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Chronic Exposure to Staphylococcal Superantigen Elicits a Systemic Inflammatory Disease Mimicking Lupus

Vaidehi R. Chowdhary,* Ashenafi Y. Tilahun,† Chad R. Clark, † Joseph P. Grande,‡ and Govindarajan Rajagopalan†

Chronic nasal and skin colonization with superantigen (SAg)-producing *Staphylococcus aureus* is well documented in humans. Given that *trans*-mucosal and *trans*-cutaneous absorption of SAg can occur, we determined whether chronic exposure to small amounts of SAg per se could activate autoreactive CD4+ and CD8+ T cells and precipitate any autoimmune disease without further external autoantigenic stimulation. Because HLA class II molecules present SAg more efficiently than do mouse MHC class II molecules, HLA-DQ8 transgenic mice were implanted s.c. with mini-osmotic pumps capable of continuously delivering the SAg, staphylococcal enterotoxin B (total of 10 µg/mouse), or PBS over 4 wk. Chronic exposure to staphylococcal enterotoxin B resulted in a multisystem autoimmune inflammatory disease with features similar to systemic lupus erythematosus. The disease was characterized by mononuclear cell infiltration of lungs, liver, and kidneys, accompanied by the production of anti-nuclear Abs and deposition of immune complexes in the renal glomeruli. The inflammatory infiltrates in various organs predominantly consisted of CD4+ T cells bearing TCR Vβ8. The extent of immunopathology was markedly reduced in mice lacking CD4+ T cells and CD28, indicating that the disease is CD4+ T cell mediated and CD28 dependent. The absence of disease in STAT4-deficient, as well as IFN-γ-deficient, HLA-DQ8 mice suggested the pathogenic role of Th1-type cytokines, IL-12 and IFN-γ. In conclusion, our study suggests that chronic exposure to extremely small amounts of bacterial SAg could be an etiological factor for systemic lupus erythematosus. *The Journal of Immunology*, 2012, 189: 000–000.

The existence of autoreactive T and B lymphocytes, even in healthy individuals, is well documented. In genetically predisposed individuals, activation of such ignorant autoreactive cells under appropriate conditions could precipitate an autoimmune disease (1). In this regard, bacterial superantigens (SAgs) are attractive candidates as initiators/propagators of autoimmune diseases because of their unique biological properties (1, 2). SAg are the most potent, naturally occurring biological activators of T lymphocytes. Unlike conventional Ags, SAg bind directly to cell surface MHC class II molecules outside of the peptide-binding groove and subsequently cause an MHC class II-dependent (but MHC-unrestricted), TCR Vβ-specific (but Ag-nonspecific) activation of both CD4+ and CD8+ T cells (3). The broader specificity of the SAg only to the TCR Vβ region, but not to the classical recombinatorial product of the TCR α- and β-chains, results in a robust activation of a significantly larger pool of T cells: 50–60% of the CD4+ and CD8+ T cells for SAg compared with ~1 in 10^5–10^6 cells for a conventional antigenic epitope. SAg can also activate the non-T cell compartment directly, though MHC class II (4), or indirectly, through several molecular mediators, such as cytokines and chemokines.

SAgs are produced predominantly by *Staphylococcus aureus* and *Streptococcus pyogenes*, whereas *Mycoplasma arthritidis* and *Yersinia pseudotuberculosis* are also known to produce SAg. Acute exposure to SAgs, produced, for example, during serious infections, sepsis, pneumonia, or menstrual/nonmenstrual toxic shock syndromes, results in a robust systemic immune activation leading to a sudden and massive release of several cytokines and chemokines. This process, termed systemic inflammatory response syndrome (SIRS), leads to multiple organ dysfunction syndrome and culminates in death, if not treated promptly (5, 6). Conversely, it is believed that chronic exposure to small nonlethal amounts of SAg contributes to autoimmunity, and such a mode of exposure to SAg can occur naturally in *S. aureus* carriers. About 20–30% of the normal human population are natural asymptomatic carriers of *S. aureus*, either intermittently or chronically, in their upper airways and/or skin (7–10). Molecular typing of *S. aureus* strains isolated from such asymptomatic carriers showed that a significant percentage of these strains harbor genes encoding for SAg (7, 11). In addition, the SAg gene transcripts, as well as their translational products, were demonstrated in individuals with staphylococcal carriage, strengthening the possibility that chronic/recurrent exposure to SAg can occur in such individuals (12–14). Given that SAg can be efficiently absorbed through nasal mucosa and skin (15–18), either directly or facilitated through other exotoxins, such as cytolsins (19–21), recurrent or chronic systemic exposure to extremely small amounts of...
SAg is possible in *S. aureus* carriers. This could lead to activation of the autoreactive T and B lymphocytes that exist in those individuals. Because SAgs can also activate APCs, either directly or indirectly, SAgs might provide the necessary inflammatory milieu for continued expansion of pathogenic autoreactive clones, break immune tolerance, and, thereby, contribute to autoimmunity.

Human studies showed that *S. aureus* carriage is associated with certain autoimmune diseases, such as granulomatosis with polyangiitis, multiple sclerosis, and rheumatoid arthritis, through their SAgs (22–26). However, no direct experimental evidence exists to prove that staphylococcal SAgs (SSAgs) by themselves (without the use of exogenous Ags) are capable of inducing any spontaneous autoimmune disease. Conventional laboratory mice are not suitable for such investigations because SSAg bind weakly to mouse MHC class II molecules. However, it is well established that SSAg bind more efficiently to human MHC (HLA) class II molecules (27). Therefore, we and other investigators showed that transgenic mice expressing HLA class II molecules, such as HLA-DQ6, -DQ8, or -DR3, mount a strong immune response to SSAgs and are excellent tools with which to study the immunopathogenesis of diseases caused by SAgs (15, 28–35). Because several additional knockout mice are available on the HLA-DQ8 background (15), using the HLA-DQ8 transgenic mouse model, we explored whether chronic exposure to extremely small nonlethal amounts of SSAg by itself can precipitate any autoimmune disease without immunization with any autoantigens.

**Materials and Methods**

**Mice**

HLA-DQ8 transgenic mice and HLA-DQ8 transgenic mice lacking CD4+ T cells (HLA-DQ8.CD4g), CD8+ T cells (HLA-DQ8.CD8g), STAT4 (DQ8.STAT4g), STAT6 (DQ8.STAT6g), and CD28 (DQ8.CD28g) were described previously (30). DQ8 transgenic mice deficient for IFN-γ (DQ8.IFN-γ) or the absence of the IFN-γ gene, and the presence of transgenic HLA-DQ8 molecules. Mice of the required genotype were intercrossed for several generations to establish the DQ8.IFN-γ line. Mice were bred within the barrier facility of the Mayo Clinic Immunogenetics Mouse Colony and moved to a conventional facility after weaning. All of the individuals within the barrier facility of the Mayo Clinic Immunogenetics Mouse Colony were typed for the absence of endogenous mouse MHC class II molecules, the absence of mouse MHC class II molecules, the absence of the IFN-γ gene, and the presence of transgenic SSAg molecules (27). Therefore, we and other investigators showed that transgenic mice expressing HLA class II molecules, such as HLA-DQ6, -DQ8, or -DR3, mount a strong immune response to SSAgs and are excellent tools with which to study the immunopathogenesis of diseases caused by SAgs (15, 28–35). Because several additional knockout mice are available on the HLA-DQ8 background (15), using the HLA-DQ8 transgenic mouse model, we explored whether chronic exposure to extremely small nonlethal amounts of SSAg by itself can precipitate any autoimmune disease without immunization with any autoantigens.

**Immunofluorescence**

Immediately following sacrifice as discussed above, tissues from mice were also collected in optimal cutting compound (Sakura Finetek Tissue-Tek) and stored frozen at −80°C. Five-micrometer sections were cut using a cryostat, fixed in cold acetone, and stained with fluorochrome-conjugated Abs as per standard techniques. Sections were mounted using SlowFade Gold Antifade Reagent with DAPI (Invitrogen) and were analyzed using an Olympus AX70 research microscope (Olympus America, Center Valley, PA). Images were acquired using an Olympus DP70 camera.

**Detection and quantification of autoantibodies**

Anti-nuclear Abs were determined using HEp-2 cells, per the manufacturer’s protocol (Bio-Rad), at 1 in 50 dilution. Binding of mouse autoantibodies was detected using a FITC-conjugated goat anti-mouse Ab (Jackson ImmunoResearch). Abs to dsDNA, Smith Ag (Sn), and ribonucleoprotein were determined using separate ELISA kits for each of these autoantigens (Alpha Diagnostic International, San Antonio, TX), following the protocol provided by the supplier.

**Statistics**

All analyses were performed using GraphPad Prism (version 3.0a; San Diego, CA). Parametric testing between two unmatched groups was performed by the unpaired t test; p values < 0.05 were considered significant.

**Results**

**Chronic immune activation with SEB results in production of autoantibodies, immune complex glomerulonephritis, and multiorgan pathology**

All sera from HLA-DQ8 transgenic mice (8/8) implanted with SEB pumps contained anti-nuclear Abs when tested using HEp-2 cells (Fig. 1A), whereas sera from mice implanted with PBS pumps showed no positive staining (0/6 mice). 5.7M mice implanted with SEB pumps also had significantly elevated levels of anti-dsDNA and anti-Sm Abs but did not develop anti-ribonucleoprotein Abs (Fig. 1B). Kidneys from mice implanted with SEB pumps showed pathological changes in the glomeruli, consistent with the World Health Organization class II classification for human lupus nephritis. In addition, mononuclear cell infiltration into periglomerular, perivascular, and tubulointerstitial regions was seen (Fig. 1C). Lungs also showed infiltration with mononuclear cells in peribronchial and perivascular regions (Fig. 1C). Liver sections showed extensive perivascular, as well as periportal, infiltration (Fig. 1C). As expected, organs from PBS pump mice were devoid of any inflammatory infiltrates (Fig. 1C). The mean organ pathology in SEB...
pump-implanted HLA-DQ8 mice was 1.5 (Supplemental Fig. 4). Immunohistochemical staining showed that the organ infiltrates largely consisted of CD4+ T cells, followed by CD8+ T cells, few CD11b+ cells, and few to no B cells in the liver (Fig. 2A), kidneys (Fig. 2C), and lungs (data not shown). A significant majority of the CD4+ T cells expressed TCR V\beta8, indicating that these T cells

FIGURE 1. Chronic exposure to SAg elicits autoantibody production and multiorgan pathology. HLA-DQ8 transgenic mice implanted s.c. with 28-d mini-osmotic pumps delivering either PBS or SEB (10 μg) were killed 30 d after implantation. Sera were tested for the presence of anti-nuclear Abs using HEp-2 cells followed by staining with FITC-conjugated goat-antimouse IgG [representative images are shown in (A)] and for the presence of specific autoantibodies by ELISA (B). (C) Sections from kidneys, lungs, and livers were stained with H&E and evaluated microscopically. Representative images at lower (left panels) and higher (right panels) magnifications are shown. Scale bars, 100 μM.

FIGURE 2. Chronic exposure to SAg causes T lymphocyte infiltration in liver and kidney. Frozen liver (A, B) and kidney (C, D) sections from HLA-DQ8 transgenic mice, implanted with 28-d mini-osmotic pumps delivering either PBS or SEB (10 μg), obtained 30 d after implantation were stained with the indicated Abs. (A) Representative images from PBS- and SEB-treated mice are shown. (B) Liver sections were stained with anti-CD4 (RPE) and anti-TCR V\beta8 (FITC), and the images were overlaid. (C) Kidney sections showing periglomerular (left panels) and perivascular (right panels) infiltration with CD4+ and CD8+ T cells in SEB pump-, but not PBS pump-, implanted mice. (D) Kidney sections from SEB pump-implanted mice showing complement, C3, and immune complex deposition. Scale bars, 100 μM.
were activated by SEB (Fig. 2B). Kidneys from mice implanted with SEB pumps also showed deposition of IgG and C3 complement in the glomeruli (Fig. 2D). Overall, chronic exposure to SEB elicited autoantibody production and a systemic inflammatory disease.

**Chronic exposure to SEB results in expansion of TCR Vβ8+ T cells**

HLA-DQ8 transgenic mice implanted with SEB pumps showed splenomegaly and had significantly elevated total splenocyte numbers compared with HLA-DQ8 mice implanted with PBS pumps (160 ± 20 and 83.8 ± 8.8 million splenocytes in SEB and PBS pump-implanted mice, respectively, n = 6). The percentages, as well as the absolute numbers, of total CD4+ T cells (Fig. 3A, 3B) were increased in SEB pump-implanted mice. The absolute numbers of SEB-reactive CD4+ TCR Vβ8+ T cells were increased by 10-fold, with no significant changes in the SEB nonreactive CD4+ TCR Vβ6+ T cell subset. With respect to the CD8+ T cell population, the total and TCR Vβ6+ CD8+ T cells were similar between PBS- and SEB-treated mice; surprisingly, the percentage and absolute numbers of TCR Vβ8+ CD8+ T cell subsets were significantly reduced in the spleens of SEB-treated mice. Although the CD8+ T cell numbers were lower in the spleens of SEB-treated mice, they were still seen in the organs (Fig. 2). This could be attributed to preferential migration of activated CD8+ T cells to the organs (40). Mice implanted with SEB pumps also had more B cells and macrophages in the spleens, but this difference was not statistically significant (Fig. 3C). We also enumerated Foxp3+ cells, a marker for regulatory T cells (Tregs), by flow cytometry. As shown in Supplemental Fig. 1, chronic stimulation with SEB also resulted in a significant expansion of total CD4+Foxp3+ T cells, as well as TCR Vβ8+ Foxp3+ Tregs, in the spleen.

**Activation profiles of T cells**

Concomitant with the observed pathology and increase in splenocyte count, the expression profiles of certain activation markers (such as CD44 and GL7) on T cells were appreciably increased in the SEB-treated group, suggesting the presence of significantly higher numbers of activated/memory T cells in these mice (Supplemental Fig. 1B). However, the expression profiles of PD-1 and several chemokine receptors, such as CXCR3, CXCR4, CXCR5, and CCR7, were not significantly different between PBS and SEB pump-implanted mice (data not shown).

**Serum cytokine profile in mice chronically exposed to SEB shows a trend toward increased IL-12 p40**

We showed on several occasions that acute exposure to a single dose of SEB (10 μg) through different routes results in a significant elevation in systemic levels of multiple cytokines/chemokines (SIRS), and such animals display symptoms of toxic shock syndrome (15, 28, 29, 31, 33). We anticipated that administration of the same dose of SEB chronically over a 4-wk period would not elicit a similar spike in systemic cytokine/chemokine levels. Consistent with this hypothesis, we did not see any marked elevation in the systemic levels of various cytokines and chemokines in mice implanted with SEB pumps. However, there was a trend toward an increase in serum levels of IL-12p40 (p = 0.06) (Supplemental Fig. 1C). As expected, mice implanted with SEB pumps also did not display any apparent symptoms of SIRS or toxic shock syndromes (e.g., hypothermia, weight loss, diarrhea, or mortality).

**Critical role for CD4+ T cells in disease pathogenesis**

SAgs are known to activate both CD4+ and CD8+ T cell subsets as long as they express the appropriate TCR Vβ. However, it is well known that CD4+ and CD8+ T cells perform different functions. Therefore, we next performed a series of experiments to delineate the roles of CD4+ and CD8+ T cell subsets in the pathogenesis of systemic inflammatory disease caused by chronic exposure to SEB. DQ8.CD4- mice implanted with SEB pumps had pronounced inflammatory infiltrates in the liver (Fig. 4A), kidneys (Fig. 4B), and lungs (data not shown). Immunohistohemical analyses showed an intense infiltration of CD4+ T cells in the liver (Fig. 4A), kidney (Fig. 4B), and lungs (data not shown) in DQ8.CD8+ mice. In the kidneys, periglomerular and perivascular infiltrates were seen along with tubular necrosis (Fig. 4B). Surprisingly, DQ8.CD4+ mice showed little or no organ inflammation (Fig. 4) and only very few or no CD8+ T cells were seen in the organs of mice.
DQ8.CD4˚ mice in concordance with minimal histopathological changes (Fig. 4, data not shown). The cumulative organ pathology in SEB pump-implanted DQ8.CD4˚ and DQ8.CD8˚ mice is shown in Supplemental Fig 4. In addition, in DQ8.CD8˚ mice, significant mononuclear cell infiltration could be seen in the thyroid and the heart (Fig. 5). In the heart, perivascular and diffuse mononuclear infiltration within the myocardium was seen (Fig. 5).

With respect to the T cell numbers in the spleen, SEB-treated DQ8.CD8˚ mice had significant splenomegaly (Supplemental Fig. 2A, inset) and showed significant expansion of TCR Vβ8˚ CD4˚ T cells. However, in SEB-treated DQ8.CD4˚ mice, although the total and TCR Vβ8˚ CD8˚ T cells were increased, the extent of splenomegaly and the fold increase in TCR Vβ8˚ T cells were not as pronounced (Supplemental Fig. 2A). Sera from DQ8.CD4˚ and DQ8.CD8˚ mice implanted with PBS or SEB pumps were tested for anti-nuclear Abs using HEp-2 cells. Although sera from SEB-treated DQ8.CD8˚ mice showed strong reactivity to HEp-2 cells, sera from SEB-implanted DQ8.CD4˚ mice showed no reactivity. Overall, there was a good concordance with the ANA profiles and immunopathology findings (Supplemental Fig. 3A). Taken together, we conclude that, although SEB is capable of activating both CD4˚ and CD8˚ T cell subsets, CD4˚ T cells are required for autoantibody production and to manifest the systemic inflammatory disease, whereas CD8˚ T cells are dispensable.

**IL-12- and IFN-γ-dependent pathways play a major pathogenic role in multiorgan pathology following chronic stimulation with SEB**

We next determined the role of Th1 and Th2 cytokine pathways in disease pathogenesis using DQ8.STAT4˚ and DQ8.STAT6˚ mice. STAT4˚ mice are known to have major defects in Th1-type T cell responses and to have exaggerated Th2-type T cell responses. Conversely, STAT6˚ mice have major defects in mounting Th2-type T cell responses and, hence, mount exaggerated Th1-type T cell responses (41).

Interestingly, liver and kidney sections from SEB-treated DQ8.STAT6˚ mice showed extensive inflammation, similar to DQ8.CD8˚ mice, whereas DQ8.STAT4˚ mice showed little or no inflammation, similar to DQ8.CD4˚ mice (Fig. 6). The cumulative organ pathology in SEB pump-implanted DQ8.STAT4˚ and DQ8.STAT6˚ mice is shown in Supplemental Fig 4. Immunofluorescent staining revealed the presence of large numbers of CD4˚ T cells and fewer CD8˚ T cells or B cells in the liver and kidneys from

**FIGURE 4.** Role of T cell subsets in multiorgan pathology elicited by chronic exposure to SAg. HLA-DQ8, HLA-DQ8.CD4˚, and HLA-DQ8.CD8˚ transgenic mice implanted s.c. with 28-d mini-osmotic pumps delivering either PBS or SEB (10 μg) were killed 30 d after implantation. Sections from livers (A) and kidneys (B) were stained with H&E, or cryosections of these tissues were stained with the indicated Abs. Representative images are shown. Scale bars, 100 μM.

**FIGURE 5.** Myocarditis and thyroiditis following chronic exposure to SAg. Formalin-fixed or frozen sections from thyroid and heart tissues from HLA-DQ8.CD8˚ transgenic mice implanted with 28-d mini-osmotic pumps delivering either PBS or SEB (10 μg) were stained with H&E or the indicated Abs. Representative images are shown. Scale bars, 100 μM.
DQ8.STAT6° mice. In contrast, DQ8.STAT4° liver/kidney showed very little or no lymphocyte infiltration (Fig. 6, data not shown). In support of these findings, SEB-treated DQ8.STAT6° mice had profound splenomegaly (Supplemental Fig. 2B, inset), along with significant expansion of CD4° TCR Vβ8° T cells, whereas spleens from SEB-treated DQ8.STAT4° mice were not significantly enlarged and showed only a moderate increase in TCR Vβ8° T cells (Supplemental Fig. 2B). Sera from DQ8.STAT6° mice implanted with SEB, but not PBS, pumps showed strong reactivity to HEp-2 cells, whereas sera from SEB-implanted DQ8.STAT4° mice showed no reactivity to HEp-2 cells, showing good concordance with the histopathological findings (Supplemental Fig. 3A).

The absence of autoantibodies and minimal multiorgan pathology in STAT4 knockout mice suggested that IL-12 plays a very important role in the immunopathogenesis. Because IFN-γ is the key Th1-type cytokine induced by the IL-12/STAT4 pathway, we next investigated the role of IFN-γ in the chronic systemic inflammatory disease using DQ8.IFN-γ° mice. As can be seen from Supplemental Fig. 3B, the absence of kidney and liver pathology in DQ8.IFN-γ° mice is consistent with the results in DQ8.STAT4° mice. Taken together, these data suggested that Th1 cytokines (IFN-γ) and STAT4-dependent proinflammatory signals are required for driving T cell expansion and autoantibody production and for eliciting immunopathology, whereas STAT6-dependent signals are anti-inflammatory.

**CD28 costimulation is required for pathology**

We next investigated the role of CD28 costimulation in eliciting organ pathology following chronic immune activation with SSAgs. Organ pathology was absent in DQ8.CD28° mice chronically exposed to SEB, similar to DQ8.CD4°, DQ8.STAT4°, and DQ8.IFN-γ° mice (Supplemental Fig. 3B), indicating that the disease is also dependent on CD28 costimulation. SEB-induced expansion of TCR Vβ8° T cells was also severely curtailed in the absence of CD28 costimulation (data not shown), suggesting an important role for CD28 costimulation in this process.

Overall, our results showed that chronic exposure to the SSAg, SEB, resulted in a CD4° T cell-dependent, CD28-dependent, IFN-γ-dependent, Th1-mediated multisystem autoimmune disease mimicking human systemic lupus erythematosus (SLE) (Fig. 7).

**Discussion**

SAs, both bacterial and viral, are attractive candidates as inducers/propagators of autoimmunity because of their unique properties (42). Among them, SSAgs are particularly important because *S. aureus* strains capable of producing SAg not only colonize apparently healthy individuals, they even produce SAg locally (12–14), thereby strongly substantiating the possibility that chronic or recurrent exposure to SSAg can occur naturally and contribute to autoimmunity. Several human studies support a role for *S. aureus*
carriage and their SAg in the etiopathogenesis of certain autoimmune diseases (22–26, 43, 44). However, a direct causal role for SSAGs per se as triggers for autoimmune diseases has not been unequivocally established. Our study shows for the first time, to our knowledge, that chronic exposure to SSAgs by itself, without the administration of any exogenous Ags, is sufficient to trigger a systemic autoimmune inflammatory disease.

We postulate that, in our model, SEB released continuously from the mini-osmotic pump binds to HLA-DQ8 molecules on various APC and chronically activates Vβ8-bearing CD4+ and CD8+ T cells of diverse Ag specificities, including the self-reactive clones. When such SAg-primed, self-reactive T cells encounter their cognate self-antigens presented in a classical manner by professional APCs, they become effector cells, release proinflammatory cytokines (e.g., IFN-γ and IL-12), and cause immunopathology. They also provide help to autoreactive B cells to produce auto-antibodies in the following manner (Fig. 7). B cells are excellent presenters of SAg because they express high levels of MHC class II molecules. If the B cells presenting SEB happen to be self-reactive, then they would receive primary activation signals through their BCR when they bind to self-antigens (e.g., dsDNA, Sm) and, at the same time, receive costimulatory signals through CD40–CD40L and B7–CD28 pathways from the Vβ8-bearing CD4+ T cells that are concomitantly recognizing HLA–DQ8–SEB complexes presented by such B cells (45, 46). This would lead to full-fledged activation of these B cells and their differentiation into plasma cells, as well as production of auto-antibodies. However, non-self-reactive B cells presenting the SAg will not undergo such activation and differentiation into Ab-producing plasma cells, because they fail to receive the primary activating signals through their BCR. Hence, there is a preferential activation and expansion of autoreactive T and B cells driven by SSAgs and self-antigens (Fig. 7). Because it is a characteristic of activated T cells to migrate into tissues in search of their cognate Ags (47), there is extensive infiltration of multiple organs with T lymphocytes in our model.

SAgs are known to directly signal through MHC class II molecules on APCs, such as macrophages and dendritic cells, resulting in IL-1 and IL-12 production, thereby driving the Th1 pathway (4, 48, 49). Increased production of IL-12 and other Th1 cytokines, such as IFN-γ, is well documented in systemic autoimmune inflammatory diseases, such as lupus (50). More pronounced im-

**FIGURE 7.** Model depicting how chronic exposure to SAg could elicit an autoimmune disease. Chronic colonization with SAg-producing *S. aureus* strains occurs in many individuals. Under suitable conditions, such colonizing strains produce one or more SAgs, which will be absorbed and bind to HLA class II molecules on B cells, as well as other APCs. This leads to activation of CD4+ and CD8+ T cells of diverse Ag specificities, including the self-reactive clones that are present in most individuals, as long as they express the appropriate TCR Vβ family preferred by those SAgs. Self-reactive T clones present among this population undergo additional rounds of activation when they encounter their cognate self-antigens presented in a classical manner by the professional APCs, amplifying autoimmunity. Because SAgs can directly signal through MHC class II molecules on professional APCs, they can induce IL-1 and IL-12 production, thereby skewing toward the proinflammatory Th1-type pathway. Self-reactive B cells presenting SAg would get signals through their BCR, as well as receive costimulatory signals through the CD40–CD40L and B7–CD28 pathways from the CD4+ T cells recognizing the SAg. Such B cells would proliferate, differentiate into plasma cells, and secrete auto-antibodies. Non-self-reactive B cells presenting SAgs would fail to undergo such activation and differentiation into plasma cells, because they would not receive signaling through their BCR. Several bacterial (e.g., qualitative and quantitative nature of the SAg produced, chronicity of colonization), host (genetic and nongenetic factors, such as providing conducive milieu to the colonizing strains to elaborate SAg), and other environmental factors may determine the overall outcome.
mucopathology in DQ8.8TAT6 mice and the absence of disease in DQ8.8TAT4 and DQ8.8FN-γ mice support this hypothesis. Because SAg binds to HLA class II molecules with much higher affinity than to mouse MHC class II molecules, SAg might be able to activate APCs and T cells more efficiently in humans (and in HLA class II transgenic mice), create a more proinflammatory milieu, and contribute to autoimmunity, rather than induce anergy. The unique signaling pathways used by SAgS may also promote the development of pathogenic T cells and render them less susceptible to Treg-mediated immune regulation (51).

SEB and other SAgS activate both CD4+ and CD8+ T cells. However, in our model, CD4+, but not CD8+, T cells are pathogenic and might shape the final outcome. Autoimmune diseases and that several host and bacterial determinants of CD8+ T cells is known to worsen the severity of SLE and several autoimmune diseases (54, 55). CD8+ T cells could downregulate an immune response by several mutually nonexclusive mechanisms (56). These include the CD8+CD122+ Tregs, CD8+ inhibitory T cells (54), and Qa-1-restricted CD8+ T cells (57). We are currently exploring these pathways in our model.

In conclusion, we demonstrate in this study that chronic exposure to SAgS, which could occur in certain S. aureus carriers, can lead to a systemic inflammatory disease characterized by lymphoproliferation, autoantibody production, and immune complex-mediated nephritis. Interestingly, these features are characteristic of SLE in humans. In this context, an association between S. aureus and human lupus was documented in many studies. Lupus patients have high levels of Abs to staphylococcal lipoteichoic acid (58) and staphylococcal DNA (59). Induction or flare of SLE and cutaneous allergic skin inflammation.

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Disclosures

The authors have no financial conflicts of interest.

References


**Supplementary Figure Legend:**

**Supp. Fig 1.** Treg cells, T cell activation markers and serum cytokine/chemokine profiles in mice chronically exposed to SAg. HLA-DQ8 transgenic mice implanted with 28-day mini osmotic pumps delivering either PBS or SEB (10 μg) were killed 30 days after implantation. (A) Distribution of FoxP3+ T cells in the spleens. Top and bottom panels represent percentage and absolute numbers, respectively. (B) Expression profiles of activation markers in splenic CD4+ and CD8+ T cell subsets. (i) CD44 and (ii) GL7 or isotype control (IC). (C) Serum cytokine and chemokines profiles as quantified by multiplex assay. Each bar represents mean±SE from 4-6 mice per group.

**Supp. Fig 2.** CD4+ and CD8+ T cell homeostasis in mice chronically administered SAg and the role of STAT4, STAT6 signaling. (A) HLA-DQ8, HLA-DQ8.CD4o and HLA-DQ8.CD8o transgenic mice were subcutaneously implanted with mini osmotic pumps delivering either PBS or SEB (10 μg) over a 4-week window. Thirty days after implantation, spleens were collected and distribution of different cell types was determined by flow cytometry. (B) HLA-DQ8, HLA-DQ8.STAT4o and HLA-DQ8.STAT6o transgenic mice were treated with SEB or PB as above and splenocytes analyzed. Each bar represents mean±SEM from 4-6 mice per group. Insert shows spleen sizes from one experiment. * represents p<0.05 when compared to corresponding PBS-treated group.
**Supp. Fig 3.** Role of T cell subsets, STAT and CD28 signaling in systemic inflammatory disease caused by chronic exposure to SEB. (A) Sera collected from HLA-DQ8, HLA-DQ8.CD4°, HLA-DQ8.CD8°, HLA-DQ8.STAT4° and HLA-DQ8.STAT6° mice implanted with 4-week SEB or PBS pumps were tested for autoantibodies using HEp-2 cells. Human positive and negative controls supplied along with kit were also used. Representative images are shown. (B) Paraffin embedded sections from HLA-DQ8.CD28° and HLA-DQ8.IFN-γ° transgenic mice implanted with 28-day mini osmotic pumps delivering SEB (10 μg) were stained with H&E. Sections from SEB-treated DQ8 mice are shown for comparison.

**Supp. Fig 4.** Scoring system to quantify the extent of inflammatory changes in selected organs and the cumulative organ inflammatory scores in SEB pump implanted mice. (A) The extent of inflammatory changes in selected organs was scored by reviewing H&E stained sections. Liver was scored from 0 to 4, with 0 indicating no infiltrates and 4 indicating more than 50% of the field showing infiltration. Lungs and kidneys were scored from 0 to 3 in a similar manner with 3 being the most intense infiltration. Several fields from each tissue section and tissues from three to four individual mice in each group were evaluated for scoring. (B) Graphical representation of inflammatory scores in SEB pump implanted mice.