

HLA-DR polymorphisms influence *in vivo* responses to staphylococcal toxic shock syndrome toxin-1 in a transgenic mouse model

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Key words

HLA-DR polymorphisms; HLA-DR transgenic mice; *Staphylococcus aureus*; superantigen; T lymphocytes; toxic shock syndrome toxin-1

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Abstract

Toxic shock syndrome toxin-1 (TSST-1) is a potent superantigen produced by *Staphylococcus aureus*. In addition to menstrual and nonmenstrual toxic shock syndromes, TSST-1 is also implicated in the immunopathogenesis of pneumonia, infective endocarditis, neonatal exanthematous disease, and atopic dermatitis among others. Superantigens first bind to major histocompatibility complex (MHC) class II molecules and then activate a large proportion of T cells by cross-linking their T cell receptor. As binding to MHC class II molecules is a critical step in the robust activation of the immune system by TSST-1 and other superantigens, polymorphic variations between different HLA-DR alleles could potentially influence the magnitude of immune activation and immunopathology caused by TSST-1. As TSST-1 is highly toxic to humans and given that multiple variations of alleles of HLA-DR and HLA-DQ are expressed in each individual, it is difficult to determine how HLA-DR polymorphisms quantitatively and qualitatively impact immune activation caused by TSST-1 in humans. However, such investigations can be conducted on transgenic mice lacking all endogenous MHC class II molecules and expressing specific HLA class II alleles. Therefore, transgenic mice expressing different HLA-DRB1 alleles (*HLA-DRB1*15:01*, *HLA-DRB1*15:02*, *HLA-DRB1*03:01*, *HLA-DRB1*04:01*), and sharing *HLA-A1*01:01* chain, were systemically challenged with purified TSST-1 and multiple immune parameters were assessed. Among the HLA-DR alleles, mice expressing *HLA-DRB1*15:01* allele elicited a significantly higher serum cytokine/chemokine response; greater splenic T cell expansion and most severe organ pathology. Our study highlights the potential utility of human leukocyte antigen (HLA) transgenic mice in understanding the impact of HLA polymorphisms on the outcomes of diseases caused by TSST-1 and other superantigens.

Introduction

Staphylococcus aureus, a gram-positive pathogen, is capable of causing a spectrum of diseases, from benign skin infections to lethal diseases such as pneumonia, sepsis, and toxic shock syndrome (TSS) (1, 2). TSS, either menstrual or nonmenstrual in origin, is clinically highly significant because of their severity and their potential to be associated with high mortality (3, 4). According to a study published in 2011, the average annual

incidences of all, menstrual, and nonmenstrual TSS were 0.52 (95% CI, 0.32–0.77), 0.69 (0.39–1.16), and 0.32 (0.12–0.67), respectively, per 100,000 (3) and it has been reported that the mortality associated with nonmenstrual TSS was significantly higher than menstrual TSS (5). TSS is primarily caused by a unique family of staphylococcal exotoxins called superantigens (6, 7). Staphylococcal superantigens (SSAg) are the most potent activators of the immune system known to date. SSAg in their native form bind directly to major histocompatibility complex (MHC) class II molecules outside of the peptide-binding groove. Some SSAg bind to the α chain and some to the β

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chain, while some members bind to both α and β chains of the MHC class II molecules (8). MHC class II-bound SSAg subsequently interact with specific variable region α (V α) (only for staphylococcal enterotoxin H) or β (V β) (for all other SSAg) regions of the T cell receptors (TCRs) expressed on CD4⁺ and CD8⁺ T cells (9). This leads to cross-linking of the TCR and robust activation of the target T cells. This process can also lead to activation of the antigen presenting cells (APCs) that are presenting the SSAg through cross-linking of MHC class II molecules and through engagement of costimulatory molecules between T cells and the APC (10). Activated T cells and APC rapidly produce large amounts of Th1- and Th17-type cytokines, chemokines as well as other molecular mediators. This condition, referred to as 'systemic cytokine/chemokine storm' often leads to severe inflammation and subsequent failure of several vital organs, which may ultimately result in death (9, 11).

S. aureus produces several SSAg which include the classical staphylococcal enterotoxins (SE), staphylococcal enterotoxin-like (SEL) toxins, and TSS toxin-1 (TSST-1) (9, 12, 13). TSST-1 is distinct from the enterotoxigenic SSAg because TSST-1 is not associated with food poisoning. However, TSST-1 can readily cross the mucosal lining of the female reproductive tract and cause robust immune activation resulting in menstrual TSS (12). However, epidemiological and molecular characterization studies have shown that even the strains of *S. aureus* that are associated with lethal pneumonia, both in the community as well as hospital settings, and infective endocarditis, also express high levels of TSST-1 (14–17). Therefore, in addition to menstrual TSS, *S. aureus* strains producing TSST-1 may be associated with several other acute serious diseases such as pneumonia, infective endocarditis, exanthematous disease, nonmenstrual TSS (18, 19), or even chronic diseases such as atopic dermatitis (20). Therefore, it is important to completely comprehend the pathogenic effects of TSST-1 as well as to identify the factors that can determine the magnitude of immune activation caused by TSST-1. Identification of these factors and determination of the mechanisms by which these factors impact the extent of immune activation and immunopathology by TSST-1 are crucial for development of effective therapies for these serious diseases as well as to offer accurate clinical prognosis. This individualized approach to treatment based on the magnitude of immune activation could be more effective than the current universal treatment for TSS, which consists of antibiotics, intravenous immunoglobulins (IVIg) to neutralize superantigens, and other supportive therapy.

As TSST-1, as well as all other SSAg, have to first bind to human leukocyte antigen (HLA) class II molecules to cause immune activation, polymorphisms within the HLA class II molecules could be an important factor that can influence the binding of TSST-1 to HLA class II molecules, and thereby determine the extent to which TSST-1 activates the immune system in humans (21, 22). Presence of different amino acid

residues in the TSST-1 binding site on HLA class II molecules dictated by polymorphic variations can directly affect binding of TSST-1. It should be noted that TSST-1 makes contact with the peptide bound to the peptide-binding groove of MHC class II molecules. HLA class II polymorphisms can affect the repertoire of peptides bound to the peptide-binding groove. Therefore, HLA class II polymorphisms can indirectly affect TSST-1 binding by determining the repertoire of peptides presented by the given HLA class II allele (23–27). Experiments on transgenic mice expressing different HLA class II alleles have confirmed that HLA class II polymorphisms can modulate the outcome of diseases caused by certain SSAg such as SEA, SEB (22, 28), superantigens produced by *Streptococcus pyogenes*, another gram-positive organism similar to *S. aureus* (29, 30), and even *Mycoplasma arthritides in vivo* (31). However, the impact of HLA-DR polymorphisms on the magnitude of immune activation and immunopathology caused by TSST-1 *in vivo* is not known and cannot be directly investigated in humans because of extreme toxicity of TSST-1 and because of expression of multiple HLA-DR and HLA-DQ alleles in each individual. Hence, to specifically determine the impact of HLA-DR polymorphisms on the responses to TSST-1 *in vivo*, we used transgenic mice expressing different HLA-DR alleles in the absence of potentially confounding endogenous mouse MHC class II molecules.

Materials and methods

Mice

Transgenic mice expressing the following HLA-DR α and β chains (*HLA-DRA1*01:01* and *HLA-DRB1*15:01*, *HLA-DRA1*01:01* and *HLA-DRB1*15:02*, *HLA-DRA1*01:01* and *HLA-DRB1*03:01*, and *HLA-DRA1*01:01* and *HLA-DRB1*04:01*) on C57Bl/6 background have been previously described (32–37). It should be noted that these mice do not express any endogenous MHC class II molecules and numerous studies from our HLA class II transgenic immunogenetics laboratory have shown that these mice express comparable levels of functionally competent HLA-DR molecules (32, 36, 38–43). All mice were bred within the barrier facility of Mayo Clinic Immunogenetics Mouse Colony (Rochester, MN) and moved to a conventional facility after weaning. Experiments were conducted on mice of either sex when they were 8–12 weeks old. All experiments were approved by the Institutional Animal Care and Use Committee.

Experimental procedures

Injection of TSST-1

Each experimental mouse received 100 μ g of highly purified, endotoxin-reduced, TSST-1 (Toxin Technology, Sarasota, FL) dissolved in 200 μ l of PBS by intraperitoneal route while control mice received 200 μ l of PBS alone. Mice were closely

monitored for symptoms of shock such as hunched posture, ruffled fur, diarrhea, hypothermia, and reduced movement.

Measurement of serum cytokines and chemokines

All mice were bled 3 h after injection of TSST-1 or PBS by cutting the tail vein. About 100 µl of blood was collected in serum separation tubes, spun, sera separated, and stored at -80°C until assayed. Concentrations of various cytokines and chemokines in the sera were determined using a mouse cytokine/chemokine magnetic bead multiplex assay following the manufacturer's guidelines (Bio-Rad, Hercules, CA) using MAGPIX multiplexing platform (Luminex, Austin, TX).

Flow cytometry

Spleens and thymii were aseptically collected from the mice after euthanasia. Mononuclear cells were prepared and stained with PerCP-conjugated anti-CD4 (Clone GK1.5), APC-conjugated anti-CD8 (Clone 53-6.7), and the following PE-conjugated anti-mouse TCR V β antibodies. V β 2 (Clone B20.6), V β 3 (Clone KJ25), V β 4 (Clone KT4), V β 5 (Clone MR9-4), V β 6 (Clone RR4-7), V β 7 (Clone TR310), V β 8 (Clone F23.1), V β 9 (Clone MR10-2), V β 10 (Clone B21.5), V β 11 (Clone RR3-15), V β 12 (Clone MR11-1), V β 13 (Clone MR12-3), V β 14 (Clone 14-2), and V β 17 (Clone KJ23). All antibodies are from BD BiosciencesTM, San Diego, CA. Flow data were analyzed using FLOWJO software V10.0.8 (Tree Star, Ashland, OR).

Histopathology

Immediately following euthanasia, small pieces of livers and lungs were collected in 10% buffered formalin and embedded in paraffin. Thin 5 µm sections prepared from these blocks were stained with hematoxylin and eosin and were objectively evaluated in a blinded fashion. A score of 0–3 were given to each slide based on the extent of tissue involvement. Score 0 – no inflammation, score 1 – less than 25% of the tissue shows inflammatory changes, score 2 – 25%–50% of the tissue shows inflammatory changes, and score 3 – more than 50% of the tissue shows inflammatory changes. Representative sections were photographed using an Olympus AX70 research microscope fitted with an Olympus DP70 camera (Olympus America, Center Valley, PA).

Statistical analyses

Kruskal–Wallis test was used to determine the significance of each parameter where indicated. Subsequently, Dunn's multiple comparisons test was used for pairwise comparison of all the groups against each other using GraphPad prism Version 6 (GraphPad Prism, Inc., San Diego, CA). *P*-values less than 0.05 were considered statistically significant.

Results and discussion

HLA-DR polymorphisms influence the systemic cytokine/chemokine response to TSST-1

To determine the extent to which HLA-DR polymorphisms impact the systemic cytokine/chemokine response to TSST-1 *in vivo*, different HLA-DR transgenic mice were challenged with TSST-1, bled 3 h later and the serum concentrations of representative Th₁, Th₂, and Th₁₇ cytokines and a few inflammatory chemokines were determined. The results are summarized in Figure 1. The 3-h time point was chosen based on our prior published studies in which we have shown that following a systemic SSAg challenge, the serum cytokine/chemokine levels peak by 3–4 h and begin to decline at subsequent time points (37, 44).

First of all, in this study, sera from all naïve mice had comparably low or undetectable levels of indicated cytokines or chemokines irrespective of the HLA class II allele they expressed. Hence, data from all naïve mice were pooled and compared with data obtained from TSST-1 challenged mice. As T cells are the primary targets for activation by TSST-1 (and other superantigens), a rapid elevation in systemic levels of IL-2, a T cell-derived cytokine, is the hallmark of immune activation by SSAg. Among the HLA-DR alleles, mice expressing *HLA-DRB1*15:01* had the highest concentration of IL-2 in sera ($P < 0.01$) followed by mice expressing *HLA-DRB1*15:02* allele ($P < 0.05$). Even though *HLA-DRB1*03:01* and *HLA-DRB1*04:01* transgenic mice had significantly elevated levels of IL-2 in their sera when compared individually with naïve mice, this significance did not stand when Dunn's correction for multiple comparisons was applied. Similar pattern applied to IL-6 (Figure 1). With respect to IL-12(p40), only mice expressing *HLA-DRB1*15:01* allele had significantly higher levels compared with naïve mice among the HLA-DR alleles (Figure 1). Serum levels of IL12(p70), IL-17, and IFN- γ were also highest in *HLA-DRB1*15:01* mice but did not attain statistical significance for any HLA-DR allele. Interestingly, serum levels of IL-5, a potent Th₂ cytokine, was the lowest in *HLA-DRB1*15:01* transgenic mice correlating with elevated levels of pathogenic Th₁ cytokines and inflammatory chemokines in this group compared with other mice even though it did not reach statistical significance. Elevated level of IL-10 has been shown to be associated with poor outcome in patients in several models of sepsis including those caused by *S. aureus* (45, 46). Hence elevated levels of IL-10 in *HLA-DRB1*15:01* mice, although not statistically significant, correlated with elevated levels other pathogenic cytokines/chemokines and other inflammatory changes in these mice (discussed later). All the chemokines tested, MIP-1 β , eotaxin, MCP-1, G-CSF, KC, and RANTES, were significantly elevated in *HLA-DRB1*15:01* transgenic mice, while only MCP-1, G-CSF, and KC were elevated in *HLA-DRB1*15:02* mice. None of the chemokines were elevated significantly in *HLA-DRB1*03:01* or *HLA-DRB1*04:01* transgenic mice.

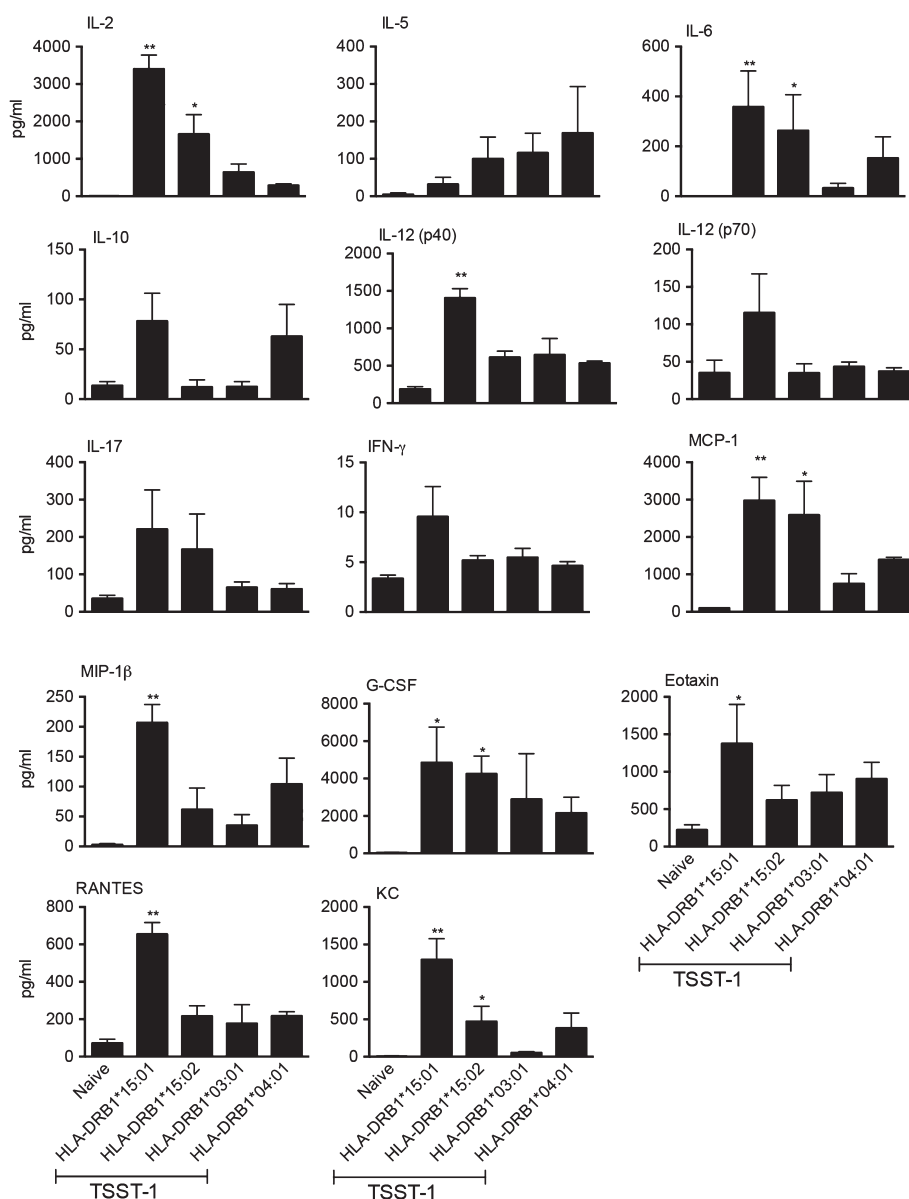


Figure 1 HLA-DR polymorphisms modulate systemic cytokine/chemokine responses to toxic shock syndrome toxin-1 (TSST-1). Transgenic mice expressing indicated HLA-DR alleles were challenged with TSST-1 through intraperitoneal route. Three hours after challenge, mice were bled, sera collected, and levels of indicated cytokines/chemokines were determined by multiplex assay (Bio-Rad). Data from all naïve mice (12–15 mice) were pooled, as they were similar. Each bar represents mean $\pm$ SE from four mice/group. ** and * represent $P < 0.01$ and $P < 0.05$, respectively, when compared with naïve mice.

Overall, HLA-DR polymorphisms seemed to impact the systemic cytokine/chemokine response to TSST-1 and among the HLA-DR alleles tested, *HLA-DRB1*15:01* elicited the strongest response with several inflammatory cytokines and chemokines significantly elevated in this group.

The impact of HLA-DR polymorphisms on TCR V β repertoire selection, TSST-1-induced expansion of mature splenic T lymphocytes and deletion of immature thymocytes

As discussed earlier, TSST-1 like other SSAG binds to MHC class II molecules and subsequently activates both CD4⁺ and CD8⁺ T cells expressing certain TCR V β families. In humans, TSST-1 activates T cells expressing TCR V β 2; and TCR V β 3, 10 and 17 in mice (8, 47). However, to our knowledge, which

murine TCR V β families are activated by TSST-1 when it is presented by human MHC class II (HLA-DR) molecules has not been described. It is also not known to what extent do polymorphisms in HLA-DR molecules impact the extent of T cell activation by TSST-1 *in vivo*. To answer these questions, mice expressing different HLA-DR were challenged with TSST-1, killed 3 days later when SSAG-induced T cell expansion is at its peak (37, 48), and the TCR V β profiles in TSST-1 treated mice was compared with respective naïve mice.

It is well established that the TCR V β repertoire in any individual is determined by MHC polymorphisms and that polymorphisms within the class II molecules would primarily determine the TCR V β repertoire of CD4⁺ T cells. However, TSST-1 activates both CD4⁺ as well as CD8⁺ T cells like other superantigens. Therefore, we first determined the distribution

of major TCR V β families in CD4⁺ as well as CD8⁺ T cell subsets in naïve mice expressing various HLA-DR alleles by flow cytometry. The frequency of T cells expressing various TCR V β families was different in each transgenic line within the CD4⁺ T cell subset as expected (Figure 2). The TCR V β repertoire within the CD8⁺ T cell population was also different between mice expressing different HLA-DR alleles and more closely resembled the pattern in CD4⁺ T cells even though all these mice express the same mouse MHC class I molecule (K^b allele). This is also expected because expression of rearranged TCR β chain occurs much early during thymic selection at the CD4CD8 double negative stage independent of MHC class I or class II molecules (49). However, fine-tuning of the T cell repertoire continues thereafter when thymocytes interact with self MHC-peptide complexes presented by various thymic APC (50).

In *HLA-DRB1*15:01* and *HLA-DRB1*04:01* transgenic mice, the frequency of CD4⁺ and CD8⁺ T cells expressing TCR V β 3, which is known to bind to TSST-1, were slightly elevated compared with *HLA-DRB1*15:02* and *HLA-DRB1*03:01* transgenic mice, but were not statistically significant. However, the frequency of CD4⁺ and CD8⁺ T cells expressing TCR V β 10, the other TCR V β shown to react with TSST-1 (47), were increased in *HLA-DRB1*15:02* and *HLA-DRB1*03:01* transgenic mice compared with the other two transgenic lines. The frequency of T cells expressing TCR V β 17, the other TCR V β in mice also known to react with TSST-1 was comparable between all the strains.

Having established the baseline TCR V β profile, we then analyzed the changes in TCR V β profile following *in vivo* TSST-1 challenge. The data is expressed as fold-change in individual TCR V β families with respect to naïve mice and the results are summarized in Figure 3. To simplify the overall changes in TCR V β profile, 11 of 14 TCR V β families tested (including TCR V β 3) were increased (by more than onefold) in both CD4⁺ and CD8⁺ T cells in *HLA-DRB1*15:01* mice, three of 14 increased in both CD4⁺ and CD8⁺ T cells in *HLA-DRB1*15:02* mice, two of 14 and one of 14 were increased in CD4⁺ and CD8⁺ T cells, respectively, in *HLA-DRB1*03:01* mice and eight of 14 and five of 14 were increased in CD4⁺ and CD8⁺ T cells, respectively, in *HLA-DRB1*04:01* mice. Overall, HLA class II polymorphisms clearly dictated which TCR V β family was expanded in response to TSST-1 and *HLA-DRB1*15:01* mice showed the most increase in various TCR V β families following *in vivo* TSST-1 challenge followed by *HLA-DRB1*04:01*, *DRB1*15:02*, and *HLA-DRB1*03:01* mice.

Mature thymocytes as well as peripheral T cells express either the CD4 or the CD8 coreceptor and hence are called single positive (SP) T cells. However, immature thymocytes, during certain developmental stage in thymus, express both CD4 and CD8 coreceptors and are called double positive or DP cells. Unlike the mature peripheral CD4⁺ or CD8⁺ SP T cells which undergo activation and expansion following stimulation by SSAg, immature CD4⁺CD8⁺ DP thymocytes undergo

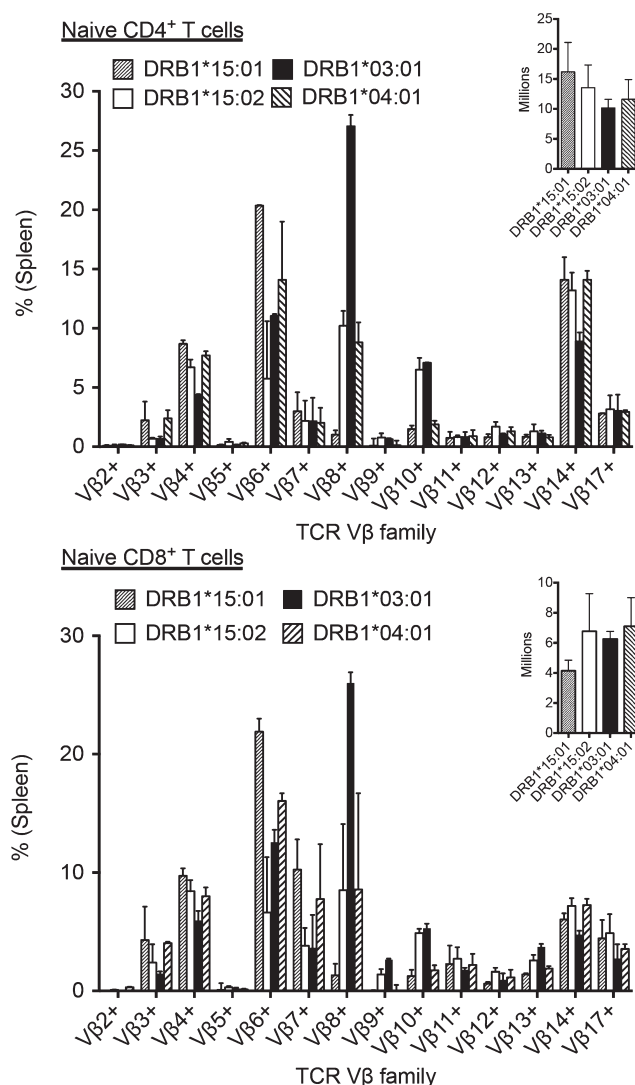


Figure 2 Baseline T cell receptor (TCR) V β repertoire in transgenic mice expressing different HLA-DR alleles: Distribution of major TCR V β families within the CD4⁺ and CD8⁺ T cell subsets in the spleens of age-matched naïve mice expressing indicated HLA-DR alleles was determined by flow cytometry. Each bar represents mean values from four mice/group.

deletion following activation by superantigens. Therefore, as another measure of T cell activation *in vivo*, we collected thymii from naïve and TSST-1 challenged mice at the time of kill and analyzed the distribution of various thymocyte subsets by flow cytometry. As shown in Figure 4, compared with naïve mice, the percentage of CD4CD8 DP thymocytes were decreased in all HLA-DR transgenic mice challenged with TSST-1. However, the extent of deletion was most pronounced in *HLA-DRB1*15:01* mice (a 30% reduction in DP subsets following TSST-1 challenge), which correlated well with serum cytokine levels and splenic T cell expansion. The mean reductions in DP subsets in TSST-1 challenged over naïve mice were

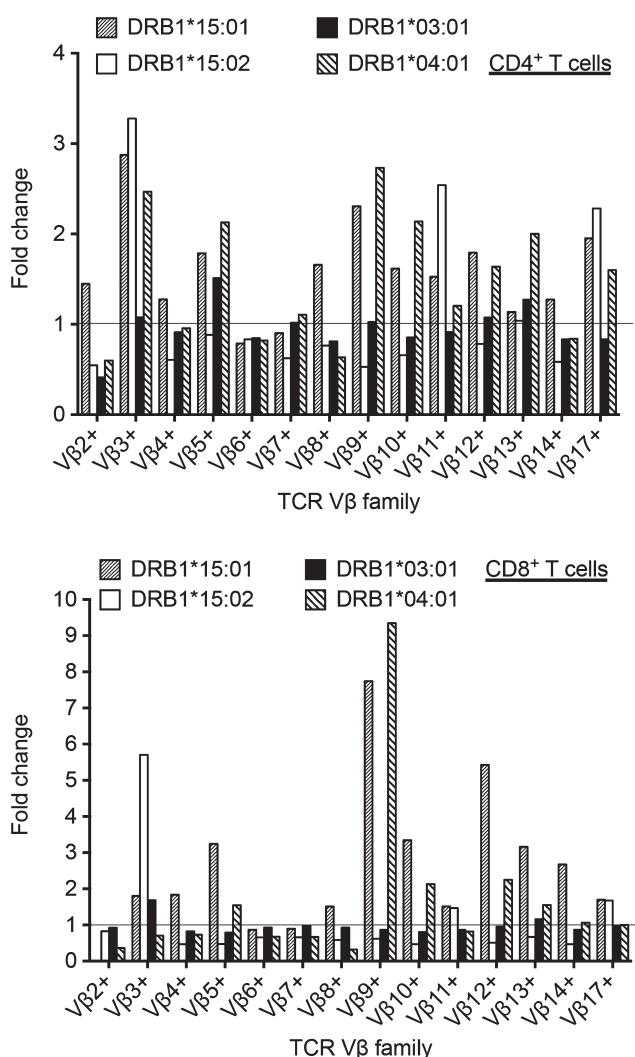


Figure 3 HLA-DR polymorphisms determine the magnitude of splenic T cell expansion in response to systemic Toxic shock syndrome toxin-1 (TSST-1) challenge. Transgenic mice expressing different HLA-DR alleles were challenged with TSST-1. Three days later, mice were euthanized, splenic mononuclear cells were prepared and the distribution of CD4⁺ and CD8⁺ T cells expressing indicated T cell receptor (TCR) Vβ families was determined by flow cytometry. Data is represented as fold-change in percentages of CD4⁺ (top panel) and CD8⁺ (bottom panel) T cells for each TCR Vβ family in TSST-1 challenged mice compared with naïve mice from that group. Each bar represents mean values from four mice/group. Horizontal lines in the chart correspond to fold change of one.

15.7, 14, and 15.3 in *HLA-DRB1*15:02*, *HLA-DRB1*03:01*, and *HLA-DRB1*04:01* mice, respectively.

TSST-1-mediated organ pathology in mice expressing different HLA-DR alleles

While all TSST-1 treated mice displayed some external signs of distress such as ruffled fur and hunched posture, these signs were not appreciably different between mice expressing

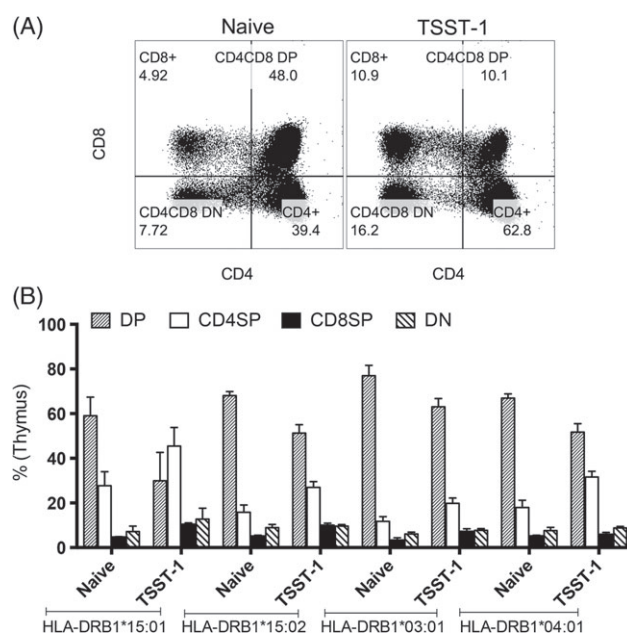


Figure 4 Impact of HLA-DR polymorphisms on toxic shock syndrome toxin-1 (TSST-1)-induced deletion of immature thymocytes. HLA-DR transgenic mice were challenged with TSST-1. Three days later, mice were euthanized, thymii were collected, and single cell suspensions were prepared. The distribution of various thymocyte subsets was determined by flow cytometry. (A) Represents representative dot plot from naïve and TSST-1 challenged *HLA-DRB1*15:01* mice. (B) Represents mean $\pm$ SE values of indicated populations from four mice/group. DP – CD4, CD8 double positive, CD4SP – CD4 single positive, CD8SP – CD8 single positive and DN – CD4, CD8 double negative.

different HLA-DR alleles. Hence, we decided to investigate the extent of organ pathology in these mice as inflammation of multiple vital organs and ultimately their failure is the hallmark of TSST-1-induced TSSs (11, 37). To determine to what extent HLA-DR polymorphisms influence organ pathology induced by TSST-1, H&E stained sections from livers and lungs from all HLA-DR transgenic mice were examined microscopically. As expected, naïve tissues from all transgenic mice were normal and devoid of any notable inflammation (Figure 5). However, liver and lung sections from all HLA-DR transgenic mice challenged with TSST-1 showed signs of inflammation with perivascular as well as interstitial lymphocytic and monocytic infiltration. However, the extent of inflammation was highest in *HLA-DRB1*15:01* mice (mean pathology scores for livers and lungs were 2.5 and 2, respectively). However, organs from *HLA-DRB1*04:01* mice, *HLA-DRB1*15:02*, and *HLA-DRB1*03:01* mice showed comparable pathology with the average pathology scores in livers and lungs being 1.5. Because of limited number of tissue sections evaluated, we were not able to perform statistical analyses. Overall, organ pathology appeared to correlate with the serum cytokine/chemokine levels and extent of splenic T cell expansion particularly in *HLA-DRB1*15:01* mice.

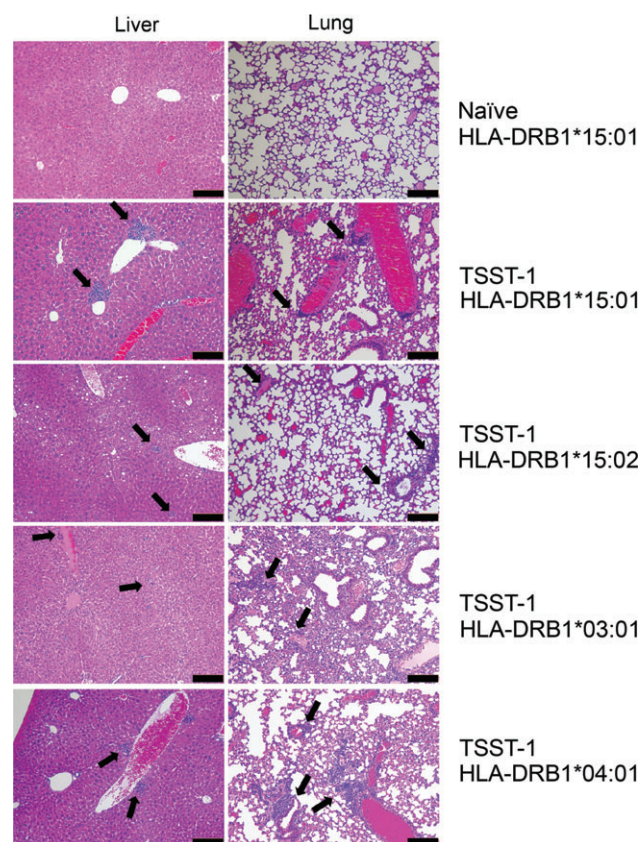


Figure 5 The impact of HLA-DR polymorphisms on toxic shock syndrome toxin-1 (TSST-1)-induced inflammation of lungs and livers. Transgenic mice expressing indicated HLA-DR alleles were challenged with TSST-1. Three days later, mice were euthanized, organs were collected, formalin fixed, and paraffin embedded. H&E stained sections prepared from these tissue blocks were evaluated by light microscopy. Black arrows indicate sites of mononuclear cell infiltration and horizontal black bar inserts at right bottom corner correspond to 100 µm. Representative images are shown.

It should be noted that TSST-1 binds to the α chain of HLA class II molecules (51). As the α chain of HLA-DR molecules is shared among HLA-DR alleles tested, TSST-1 is expected to elicit comparable immune responses in all mice irrespective of the HLA-DR allele that they express. However, the observed differences in responses between mice expressing different HLA-DR alleles could be because of two major reasons. One, differences in the TCR repertoire between different HLA-DR transgenic mice as discussed earlier and two, the unique MHC class II binding property of TSST-1. Elegant *in vitro* studies using APC defective in peptide presentation (27) as well as structural studies (52) have shown that TSST-1 makes significant contact with the peptides bound to the peptide-binding groove of HLA-DR molecules and as a result, the MHC class II-bound peptides can significantly influence the extent of immune activation caused by TSST-1 (25–27). Therefore, even though the α chain of HLA-DR molecules is shared among

different HLA-DR alleles, the peptide pool presented by different HLA-DR allele will be extremely diverse, constantly changing *in vivo* and influenced by numerous other factors including polymorphisms in HLA-DR β chain (53). Therefore, HLA class II polymorphisms could potentially impact the *in vivo* responses to TSST-1 by determining the TCR repertoire as well as by influencing the peptide repertoire presented by them. Our transgenic mouse models can be used to further investigate how various peptides determine the extent of immune activation/immunopathology and whether this can be manipulated to favorably modify the outcome of diseases caused by TSST-1.

A recent human study has shown that HLA class II polymorphisms can determine the outcome of certain staphylococcal infections (54). While this study did not specifically investigate the impact of HLA class II polymorphisms on the outcome of TSS or other diseases caused by SSaG *per se*, they did find some association between certain HLA class II alleles and staphylococcal infections. Given the observations that HLA class II polymorphisms can determine the outcome of invasive diseases caused by streptococcal superantigens (30) and the results from this study, it is possible that HLA class II polymorphisms might also determine the outcome of diseases caused by staphylococcal SAGs including TSS caused by TSST-1. A bigger clinical study involving large number of patients is needed to clearly establish this association in humans.

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Conflict of interests

The authors have declared no conflicting interests.

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