Published in final edited form as:
J Immunol. 2012 August 15; 189(4): 2054–2062. doi:10.4049/jimmunol.1201097.

Chronic Exposure to Staphylococcal Superantigen Elicits a Systemic Inflammatory Disease Mimicking Lupus[1]

Vaidehi R. Chowdhary, MD[†,*], **Ashenafi Y. Tilahun**[‡], **Chad R. Clark**[‡], **Joseph P. Grande, MD, PhD**[§], and **Govindarajan Rajagopalan, DVM, PhD**[‡,*]

[†]Division of Rheumatology, Department of Medicine, Mayo Clinic, Rochester, MN

[‡]Department of Immunology, Mayo Clinic, Rochester, MN

[§]Division of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN

Abstract

Chronic nasal and skin colonization with superantigen (SAg)[2]-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. Since human leukocyte antigen (HLA) class II molecules present SAg more efficiently than mouse MHC class II molecules, HLA-DQ8 transgenic mice were subcutaneously implanted with miniosmotic pumps capable of continuously delivering the SAg, staphylococcal enterotoxin B (SEB, total of 10 μg/mouse) or phosphate buffered saline (PBS), over 4 weeks. Chronic exposure to SEB resulted in a multisystem autoimmune inflammatory disease with features similar to systemic lupus erythematosus (SLE). The disease was characterized by mononuclear cell infiltration of lungs, livers and kidneys, accompanied by the production of antinuclear antibodies and deposition of immune complexes in the renal glomeruli. The inflammatory infiltrates in various organs predominately consisted of $CD4^+$ T cells bearing TCR Vβ8. The extent of immunopathology was markedly reduced in mice lacking $CD4^+$ T cells and CD28, indicating 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 SLE.

Keywords

HLA class II transgenic mice; Superantigen; autoimmunity; T lymphocytes

[1]This study was supported by NIH award 5K23AR057815-02, ACR/REF career development bridge funding award and Ronald F. Kinney Executive Dean for Research Career Development Award from Mayo Foundation to VC, and Mayo Clinic Research Early Career Development Award to GR.

[2]**Abbreviations:** MODS, multiple organ dysfunction syndrome, SAg, superantigen, SSAg, Staphylococcal superantigen, SEB, staphylococcal enterotoxin B, SIRS, systemic inflammatory response syndrome, SLE, systemic lupus erythematosus, Sm, Smith antigen

[*]Corresponding Authors: Dr. Vaidehi R. Chowdhary, MD, Division of Rheumatology, Department of Medicine, Mayo Clinic, 200 First Street, SW, Rochester, MN 55905, USA. Phone: 507-284-2970, Fax: 507-284-0534, chowdhary.vaidehi@mayo.edu. Dr. Govindarajan Rajagopalan, DVM, PhD, Department of Immunology, Mayo Clinic, 200 First Street, SW, Rochester, MN 55905, USA. Phone: 507-284-4562, Fax: 507-284-1637, rajagopalan.govindarajan@mayo.edu.

Introduction

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 (SAg) are attractive candidates as initiators/propagators of autoimmune diseases because of their unique biological properties (1, 2). Superantigens (SAg) are the most potent, naturally occurring biological activators of T lymphocytes. Unlike conventional antigens, 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 antigen-nonspecific), activation of both $CD4^+$ and $CD8^+$ T cells (3). The broader specificity of the SAgs 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 to approximately 1 in 10^5 to 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.

Superantigens are produced predominantly by *Staphylococcus aureus* and *Streptococcus pyogenes,* while *Mycoplasma arthritidis* and *Yersinia pseudotuberculosis* are also known to produce SAg. Acute exposure to SAg, produced during serious infections, sepsis, pneumonia or menstrual/non-menstrual toxic shock syndromes (TSS) etc., results in a robust systemic immune activation leading to a sudden and massive release of several cytokines and chemokines. This process, termed as systemic inflammatory response syndrome (SIRS), leads to multiple organ dysfunction syndrome (MODS) and culminates in death, if not intervened promptly (5, 6). Conversely, it is believed that chronic exposure to small non-lethal 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 is 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 has shown 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 have been demonstrated in individuals with staphylococcal carriage, strengthening the possibility of 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 cytolysins (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. Since SAg can also activate the APC, either directly or indirectly, SAg might provide the necessary inflammatory milieu for continued expansion of pathogenic autoreactive clones, break immune tolerance and thereby contribute to autoimmunity.

Human studies have shown that *S. aureus* carriage is associated with certain autoimmune diseases such as granulamatosis with polyangiitis, multiple sclerosis and rheumatoid arthritis through their SAg (22–26). However, to date no direct experimental evidence exists to date to prove that staphylococcal SAg (SSAg) by themselves (without the use of exogenous antigens) are capable of inducing any spontaneous autoimmune disease. Conventional laboratory mice will not be suitable for such investigation 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 others have shown that transgenic mice expressing HLA class II molecules such as, HLA-DQ6, -DQ8 or

-DR3, mount a strong immune response to SSAg and are excellent tools to study the immunopathogenesis of diseases caused by SAg (15, 28–35). As several additional knockout mice are available on the HLA-DQ8 background (15), using HLA-DQ8 transgenic mouse model, we explored whether chronic exposure to extremely small non-lethal amounts of staphylococcal SAg by itself can precipitate any autoimmune disease without immunization with any autoantigens.

MATERIALS AND METHODS

Mice

HLA-DQ8 transgenic mice, HLA-DQ8 transgenic mice lacking $CD4^+$ T cells (HLA-DQ8.CD4°), $CD8^+$ T cells (HLA-DQ8.CD8°), STAT4 (DQ8.STAT4°), STAT6 (DQ8.STAT6°) and CD28 (DQ8.CD28°) mice have been described previously (30). DQ8 transgenic mice deficient for IFN-γ (DQ8.IFN-γ°) were generated by standard mating and genotyping procedures. Briefly, HLA-DQ8 and IFN-γ-deficient mice on a B6 background (Jackson Laboratory) were mated. Heterozygous offspring were intercrossed, their pups were typed for the absence of endogenous mouse MHC class II molecules, absence of *IFN-γ* gene and presence of transgenic HLA-DQ8 molecules. Mice of required genotype were intercrossed for several generations to establish the DQ8.IFN-γ° line. Mice were bred within the barrier facility of Mayo Clinic Immunogenetics Mouse Colony (Rochester, MN) and moved to a conventional facility after weaning. All the experiments were approved by the Mayo Clinic Institutional Animal Care and Use Committee.

Reagents, antibodies and Flow cytometry

Endotoxin-reduced, highly purified staphylococcal enterotoxin B (SEB, Toxin Laboratories, Sarasota, FL) was dissolved in PBS at 1 mg/ml and stored frozen at −80°C in aliquots. The purity of SEB was verified by SDS-PAGE followed by Coomassie blue staining and the absence of certain other staphylococcal SAg was verified using staphylococcal enterotoxin identification visual immunoassay (SET VIA™, 3M, MN, USA). The following antibodies were used for flow cytometry (BD biosciences) CD4 - GK1.5, CD8 - 53-6.7, TCR Vβ6 - RR4-7, TCR Vβ8 - F23.1, CD19 -1D3, B220 - RA3- 6B2, Mac-1 - M 1/70, CD44 - M7, CD62L - MEL-14, CD69 - H1.2F3, CD40 - 3/23, CD40L - MR1, PD-1 - TY25, CXCR3 - 1C6, CXCR4 - 2B11, CXCR5- 2G8, CCR7-3D12, GL7- GL7 and isotype control. $FoxP3^+$ T cells were enumerated using the intracellular staining kit from eBioscience (San Diego, CA). C3 and IgG deposition in kidneys were determined using anti-mouse C3 (Clone RmC11H9, Cedarlane Laboratories Ltd, Burlington, Ontario, Canada) and goat anti-mouse IgG antibodies (Jackson Immunoresearch, West Grove, PA), respectively.

Implantation of miniosmotic pump

Miniosmotic pumps (Alzet Corporation, Cupertino, CA) capable of continuous constant delivery of liquids over 4-weeks were filled with either PBS or SEB (10 μg, dissolved in PBS) as per supplier's protocol. PBS- or SEB-filled pumps were implanted subcutaneously into surgically prepared experimental mice of either sex (8 to 12-weeks old) under anesthesia as per standard procedure. Surgical incisions were closed with stainless steel wound clips, which were removed 7–10 days later.

Serum cytokine quantification and histopathology

Groups of HLA-DQ8 transgenic mice implanted with either SEB or PBS pumps were sacrificed at the end of experimental period. At the time of sacrifice, blood was collected from the mice into serum separation tubes (BD Biosciences, San Jose, California). The serum was separated and then stored frozen in aliquots at −80°C. Concentration of cytokines

was determined in the serum samples by using a multiplex bead assay using the manufacturer's protocol and their software and hardware (Bio-Plex; Bio-Rad, Hercules, California).

Tissues collected in buffered formalin were paraffin embedded, cut, and stained with H&E per standard procedure for histopathologic analysis (JG). The extent of organ inflammation was semi-quantitatively determined using a scoring system based on the percentage involvement the tissue being examined as described previously, with slight modifications (36–39) (Supp. Fig 4). For liver, H&E stained sections were viewed under the low power objective (10x) and the extent of inflammatory infiltration (periportal as well as perivenular) relative to the field size 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 on a scale of 0 to 3 in a similar manner with 3 being the most intense infiltration (Supp. Fig 4). Several fields from each tissue section and tissues from three to four individual mice in each group were evaluated for scoring.

Immunofluorescence

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

Detection and quantification of autoantibodies

Antinuclear antibodies were determined using HEp-2 cells as per manufacturer's protocol (Bio-Rad, Hercules, CA) at 1 in 50 dilution. Binding of mouse autoantibodies was detected using a FITC-conjugated goat anti-mouse antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Antibodies to double stranded (dsDNA), Smith antigen (Sm) and ribonucleoprotein (RNP) 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 unpaired t test. P values below 0.05 were considered significant.

RESULTS

Chronic immune activation with SEB results in production of autoantibodies, immune complex glomerulonephritis and multi-organ pathology

All sera from HLA-DQ8 transgenic mice (8/8) implanted with SEB pumps contained antinuclear antibodies when tested using HEp2 cells (Fig 1, panel A), while sera from mice implanted with PBS pumps showed no positive staining (0/6 mice). Mice implanted with SEB pumps also had significantly elevated levels of anti-dsDNA and anti-Sm antibodies but did not develop anti-RNP antibodies (Fig 1, panel B). Kidneys from mice implanted with SEB pumps showed pathological changes in the glomeruli consistent with WHO class II classification for human lupus nephritis. In addition, mononuclear cell infiltration in periglomerular, perivascular and tubulo-interstitial region were seen (Fig 1, panel C). Lungs also showed infiltration with mononuclear cells in peribronchial and perivascular region (Fig

1, panel C). Liver sections showed extensive perivenular as well as periportal infiltration (Fig. 1, panel C). As expected, organs from PBS pump mice were devoid of any inflammatory infiltrates (Fig 1, panel C). The mean organ pathology in SEB pump implanted HLA-DQ8 mice was 1.5 (Supp. 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 (Not shown). A significant majority of the $CD4^+$ T cells expressed TCR Vβ8 indicating that these T cells 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 $V\beta8^+$ T cells

HLA-DQ8 transgenic mice implanted with SEB pumps showed splenomegaly and had significantly elevated total splenocyte numbers compared to 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 and B) were increased in SEB pump implanted mice. The absolute numbers of SEB-reactive $CD4^+$ TCR $V\beta8^+$ T cells were increased by 10-folds with no significant changes in the SEB non-reactive $CD4^+$ TCR $V\beta6^+$ T cell subset. With respect to the $CD8^+$ T cell population, the total and TCR $V\beta6^+$ $CD8^+$ T cells were similar between PBS and SEB treated mice but surprisingly, the percentage and absolute numbers of TCR $V\beta8^+$ $CD8^+$ T cell subsets were significantly reduced in the spleens of SEB treated mice. Even though 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 (Treg) cells, by flow cytometry. As shown in Supp. Fig 1, chronic stimulation with SEB also resulted in a significant expansion of total $CD4^+FoxP3^+$ T cells as well as TCR $V\beta8^+$ $FoxP3^+$ Treg cells 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 SEB treated group suggesting the presence of significantly higher numbers of activated/memory T cells in these mice (Supp. Fig 1B). However, 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 towards increased IL-12 p40

We have shown 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-week period would not elicit a similar spike in systemic cytokine/chemokine levels. Consistent with this hypothesis, we did not see any marked elevation in systemic levels of various cytokines and chemokines in mice implanted with SEB pumps. However, there was a trend towards increase in serum levels of IL-12p40 ($p=0.06$) (Supp. Fig 1C). As expected, mice implanted with SEB pumps also did not display any apparent symptoms of SIRS and TSS (such as hypothermia, weight loss, diarrhea including any mortality).

Critical role for CD4+ T cells in the disease pathogenesis

SAg 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 $CD4^+$ 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.CD8° mice implanted with SEB pumps had pronounced inflammatory infiltrates in the liver (Fig 4A), kidneys (Fig 4B) and lungs (not shown). Immunohistohemical analyses showed an intense $CD4^+$ T cells infiltration in the liver (Fig 4A), kidney (Fig 4B) as well as lungs (not shown) in DQ8.CD8° mice. In the kidneys periglomerular as well as perivascular infiltrates were seen along with tubular necrosis (Fig 4B). Surprisingly, DQ8.CD4° mice showed little or no organ inflammation (Fig 4A and B) and only very few or no $CD8^+$ T cells were seen in the organs of DQ8.CD4° mice in concordance with minimal hispathological changes (Fig 4A and B, additional data not shown). The cumulative organ pathology in SEB pump implanted DQ8.CD4° and DQ8.CD8° mice are shown in Supp. Fig 4. In addition, in DQ8.CD8° mice, significant mononuclear cell infiltration could be seen in the in the thyroid as well as the heart (Fig 5). In the heart, perivascular as well as 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 (Supp. Fig 2A, insert) and showed significant expansion of TCR $V\beta8^+$ $CD4^+$ T cells. However, in SEB-treated DQ8.CD4° mice, even though the total as well as TCR $V\beta8^+$ $CD8^+$ T cells were increased, the extent of splenomegaly and fold-increase in TCR $V\beta8^+$ T cells were not as pronounced (Supp. Fig 2A). Sera from DQ8.CD4° and DQ8.CD8° mice implanted with PBS or SEB pumps were tested for antinuclear antibodies using HEp2 cells. While sera from SEB treated DQ8.CD8° mice showed strong reactivity to HEp2 cells, sera from SEB-implanted DQ8.CD4° mice showed no reactivity. Overall, there was a good concordance with the ANA profiles and immunopathology findings (Supp. Fig 3A). Taken together, we could conclude that while the SEB is capable of activating both $CD4^+$ and $CD8^+$ T cell subsets, the $CD4^+$ T cells are required for autoantibody production and to manifest the systemic inflammatory disease whereas the $CD8^+$ T cells are dispensable.

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

We next determined the role of Th1 and Th2 cytokine pathways in the disease pathogenesis using DQ8.STAT4° and DQ8.STAT6° mice. STAT4° mice are known to have major defects in Th1-type T cell responses and 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 are shown in Supp. 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 as well as kidneys from DQ8.STAT6° mice. On the other hand, DQ8.STAT4° liver/kidney showed very few or no lymphocytes infiltration (Fig 6 and additional data not shown). In support of these findings, SEB treated DQ8.STAT6° mice had profound splenomegaly (Supp Fig 2B insert), along with significant expansion of $CD4^+$ TCR $V\beta8^+$ T cells whereas spleens from SEB treated DQ8.STAT4° mice were not significantly enlarged and showed only moderate increase in TCR $V\beta8^+$ T

cells (Supp Fig 2B). Sera from DQ8.STAT6° mice implanted with SEB, but not PBS, pumps showed strong reactivity to HEp2 cells whereas sera from SEB-implanted DQ8.STAT4° mice showed no reactivity to HEp2 cells, showing good concordance with the histopathological findings (Supp. Fig 3A).

Absence of autoantibodies and minimal multiorgan pathology in STAT4 KO mice suggested that IL-12 plays a very important role in the immunopathogenesis. Since 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 Supp. Fig 3B, the absence of kidney and liver pathology in DQ8.IFN-γ° mice is consistent with the results with DQ8.STAT4° mice. Taken together, these data suggested that Th1 cytokines (IFN-γ) and STAT4-dependent pro-inflammatory signals are required for driving the T cell expansion, 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 SSAg. Organ pathology was absent in DQ8.CD28° mice chronically exposed to SEB similar to DQ8.CD4°, DQ8.STAT4° mice and DQ8.IFN.γ° mice (Supp. Fig 3B), indicating that the disease is also dependent on CD28 costimulation. SEB-induced expansion of TCR $V\beta8^+$ T cells was also severely curtailed in the absence of CD28 costimulation (Data not shown), suggesting the important role for CD28 costimulation in this process.

Overall, our results showed that chronic exposure to the staphylococcal SAg, SEB, resulted in a $CD4^+$ T cell-dependent, CD28-dependent, IFN.γ-dependent, Th1-mediated multi-system autoimmune disease mimicking human systemic lupus erythematosus (SLE).

DISCUSSION

Superantigens, both bacterial and viral, are attractive candidates as inducers/propagators of autoimmunity because of their unique properties (42). Among them, the staphylococcal SAg (SSAg) 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 SSAg *per se* as triggers for autoimmune diseases has not been unequivocally established. Our study shows for the first time that chronic exposure to SSAg by itself, without the administration of any exogenous antigens, 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 antigen 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 the professional APC, they become effector cells, release pro-inflammatory cytokines, such as IFN-γ and IL-12 and cause immunopathology. They also provide help to autoreactive B cells to produce autoantibodies 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 B cell receptor when they bind to self-antigens (e.g., such as dsDNA, Sm etc) and at the same time, receive costimulatory signals through CD40-CD40L and B7-CD28 pathways from the Vβ8-bearing $CD4^+$ T cells which 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, their differentiation into plasma cells and production of autoantibodies. However, non self-reactive B cells presenting the SAg will not undergo such activation and differentiation into antibody-producing plasma cells, as they fail to receive the primary activating signals through their B cell receptor. Hence, there is a preferential activation and expansion of autoreactive T and B cells driven by SAg and self-antigens (Fig 7). Since it is the property of the activated T cells to migrate into tissues in search of their cognate antigens (47), there is extensive infiltration of multiple organs with T lymphocytes in our model.

SAg are known to directly signal through MHC class II molecules on APC 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 immunopathology in DQ8.STAT6° mice and the absence of disease in DQ8.STAT4° and DQ8.IFN-γ° mice support this hypothesis. Since SAg binds to HLA class II molecules with much higher affinity than to mouse MHC class II molecules, SAg might be able activate APC and T cells more efficiently in humans (and in HLA class II transgenic mice), create a more pro-inflammatory milieu and contribute to autoimmunity, rather than inducing anergy. The unique signaling pathways utilized by SAg may also promote the development of pathogenic T cells and render them less susceptible to Treg cell-mediated immune regulation (51).

SEB and other SAg activate both $CD4^+$ as well as $CD8^+$ T cells. However, in our model, $CD4^+$ but not $CD8^+$, T cells are pathogenic. Given the importance of “helper functions” of $CD4^+$ T cells in sustaining an immune response elicited by both B cells (52) and $CD8^+$ T cells (53), it is not surprising that SAg are unable to sustain an immune response and are not able to mediate immunopathology in CD4-deficient mice. At the same time, a lack of $CD8^+$ T cells is known to worsen the severity of SLE and several autoimmune diseases (54, 55). $CD8^+$ T cells could down regulate an immune response by several mutually non-exclusive mechanisms (56). These include the $CD8^+CD122^+$ T regulatory cells, 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 SSAg, 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 has been documented in many reports. Lupus patients have high levels of antibodies to staphylococcal lipoteichoic acid (58) and staphylococcal DNA (59). Induction or flare of SLE and cutaneous lesions has been described following an infection with *S. aureus* (60). Some patients with chronic cutaneous lupus show expansion of $CD3^+$ T cell bearing TCR Vβ8.1 and Vβ13.3 in the lesional skin suggesting the involvement of SSAg at least in a subset of lupus patients (61). Nonetheless, to date a systematic study of the *S. aureus* colonization rates and the SAg profile of the colonizing strains in SLE patients has not been conducted. Our study suggests that *S. aureus* carriage might play a role in the pathogenesis of lupus or other autoimmune diseases and several host as well as bacterial determinants might shape the final outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to Prof. Chella David for HLA class II transgenic mice. We are thankful to Dr. Adam Schrum and Prof. Richard Goldsby, for a critical review of the manuscript and Dr. Shu Man Fu, for his critical comments.

References

1. Sfriso P, Ghirardello A, Botsios C, Tonon M, Zen M, Bassi N, Bassetto F, Doria A. Infections and autoimmunity: the multifaceted relationship. Journal of Leukocyte Biology. 2010; 87:385–395. [PubMed: 20015961]
2. Schiffenbauer J, Soos J, Johnson H. The possible role of bacterial superantigens in the pathogenesis of autoimmune disorders. Immunol Today. 1998; 19:117–120. [PubMed: 9540270]
3. Fraser DJ, Proft T. The bacterial superantigen and superantigen-like proteins. Immunol Rev. 2008; 225:226–243. [PubMed: 18837785]
4. Kissner TL, Ruthel G, Alam S, Ulrich RG, Fernandez S, Saikh KU. Activation of MyD88 signaling upon staphylococcal enterotoxin binding to MHC class II molecules. PLoS ONE. 2011; 6:e15985. [PubMed: 21283748]
5. Murray RJ. Recognition and management of *Staphylococcus aureus* toxin-mediated disease. Intern Med J. 2005; 35:S106–S119. [PubMed: 16271055]
6. Lappin E, Ferguson AJ. Gram-positive toxic shock syndromes. Lancet Infect Dis. 2009; 9:281–290. [PubMed: 19393958]
7. Graham PL 3rd, Lin SX, Larson EL. A U.S. population-based survey of *Staphylococcus aureus* colonization. Ann Intern Med. 2006; 144:318–325. [PubMed: 16520472]
8. Sivaraman K, Venkataraman N, Cole AM. *Staphylococcus aureus* nasal carriage and its contributing factors. Future Microbiology. 2009; 4:999–1008. [PubMed: 19824791]
9. Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR. The Human Nasal Microbiota and *Staphylococcus aureus* Carriage. PLoS ONE. 2010; 5:e10598. [PubMed: 20498722]
10. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev. 1997; 10:505–520. [PubMed: 9227864]
11. Thomas D, Chou S, Dauwalder O, Lina G. Diversity in *Staphylococcus aureus* enterotoxins. Chem Immunol Allergy. 2007; 93:24–41. [PubMed: 17369698]
12. Seiberling KA, Conley DB, Tripathi A, Grammer LC, Shuh L, Haines GK 3rd, Schleimer R, Kern RC. Superantigens and chronic rhinosinusitis: Detection of staphylococcal exotoxins in nasal polyps. Laryngoscope. 2005; 115:1580–1585. [PubMed: 16148698]
13. Van Zele T, Gevaert P, Watelet JB, Claeys G, Holtappels G, Claeys C, van Cauwenberge P, Bachert C. *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. J Allergy Clin Immunol. 2004; 114:981–983. [PubMed: 15480349]
14. Burian M, Grumann D, Holtfreter S, Wolz C, Goerke C, Bröker B. Expression of staphylococcal superantigens during nasal colonization is not sufficient to induce a systemic neutralizing antibody response in humans. Eur J Clin Microbiol Infect Dis. 2012; 31:251–256. [PubMed: 21625994]
15. Rajagopalan G, Sen M, Singh M, Murali N, Nath KA, Iijima K, Kita H, Leontovich AA, Unnikrishnan G, Patel R, David CS. Intranasal exposure to staphylococcal enterotoxin B elicits an acute systemic inflammatory response. Shock. 2006; 25:647–656. [PubMed: 16721274]
16. Rajagopalan G, Smart MK, Murali N, Patel R, David CS. Acute systemic immune activation following vaginal exposure to staphylococcal enterotoxin B--implications for menstrual shock. J Reprod Immunol. 2007; 73:51–59. [PubMed: 17070600]
17. Rajagopalan G, Smart MK, Patel R, David CS. Acute systemic immune activation following conjunctival exposure to staphylococcal enterotoxin B. Infect Immun. 2006; 74:6016–6019. [PubMed: 16988282]

18. Laouini D, Kawamoto S, Yalcindag A, Bryce P, Mizoguchi E, Oettgen H, Geha RS. Epicutaneous sensitization with superantigen induces allergic skin inflammation. J Allergy Clin Immunol. 2003; 112:981–987. [PubMed: 14610492]
19. Schlievert PM, Strandberg KL, Lin YC, Peterson ML, Leung DY. Secreted virulence factor comparison between methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*, and its relevance to atopic dermatitis. J Allergy Clin Immunol. 2010; 125:39–49. [PubMed: 20109735]
20. Iwatsuki K, Yamasaki O, Morizane S, Oono T. Staphylococcal cutaneous infections: Invasion, evasion and aggression. J Dermatol Sci. 2006; 42:203–214. [PubMed: 16679003]
21. Nishifuji K, Sugai M, Amagai M. Staphylococcal exfoliative toxins: Molecular scissors of bacteria that attack the cutaneous defense barrier in mammals. Journal of Dermatological Science. 2008; 49:21–31. [PubMed: 17582744]
22. Stegeman CA, Tervaert JW, Sluiter WJ, Manson WL, de Jong PE, Kallenberg CG. Association of chronic nasal carriage of *Staphylococcus aureus* and higher relapse rates in Wegener granulomatosis. Ann Intern Med. 1994; 120:12–17. [PubMed: 8250451]
23. Popa ER, Stegeman CA, Abdulahad WH, van der Meer B, Arends J, Manson WM, Bos NA, Kallenberg CGM, Cohen Tervaert J-W. Staphylococcal toxic-shock-syndrome-toxin-1 as a risk factor for disease relapse in Wegener's granulomatosis. Rheumatology. 2007; 46:1029–1033. [PubMed: 17409134]
24. Mulvey MR, Doupe M, Prout M, Leong C, Hizon R, Grossberndt A, Klowak M, Gupta A, Melanson M, Gomori A, Esfahani F, Klassen L, Frost EE, Namaka M. *Staphylococcus aureus* harbouring Enterotoxin A as a possible risk factor for multiple sclerosis exacerbations. Multiple Sclerosis Journal. 2011; 17:397–403. [PubMed: 21212089]
25. Jackson MS, Bagg J, Gupta MN, Sturrock RD. Oral carriage of staphylococci in patients with rheumatoid arthritis. Rheumatology. 1999; 38:572–575. [PubMed: 10402081]
26. Tabarya D, Hoffman WL. *Staphylococcus aureus* nasal carriage in rheumatoid arthritis: antibody response to toxic shock syndrome toxin-1. Ann Rheum Dis. 1996; 55:823–828. [PubMed: 8976639]
27. DaSilva L, Welcher BC, Ulrich RG, Aman MJ, David CS, Bavari S. Humanlike immune response of human leukocyte antigen-DR3 transgenic mice to staphylococcal enterotoxins: a novel model for superantigen vaccines. J Infect Dis. 2002; 185:1754–1760. [PubMed: 12085321]
28. Tilahun AY, Holz M, Wu TT, David CS, Rajagopalan G. Interferon gamma-dependent intestinal pathology contributes to the lethality in bacterial superantigen-Induced toxic shock syndrome. PLoS ONE. 2011; 6:e16764. [PubMed: 21304813]
29. Tilahun AY, Marietta EV, Wu TT, Patel R, David CS, Rajagopalan G. Human leukocyte antigen class II transgenic mouse model unmasks the significant extrahepatic pathology in toxic shock syndrome. Am J Pathol. 2011; 178:2760–2773. [PubMed: 21641398]
30. Rajagopalan G, Iijima K, Singh M, Kita H, Patel R, David CS. Intranasal exposure to bacterial superantigens induces airway inflammation in HLA class II transgenic mice. Infect Immun. 2006; 74:1284–1296. [PubMed: 16428778]
31. Rajagopalan G, Polich G, Sen MM, Singh M, Epstein BE, Lytle AK, Rouse MS, Patel R, David CS. Evaluating the role of HLA-DQ polymorphisms on immune response to bacterial superantigens using transgenic mice. Tissue Antigens. 2008; 71:135–145. [PubMed: 18086265]
32. Rajagopalan G, Smart MK, Cheng S, Krco CJ, Johnson KL, David CS. Expression and function of HLA-DR3 and DQ8 in transgenic mice lacking functional H2-M. Tissue Antigens. 2003; 62:149–161. [PubMed: 12889995]
33. Rajagopalan G, Smart MK, Krco CJ, David CS. Expression and function of transgenic HLA-DQ molecules and lymphocyte development in mice lacking invariant chain. J Immunol. 2002; 169:1774–1783. [PubMed: 12165499]
34. Sriskandan S, Unnikrishnan M, Krausz T, Dewchand H, Van Noorden S, Cohen J, Altmann DM. Enhanced susceptibility to superantigen-associated streptococcal sepsis in human leukocyte antigen-DQ transgenic mice. J Infect Dis. 2001; 184:166–173. [PubMed: 11424013]
35. Chau TA, McCully ML, Brintnell W, An G, Kasper KJ, Vines ED, Kubes P, Haeryfar SM, McCormick JK, Cairns E, Heinrichs DE, Madrenas J. Toll-like receptor 2 ligands on the

staphylococcal cell wall downregulate superantigen-induced T cell activation and prevent toxic shock syndrome. Nat Med. 2009; 15:641–648. [PubMed: 19465927]

36. Whary MT, Morgan TJ, Dangler CA, Gaudes KJ, Taylor NS, Fox JG. Chronic active hepatitis induced by Helicobacter hepaticus in the A/JCr mouse is associated with a Th1 cell-mediated immune response. Infect Immun. 1998; 66:3142–3148. [PubMed: 9632578]
37. Skerrett SJ, Liggitt HD, Hajjar AM, Ernst RK, Miller SI, Wilson CB. Respiratory epithelial cells regulate lung inflammation in response to inhaled endotoxin. American Journal of Physiology - Lung Cellular and Molecular Physiology. 2004; 287:L143–L152. [PubMed: 15047567]
38. Reilly CM, Farrelly LW, Viti D, Redmond ST, Hutchison F, Ruiz P, Manning P, Connor J, Gilkeson GS. Modulation of renal disease in MRL/lpr mice by pharmacologic inhibition of inducible nitric oxide synthase. Kidney Int. 2002; 61:839–846. [PubMed: 11849435]
39. Chowdhary VR, Grande JP, Luthra HS, David CS. Characterization of haemorrhagic pulmonary capillaritis: another manifestation of Pristane-induced lupus. Rheumatology. 2007; 46:1405–1410. [PubMed: 17576695]
40. Gebhardt T, Whitney PG, Zaid A, Mackay LK, Brooks AG, Heath WR, Carbone FR, Mueller SN. Different patterns of peripheral migration by memory CD4+ and CD8+ T cells. Nature. 2011; 477:216–219. [PubMed: 21841802]
41. Wurster AL, Tanaka T, Grusby MJ. The biology of Stat4 and Stat6. Oncogene. 2000; 19:2577–2584. [PubMed: 10851056]
42. Torres BA, Johnson HM. Modulation of disease by superantigens. Current Opinion in Immunology. 1998; 10:465–470. [PubMed: 9722924]
43. Paliard X, West S, Lafferty J, Clements, Kappler J, Marrack P, Kotzin B. Evidence for the effects of a superantigen in rheumatoid arthritis. Science. 1991; 253:325–329. [PubMed: 1857971]
44. Kallenberg CG. Pathophysiology of ANCA-associated small vessel vasculitis. Curr Rheumatol Rep. 2010; 12:399–405. [PubMed: 20878509]
45. Tumang JR, Zhou JL, Gietl D, Crow MK, Elkon KB, Friedman SM. T helper cell-dependent, microbial superantigen-mediated B cell activation in vivo. Autoimmunity. 1996; 24:247–255. [PubMed: 9147583]
46. Stohl W, Elliott JE, Linsley PS. Human T cell-dependent B cell differentiation induced by staphylococcal superantigens. J Immunol. 1994; 153:117–127. [PubMed: 7515921]
47. Berstein G, Abraham RT. Moving out: mobilizing activated T cells from lymphoid tissues. Nat Immunol. 2008; 9:455–457. [PubMed: 18425096]
48. Bright J, Xin Z, Sriram S. Superantigens augment antigen-specific Th1 responses by inducing IL-12 production in macrophages. J Leukoc Biol. 1999; 65:665–670. [PubMed: 10331496]
49. Kato M, Nakamura Y, Suda T, Ozawa Y, Inui N, Seo N, Nagata T, Koide Y, Kalinski P, Nakamura H, Chida K. Enhanced anti-tumor immunity by superantigen-pulsed dendritic cells. Cancer Immunol Immunother. 2011; 60:1029–1038. [PubMed: 21519830]
50. Tucci M, Lombardi L, Richards HB, Dammacco F, Silvestris F. Overexpression of interleukin-12 and T helper 1 predominance in lupus nephritis. Clin Exp Immunol. 2008; 154:247–254. [PubMed: 18795942]
51. Bueno C, Lemke CD, Criado G, Baroja ML, Ferguson SS, Rahman AK, Tsoukas CD, McCormick JK, Madrenas J. Bacterial superantigens bypass Lck-dependent T cell receptor signaling by activating a Galpha11-dependent, PLC-beta-mediated pathway. Immunity. 2006; 25:67–78. [PubMed: 16860758]
52. Vinuesa CG, Tangye SG, Moser B, Mackay CR. Follicular B helper T cells in antibody responses and autoimmunity. Nat Rev Immunol. 2005; 5:853–865. [PubMed: 16261173]
53. Sun JC, Bevan MJ. Defective $CD8^+$ T cell memory following acute infection without $CD4^+$ T cell help. Science. 2003; 300:339–342. [PubMed: 12690202]
54. Singh RP, La Cava A, Wong M, Ebling F, Hahn BH. CD8+ T cell-mediated suppression of autoimmunity in a murine lupus model of peptide-induced immune tolerance depends on Foxp3 expression. J Immunol. 2007; 178:7649–7657. [PubMed: 17548601]
55. Lu JW, Wang H, Yan-Li J, Zhang C, Ning H, Li XY, Zhang H, Duan ZH, Zhao L, Wei W, Xu DX. Differential effects of pyrrolidine dithiocarbamate on TNF-[alpha]-mediated liver injury in two different models of fulminant hepatitis. J Hepatol. 2008; 48:442–452. [PubMed: 18215436]

56. Lu L, Cantor H. Generation and regulation of CD8+ regulatory T cells. Cell Mol Immunol. 2008; 5:401–406. [PubMed: 19118505]
57. Kim HJ, Verbinnen B, Tang X, Lu L, Cantor H. Inhibition of follicular T-helper cells by CD8+ regulatory T cells is essential for self tolerance. Nature. 2010; 467:328–332. [PubMed: 20844537]
58. Larkinkari U, Leirisalo M, Pentikkinen PJ, Turnen U, Pikkarainen P, Vuoristo M, Lumio J, Rnsnen T, Valtonen VV. Occurrence of antibodies to teichoic acid in patients with diseases other than staphylococcal infection. J Med Microbiol. 1983; 16:45–52. [PubMed: 6401814]
59. Wu ZQ, Drayton D, Pisetsky DS. Specificity and immunochemical properties of antibodies to bacterial DNA in sera of normal human subjects and patients with systemic lupus erythematosus (SLE). Clin Exp Immunol. 1997; 109:27–31. [PubMed: 9218820]
60. Wada Y, Tomikawa M, Kubo M, Etoh Y. A boy diagnosed SLE after *Staphylococcus aureus* infection over a long period. Nihon Rinsho Meneki Gakkai Kaishi. 2001; 24:48–56. [PubMed: 11280901]
61. Furukawa F, Tokura Y, Matsushita K, Iwasaki-Inuzuka K, Onagi-Suzuki K, Yagi H, Wakita H, Takigawa M. Selective expansions of T cells expressing V beta 8 and V beta 13 in skin lesions of patients with chronic cutaneous lupus erythematosus. J Dermatol. 1996; 23:670–676. [PubMed: 8973031]

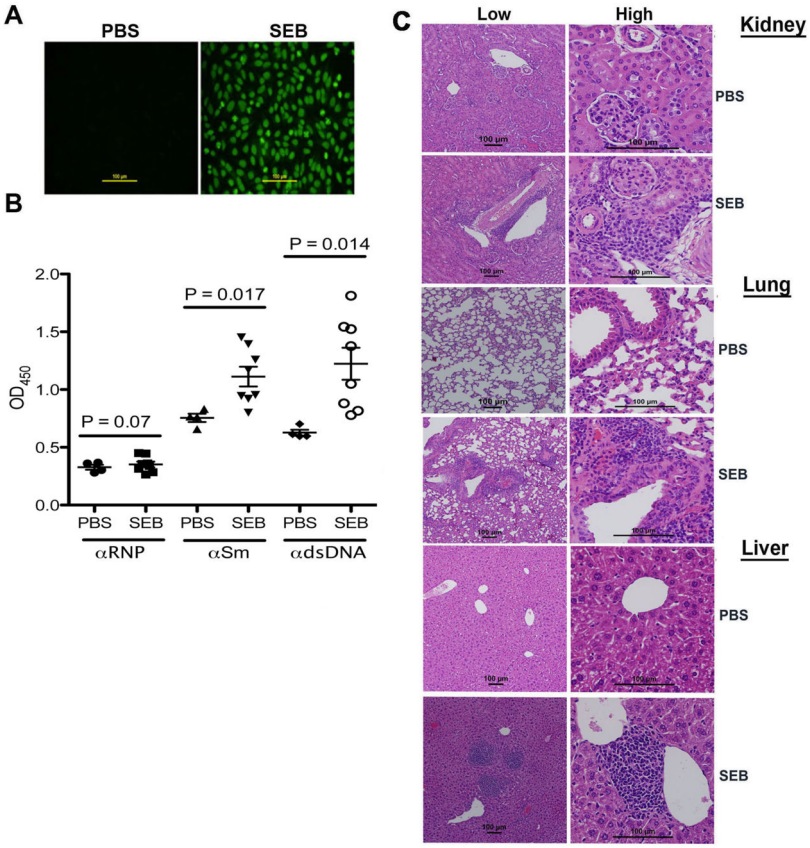

Figure 1. Chronic exposure to SAg elicits autoantibody production and multi organ pathology

HLA-DQ8 transgenic mice subcutaneously implanted with 28-day mini osmotic pumps delivering either PBS or SEB (10 μg) were killed 30 days after implantation. Sera were tested for antinuclear antibodies using Hep-2 cells (panel A depicts representative samples) and autoantibodies by ELISA (panel 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 in panel C.

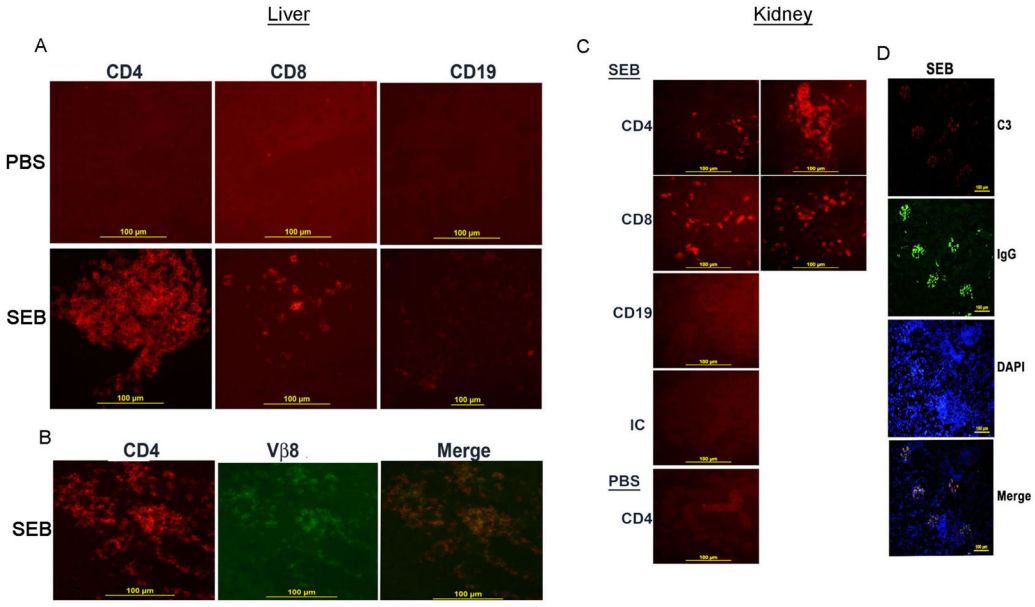

Figure 2. Chronic exposure to SAg causes T lymphocyte infiltration in liver and kidney

Frozen liver (panels A and B) and kidney (panels C and D) sections from HLA-DQ8 transgenic mice implanted with 28-day mini osmotic pumps delivering either PBS or SEB (10 μg) obtained 30 days after implantation were stained with indicated antibodies. (A) Representative images from PBS and SEB treated mice are shown. (B) Liver sections were stained with anti-CD4 (RPE), anti-TCR Vβ8 (FITC) and the images were overlaid. (C) Kidney sections showing periglomerular (left) as well as perivascular (right) 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.

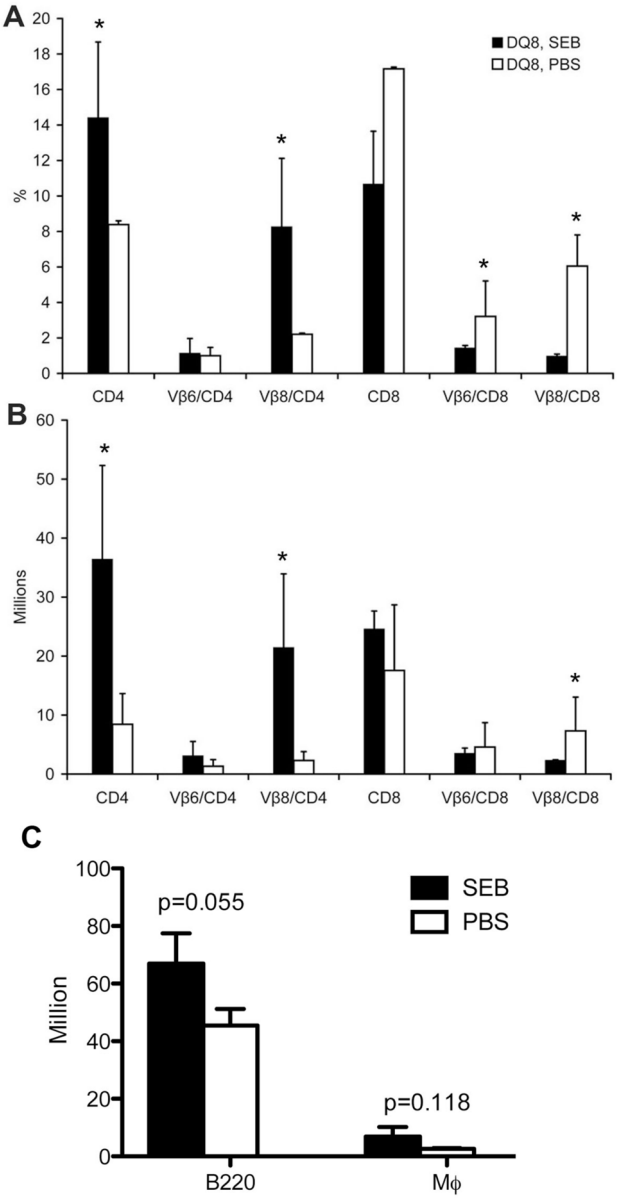

Figure 3. Chronic exposure to SAg causes expansion of T lymphocyte

HLA-DQ8 transgenic mice were implanted with 28-day mini osmotic pumps delivering either PBS or SEB (10 μg). Spleens were collected at the time of sacrifice (30 days after implantation) and distribution of different cell types was determined by flow cytometry. Panels A and B depict percentage and absolute numbers of different T cell subsets, respectively, and panel C depicts the number of B cells and macrophages. Each bar represents mean±SEM from 4–6 mice per group. * represents $p<0.05$ when compared to corresponding PBS-treated group.

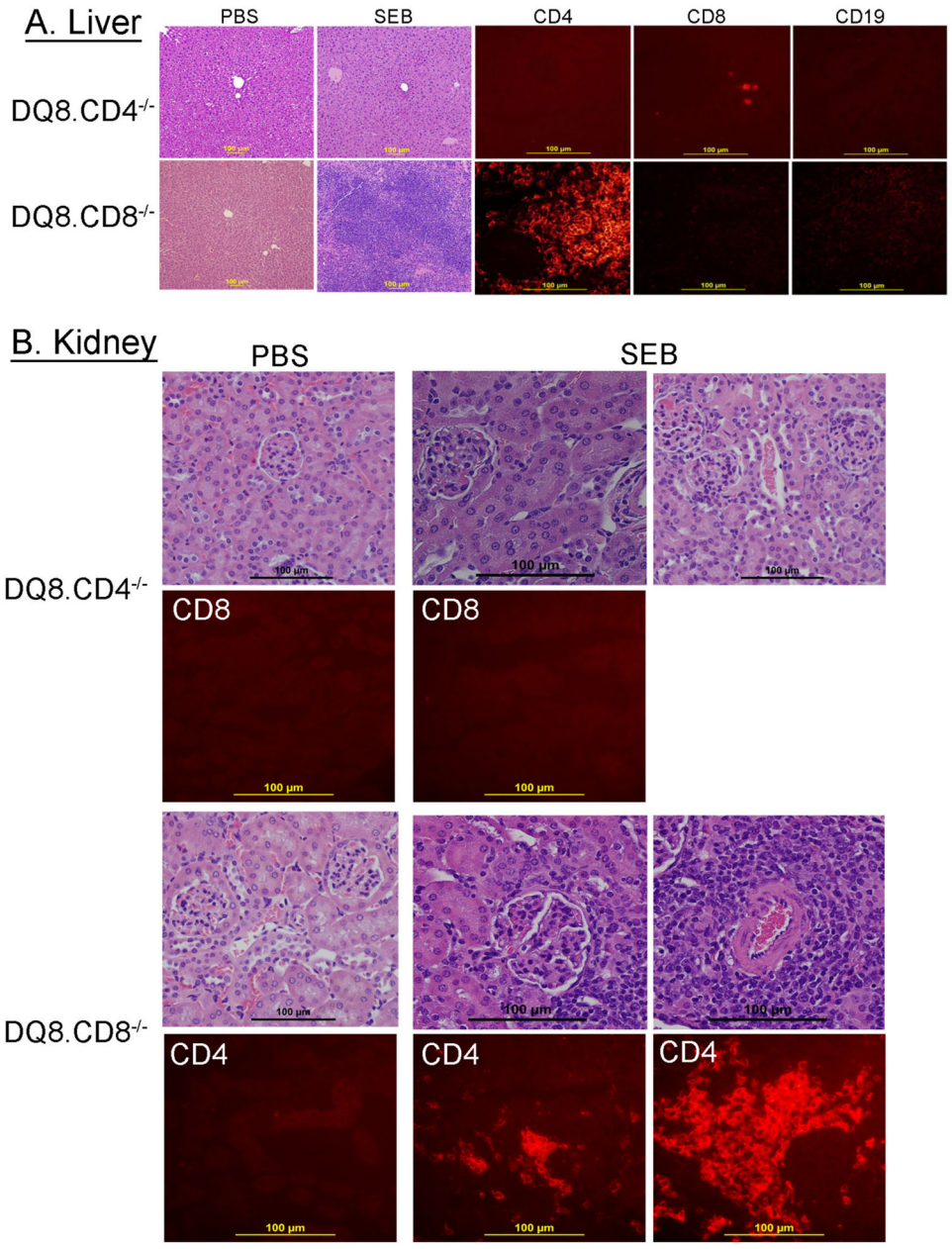

Figure 4. Role of T cell subsets in multi organ pathology elicited by chronic exposure to SAg

HLA-DQ8, HLA-DQ8.CD4° and HLA-DQ8.CD8° transgenic mice subcutaneously implanted with 28-day mini osmotic pumps delivering either PBS or SEB (10 μg) were killed 30 days after implantation. Sections from livers (A) and kidneys (B) were stained with H&E or cryosections of these tissues were stained with indicated antibodies. Representative images are shown.

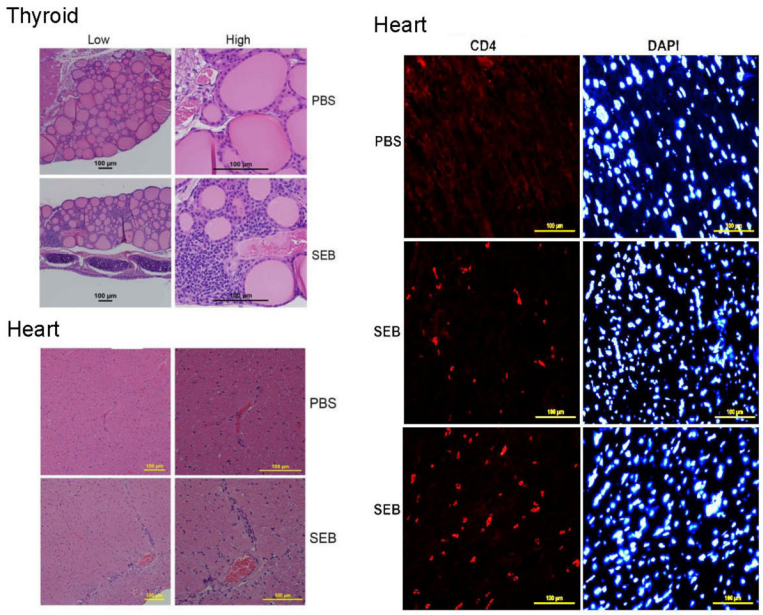

Figure 5. Myocarditis and thyroiditis following chronic exposure to SAg

Formalin fixed or frozen sections from thyroid and heart tissues from HLA-DQ8.CD8° transgenic mice implanted with 28-day mini osmotic pumps delivering either PBS or SEB (10 μg) were stained with H&E or indicated antibodies. Representative images are shown.

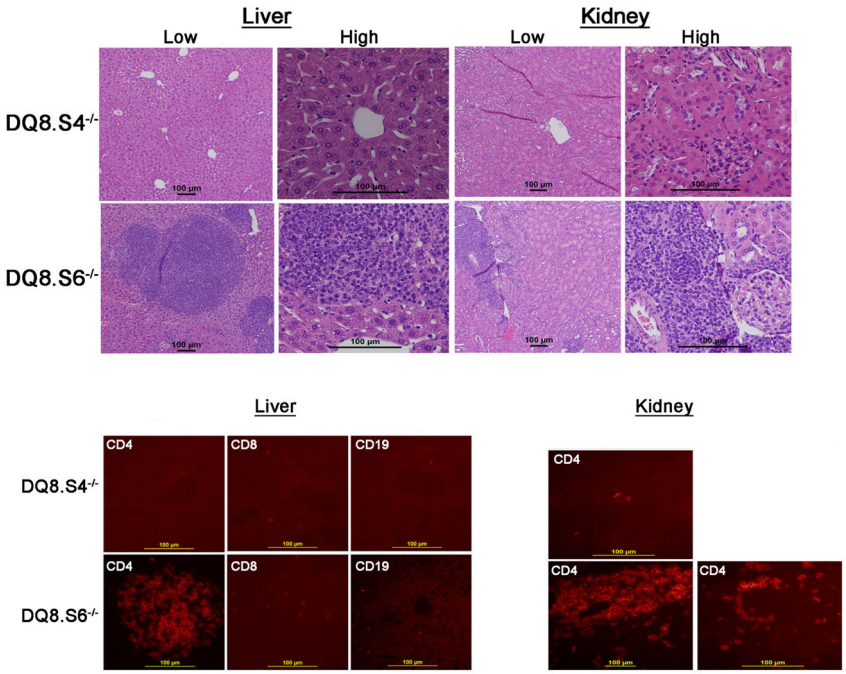

Figure 6.

Modulation of chronic SAg-induced multi organ pathology by STAT4 and STAT6. HLA-DQ8.STAT4° and HLA-DQ8.STAT6° transgenic mice were subcutaneously implanted with 28-day mini osmotic pumps delivering either PBS or SEB (10 μg) were killed 30 days after implantation. Sections from livers and kidneys were stained with H&E or frozen sections were stained with indicated antibodies. Representative images are shown.

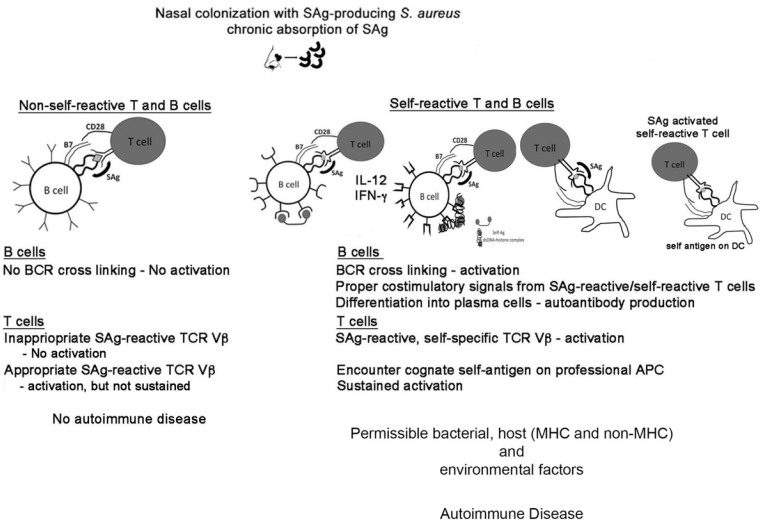

Figure 7.

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 will produce one or more SAg, which will be absorbed and bind to HLA class II molecules on B cells as well as other APC. This leads to activation of $CD4^+$ and $CD8^+$ T cells of diverse antigen specificities, including the self-reactive clones that are present in most individuals, as long as they express appropriate TCR Vβ family preferred by those SAg. Self-reactive T clones present among this population will undergo further rounds of activation when they encounter their cognate self-antigens presented in a classical manner by the professional APC, amplifying autoimmunity. As SAg can directly signal through MHC class II molecules on professional APC, they could induce IL-1 and IL-12 production, thereby skewing towards the pro-inflammatory Th1-type pathway. Self-reactive B cells presenting SAg would get signals through their BCR as well as receive costimulatory signals through CD40-CD40L, B7-CD28 pathways from the $CD4^+$ T cells recognizing the SAg. Such B cells would proliferate, differentiate to plasma cells and secrete autoantibodies. Non-self reactive B cells presenting SAg would fail to undergo such activation and differentiation into plasma cells, as they would not receive signaling through their BCR. Several bacterial (such as qualitative and quantitative nature of the SAg produced, chronicity of colonization etc.,), host (genetic and non-genetic factors, such as providing conducive milieu to the colonizing strains to elaborate SAg) and other environmental factors may determine the overall outcome.