The globally prominent pathogen *Streptococcus pyogenes* secretes potent immunomodulatory proteins known as superantigens (SAgs), which engage lateral surfaces of major histocompatibility class II molecules and T-cell receptor (TCR) β-chain variable domains (Vβs). These interactions result in the activation of numerous Vβ-specific T cells, which is the defining activity of a SAg. Although streptococcal SAgs are known virulence factors in scarlet fever and toxic shock syndrome, mechanisms by which SAgs contribute to the life cycle of *S. pyogenes* remain poorly understood. Herein, we demonstrate that passive immunization against the Vβ8-targeting SAg streptococcal pyrogenic exotoxin A (SpeA), or active immunization with either wild-type or a nonfunctional SpeA mutant, protects mice from nasopharyngeal infection; however, only passive immunization, or vaccination with inactive SpeA, resulted in high-titer SpeA-specific antibodies in vivo. Mice vaccinated with wild-type SpeA rendered Vβ8+ T cells poorly responsive, which prevented infection. This phenotype was reproduced with staphylococcal enterotoxin B, a heterologous SAg that also targets Vβ8+ T cells, and rendered mice resistant to infection. Furthermore, antibody-mediated depletion of T cells prevented nasopharyngeal infection by *S. pyogenes*, but not by *Streptococcus pneumoniae*, a bacterium that does not produce SAgs. Remarkably, these observations suggest that *S. pyogenes* uses SAgs to manipulate Vβ-specific T cells to establish nasopharyngeal infection.

**Significance**

Superantigen toxins were defined over 25 years ago for their ability to activate T cells in a T-cell receptor β-chain variable domain-dependent manner. This “Vβ-specific” T-cell activation is the hallmark feature of the superantigen, and although these toxins can mediate dangerous human disease such as toxic shock syndrome, mechanisms that explain why bacteria produce superantigens have remained enigmatic. Herein, we provide evidence that *Streptococcus pyogenes* utilizes superantigen to target functional, Vβ-specific T cells to promote a state of colonization providing a mechanism that helps explain why bacteria produce toxins that specifically activate T cells of the adaptive immune system. This work also implicates the superantigen exotoxins as potential vaccine candidates against this globally important, human-specific pathogen.
organism, *Streptococcus pneumoniae*. This work supports the use of toxoid SAgS as potential vaccine candidates against *S. pyogenes* nasopharyngeal infection and indicates that SAgS specifically target and manipulate Vβ-specific T-cell subsets to promote the initiation of infection.

**Results**

**Passive Immunization with SAg-Neutralizing Antibodies Protects Mice from *S. pyogenes* Nasopharyngeal Infection.** The human upper respiratory tract represents the major ecological niche for many strains of *S. pyogenes* (18), and intranasal inoculation of mice has been used to model this environment (16, 19). Previously, we demonstrated that mouse expression of HLA class II molecules (referred to as B6.HLA mice), and *S. pyogenes* MGASS232 expression of SpeA, were critical host and bacterial factors, respectively, that enhanced nasopharyngeal infection by up to four orders of magnitude (17). It was also demonstrated that vaccination of these mice with a SpeA MHC-II binding mutant (SpeA100A) was protective during nasopharyngeal challenge with *S. pyogenes* MGASS232, a phenotype that was linked to anti-SpeA antibodies (17).

To confirm the protective nature of the anti-SAg humoral response, we passively immunized B6.HLA mice with antisera prepared in rabbits that had been vaccinated with SpeA (Fig. 1A). As a control, we passively immunized B6.HLA mice with anti-SpeC rabbit serum since deletion of specC from *S. pyogenes* MGASS232 had no measurable impact on nasopharyngeal infection (17). Following treatment with anti-SpeA serum, quantitating bacterial colony-forming units (cfus) from the complete nasal turbinates (cNTs) demonstrated a dramatic reduction in bacterial burden compared with the control anti-SpeC serum group (Fig. 1B). Furthermore, Western blot analysis demonstrated that the anti-SAg sera were specific for their intended toxin (Fig. 1C), and SAg-specific antibodies were recovered from the serum of treated mice as determined by ELISA (Fig. 1D and E). These data indicate that humoral immunity against specific SAgS can be protective during experimental *S. pyogenes* nasopharyngeal infection.

**Active Vaccination with Wild-Type or Toxoid SAg Reduces *S. pyogenes* Nasopharyngeal Infection.** Our previous experiments demonstrated that SpeA100A could elicit protection when used as a vaccine; however, this SpeA mutant still maintained residual superantigenic activity in vitro at high concentrations (i.e., 1 µg mL⁻¹) (17). We therefore desired to generate a fully inactivated SpeA toxoid. Previous research implicated two leucines (Leu⁴¹ and Leu⁴²) as critical residues for the interaction of SpeA with the MHC-II α-chain, and mutants containing substitutions at these positions have been used in vaccination studies (20, 21). Consistent with this, a model of SpeA complexed with HLA-DR08 predicted Tyr⁴⁰ would hydrogen bond with the conserved MHC-II α-chain Lys⁹ (Fig. 2A), while the SpeA side chains of Leu⁴¹ and Leu⁴² were predicted to extend into a pocket formed by the MHC-II α-domain (Fig. S1). Based on this analysis, we generated a triple mutant containing alanine substitutions at all three positions (SpeA141A/L42A/Y100A), henceforth known as SpeA₃⁵ (Fig. 2B). SpeA₃⁵ was attenuated at all concentrations tested for activating B6.HLA mouse splenocytes compared with wild-type SpeA (Fig. 2C). Next, we used SpeA₃⁵ in our vaccination regimen (Fig. 2D) in parallel with wild-type SpeA, or a vehicle (sham) control. Interestingly, mice vaccinated with wild-type SpeA and SpeA₃⁵ were both protected from nasopharyngeal infection compared with sham-vaccinated mice (Fig. 2E); however, only SpeA₃⁵-vaccinated mice generated significant anti-SpeA IgM antibody titers (Fig. 2F). Low levels of anti-SpeA IgM were only detected in the SpeA₃⁵-vaccinated mice, while anti-SpeA IgA were not detectable from any group (Fig. S2). The SpeA₃⁵-vaccinated mice supported our previous conclusion that anti-SAg antibody could be protective, yet the lack of anti-SpeA antibodies in the wild-type SpeA-vaccinated mice was puzzling. Knowing that SAgS target T cells based on expression of specific Vβ T-cell receptors, we hypothesized that protection in the wild-type SpeA-vaccinated mice may be independent of humoral immunity but related to the T-cell response to the vaccine. To test this idea, we used wild-type staphylococcal enterotoxin B (SEB), a SAg that targets mouse Vβ⁸ TCRs (22), similar to SpeA (23). As an additional control, we used wild-type SpeC, a SAg that does not activate mouse T cells (24). Recombiant SEB and SpeC were purified (Fig. 2B), and it was demonstrated that SEB could stimulate B6.HLA splenocytes similar to wild-type SpeA, while SpeC was unable to do so (Fig. 2C). Following vaccination, mice that received SEB had significantly reduced *S. pyogenes* bacterial numbers, whereas SpeC-treated mice were comparable to sham-treated mice (Fig. 2E). As expected, SEB or SpeC vaccination did not elicit detectable anti-SpeA antibodies (Fig. 2F), further indicating that SEB-induced protection was not mediated by humoral immunity.

**Wild-Type SpeA- and SEB-Vaccinated Mice Have Poorly Responsive Vβ8 T Cells.** Since the protective phenotype from wild-type SpeA- and wild-type SEB-vaccinated mice was not likely due to neutralizing antibodies, we examined if this protective phenotype stemmed from an impact on the specific T-cell subset that is targeted by both SpeA and SEB (i.e., Vβ⁸ T cells). To assess this, B6.HLA mice were vaccinated with either a vehicle control (sham), wild-type SpeA, or wild-type SEB, killed on day 43.

![Fig. 1. Passive immunization with anti-SpeA serum reduces the burden of *S. pyogenes* in the nasopharynx.](image-url)
T Cells Are Required for Efficient Nasopharyngeal Infection by \textit{S. pyogenes} MGAS8232. Since our wild-type SAg vaccination studies suggested a role for SAg-responsive T cells during \textit{S. pyogenes} infection, we sought to deplete T cells from the murine infection model and determine the impact on nasopharyngeal infection. We used a previously described T-cell depletion protocol (28) to deplete CD4+ or CD8+ T cells, or both T-cell subsets concurrently, followed by nasopharyngeal infection with \textit{S. pyogenes} MGAS8232 (Fig. 4A). T-cell depletion was confirmed by flow cytometric analysis of the lymphocyte population from cervical lymph nodes compared with the isotype control-treated mice (Fig. 4B and C). Removal of either CD4+ T cells alone, or the removal of both CD4+ and CD8+ T cells, significantly reduced the nasopharyngeal burden of \textit{S. pyogenes} MGAS8232 in B6I.IL2a(-/-) mice (Fig. 4D). We also evaluated \textit{Streptococcus pneumoniae}, which is another human pathogen of the upper respiratory tract that is not known to produce SAg. First, we tested nasopharyngeal infection in both conventional B6 mice and B6I.IL2a(-/-) mice, and \textit{S. pneumoniae} infected both mice backgrounds at similar levels (Fig. 4E). This further suggests \textit{S. pneumoniae} does not produce a human-specific SAg, whereas \textit{S. pyogenes} cannot efficiently infect B6 mice lacking human MHC-II (17). Next, we tested nasopharyngeal infection with \textit{S. pneumoniae} in isotype-treated, and CD4/CD8 T-cell-depleted mice. Removal of both

\textit{S. pyogenes} MGAS8232 postvaccination with indicated treatments (control, black; SpeA, red; SpeA\textsubscript{rl}, pink; SEB, blue; and SpeC, green). Data points represent cfus from the complete nasal turbinates (cNTs) of individual mice at 48 h. Horizontal bars represent the geometric mean. The horizontal dotted line indicates theoretical limit of detection. (F) Serum IgG antibody titers determined using ELISA from B6I.IL2a mice vaccinated with indicated treatment (control, black; SpeA, red; SpeA\textsubscript{rl}, pink; SEB, blue; and SpeC, green). Data points represent the mean ± SEM. Significance was determined by one-way ANOVA with Dunn’s multiple comparison post hoc test (***P < 0.001).

**Fig. 3.** SpeA- and SEB-vaccinated mice have poorly functional V\textit{β}8+ T cells. (A) Vaccination protocol. (B and C) Flow cytometric analysis of splenocytes at day 43 postsuperantigen vaccination (n = 4 for each group). (B) Representative flow plots for each treatment group stained for CD3 (APC) and either V\textit{β}3 (FITC) or V\textit{β}8 (PE). (C) Percentage of CD3\textsuperscript{+}V\textit{β}3\textsuperscript{+} or CD3\textsuperscript{+}V\textit{β}8\textsuperscript{+} T-cell subset for each treatment group (control, black; SpeA, red; and SEB, blue). Data are shown as the mean ± SEM. Significance was determined by two-way ANOVA with Dunnett’s multiple comparison post hoc test (***P < 0.001). (D–F) B6I.IL2a mouse splenocyte IL-2 assay postvaccination with control (black circle), SpeA (red circle), or SEB (blue triangle) (n = 3 for each group). Treated mouse splenocytes were stimulated with increasing concentrations of SAg targeting specific T-cell variable \textit{β}-chain (V\textit{β}) subsets (D) SpeA, V\textit{β}8; (E) SEB, V\textit{β}8; and (F) SmeZ, V\textit{β}11. Stimulation occurred for 18 h and culture supernatants were analyzed for IL-2 using ELISA as a readout for T-cell activation. Data are shown as the mean ± SEM. Significance was determined by two-way ANOVA with Tukey’s post hoc test on the highest (10\textsuperscript{6} pg mL\textsuperscript{-1}) concentration tested (***P < 0.001; **P < 0.01).

**Fig. 2.** Vaccination with specific SAg proteins induces antibody-mediated, and antibody-independent protection from nasopharyngeal infection by \textit{S. pyogenes}. (A) Ribbon diagram model of SpeA (blue) in complex with the TCR (\textit{α}-chain, light blue; \textit{β}-chain, yellow) and MHC-II (\textit{α}-chain, red; \textit{β}-chain, green). Inset image shows amino acid residues mutated in SpeA\textsubscript{rl} (blue) and the conserved lysine 39 on MHC-II (red). (B) Recombinant SAgS visualized on a 15% SDS-PAGE. (C) SAg activation of B6I.IL2a mouse splenocytes (2 × 10\textsuperscript{5} cells per well) using SpeA (red), SpeA\textsubscript{rl} (pink), SEB (blue), and SpeC (green) at the indicated concentrations using murine IL-2 as a readout. Bars represent the mean ± SEM. (D) SAg vaccination protocol. (E) Nasal challenge of B6I.IL2a mice with ∼10\textsuperscript{6} cfus of \textit{S. pyogenes} MGAS8232 postvaccination with indicated treatments (control, black; SpeA, red; SpeA\textsubscript{rl}, pink; SEB, blue; and SpeC, green). Data points represent cfus from the complete nasal turbinates (cNTs) of individual mice at 48 h. Horizontal bars represent the geometric mean. The horizontal dotted line indicates theoretical limit of detection. (F) Serum IgG antibody titers determined using ELISA from B6I.IL2a mice vaccinated with indicated treatment (control, black; SpeA, red; SpeA\textsubscript{rl}, pink; SEB, blue; and SpeC, green). Bars represent the mean ± SEM. Significance was determined by one-way ANOVA with Dunn’s multiple comparison post hoc test (***P < 0.001; **P < 0.01).

**Fig. 1.** Vaccination protocol. (A) Vaccination protocol. (B) Flow cytometry analysis of splenocytes at day 43 postsuperantigen vaccination (n = 4 for each group). (C) Representative flow plots for each treatment group stained for CD3 (APC) and either V\textit{β}3 (FITC) or V\textit{β}8 (PE). (D) Percentage of CD3\textsuperscript{+}V\textit{β}3\textsuperscript{+} or CD3\textsuperscript{+}V\textit{β}8\textsuperscript{+} T-cell subset for each treatment group (control, black; SpeA, red; and SEB, blue). Data are shown as the mean ± SEM. Significance was determined by two-way ANOVA with Dunnett’s multiple comparison post hoc test (***P < 0.001). (D–F) B6I.IL2a mouse splenocyte IL-2 assay postvaccination with control (black circle), SpeA (red circle), or SEB (blue triangle) (n = 3 for each group). Treated mouse splenocytes were stimulated with increasing concentrations of SAg targeting specific T-cell variable \textit{β}-chain (V\textit{β}) subsets (D) SpeA, V\textit{β}8; (E) SEB, V\textit{β}8; and (F) SmeZ, V\textit{β}11. Stimulation occurred for 18 h and culture supernatants were analyzed for IL-2 using ELISA as a readout for T-cell activation. Data are shown as the mean ± SEM. Significance was determined by two-way ANOVA with Tukey’s post hoc test on the highest (10\textsuperscript{6} pg mL\textsuperscript{-1}) concentration tested (***P < 0.001; **P < 0.01).
MGAS8232 requires T cells to efficiently infect nasopharyngeal tissue, and additionally, the presence of SAg-responsive T cells results in a proinflammatory environment, whereas S. pneumoniae could persist in the nasopharynx regardless of T cells.

**Discussion**

T lymphocytes are central components of the adaptive immune system, and through the extreme diversity of TCRs, these cells can recognize a virtually unlimited assortment of microbial peptides when presented by MHC molecules. Despite the variability of TCRs through variable (V), diversity (D), and joining (J) segment [V(D)J] recombination, the specific and polymorphic nature of MHC-II molecules, the SAg exotoxins have managed to evolve to recognize both of these highly diverse adaptive immune receptors, forcing the activation of numerous Vβ-specific T cells, and thus altering the course of the immune response. However, mechanisms by which SAg-mediated manipulation of the adaptive immune system contributes to the benefit of S. pyogenes, and other SAg-producing microbes, is not well understood. Herein, we present evidence that S. pyogenes requires functional, Vβ-specific T-cell populations to promote an environment that dramatically enhances the early stages of nasopharyngeal infection by this globally important pathogen.

Not surprisingly, T lymphocytes are beneficial to the host in numerous infection models including *Mycobacterium tuberculosis* (29), *Haemophilus influenzae* (30), *Salmonella enterica* serovar *Typhimurium* (31), and *Listeria monocytogenes* (28). Although active immunity to *S. pneumoniae* nasopharyngeal infection has been shown to be dependent upon CD4+ T cells (32), our control T-cell depletion experiments did not overly influence *S. pneumoniae* cfus by 48 h (Fig. 4E). This was expected as the mice were naïve to *S. pneumoniae*, and this bacterium is not known to produce SAgS. However, in the absence of functional Vβ-specific T cells (Fig. 3D-F), or in the absence of both CD4+ and CD8+ T cells (Fig. 4 B and C), cfus of *S. pneumoniae* MGAS8232 were dramatically reduced by approximately three orders of magnitude (Figs. 2E and 4D). Additionally, removal of CD8+ T cells alone impaired nasopharyngeal infection. As SAgS activate both

SAg-Responsive T Cells Are Required for Nasopharyngeal Inflammation by *S. pyogenes*, but Not *S. pneumoniae*. We previously demonstrated that nasopharyngeal infection by *S. pyogenes* induces a SAg-driven inflammatory environment at 24 h within the cNT that appears to promote infection (17). To further assess differences between the T-cell-depleted mice, we conducted a cytokine/chemokine array from cNT homogenates. As predicted, in uninfected mice there was no apparent inflammatory signature (Fig. S4 and Fig. S5), whereas infection in the presence of T cells (isotype control) generated a proinflammatory environment that correlated with high bacterial load (Fig. 5B and Fig. S3). However, depletion of CD4+ or CD8+ T cells reduced the inflammatory signature, while remarkably, depletion of both CD4+ and CD8+ T cells largely resembled uninfected control mice (Fig. 5B and Fig. S3). Interestingly, infection with *S. pneumoniae* induced a comparatively moderate inflammatory environment, which was exaggerated in T-cell-depleted mice (Fig. 5C and Fig. S3). To confirm these findings, we also conducted the cytokine/chemokine array from mice vaccinated with wild-type SpeA or wild-type SEB. Similar to the T-cell depletion experiments, sham-vaccinated mice induced a strong inflammatory signature, whereas both SAg-vaccinated groups resembled the uninfected control group (Fig. 5D). Remarkably, these collective results indicate that *S. pyogenes*
CD4+ and CD8+ T cells in a Vβ-specific manner, we suspect that although both cells likely contribute to the phenotype, CD8+ T cells may be more numerically dominant within this environment (Fig. 4C). Alternatively, CD8+ T cells may be functionally more important for this phenotype. To assess how general this T-cell-dependent phenotype is for different S. pyogenes strains, we evaluated two additional strains that encode speA, including S. pyogenes 5448 and MGAS315. The M1 serotype S. pyogenes 5448, surprisingly, did not efficiently infect the B6HlaL mice (Fig. S4A), although we could not detect SpeA expression from this background (Fig. S4B), likely due to degradation from high levels of the SpeB cysteine protease produced by this strain (33). S. pyogenes MGAS315, however, which does produce SpeA (Fig. S4B), infected higher than MGAS8232, although depletion of T cells from the B6HlaL mice trended to reduce infection by only ~1 log (Fig. S4A). The B6HlaL mouse infection model, accordingly, does not have limitations where the majority of the streptococcal SAgS are not functionally active (Fig. S5), and similarly to SpeC (24), we believe this is due to the inability of most streptococcal SAgS to target mouse Vβ8s. Thus, although all S. pyogenes isolates may not require SAg-responsive T cells in this mouse model, we do predict that SAgS other than SpeA would likely contribute to human nasopharyngeal infection, and it remains to be determined if and which SAgs when targeted would afford the most protection in different environments.

SAgs have long been recognized for the ability to suppress antibody production (34, 35), which occurs in part through T-cell- and Fas–FasL-dependent apoptosis of B cells (36, 37). Although the lack of anti-SpeA antibodies in the wild-type SpeA-vaccinated mice was therefore not unexpected (Fig. 2F), we were initially surprised by the low cfus in wild-type SpeA-vaccinated mice (Fig. 2E). However, as we have detected activation of SpeA-targeted Vβ8 T cells in vivo during nasopharyngeal infection by S. pyogenes (38), and since SAg exposure is known to induce Vβ-specific T-cell unresponsiveness (25, 39), we reasoned that T cells require Vβ-specific T cells to remodel the nasopharyngeal environment. This prediction was supported by two different experimental approaches, including the wild-type SEB vaccination experiments (Fig. 2E), and the T-cell depletion experiments (Fig. 4D). These findings are also entirely consistent with our previous work where host expression of human MHC-II (HLA-DA), and expression of SpeA (17), were similarly critical for efficient infection by S. pyogenes MGAS8232.

Cytokine and chemokine analysis demonstrated that in the absence of T-cell function, when the S. pyogenes bacterial load was high (Fig. 4D), the nasopharyngeal environment was rich in proinflammatory cytokines and chemokines (Fig. 5 B and D and Fig. S3). Remarkably, in wild-type SpeA- or SEB-vaccinated mice (Fig. 5D), or CD4/CD8-depleted mice (Fig. 5B), the cytokine/chemokine profile phenocopied the infected control mice (Fig. 5A). T-cell depletion did not impact significantly on nasopharyngeal S. pneumoniae cfus, although an increased trend was noted in the T-cell–depleted mice (Fig. 4E) that was accompanied by an enhanced proinflammatory cytokine signature (Fig. 5C). Thus, the inflammatory signature was entirely consistent with the relative cfus obtained from either pathogen. If a pathogen can avoid mucociliary clearance mechanisms, one of the first steps for nasopharyngeal colonization is attachment to the underlying epithelial surfaces (40). However, binding to epithelial surfaces would be expected to engage multiple pattern recognition receptors, resulting in cytokine production (41). Thus, it appears that in the absence of SAg-driven T-cell activation, S. pyogenes cannot initiate even the earliest steps of nasopharyngeal colonization. It is tempting to speculate that this inflammatory response, per se, could provide a suitable environment that allows S. pyogenes to survive and proliferate, at least in an acute setting.

This work supports the development and testing of toxoid SAgS as vaccine candidates. The majority of previous streptococcal SAg vaccine research has focused on the generation of anti-SAg antibodies for protection against sepsis and toxic shock syndrome (20, 21, 42). This concept has had clinical implications, whereby administration of i.v. immunoglobulins, which contains SAg-neutralizing antibodies (43), have been demonstrated to reduce patient mortality in some settings (44–46). The passive immunization experiments show conclusively that anti-SAg antibodies can be protective against experimental S. pyogenes nasopharyngeal infection (Fig. 1). However, the current most promising S. pyogenes vaccines target the M protein, a surface-anchored virulence determinant and multiple variations are currently in early clinical trials (3). However, an impediment for these vaccines is the hypervariability of the M protein with over 200 streptococcal emm types and differential distributions worldwide (47), making a universally protective vaccine based solely on this molecule challenging. S. pyogenes SAgs are usually encoded on mobile, or putatively mobile, bacteriophage elements and thus different strains of S. pyogenes often encode different combinations of SAgs (48). Although streptococcal SAgs, in most cases, are immunologically distinct (17), this repertoire to date appears to be limited to 14 SAgs (5). Consequently, we believe that SAgs should receive renewed consideration for inclusion within a multicomponent vaccine.

Many important upper respiratory tract pathogens exist predominantly within a state of asymptomatic colonization (40), and thus a number of bacterial “virulence” factors have likely evolved under selective pressures outside circumstances of overt disease, and may more accurately function as “colonization” factors. Our data provide a mechanism whereby SAgs target and activate Vβ-specific T cells to remodel the nasopharyngeal environment to promote the earliest stages of colonization. Indeed, in the absence of a functional SAg, an appropriate MHC-II receptor, or functional Vβ-specific T cells, S. pyogenes fails to colonize and multiply. The specific immunological changes induced by SAgs that are beneficial to S. pyogenes infection remain to be characterized, although we favor a T-cell–driven inflammatory environment necessary for colonization that may allow for the exposure of host cells’ bindings sites, impairment of innate immune responses, and/or enhanced acquisition of nutrients in the nutrient-poor nasopharyngeal environment. Overall, this work further supports SAgs as prophylactic vaccines to target the carriage state of this important and human-specific pathogen, as well as furthers our understanding of these toxins outside of the context of severe and invasive disease.

**Materials and Methods**

**Bacteria.** S. pyogenes strains MGAS8232, 5448, and MGAS315, and S. pneumoniae strain 11/212, were used for the nasal infection experiments. Further experimental details are provided in SI Materials and Methods.

**Mice.** C57BL/6 mice expressing human major histocompatibility complex II molecules (H-2D, H-2K, and H-2DQ) have been previously described (14, 49, 50). HLA-DQ8 and HLA-DRQ8 mice were infected equally well with S. pyogenes MGAS8232 compared with C57BL/6 (Fig. S6) and henceforth, both were used in experiments and labeled B6HlaL. Further experimental details on mouse experiments are provided in SI Materials and Methods.

**Recombinant SAg and Antibody Production.** Details on protein expression and purification, and antibody production, are provided in SI Materials and Methods.

**Molecular Modeling.** Details of the molecular modeling are provided in SI Materials and Methods.

**Flow Cytometry.** Details of flow cytometry analysis are provided in SI Materials and Methods.

**Mouse Cytokine/Chemokine Array.** Details of the cytokine/chemokine array experiments are provided in SI Materials and Methods.

**Statistical Analysis.** All statistical analysis was completed using Prism software (GraphPad). Significance was calculated using, where indicated, the Student’s t test and one-way or two-way ANOVA with Dunnett’s multiple comparisons post hoc test. A P value less than 0.05 was determined to be statistically significant.
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