A B Cell Superantigen-induced Persistent “Hole” in the B-1 Repertoire

By Gregg J. Silverman, Stephen P. Cary, Denise C. Dwyer, Linda Luo, Raymond Wagenknecht, and Virginia E. Curtiss

From the Department of Medicine, University of California at San Diego, La Jolla, California 92093-0663

Abstract

The bacterial toxin protein A from Staphylococcus aureus (SpA) interacts with B cell antigen receptors encoded by variable region heavy chain (VHelvetica\textsubscript{H}) clan III genes via a V region framework surface that has been highly conserved during the evolution of the adaptive immune system. We have investigated the consequences of exposure to this prototypic B cell superantigen, and found that treatment of neonates or adults induces a T cell–independent deletion of a large superclonal set of susceptible B cells that includes clan III/VHelvetica\textsubscript{H} S107 family–expressing lymphocytes. In studies of different SpA forms, the magnitude of the induced deletion directly correlated with the VHelvetica\textsubscript{H}-specific binding affinity/avidity. Upon cessation of SpA exposure, the representation of conventional splenic (B-2 subset) lymphocytes normalized; however, we found that the VHelvetica\textsubscript{H} family–restricted deficit of peritoneal B-1 cells persisted. SpA treatment also induced a persistent loss of splenic S107-\(\mu\) transcripts, with a loss of certain natural antibodies and specific tolerance to phosphorylcholine immunogens that normally recruit protective antimicrobial responses dominated by the S107-expressing B-1 clone, T15. These studies illustrate how a B cell superantigen can exploit a primordial Achilles heel in the immune system, for which B-1 cells, an important source of natural antibodies and host immune responses, have special susceptibility.

Key words: tolerance • repertoire • clonal selection • immunoglobulin genes • host immunity

Introduction

Superantigens (SAgs)\(^1\) are products of microbial pathogens that have the capacity to adversely affect immune responsiveness by interactions with highly represented conserved sites in the V regions of the antigen receptors of host lymphocytes. These proteins have been studied to elucidate their possible roles in pathogenesis of inflammatory and autoimmune diseases, and these nonimmune binding interactions have also provided an invaluable window into the signaling, cytokine, and cognate parameters of T lymphocyte antigen receptor (TCR)–mediated stimulation. Analogous in vitro interactions of several naturally occurring proteins with B cell antigen receptors (BCRs) have also been described (for a review, see reference 1); however the implications for in vivo exposure to a B cell SAg are largely unexplored.

Of all putative B cell SAgs, protein A of Staphylococcus aureus (SpA) has been the best characterized. Despite the fact that this 42-kD secreted membrane protein does not appear to play an essential role in the metabolism or survival of the bacterium, SpA is produced by most (or all) clinical isolates (2). Consequently, it has been postulated that the highly refined Ig binding properties of SpA evolved to play a role in the host–pathogen relationship. Staphylococcal virulence has been shown to be enhanced by SpA in experimental models (3) but the responsible pathophysiologic mechanism(s) have not been determined. The immunomodulatory activities of SpA are likely aided by its oligovalent organization. It is composed of five 56–61-amino acid homologous extramembrane domains in tandem (4), and each domain possesses both the well-known Fc\(\gamma\) binding specificity and a separate binding site that is specific for Fab-containing VHelvetica\textsubscript{H} regions from the structurally related clan III families (5, 6). Furthermore, in vitro stimulation with S. aureus has been reported to prefer-
entially select for human B cells expressing genes from the $V_{H}3$ family (7).

The special molecular features of the Fab-binding specificity of SpA, which were first identified in correlation with antibody sequence usage (1, 8, 9), have recently been elucidated in crystallographic analyses of a human IgM Fab--SpA domain cocomplex. This interaction was shown to be mediated by a clan III–restricted surface, distant from the CDR loops responsible for the recognition of conventional antigens (10), which involves 13 contact residues in the $V_{H}$ framework (FR )1 and FR 3 subdomains that have been conserved during the evolution of the adaptive immune system (11, 12). As a direct consequence, this unconventional type of $V_{H}$-restricted BCR–mediated binding activity is highly represented in immune systems of diverse mammalian species, including the human system in which the $V_{H}3$ family of clan III composes nearly half of all inherited $V_{H}$ genes. It is also prevalent in amphibian and avian species that have been studied (12). In the mouse, the homologous clan III families S107, J606, 7183, and DNA4 commonly convey this binding activity although the affinities of these interactions vary (12–14).

In a recent report, we showed that >5% of mature B cells in naïve BALB/c mice possess this nonimmune binding activity, and it is also displayed by ~12% of constitutively IgM-secreting splenic cells and a comparable proportion of circulating natural IgM (15). Most importantly, we found that neonatal exposure to a chemically modified form of SpA that is devoid of Fc-binding activity induced an acute loss of >80% of SpA-reactive splenic B cells. Although this cellular representation in the spleen later normalized, there was still a long-lasting loss of SpA-reactive IgM-secreting cells (ISCs) and an equivalent loss of circulating SpA-reactive IgM, which persisted, despite the presence of SpA-specific T cells, when evaluated >1 yr later (15). However, these studies did not identify which B cells are susceptible to SAg-mediated deletion, and the implications for host immune responsiveness were not further considered.

We have now investigated the molecular and cellular mechanisms responsible for the immunomodulatory activities of this model B cell SA g. To determine the functional features responsible for its immunological properties, we have compared the host’s response to native SpA to treatment with several forms of SpA that vary in their Fc- and Fab-binding activities. Within these studies, we have also identified the $V_{H}$-defined supraclonal B cell set most affected by treatment. Moreover, we found that SpA exposure adversely affected the levels of certain natural antibodies and caused a selective tolerance to immunogens important for host defense against many bacterial as well as protozoan, fungal, and nematode pathogens (16). Based on these findings, we present a model explaining how this microbial toxin can cause an immunosuppression in the host B cell compartment based on $V_{H}$ usage, and discuss why B-1 cells are especially susceptible to the induction of long-lasting effects.

**Materials and Methods**

M utageneration and C loning of D omain D ’ D erivatives. To create novel recombinant forms of SpA, the L17D and 131A mutations (17) were introduced into the gene for domain D (DD’ ) of SpA in the pDOMD’ plasmid (5), and termed mDOMD’. By overlap PCR methods, a two domain product, dimeric mDD’ (dimDD’), and four domain product, tetra-mutant domainD’ (tetmDD’), were created in the pRSET system (Invitrogen) (Silverman, G., manuscript in preparation).

M ice and Immunogens. M ice were obtained from The Jackson Laboratory and bred under specific pathogen-free conditions under the supervision of the University of California San Diego Animal Subjects Program. Applying a previously reported protocol (15), beginning within 24 h of birth mice were treated with either hen egg lysozyme (HEL; Sigma-Aldrich), OVA (SigmaAldrich), tetmDOMD’, domain D’, rSpA (R epligen), or SpA that was iodinated (MSpA) to selectively ablate Fc-binding activity (18, 19), which had been purified to remove endotoxin. These neonatal mice received PBS or 100 μg of protein in PBS, intraperitoneally every other day for the first 2 wk of life (eight doses of 100 μg protein). Adult mice, at least 6 wk of age, received 1 mg of protein every other day for five doses. In all experiments, mice were age and sex matched.

To assess immune responsiveness, certain groups of BALB/c × C57BL/6 F1 mice were challenged with the phosphorylcholine (PC)-KLH conjugate (50 μg/dose; Biosearch Technologies), whereas others received the thymus-independent type 2 (TI-2) immunogens of B1355 dextran (50 μg/dose; gift of Norman Klinman, The Scripps Research Institute, La Jolla, CA) or pronase-treated extract of 10 9 CFU of heat-killed R 36A Streptococcus pneumoniae (gift of David Briles, University of Alabama at Birmingham, Birmingham, AL) in CFA (Difco). Alternatively, mice were challenged intravenously with 2 μg of pneumococcal cell wall polysaccharide (C-PS; Serumstadinstitut) in saline. Blood was collected 10 d later for serologic assays, as described (15).

Immunooasays of Antibody Responses. The antibody response to MSpA and control antigens was quantitated as described previously (15). In brief, microtiter wells were coated overnight with protein, dextran, or C-PS at 5 μg/ml in PBS. After blocking with 2% BSA/PBS, serum samples diluted in block were incubated for 4 h at room temperature. The amount of bound antibody was determined by incubation with horseradish peroxidase (HRP)-labeled affinity-purified goat F(ab)2 anti–mouse IgG, IgG3, IgG-, or IgG subclass-specific reagents (Jackson Immunoresearch Laboratories), with values obtained after incubation of substrate for 15 min. The anti-PC response from the SI707/clan III–encoded T15 set was measured by development with a saturating concentration of the T15 clone–specific rat IgG2a, T139.2 (20; gift of Matthew Scharff, Albert Einstein College of Medicine, New Hyde Park, NY), or with the T15–specific biotinylated mouse IgG1, AB1–2 (21; gift of John Kearney, University of Alabama at Birmingham, Birmingham, AL), with the recombinant avian mAb, LJ–26, which has restricted recognition of clan III products (including 7183, J606, SI707, and DNA4) but not clan I or II products (12). For quantitation, we used a calibration curve of a T15 IgM mAb. The J558/clan I–encoded antibody response to α1–3 dextran was ascertained using an HRP–anti-λ (Jackson Immunoresearch Laboratories), and the IgM λ, M 104E, was used in a standard curve.

To compare relative Fab-binding activity of the SpA forms, 100 μl aliquots of an mAb at fixed concentration were diluted in 1% BSA/PBS with 10 μg/ml of Fc (Jackson Immunoresearch
Laboratories) and incubated for 4 h at room temperature with one of the SpA forms at a range of concentrations; these were later incubated for 1 h in wells coated with tetmDomD. Plates were developed with HRP-conjugated anti–mouse IgG or IgM (Jackson Immunoresearch Laboratories), as appropriate. To evaluate relative Fc-binding activity, wells were coated with SpA, and proteins were incubated with a fixed concentration of biotinylated IgG Fc and later developed using streptavidin-alkaline phosphatase (Kirkegaard & Perry Laboratories). Relative inhibition values were determined by comparison to a standard curve of the protein without inhibitor.

Enzyme-linked immunoassays. The frequencies of Ig- and specific antibody-secreting splenocytes and bone marrow were quantitated as described previously (15). In parallel studies, wells were coated with either goat affinity-purified anti–mouse IgM or IgG (Jackson Immunoresearch Laboratories), M SpA, control protein antigens of OVA, HEL, or FCS, Lj26 (12), AB1-2, T139.2, Tc54.8 (Fig. 1), or isotype controls.

Flow Cytometry Analysis. Adapting previously reported methods (15, 19, 22) to quantify the representation of SAgsusceptible B cells, cells from a mononuclear cell scatter gate were evaluated. For studies of T15i mice, we then gated on all allogeneic B220+ cells (clone RA3-6B2), and then evaluated the representation of surface peridinin chlorophyll protein of PerCP was found to confirm equivalent Ig expression.

Figure 1. A scheme of sets of murine V\textsubscript{H} genes and SAgs reactivity. In many strains, genes from clan I and clan II are the source of most of the repertoire, whereas clan III represents a nonoverlapping set. Most clan III products interact with the Fab-binding site of SpA and also with Lj26, a recombinant avian mAb. Each recognizes similar but nonidentical sets. These also identify S107-encoded antibodies, including those expressing the T15-specific VS107.1 rearrangement that generally display among the highest levels of SpA-binding affinity.
with a chemically modified form of SpA (M SpA) induced a persistent suppression of M SpA-reactive splenic ISCs (15). To better define the features responsible for B cell immunomodulatory properties of this natural bacterial toxin, we assembled a panel of different forms of SpA, and the Ig-binding properties of these proteins were compared in direct binding and inhibition studies with human and murine mAbs. In a representative study (Fig. 2), all of these proteins displayed the same clan III-specific Fab-binding specificity, although relative affinity/avidity varied greatly (Table I). Native SpA displayed the strongest Fab-binding activity, whereas M SpA exhibited about fivefold weaker activity. Compared with native SpA, the monomeric domain D' of SpA, which is capable of simultaneously binding a V\textsubscript{H}3 Fab and an F\textsubscript{C\gamma} molecule via distinct sites (5), exhibited \( \approx \)5,000-fold weaker Fab-binding activity. By introduction of the L17D and I31A mutations that are reported to ablate Fc-binding activity (17), a genetic mutant domain D' (mDD') was generated and oligomeric forms were also engineered. Although not anticipated, the mutant dimeric and tetrameric mutant SpA forms, dimDD' and tetmDD', exhibited \( \approx \)2,000–5,000-fold weaker Fab-binding activity than native SpA. In separate studies, the M SpA and mutant SpA forms were devoid of detectable Fc\gamma activity, whereas the greatest binding activity was displayed by SpA, and by comparison the domain D monomer had \( \approx \)500-fold weaker inhibitory activity (not shown).

Fab-binding Avidity Determines Level of Immunosuppression. To investigate the in vivo biologic activities of different proteins, mice were treated during the first 2 wk of life and then evaluated months later. In general, neonatal treatment with SpA or control protein antigen resulted in a lasting increase in the overall frequency of splenic constitutive ISCs (Fig. 3A; reference 15). To evaluate whether treatment altered the representation of ISCs reactive with the clan III-specific Fab-binding site of SpA (i.e., SA-g-reactive ISCs), we ascertained the frequencies of cells producing IgM that bind to M SpA-coated wells (15) and found that in naive adult mice, 12.5 ± 0.7% (mean ± SEM) of splenic ISCs were SA-g reactive and this proportion was not altered by prior treatment with a control protein antigen (HEL, 12.3 ± 1.1%; Fig. 3A) (15). Treatment with the tetrameric mutant SpA form, which has weak Fab-binding activity and no Fc-binding activity, induced a minor decrease (10.3 ± 0.7%) in the representation of SA-g-reactive ISCs that was not significantly less than in control groups (\( P = 0.095 \)). By contrast, treatment with the domain D' monomer induced a significant level of inhibition (7.9 ± 0.02%, \( P = 0.03 \)). A greater level of suppression was induced by M SpA, which has relatively strong Fab-binding activity and no Fc-binding activity (6.25 ± 0.4%, \( P < 0.001 \)). However, the greatest suppression was detected in mice treated with native SpA, which has the strongest Fab-binding activity (2.8 ± 0.4%, \( P < 0.004 \)). Essentially the same patterns of suppressive activity were also detected in bone marrow ISCs where the effects in the M SpA- and SpA-treated groups were similar (Fig. 3B). A similar effect was also demonstrated for cells in the peritoneal cavity, the treatment site (not shown). Even though total IgM levels were generally increased compared with naive mice (not shown), SpA treatment of neonatal mice also induced a decrease in the levels of M SpA-binding circulating IgM (15). Notably, a total dose of only 50 \( \mu \)g of M SpA often induced a similar level of suppression, but the responses of mice within this treatment group were more heterogeneous (not shown). Hence, the level of suprachronal suppression of SA-g-reactive ISCs directly correlated with the relative Fab-binding affinity and not the Fc-binding activity.

A adult Mice Are Also Susceptible to SA-g-induced Immunosuppression. To ascertain whether adult mice are also affected by SpA treatment, 7-wk-old BALB/c mice were given proportionately larger doses and evaluated 1 mo later. In

### Table I. Relative Activities of SpA Forms

| SpA form | MW (kD) | No. of Fab-binding domains | Fc-binding activity |
|----------|---------|---------------------------|---------------------|
| SpA      | native  | 42                        | 5                   | \( 2 \times 10^{-6} \) | \( 4 \times 10^{-7} \) |
| M SpA    | iodinated | 42                       | 5                   | \( 1 \times 10^{-5} \) | ND \(^*\) |
| D'D'     | native  | 11                        | 1                   | \( 1 \times 10^{-2} \) | \( 2 \times 10^{-4} \) |
| DimDD'   | L17D I31A | 24                      | 2                   | \( 1 \times 10^{-2} \) | ND |
| TetmDD'  | L17D I31A | 37                      | 4                   | \( 5 \times 10^{-3} \) | ND |

MW, molecular mass.

*Relative activities based on 50% inhibition studies in a solution phase enzyme immunoassay.

\(^*\) Not detected. Detection limits in these assays were \( \approx 5 \times 10^{-1} \) M.
these treated mice, the frequency of total splenic ISC s was not affected, whereas SA g-reactive splenic ISCs were significantly reduced by treatment with MS pA (4.81 ± 0.95%, \( P < 0.001 \)) or SpA (3.41 ± 0.039%, \( P < 0.0005 \)) compared with control groups (Fig. 3 C). Similar suppressive effects were also found on constitutive ISCs in the bone marrow (Fig. 3 D).

**T Lymphocytes Are Not Required for SAg-induced B Cell Suppression.** To investigate whether T lymphocytes play a critical role in SpA-induced B cell suppression, we evaluated the effect of neonatal treatment on mice with homozygous deficiencies in both TCR \( \beta \) and \( \delta \) chains (TCR-\( \beta^{-/-}\delta^{-/-} \)), which are devoid of mature T cells (25, 26). Akin to the control C57BL/6 groups, neonatal treatment of T cell–deficient mice with a protein antigen also resulted in increased overall levels of splenic ISCs while these mice were also susceptible to SpA-induced selective decreases in the frequency and relative representation of SAg-reactive ISCs in the spleen (Fig. 3 E) and bone marrow (Fig. 3 F). Compared with control groups, SpA treatment reduced by 35% the proportion of clan III–expressing splenic ISCs (\( P = 0.007 \); Fig. 3 E), identified by a broadly
reactive clan III Ig-specific marker (LJ-26 antibody) (12). The T15 B cell clonal set represents only a small component of $V_{H}$ clan III/S107-expressing cells, and using a $V_{H}$ T15-specific marker (T68) we found that in these treated mice the representation among ISCs of these products was reduced by 76% ($P = 0.0002$) in the spleen (Fig. 3E) and by 82% in bone marrow ($P = 0.026$; Fig. 3F). Compared with control groups, levels of circulating natural Ig-bearing T15-specific idiotypic markers, which included IgM, IgG2b, and IgG3 responses, were each found to be significantly reduced (not shown). These results document that SpA-induced suppression is mediated via a B cell–targeted mechanism affecting certain S107-expressing B cells, which is not dependent on T cell influences.

SAg Induces a Selective $V_{H}$ Family-targeted Deficit in the Expressing Ig R epito"
nogens that induce TI-2 responses. Compared with the vigorous responses in naive or control protein-treated groups, all of the mice neonatally treated with MSpA or SpA were tolerant to challenge with \( S. \) pneumoniae extract, whereas the mice treated with the domain D\(^9\) monomer of SpA displayed highly heterogeneous anti-PC responses (Fig. 6 A). In other challenged groups, mice treated as neonates with SpA were also nonresponsive to the PC-containing purified C-PS TI-2 immunogen (not shown), and were also tolerant to the T cell–dependent PC immunogen PC-KLH (Fig. 6 B) that also recruits a response dominated by T15 B cell clone(s) (31). Treatment of adult mice with SpA induced the same selective tolerance to the PC-containing pneumococcal immunogen (Fig. 6 C). Therefore, SAg treatment induces tolerance in an antigen-specific response requiring clan III/S107–expressing B cell clonal sets; in the absence of this response, other clonal sets are not recruited into this response, whereas a response requiring clan I/J558–expressing B cells is unaffected (Fig. 6, A and C).

SpA induces a Loss of Detectable Clan III–expressing B-1 Cells in the Peritoneal Cavity. To consider the cellular mechanisms responsible for SpA-induced defects, we have evaluated T15i transgenic "knockin" mice that have B cells expressing the canonical T15/S107 \( V_\text{H} \) rearrangement in heterozygotic mice, clonal representation of B cells can be readily tracked, as B cells expressing endogenous \( V_\text{H} \) regions bear the IgM\(^b\) allotype, whereas expression of the \( V_\text{H} \) T15 transgene is linked to the IgM\(^a\) allotype. Confirming their earlier characterization (32), in these mice most peritoneal \( V_\text{H} \) T15-expressing B cells, which coexpress diverse endoge-
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nous L chains, bear the phenotype of B-1b cells (i.e., B220\(^{low}\)IgD\(^{low}\)Mac-1\(^{+}\)CD5\(^{2}\)). Clarifying our previously reported findings in BALB/c mice, 1 d after completion of neonatal SpA treatment, heterozygous mice exhibited a >90% reduction of V\(_H\)T15 transgene–expressing splenic B cells (not shown). Furthermore, treatment of adult 6-wk-old mice with a single 0.5-mg dose of SpA also induced an acute selective loss of >60% of V\(_H\)T15-expressing splenic and peritoneal B cells when evaluated 1 d later (not shown).

To evaluate the clonal representation associated with long-lasting functional defects, we studied T15i heterozygous mice months after the last SpA exposure. At these late time points, there were no major differences in the representation of transgene-expressing splenic conventional B cells (IgD\(^{hi}\)B220\(^{+}\)) (Fig. 7 B), presumably because these B cells are continually replenished from the bone marrow. However, in the peritoneal cavity, a major anatomic residence of B-1 cells, neonatal SpA treatment resulted in a highly significant mean 78% reduction (\(P = 0.0006\)) in the frequency of V\(_H\)T15 transgene–expressing peritoneal B-1 cells (Mac-1\(^{+}\)B220\(^{+}\)) (7.9 ± 1.1%, \(n = 7\)) compared with control treated mice (36.5 ± 3.2%, \(n = 6\)) (Fig. 7 A). Using the clan III (Lj-26) marker, comparable losses of transgene-expressing B-1 cell populations were also demonstrated in the peritoneum but not the spleen (not shown). We also demonstrated similar findings in studies of adult TCR-deficient mice with polyclonal endogenous B cell populations. In the T cell–deficient mice that had received neonatal SpA treatment, the representation of clan III–expressing peritoneal B-1a (Lj-26\(^{+}\)IgD\(^{low}\)B220\(^{+}\)CD5\(^{2}\)) cells was significantly reduced (8.1 ± 0.49%, \(n = 4\), \(P < 0.016\)), representing a mean 30% reduction, compared with naive (11.5 ± 0.36%) or control treated mice (10.3 ± 0.52%). Similar significant losses were also demonstrated in the clan III–expressing B-1b (Lj-26\(^{+}\)IgD\(^{low}\)B220\(^{+}\)CD5\(^{2}\)) cells of SpA-treated mice (6.8 ± 0.48%, \(P < 0.015\)) compared with naive (9.7 ± 0.14%) and control treated mice (9.75 ± 0.65%) (Fig. 7 C). In contrast, the representation of conventional splenic B cells in these mice was not significantly affected (not shown). These findings demonstrate that a limited interval of exposure to SpA can induce a long-lasting clonal loss of clan III/S107–expressing peritoneal B-1 cells.

Discussion

We have demonstrated that in vivo exposure to staphylococcal protein A, a model B cell SAg, results in a selective targeting and immunosuppression of a suprACLonal set of B lymphocytes that express V\(_H\) clan III antibody genes, and both neonatal and mature mice are susceptible. In general, the magnitude of the induced B cell defect correlated with the relative avidity of Fab-binding interaction of the SAg. While the structural basis of this SAg–BCR interaction is no doubt distinct from those associated with binding of conventional antigens, these findings are consistent with earlier observations in which high affinity ligands are most effective at inducing B cell tolerance in classical models (33) and in transgenic Ig models (34, 35).

In surveys of the murine antibodies, many genes from clan III–related V\(_H\) families commonly encode for binding of this prototypic bacterial B cell SAg (13, 14), but certain of these genes, especially those from the S107 family, gen-
eral activity (14). These findings parallel data from earlier studies of phage-display antibody libraries, in which SpA preferentially selected for phagemid clones expressing certain human germ-line V<sub>H</sub>3 genes that conveyed greater SpA binding activity (5, 8). In the current studies, we have now demonstrated that in vivo exposure to SpA can induce a nearly complete loss of constitutively expressed splenic S107-μ transcripts and natural IgM, including anti-PC antibodies. In view of the cumulative data, we can only interpret the loss of these S107-expressing B cell clones as due, at least in part, to their higher binding affinity for SpA.

The susceptibility of T cell–deficient mice to SAg-induced immune defects indicates that this process, which is associated with long-lasting clonal loss, does not have an obligatory T cell dependence. In fact, induced PC-specific tolerance persists in the presence of SpA-specific T cells (15). Hence, SAg-induced tolerance has features that appear distinct from experimental models of B cell tolerance in which the unavailability of antigen-specific T cells is a primary determinant in whether B cell interactions with nominal antigen result in clonal anergy and/or deletion (36, 37).

The capacity to induce a persistent “hole” in the B cell repertoire is not unique to SpA, as similar outcomes have also been previously reported upon treatment with certain specific B cell ligands/antigens, or with antidiotypic antibodies that act as their surrogates (38). However, we know that in naive mice, PC-specific antibody-forming cells normally represent only an estimated 1/50,000 splenocytes (39), indicating that PC-specific B cells represent only a limited component of the large supraclonal set affected by SAg exposure. Hence, the distinction of the SpA response derives in part from the much larger scale of the induced supraclonal defect. In addition, the immunological properties of SpA are also special, as this naturally occurring “tolerizing agent” affects host responses to other immunogens by virtue of its ability to interact with supraclonal sets via V<sub>H</sub> family-specific interactions (i.e., it has superantigenic properties).

Our earlier surveys identified two distinct temporal phases in BALB/c mice during the response to neonatal treatment with this B cell SAg. Throughout both early and late phases, suppression of levels of SAg-reactive IgM was a consistent finding (15), whereas the late phase response, beginning at ~6 wk of life, was distinguished by the acquisition of SpA-specific T cell responsiveness and the induction of SAg-specific IgG1 antibody production. In the current studies, T cell–deficient mice were also found to be sensitive to the induced B cell supraclonal suppression, but the features characteristic of the late phase of the response were absent in these mice. This late phase T cell–dependent response likely involves conventional (B-2) lymphocytes in germinal center–type reactions in secondary lymphoid tissue that elaborate clonally focussed MSpA-reactive IgG1 antibody responses (Silverman, G.J., manuscript in preparation). Therefore, because these IgG1 responses from conventional B cells can reconstitute in BALB/c mice, our data indicate that it is the effects of this bacterial toxin on a separate B cell pool that is responsible for the persistent induced defects in anti-PC responses and natural antibody production.

Several compelling pieces of evidence implicate the targeting of clones within the B-1 pool as the origin of the long-lasting immunological defects. Most relevant, B-1 cells are believed to be the source of constitutively produced natural IgM antibodies that provide the primary defense against many common microbial pathogens (40, 41). Moreover, the susceptible set includes T15 B cells, the source of S107-encoded anti-PC antibodies that dominate these natural IgM and postimmunization protective responses, which adoptive transfer studies have rigorously demonstrated reside predominantly, if not solely, within the B-1 pool (42, 43). We found that SAg exposure resulted in a near complete loss of splenic S107-μ transcripts, but these findings, from RNA-based assays, are greatly influenced by the representation of antibody-forming cells that contain 100–1,000-fold greater amounts of antibody-specific transcripts than resting B cells. Hence, the dramatic reductions in S107-μ transcript levels undoubtedly reflect the loss of an SAg-reactive set of constitutive ISC’s that derive from B-1 cells to produce natural antibodies.

The special immunobiology of B-1 cells explains their susceptibility to the persistent V<sub>H</sub>1-targeted effects of SpA. For the developing murine B-1 pool, entry of newly generated lymphocytes is reportedly complete by the end of the neonatal period, and thereafter these B cells are self-sustaining and cannot be replenished by the lymphogeneic system that perpetually supplements the conventional (B-2) compartment in the adult (44–46). Also relevant, compared with splenic follicular B cells, adult peritoneal B-1 cells are poor responders to in vitro stimulation with anti-IgM, an experimental surrogate for a BCR signal. In fact, BCR-mediated apoptosis is a common outcome of high level antigenic stimulation of these extrafollicular cells (47, 48). Strong in vivo cross-linking of cell surface Igs has also been reported to induce apoptotic death of mature B cells in the peritoneal cavity (48). Presumably, these features of B-1 cells are also responsible for the persistent clonal loss that results from instillation of IgM allotype-specific antibody into allototype homozygotic mice (49). Based on these observations, it is predictable that even a limited exposure to a potent natural BCR-targeted agent would induce a supraclonal hole in the B-1 repertoire that would persist, likely indefinitely. In earlier studies, we demonstrated a transient supraclonal deletion in the spleen and bone marrow immediately after SAg treatment (15); however, the clonal representation at these sites later normalized, presumably because of replenishment of conventional B cells from the central compartment. Consistent with this hypothesis, in surveys of splenic V<sub>H</sub>1-δ rearrangements that are representative of mature naive conventional follicular B cells (i.e., IgD<sup>hi</sup>), we did not detect a lasting SAg-induced supraclonal defect (Silverman, G.J., manuscript in preparation). These findings contrast with the persistent loss of V<sub>H</sub>S107-μ transcripts characterized in the current studies, which correlates...
with the supraclonal loss of splenic constitutive ISCs that have been linked to the B-1 pool. Hence, we believe that the lasting SpA-induced loss of SAgs-reactive clan III-derived peritoneal B-1 cells reflects their special vulnerability for the induction of persistent clonal defects by this bacterial Fab-binding protein.

B-1 cells are associated with a distinct repertoire that includes well-defined clonal sets that produce natural antibodies responsible for housekeeping and antimicrobial functions. From lessons first appreciated for the T15 clone, it appears that the binding specificities of B-1 cells commonly derived from canonical antibody gene rearrangements formed in primary sequence-directed splicing events (23, 50, 51) and are subsequently subject to ligand-mediated clonal selection (52); hence, B-1 cells have been postulated to convey a repertoire naturally selected during the evolution of the adaptive immune system (53). From this viewpoint, targeting of host clan III B cells, especially those providing natural immune defenses (29, 41), may provide a great advantage to a pathogen. Clan III genes are expressed in the immune systems of phylogenetically distant species as far back as elasmobranchs, and in certain species these antibody genes are the earliest expressed during development (11). In particular, clan III V\textsubscript{H} families and SpA-binding activity are highly represented in mammalian immune systems (12), and in humans >30% of B cells are targeted by SpA (19). Moreover, the current findings, which elucidate the immunological properties of SpA, should also help us to understand the effects of other postulated B cell SAgs (54–56). In particular, our findings may provide insight into how the SA\textsubscript{G} properties of HIV-1-associated retroviral products may contribute to a predisposition to certain infections (57), especially the otherwise inexplicably high frequency of pneumococcal sepsis (58).

In summary, our findings indicate that staphylococcal produce a toxin that can impair host defenses through effects on B lymphocytes, for which B-1 cells have special vulnerability. These interactions are mediated by an unconventional binding site on clan III-associated V\textsubscript{H} region framework subdomains that have been highly conserved in evolution (10). From these findings, we speculate that this common pathogen has developed a toxin with B cell SAg properties to exploit a primordial Achilles heel in the immune system.

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Note added in proof. Confirming the importance of the T15/clan III B cell clone, in a recent report Mi et al. provided direct evidence that genetic manipulation that knocks out the S107.1/clan III gene results in greatly impaired immune defense from pneumococcal infection. (Mi, Q.S., L. Zhou, D.H. Schulze, R.T. Fischer, A. Lustig, L.J. Rezanka, D.M. Donovan, D.L. Longo, and J.J. Kenny. 2000. Proc. Natl. Acad. Sci. USA. 97:6031–6036.)

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