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Staphylococcus aureus peptidoglycan (PGN) induces pathogenic autoantibody production via autoreactive B cell receptor clonal selection, implications in systemic lupus erythematosus

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Patient and Public Involvement. It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

INTRODUCTION

Approximately 18% of healthy women tested have positive IgG anti-nuclear antibodies (ANA), and most never develop a clinical autoimmune disease [1]. In healthy individuals, most autoantibodies are naturally occurring and non-pathogenic; however, in patients with systemic lupus erythematosus (SLE or lupus), pathogenic autoantibodies are produced [e.g., lupus nephritis is partially mediated by anti-double-stranded DNA (dsDNA) antibodies] many years before clinical diagnosis [2]. However, the mechanisms underlying the stimulus for natural versus pathogenic autoantibody production remains undefined.

Translocation of *Lactobacillus reuteri* exacerbates disease progression in lupus-prone mice via toll-like receptor (TLR) 7 [3]. Translocation of commensal *Enterococcus gallinarum* from gut to the liver induced type I interferon (IFN) and anti-dsDNA antibody [4]. Previous studies from others and us indicate a possible role of plasma microbial translocation likely through a compromised mucosal barrier, in autoantibody production in SLE [4, 5]. *Staphylococcus aureus* is a commensal bacterium that mainly colonizes nasal, skin, and other mucosal membranes [6]. The percentage of *S. aureus* nasal colonization was similar in healthy controls and those with SLE (~20%), but greater in patients with skin lesions (~50%) and associated with renal disease and autoantibody positivity in patients [7, 8]. Chronic exposure to type I IFNs disrupts the skin barrier of lupus patients [8], which may promote systemic microbial translocation (e.g., *S. aureus*). Previously, we intraperitoneally (i.p.) injected non-vital *S. aureus* inducing anti-dsDNA autoantibody production, but not kidney disease in C57BL/6 mice [9]. We thus pursued studies presented in this manuscript to define mechanisms by which S. aureus induces autoantibody production.

Peptidoglycan (PGN) is a part of the bacterial cell wall and highly antigenic due to conserved structural molecular motifs unique to a bacterium [10]. PGNs from different bacteria can uniquely modulate immune activities [11]. Physiologically, without an obvious infection, PGN can be detected in the circulation of healthy individuals [12, 13].

Healthy individuals all have low levels of natural non-pathogenic autoantibodies that differ from pathogenic autoantibodies [14]. The mechanisms underlying natural autoantibodies versus induced pathogenic autoantibodies is incompletely understood. Here, we found that *S. aureus* PGN induces a sustained anti-dsDNA autoantibody response in mice with immune complex glomerulonephritis, whereas *B. subtilis* PGN induces a short-term anti-dsDNA response with no development of renal disease.

MATERIALS AND METHODS

Mice

Female C57BL/6 and MRL/lpr mice (Jackson Laboratories, Bar Harbor, ME, USA) were housed at the Medical University of South Carolina vivarium (MUSC). Mice were i.p. injected with PBS, *S. aureus* PGN, or *B. subtilis* PGN (100 μg/time) (InvivoGen, San Diego, CA). C57BL/6 mice (6-week-old) were treated with PGNs and PBS twice per week for 8 weeks; then the treatment was stopped for another 8 weeks. MRL/lpr lupus-prone mice (6-week-old) were treated with PBS and PGNs twice per week for 12 weeks.

ELISA

The methods were described previously [15]. Calf thymus dsDNA (5 μg/mL, Sigma-Aldrich, St. Louis, MO, USA) and peroxidase-labeled goat anti-mouse IgG (γ) (SeraCare, Milford, MA, USA) or anti-mouse IgG1, IgG2a, IgG2b, or IgG3 (SouthernBiotech, Birmingham, AL, USA) was used. Serum IgG ANA levels were quantified using lysates of Hep-2 laryngeal carcinoma cells (ATCC, Manassas, VA, USA).

Kidney pathology

At the end of the study in MRL/lpr mice (18-week-old), one kidney was frozen, sectioned, and stained with anti-mouse IgG or complement C3 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA). The fluorescent intensities of IgG and C3 were assessed using a microscope (Zeiss Axio Vet. A1) and Image J with 10 random high-power fields (x400) taken from each section. The other kidney was fixed with 10% formalin for H&E staining, examined in a blinded fashion, and graded for glomerular inflammation, proliferation, crescent formation, and necrosis from 0 to 3+ (0: none; 1+: mild; 2+: moderate; and 3+: severe) [16]. Scores from 0 to 3+ were assigned for each of these features in 30 different areas. The median of these areas yielded the final renal pathology score (the maximum score of 18). Crescent formation and necrosis scoring were doubled to reflect the enhanced severity of these lesions.

Flow cytometry analysis of splenic anti-dsDNA IgG+ B cells

The antibodies include anti-CD3-PerCP-Cy5.5, anti-CD4-BV510, anti-CD8a-APC-vio770, anti-F4/80-APC, anti-CD19-BV421, anti-IgG-FITC, biotinylated dsDNA (130 ng/μL), and Streptavidin-PerCP. Cells were analyzed using a BD FACSVerse and FlowJo software. All antibodies are listed in Table S1.

Autoantibody microarray

Human plasma and mouse serum samples (1:50 dilution) were analyzed for 125 autoantibodies using autoantibody microarrays in the Genomics and Microarray Core at the University of Texas Southwestern Medical Center, as described previously [9].

B cell receptor (BCR) sequencing of splenic anti-dsDNA IgG+ B cells

Splenic anti-dsDNA IgG+ B cells were sorted using flow cytometry. Total RNA was extracted [9]. Purified libraries were quantified based on a 1:10,000 (a serial 100-fold dilution) using the Ion Library TaqMan Quantitation kit (ThermoFisher, Waltham, MA). Following the Ion AmpliSeq Mouse BCR IGH-SR (RNA) protocol, equal amounts of sample were loaded on the Ion Chef System for processing, and 530 chip preparations were sequenced on the Ion Gene Studio S5 Prime (Mayo Medical, Henderson, NC, USA).

B cell class switch recombination (CSR)

Unmanipulated splenic B cells were isolated from 8- to 12-week-old female C57BL/6 mice using the Mouse B Cell negative Isolation Kit (STEMCELL Technologies, Cambridge, MA). B cells were labeled with 10 μmol CFSE [17] and stimulated with medium or PGNs (10 μg/mL) in the presence or absence of anti-TLR2 neutralizing antibody or isotype

control antibody (100 ng/mL, InvivoGen), or a positive control [LPS; 1 μg/mL of *E. coli* 055:B5 (Sigma-Aldrich, St. Louis, MO, USA) plus 2 ng/mL of TGF-β1 (R&D Systems, Minneapolis, MN, USA)] for 72 h. Cells were stained with Ghost red 780, B220, and IgG2b, and analyzed for CSR ($CFSE^{low}$IgG2b+Ghost–B220+) using flow cytometry.

PGN-induced TLR2 activity.—Different concentrations of PGNs were added to mTLR2-HEK blue Reporter cell line (50,000 cells/well) (InvivoGen) for 20 hours *in vitro*. SEAP was determined using a spectrophotometer.

Human participants

We recruited unrelated controls (n = 25) or lupus patients (n = 32) as defined by the updated American College of Rheumatology classification criteria [18]. All participants were premenopausal females (age ≥18 years). Pregnant or breastfeeding, recent severe illness, contraindications for blood withdrawals, or received antibiotics within the past 90 days were exclusionary. The clinical information is shown in Table S2. Patients were subdivided based on their serum levels of albumin (normal: 3.5–5.0 g/dL versus low: < 3.5 g/dL), urinary protein (normal: < 11.9 mg/dL versus high: ≥ 11.9 mg/dL), and ratio of urinary protein versus creatinine (normal: < 300 versus high ≥ 300).

Plasma levels of *S. aureus* DNA translocation

The method for determining plasma total bacterial DNA levels by qPCR was described previously [19]. The PCR primers for *S. aureus* were forward: 5′-TTCGCTACTAGTTGCTTA-3′ and reverse: 5′-GCACTATATACTGTTGGATC-3′.

Statistical analysis

We applied non-parametric Mann-Whitney U tests to compare differences between two groups, and Spearman's correlation tests to evaluate associations. All tests were two-sided, and $P \leq 0.05$ was considered significant.

RESULTS

S. aureus PGN induced a prolonged anti-dsDNA autoantibody response, whereas *B. subtilis* PGN induced short term production of anti-dsDNA autoantibodies.

We first confirmed the optimal concentrations of bacteria and PGN (Figure S1) [9]. Next, we found that 8-week treatment of both PGNs and inactivated *S. aureus* produced anti-dsDNA IgG antibodies in C57/B6 mice (Figure 1A). After stopping the treatment for 8 weeks, serum anti-dsDNA IgG levels returned to baseline in the *B. subtilis* PGN and whole *S. aureus* groups; however, the levels remained elevated in the *S. aureus* PGN group (2.87-fold higher versus PBS, Figure 1A). Total serum IgGs did not differ, indicating the anti-dsDNA antibodies were not a manifestation of polyclonal activation in autoreactive and non-autoreactive B cells (Figure 1B). *S. aureus* PGN increased serum IgG ANA levels starting 4 weeks after start of injections and persisting until 8 weeks after stopping the treatment (Figure S2A). Moreover, *S. aureus* PGN, but not *S. aureus* lipoteichoic acid (LTA), induced ANA production in mice, indicating not all bacterial cell wall antigens induce an anti-dsDNA response (Figure S2B). Using autoantigen array to measure more

than 100 autoantibodies in serum of C57/B6 mice, *S. aureus* PGN increased a number of IgG autoantibodies, including IgG anti-dsDNA, whereas *B. subtilis* PGN increased IgM autoantibodies, including IgM anti-dsDNA (Figure S2C). Thus, *B. subtilis* PGN induced primarily IgM or short-lived IgG autoantibody responses, whereas *S. aureus* PGN induced stable long-lasting IgG autoantibody responses. Importantly, *S. aureus* PGN-mediated antibody responses were due to limited B cell clonal responses but not overall polyclonal B cell activation, as total IgG did not differ.

S. aureus PGN increased pathogenic IgG anti-dsDNA and worsened lupus kidney pathology

IgG anti-dsDNA autoantibodies contribute to kidney damage in SLE [20, 21]. After 12-weeks of treatment in MRL/lpr mice, serum IgG anti-dsDNA levels were greater in both PGN groups than PBS (Figure 2A). Although the percentage of anti-dsDNA IgG+ splenic B cells was higher in the two PGN groups than PBS, no difference was observed between the PGNs (Figure 2B). Serum ANA IgG levels were higher in *S. aureus* PGN than PBS (Figure S3A), and total serum IgGs were unchanged (Figure 2C).

The C3 and total IgG deposition and glomerular lesion score for kidney pathology were higher in *S. aureus* PGN than the control (Figure 2D), suggesting autoantibody and complement interaction. At the end of study, the ratio of urinary albumin versus creatinine showed a strong trend towards an increase in *S. aureus* PGN versus PBS, but was not significant (Figure S3B). Notably, the two PGN groups had similar levels of serum anti-dsDNA IgG, different from the response in C57/B6 mice; only *S. aureus* PGN, however led to increased kidney pathology in MRL/lpr mice. This finding suggests a qualitative difference in anti-dsDNA antibodies induced by the two PGNs, which may reflect higher affinity maturation or more of an extrafollicular response rather than a germinal center response. We did not detect significant differences in interstitial inflammation between the PGN groups.

S. aureus PGN drives specific clonal expansion, somatic mutation, and CSR of splenic anti-dsDNA autoreactive B cells

Differences in autoantibody affinity and pathogenicity reflect variation in the sequences of the variable region of immunoglobulin heavy chain, often in the complementarity determining region (CDR) 3, primarily due to antigen driven somatic mutation [22]. To investigate the mechanisms for the differences in antibody responses of the two PGNs, we sorted splenic anti-dsDNA IgG B cells (Figure S4) from C57BL/6 mice after 8 weeks of treatment, and conducted BCR sequencing to assess usage of immunoglobulin heavy-chain variable region genes (IGHV) (Figure 3). IGHV1-45 and IGHV3-74 were the predominant IGHVs in anti-dsDNA IgG-producing splenic B cells after both PGN treatments (Figure 3A). *S. aureus* PGN induced more limited clonal expansion with *B. subtilis* PGN inducing a wider array of clonal expansions (Figure 3B). We did not study MRL mice responses due to their spontaneous autoantibody production that cound confound interpretation of sequence differences.

The BCR diversity was lower in *S. aureus* PGN than *B. subtilis* PGN treated mice (Figure 3B); CDR3 amino acid length was generally similar between PGNs, except the CDR3 length of 10 amino acids was more common in *S. aureus* PGN (Figure 3C). The somatic mutation rate of IGHV3-74, but not IGHV1-45, was higher in *S. aureus* PGN versus *B. subtilis* PGN (Figure 3D). Prior sequencing of anti-dsDNA antibodies from lupus prone mice identified a higher presence of arginine in the CDR3 variable region [23]. Although there was a trend towards increased CDR3 arginines in the *S. aureus* PGN group, it did not reach significance likely due to the small sample size.

To study CSR, we found that *S. aureus* PGN predominantly induced IgG2b anti-dsDNA in C57BL/6 mice *in vivo* (Figure 4A). Further, *S. aureus* PGN, but not *B. subtilis* PGN, promoted B-cell CSR to IgG2b (the percentage of IgG2b+ proliferating B cells [24]) via TLR2 in isolated splenic B cells from untreated C57/B6 mice *in vitro* (Figure 4B). To denature any contaminating proteins, *S. aureus* PGN was heat inactivated at 56°C for 30 min, CSR IgG2b induction was no different compared to untreated PGN (Figure S5). Both bacterial PGNs induced similar TLR2 activation (Figure 4C).

Plasma levels of *S. aureus* DNA translocation increase in patients with SLE and correlate with lupus-specific autoantibody levels

Next, we assessed plasma levels of *S. aureus* DNA (copy number/mL) and the ratio of *S. aureus* DNA versus total bacterial rDNA, and found that both were higher in patients than controls (Figure 5A). Plasma *S. aureus* DNA translocation correlated with plasma levels of various lupus-related autoantibodies, including anti-dsDNA IgG (Figure 5B). Based on clinical assessments of renal disease, *S. aureus* DNA translocation was increased in patients with low serum albumin, high serum creatine, and high urinary protein levels, versus controls (Figure S6A). Translocation of *S. aureus* DNA did not differ based on lupus treatment regimens (Figure S6B). At the time of sample collection, none of the patients had ongoing active lupus skin disease.

DISCUSSION

In this study, administration of *B. subtilis* PGN induced a short-term anti-dsDNA response, whereas *S. aureus* PGN induced a sustained anti-dsDNA response that accelerated renal disease in MRL/lpr mice. *S. aureus* PGN induced anti-dsDNA IgG B cells by specific clonal expansion, incleased somatically mutated, and inducing class switching predominantly to IgG2b. Plasma translocation of *S. aureus* DNA in patients was greater than matched controls, which correlated with plasma anti-dsDNA IgG levels, suggesting similarity between the murine and the human responses. The higher plasma *S. aureus* DNA in patients, we postulate, is one factor that induces autoantibody production and disease development in susceptible individuals.

The PGN structure is comprised of repeating disaccharide backbones of N-acetylglucosamine and β-(1–4)-N-acetylmuramic acid that are cross-linked by peptide stem chains attached to residues of β-(1–4)-N-acetylmuramic acid [25]. Among bacterial species, PGNs have different lengths of sugar polymer. *Bacillus* PGN has ~50 to 250 disaccharides,

whereas *S. aureus* PGN has ~18 disaccharides [26]. This difference in length/structure may contribute to the distinct effects of two PGNs we observed.

Anti-dsDNA antibodies can induce kidney damage in SLE, though they are not the only factor [27, 28]. Levels of IgG anti-dsDNA rise in serum before renal flares in some lupus patients [29]. Importantly, each IgG isotype differs in their biological properties. Human IgG1 and IgG3, but not IgG2 and IgG4, activate complement [30]. In mice, IgG2a, IgG2b, and IgG3 can form immune complexes with complement and contribute to nephritis. These IgG subclasses are often detected in the glomeruli of murine lupus [31–33]. Thus, IgG subclasses of autoantibodies may impact lupus nephritis, though a variety of factors interplay including genetic susceptibility and the kidney response to immune complex deposition. C57/B6 mice are more resistant to induction of lupus nephritis than other strains and the target organ susceptibility to immune insult can differ. This perhaps explains why renal disease was not seen in the B6 mice but was evident in the MRL/lpr mice. The predominant IgG subclasses in lupus patients with renal flares were IgG1 anti-DNA in the serum and IgG2 anti-nucleosome antibodies in kidney biopsies [29, 34]; autoantibodies deposited in the kidney and blood may have different antigenic specificities.

Autoreactive B cells can be activated via polyclonal nonspecific activation or antigen-specific activation. Only a few studies report the IGHV usages of anti-dsDNA antibodies [35, 36]. The IGHV1-3, IGHV3-23, and IGHV3-74 genes were dominant in human anti-dsDNA antibodies [36], and the IGHV J558 and 7183 genes were dominant in MRL/lpr mice anti-dsDNA antibodies [35]. Monoclonal anti-ds DNA autoantibodies derived from lupus mouse spleens show evidence of antigen driven somatic mutation and enrichment in VHCDR3 arginine [35]. Recent studies in both humans and mice indicate that most autoantibodies in lupus may be derived extrafollicularly with help from peripheral helper T cells [37]. Thus, likely genetic and local B cell zone factors (e.g., TLR ligands) influence B cell maturation and responses.

To understand the mechanism of the differential responses to the two PGNs, we analyzed BCR sequences of anti-dsDNA IgG+ splenic B cells, and found that IGHV1-45 and IGHV3-74 were predominant for both PGNs. The total serum IgG did not differ. Thus, neither bacterial PGNs promoted B-cell overall polyclonal activation. *S. aureus* PGN promoted a more limited clonal expansion of anti-dsDNA IgG-producing B cells, and while *B. subtilis* PGN induced a more diverse clonal expansion. The most intriguing and perhaps important difference between the two PGNs was that anti-dsDNA antibody production by *B subtilis* PGN was transient and stopped when PGN injections ended. In contrast, *S aureus* PGN induced anti-dsDNA antibody production was sustained long after PGN stopped, suggesting induction of an antigen driven autoantibody response and a break in tolerance. What makes an antibody pathogenic is not completely understood. The antibody affinity, the CDR3 region and the Fcg region of an antibody all may contribute to the pathogenicity of the antibody. We performed BCR sequencing of isolated anti-dsDNA antibody producing B cells from the spleens between the two PGN groups and there were differences in IgG subtype and in the VH CDR3 region (Figure 3). We did find IGHV3-74 mutation was higher in the *S. aureus* PGN group compared to the *B. subtilis* control group (Figure 3D), but did not find evidence of enhanced VHCDR3 charge. Moreover, we do not know the half-life

of the autoantibodies induced by *S. aureus* PGN. The anti-dsDNA response is present for a longer period of time following injection of *S. aureus* PGN than *B subtilis*. This could relate to either a longer antibody half-life versus prolonged production of anti-dsDNA antibodies by antibody producing cells. It is more likely that the induced response is prolonged rather than the antibodies have a longer half-life.

Our study has several limitations. The main limitation is the potential contamination in the *S. aureus* PGN preparation that could contain other *S. aureus* cell wall components (e.g., surface protein A, *Staphylococcal* protein A [a known B cell superantigen], LTA, lipoproteins/peptides). Thus, the TLR2 stimulating capacity of *S. aureus* PGN may come from non-PGN cell wall contaminants [26, 38, 39]. As bacterial DNA alone does not induce autoantibody production, except when administered in Freund's adjuvant [40, 41], contaminating DNA likely did not contribute to this effect. There is strong evidence supporting a role for *S. aureus* PGN on autoantibody production: 1) a TLR2 inhibitor blocked the PGN effects; 2) CSR induction did not differ in heat inactivation and untreated *S. aureus* PGNs; 3) PGNs can be detected in the circulation in healthy humans [12, 13]; 4) *S. aureus* LTA predominately induces pro-inflammatory cytokines in myeloid cells where PGN has limited effects [42–44]. Further LTA i.p. injection did not induce autoantibodies in C57/B6 mice; 5) structural difference between *S. aureus* and *Bacillus* PGNs impacts immune responses [26]; and 6) Staph Protein A and Staphylococcus Enterotoxin B reduce disease severity in lupus-prone mice [45, 46]. Additionally, only 20% of patients had skin lesions in the current study. However, *S aureus* is not limited to the skin but can be found in the other mucosal areas. Importantly, levels of *S. aureus* DNA translocation were increased in patients with high urinary protein levels compared to controls, suggesting an association between *S. aureus* translocation and renal involvement in SLE.

Finally, the serum IgG anti-DNA levels were higher in the *S aureus* group, but the number of B cells producing anti-dsDNA were the same in the two PGNs; *S. aureus* PGN-mediated CSR was via TLR2, and TLR2 induced activity was similar between the two PGNs. These results suggest that *S. aureus* PGN induces both higher quantity and quality of IgG anti-dsDNA per B cell. *S. aureus* PGN-mediated specific B cell clone selection may be due to different coreceptor usage (e.g., TLR2-TLR1, TLR2-TLR6) or other mechanisms, deserving further investigations. Nonetheless, this study provides novel insight into the potential role of one bacterial cell wall component that is present systemically in humans in inducing pathogenic autoantibodies. Therapeutics aimed at blocking TLR2 or staph colonization may have a role in treating lupus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data sharing statement.

Data are available in Mendeley Data (doi:10.17632/m8sz7s7zgz.1).

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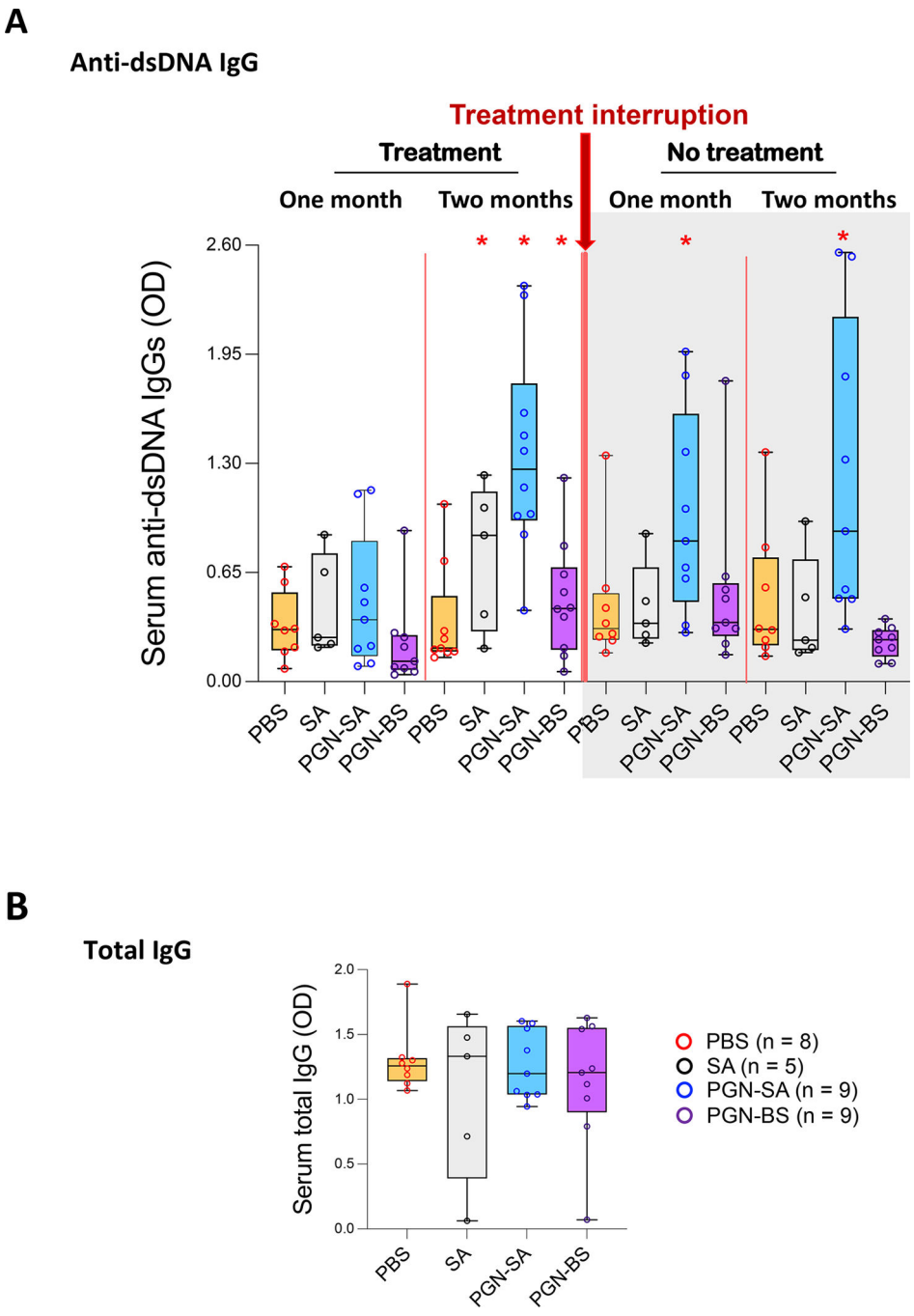


Figure 1.

S. aureus PGN but not *B. subtilis* PGN induces production of stable IgG anti-dsDNA autoantibodies in C57/B6 mice. C57/B6 mice were treated with PBS (0.2 mL), *S. aureus* (PGN-SA) or *B. subtilis* PGN (PGN-BS) (100 μg/mouse/injection), or whole inactivated *S. aureus* (5×10^7 CFU/mouse/injection) via i.p. injection twice per week for 8 weeks. Then treatment was stopped for 8 weeks. (A) Serum levels of IgG anti-dsDNA and (B) endpoint serum levels of total IgG in mice at 22 weeks of age. Median ± Interquartile range. $*P <$ 0.05 compared to the PBS group.

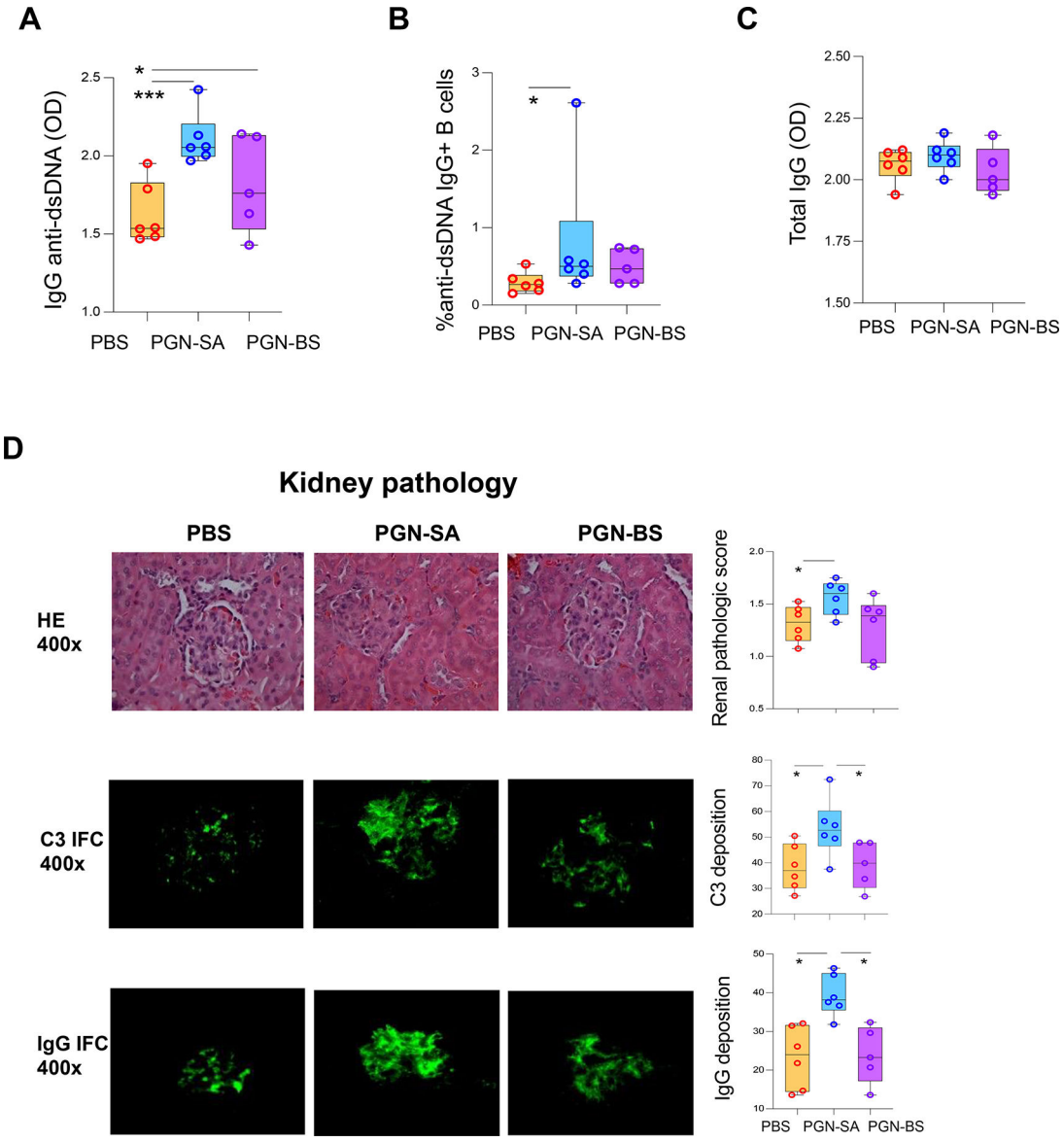
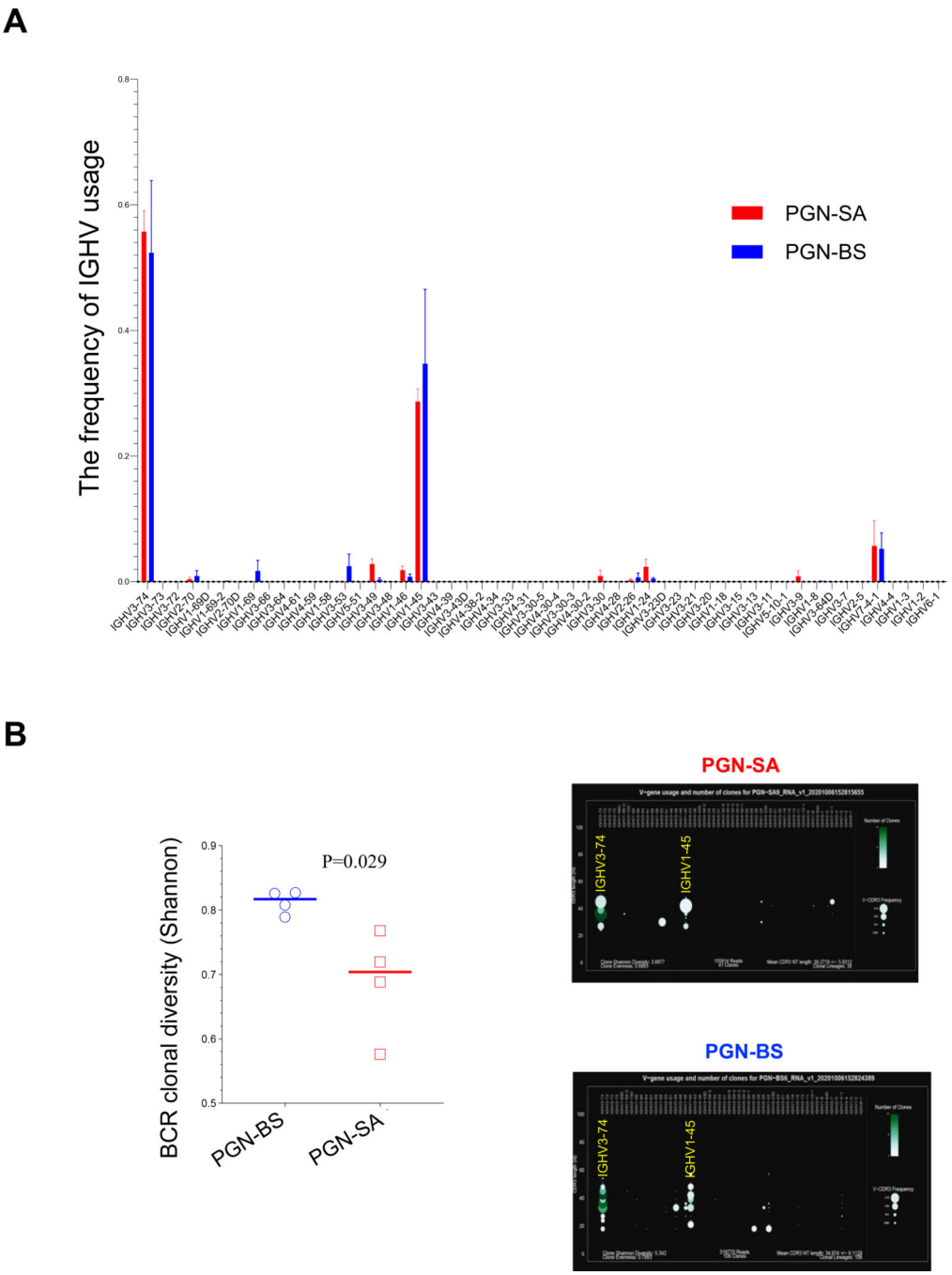


Figure 2.

S. aureus PGN accelerates autoantibody production and kidney pathology in MRL/lpr mice. MRL/Lpr mice were treated with PBS, *S. aureus* PGN (PGN-SA), or *B. subtilis* PGN (PGN-BS) (100 μg/mouse/injection) via i.p. injection twice per week for 12 weeks. Mice were euthanized and blood and tissue samples were collected at 18 weeks of age. (A) Serum levels of IgG anti-dsDNA. (B) Percent of anti-dsDNA IgG+ B cells in spleen (gating on B cells). (C) Serum levels of total IgG at the end of the study. (D) Representative kidney H&E imaging and collective glomerular lesion index. Representative kidney C3 and total IgG staining and collective quantification of C3 and IgG deposition.



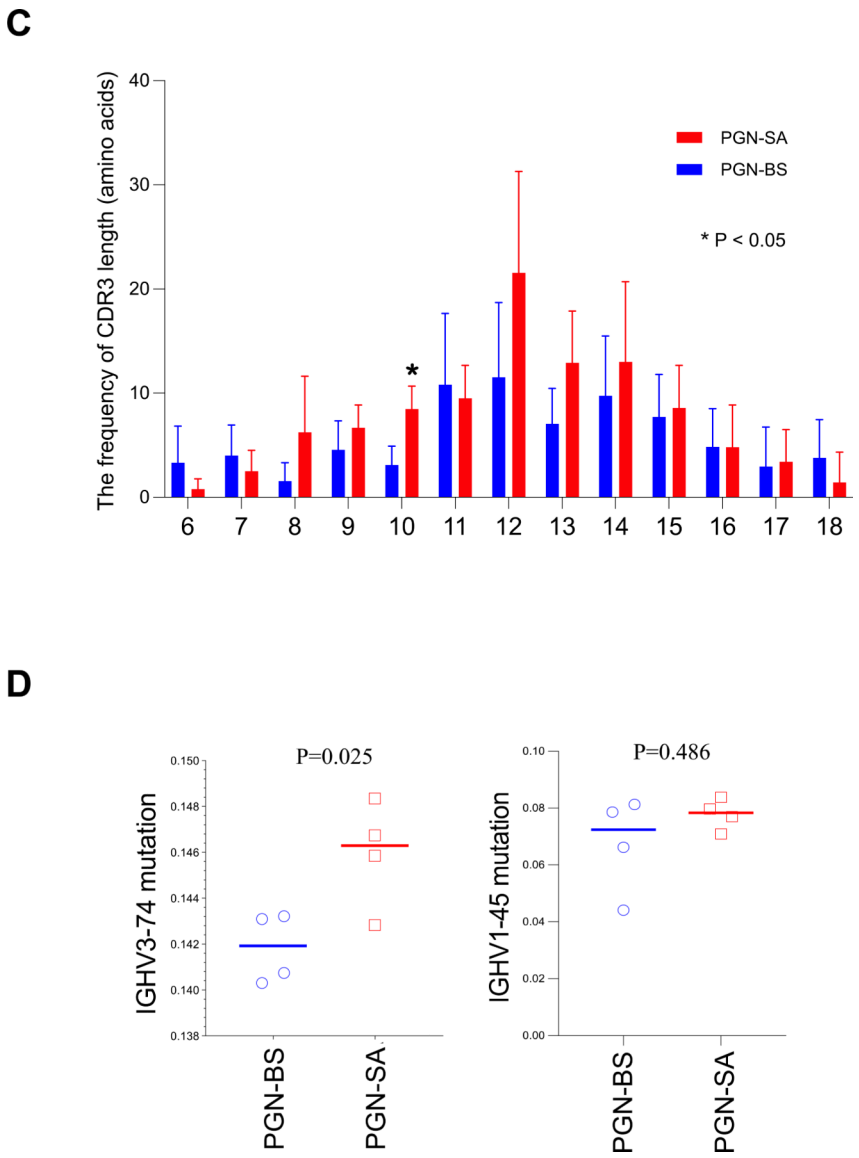


Figure 3.

Characteristics of BCR repertoire from anti-dsDNA IgG+ B cells from spleen. C57/B6 mice were treated with *S. aureus* or *B. subtilis* PGNs via i.p. injection twice per week for 8 weeks. At the end of study, B cells from mice at 14 weeks of age were isolated from spleen and anti-dsDNA IgG+ B cells were sorted using flow cytometry. RNA was extracted, and BCR sequencing was conducted. BCR clonality was analyzed in C57/B6 mice treated with *S. aureus* (SA) and *B. subtilis* (BS) PGN (n = 4 per group). (A) The frequency of IGHV usage. (B) BCR clonal diversity. (C) The frequency of CDR3 length. (D) Somatic mutation of IGHV3-74 and IGHV1-45. Statistical analysis used non-parametric Mann-Whitney U tests.

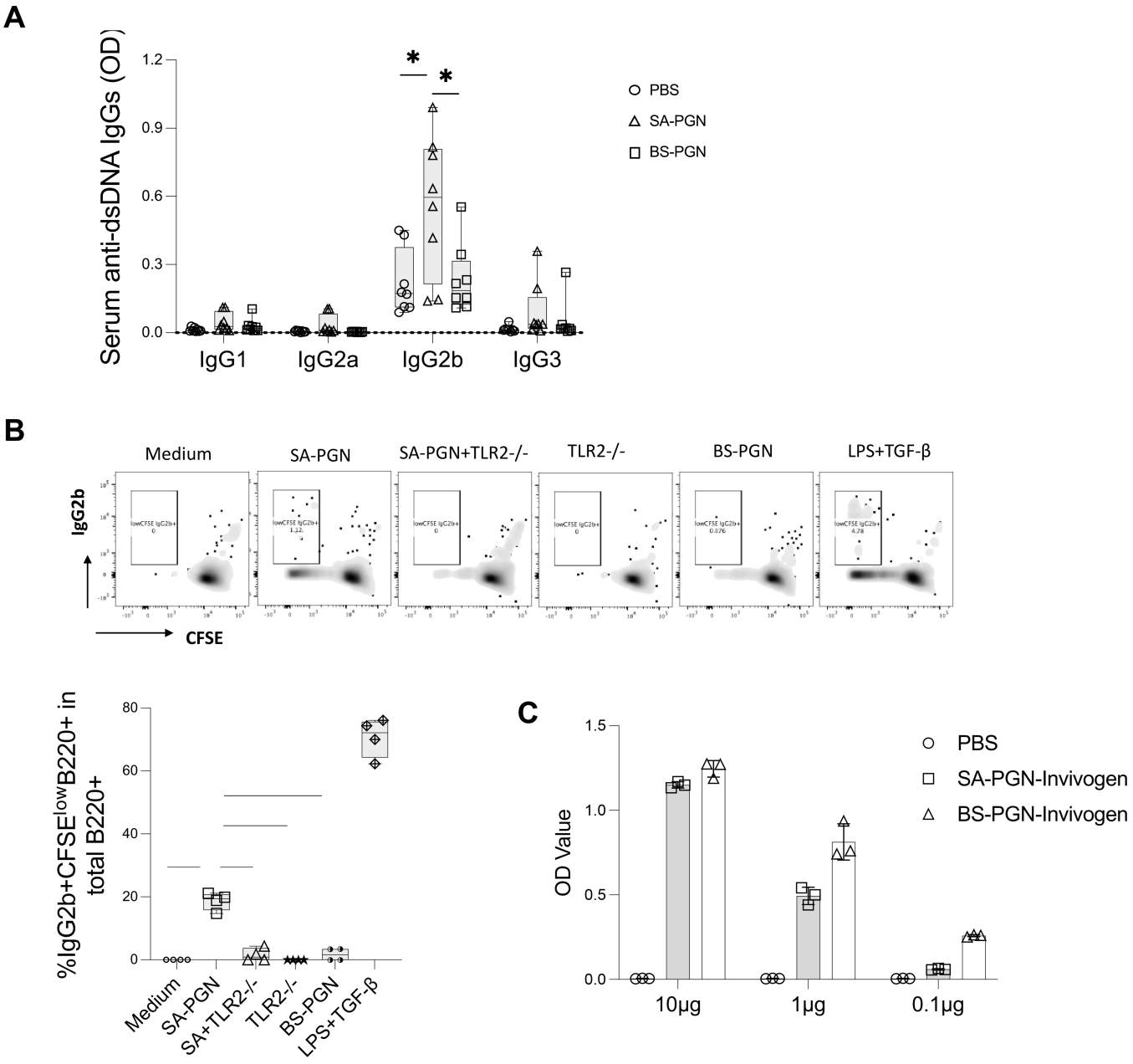
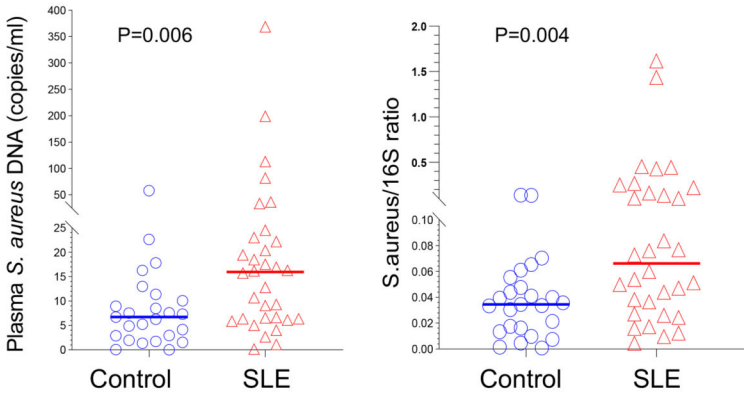


Figure 4.

IgG2b CSR mediated by *S. aureus* PGN. (A) C57/B6 mice were treated with *S. aureus* or *B. subtilis* PGNs via i.p. injection twice per week for 8 weeks. At the end of study, subclasses of serum anti-dsDNA IgG were evaluated in mice at 14 weeks of age. (B) B cells were isolated from spleen of untreated C57/B6 mice, cultured with basal medium, LPS plus TGF-β1 (a positive control), *S. aureus* (SA) or *B. subtilis (BS)* PGN in the presence of absence of TLR2 mAb or isotype Ab for 72 h. The percentages of proliferating IgG2b+ B cells (%$CFSE^{low}$IgG2b+ in IgG2b+B220+ cells) were calculated. (C) The TLR2 induced activity by S. aureus and B. subtilis PGN (P = 0.25). Statistical analysis used non-parametric Mann-Whitney U tests.

A



B

Direct correlations between *S. aureus* DNA vs autoIgGs	r	P value
Anti-Chromatin	0.326	0.013
Anti-Heparin	0.284	0.032
Anti-KU (P70/P80)	0.305	0.021
Anti-Nucleosome antigen IgG	0.296	0.025
Anti-Ro-52/SSA	0.296	0.025
Anti-ssDNA	0.311	0.018
Anti-U1-snRNP-68	0.303	0.022
Anti-U1-snRNP-C	0.327	0.013
Anti-complement C3a	0.409	0.002
Anti-dsDNA	**0.340**	**0.010**
Anti-Ribo phasphoprotein P0	0.378	0.004
Anti-SmD1	0.340	0.010
Anti-ssRNA	0.448	0.001
Anti-U1-snRNP-BB'	0.471	<0.001
Anti-*S. aureus* PGN	0.471	<0.001

Figure 5.

Increased plasma *S. aureus* DNA levels in patients with SLE and their correlation with lupus-related autoantibody levels. (A) Presence of plasma *S. aureus* DNA (absolute copy number per mL blood or ratio of *S. aureus* DNA versus total 16S rDNA) measured by qPCR in extracted microbial DNA from plasma samples of patients with SLE (n = 32) and healthy controls (n = 25). (B) Spearman correlation coefficient (r) and *P* values of *S. aureus* DNA copy number per mL blood and levels of each lupus-related autoantibody. Autoantibody levels were evaluated in plasma samples by ELISA.