textsubscript{\textkappa} genes, which vary considerably in the canonical structures of their CDR (34). Presumably, there has been selective pressure for the retention of these diverse inherent basic building blocks for the formation of Ag receptors that can bind very different types of Ags. In fact, a deficiency in the inheritance of even a single specific V\textsubscript{\textkappa} germline gene can result in impaired immune defenses and serious and recurrent infections from a common bacterial pathogen (35).

Our findings support emerging evidence that a B cell superantigen can effectively target a large genetically and structurally defined set of V\textsubscript{\textkappa}-expressing adaptive immune cells for depletion. We have previously reported that in vivo exposure to a superantigen can induce preferential targeted depletion of innate-like marginal zone B cells and B-1 cells, which can significantly impair immune responses to certain microbial Ags (14, 15). A B cell superantigen can cause activation-induced apoptosis in vivo (10, 13–15) by a process that requires the induction of the proapoptotic Bim member of the Bcl-2 family (12). Hence these microbial products appear to have coevolved with the host’s immune system to enable the hijacking of BCR-associated signaling pathways, in a manner similar to that shown in transgenic mouse models of regulated negative selection of autoreactive B cells by introduction of neoantigens (28, 36) or a synthetic Ig\textsubscript{\textkappa}-reactive macro-self-Ag (37). Notably, in the current studies, adapting a regimen widely used to evaluate responses to putative B cell superantigens (10–15), mice received two 500-\mu g doses of PpL. However, with another superantigen, SpA, systemic doses as low as 2 \mu g were shown to significantly decrease the in vivo frequency of superantigen-binding B cells (38). Although the level of in vivo production of this bacterial product during colonization or active
infection is currently unknown, based on the dramatic effects in this study demonstrated for systemic doses of PpL, we postulate that even proportionately smaller doses locally produced during invasive infection could have substantial local effects in draining lymph nodes wherein defensive immune responses are initiated. This investigation therefore contributes to emerging data that a B cell superantigen bacterial product can induce a targeted supraclonal hole in the repertoire (15). Such superantigens can represent key virulence factors necessary for the persistence of the pathogen in host tissue (39), and this effect may facilitate the microorganism’s escape from host immune recognition. In accordance, in experimental infection models with *S. aureus*, one of the most common causes of invasive and life-threatening infections, immunization of mice with a modified nontoxogenic form of the SpA superantigen was shown to result in induction of neutralizing Abs and protection against highly virulent staphylococcal strains (40). Therefore, microbial expression of a B cell superantigen may provide advantages to the pathogen by blocking the capacity of the immune system to limit a bacterial infection and to form recall responses that protect from reinfection, whereas neutralization of this superantigen influence can restore immune defenses (40, 41). Yet, although both *S. aureus* and *F. magna* are usually commensal species and part of the microbiome in healthy individuals, nonetheless each can become invasive pathogens, presumably after local breaches in defenses.

The microbiome refers to the vast collection of symbiotic microorganisms in the human body and their collective interacting genomes. It performs numerous key biochemical functions for the host, and disorders of the microbiome are associated with many human disease processes (42). In a recent report, Matzinger and

**FIGURE 4.** Igκ V–J repertoire in C57BL/6 mice with and without exposure to the superantigen PpL. Results are depicted for the frequency of clonotypes with a specific V–J pairing in four control mice (libraries 1–4) and four PpL exposed mice (libraries 5–8) either when evaluated for treatment groups or individually. Underlined Vk subgroups were predicted to have PpL interactions with products of one or more associated members. ND represents the small number of clonotypes that could not be assigned to a specific Vk subgroup or Jκ gene. The results are based on analyses of a total of 84,771 unique clonotypes from the eight libraries.
coworkers showed that immune homeostasis depends on a three-way interaction between the B cell compartment of the immune system, gut-associated microbiota, and innate responses from the intestinal epithelium (43). In the absence of B cells, or of their IgA products, which is the predominant Ab isotype of secreted Abs in the gut, the intestinal epithelium launches its own protective mechanisms after exposure to the microbiota (43). This results in upregulation of IFN-inducible immune response pathways and altered metabolic functions (43). Moreover, changes in the Ig repertoire expressed in GALT can alter the balance among species within the microbiome, and thereby may affect global immune homeostasis. The intestinal microbiome is dominated by anaerobic microbes, and the true prevalence of different species in health and during infections is now being reconsidered in light of recently developed culture-independent methods of identification (44). Knight and coworkers (45) have shown that intestinal bacterial species may commonly express proteins with superantigen properties, and when introduced into an otherwise sterile gut, such microbial isolates can induce B cell proliferation in GALT and contribute to the development and expansion of a new “pre-immune” repertoire. Therefore, although in some settings the B cell targeted effects of a superantigen can represent a pathogenic factor that interferes with host defenses during infection, these or related factors may also provide a pathway by which intestinal flora may selectively mold the physiologic immune repertoire (46). The outcome of immune exposure to a superantigen is likely to be highly dependent on the context of intestinal colonization and the balance among commensals within the overall microbiome. However, as part of an invasive infection, secretion of a superantigen, together with potentially stimulatory coexpressed factors, may instead impair immune defenses.

In our investigations, we have applied recently developed technologic approaches that incorporate high-throughput sequencing and in-house developed bioinformatics tools to investigate the representation of expressed L chain rearrangements. Because the immune system contains >10^{11} B and T lymphocytes that derive from a large number of parental clones, with an estimated 3 to 9 million B cell clones with different Ig H chains in the circulation at any given moment (47), traditional Sanger DNA sequencing technology can provide only limited insights into the true clonal diversity within the repertoire. By contrast, next-generation sequencing platforms have greatly expanded our capacity to more accurately assess the complex diversity of gene sequences that encode for lymphocyte Ag receptors (47–53). Our studies have provided a more complete insight into the effects on B cell clonal diversity of exposure to a single microbial factor.

These findings therefore shed new light on the ability of pathogens to globally disrupt or bias the clonal distribution within the B cell compartment that affects host defenses. Furthermore, our methodologic approach, which applied one of the most rigorous methods currently available, provided a practical means to survey for potential effects on lymphocyte clonal diversity within host defenses. In the future, high-throughput sequencing of immune repertoires should be extensively used to advance our understanding of the microbiome–host relationship and to guide the development of new and more effective vaccines, including those against pathogens that produce superantigens.

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Disclosures

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