Humanlike Immune Response of Human Leukocyte Antigen–DR3 Transgenic Mice to Staphylococcal Enterotoxins: A Novel Model for Superantigen Vaccines

Luis DaSilva,1 Brent C. Welcher,1 Robert G. Ulrich,1 M. Javad Aman,1 Chella S. David,2 and Sina Bavari1

This study examined the biologic responses of transgenic mice expressing human leukocyte antigen (HLA)–DR3 and human CD4 molecules, in the absence of murine major histocompatibility complex (MHC) class II molecules (Ab0), to staphylococcal enterotoxins (SEs) and evaluated protective immunity of a nonsuperantigen form of SEB against wild-type holotoxin. HLA-DR3 transgenic mice responded to several log lower concentrations of SEs and secreted higher levels of proinflammatory cytokines than did wild-type mice. Vaccination of transgenic mice with a nonsuperantigenic form of SEB induced higher levels of neutralizing anti-SEB antibodies, which protected the mice from a surge in proinflammatory cytokine secretion after SEB challenge. The humanlike responses of the transgenic mice to SEs support the hypothesis that these mice represent an appropriate model to examine vaccines and therapeutics against SEs. This is thought to be the first report of examination of a vaccine against SEB in the context of human MHC class II receptors.

The primary function of major histocompatibility complex (MHC) class II molecules is to capture antigenic peptides in acidic endocytic compartments and to present them to T cells. Optimum antigen recognition by CD4-carrying T cells occurs after interaction of T cell receptor (TCR) Vα and Vβ chains with MHC-bound peptides, in conjunction with costimulatory molecules provided by antigen-presenting cells (APCs) and their counter receptors on responding T cells. Various pathogenic bacteria, such as Staphylococcus aureus, produce exotoxins that are virulence determinant factors, which bypass this integral interaction between T cells and MHC class II molecules [1–3]. These exotoxins are capable of inducing acute and chronic immunologic disorders [4–8].

Staphylococcal enterotoxins (SEs), a family of many exotoxins produced by S. aureus, have been termed superantigens (SAgs) because of their ability to massively stimulate the immune system [3, 9–12]. Bacterial SAgs divert immune responses by blocking T cell recognition of peptides bound to MHC class II molecules on the surface of APCs as one of their major physiologic receptors. These bacterial exotoxins cross-link MHC class II molecules and TCR β chains independently of MHC class II restriction and cause large numbers of T cells to participate in responses to bacterial SAgs. The resulting T cell activation is a major contributing factor to several serious disease states, including toxic shock syndrome [13, 14]. The functional activity of bacterial SAgs is characterized by robust proinflammatory cytokine responses, demonstrated by the secretion of interferon (IFN)–γ, a Th1 type cytokine [15, 16]. The exact mechanism by which Th1 type cytokines dominate the overall responses to bacterial SAgs is not well understood; however, the overall avidity of SAg interaction with receptors of immune cells may dictate the profile of cytokine production.

T cell binding to the MHC class II–SAg binary complex is mainly controlled by TCR Vβ and results in the stimulation of a high proportion of circulating T cells [15]. Like conventional antigen stimulation, the stimulated T cells are removed from circulation by apoptosis, resulting in the depletion of specific TCRs. For some SAgs, high-affinity interactions between the toxin and MHC class II receptors have been identified, and this presentation may resemble high-affinity antigenic peptide recognition by TCRs [17].

Bacterial SAgs have lower affinity for mouse MHC class II than for human MHC class II molecules [18, 19]. Because of this biologic characteristic, SAg-induced toxicity is much lower in mouse models than it is in humans. The objective of this work was to characterize the biologic responses of human MHC class II/human CD4 transgenic mice lacking endogenous murine MHC class II and murine CD4 expression (HLA-DR3,CD4,Ab0) to bacterial SAgs and to examine the efficacy of a vaccine against bacterial SAg SEB in the context of human MHC class II molecules [20–22]. These transgenic mice may provide an opportunity for developing bacterial SAg-experimental animal models that closely resemble human responses.
Materials and Methods

**Mice.** BALB/c mice (H-2b) were obtained from Harley Sprague-Dawley. HLA-DR3 and HLA-DR2β/IEα human CD4 transgenic mice were generated as described elsewhere [20–22]. The transgenic mice were bred onto the mouse class II knockout (Ab-β) background [23]. In brief, the transgenic mice were developed by coinjecting the HLA-DRA1*0101 and the DRB1*0301 gene fragments into (C57BL/6 x DBA/2) F1 x C57BL/6 embryos and backcrossing to B10.M mice to produce DR3.B10.M mice. HLA-DR3 transgenic mice were then altered into class II–negative mice by mating the H-2–negative strain (H-2 aβ0) with DR3.B10.M mice. Human CD4.Aβ mice were similarly bred and subsequently crossed with DR3.B10.M mice to produce the HLA-DR3.CD4.Aβ0 mouse. A similar strategy was used to breed HLA-DR2β/IEα. All mice used in this study were bred and maintained in the pathogen-free mouse colony of the US Army Medical Research Institute of Infectious Diseases, according to institutional and National Institutes of Health guidelines.

**Bacterial SAgS and SEB vaccine.** Recombinant toxins were produced from Toxin Technology. Engineered recombinant SEB vaccine containing 3 site mutations (SEB L45R/Y89A/Y94A) was prepared in our laboratory, as described elsewhere [24]. In brief, the wild-type SEB gene was isolated from S. aureus, and site-specific mutations were created. The final construct had the following mutations: SEB L45R/Y89A/Y94A (referred to as SEB vaccine). The vaccine was purified by ion-exchange chromatography after bacterial lysis. The SEB vaccine was > 95% pure, as determined by SDS-PAGE. Binding assays that used human B cell lines and flow cytometry indicated that this protein lacked binding to MHC class II receptors on the surface of the APC and did not stimulate human T cells [24]. The bacterial toxins and the SEB vaccine contained no detectable endotoxin, as determined by limulus assay.

**Polymerase chain reaction (PCR) analysis.** Before all experiments, we determined that all mice contained the appropriate transgene in their genome, as described below. The presence of the HLA-DRB*0301 gene in the transgenic mice was detected by PCR amplification by use of the following primer set: DR3 sense 5′-CCG TTC GAC AGC GAC CAC-3′ and DR3 antisense 5′-GAC AAA TCC ACA CTC CAC-3′. DNA (200 ng) was extracted from peripheral blood mononuclear cells (PBMC) by use of the Isoquick nucleic acid extraction kit (Orca Research). Purified DNA was then used for PCR amplification in the presence of 0.8 μM primers, 200 μM each dNTP, and 2.5 U of AmpliTaq (PE Biosystems). Cycling conditions consisted of 30 s at 94°C, 1 min at 50°C, and 1 min at 72°C.

**Flow cytometry.** Before initiation of experiments, the expression of HLA-DR on PBMC was determined by flow cytometry, as described below. Peripheral blood (300 μL) was collected from the transgenic mice and BALB/c control mice. The mononuclear cell fraction was isolated by centrifugation over a ficoll-hypaque gradient. Cells then were washed in RPMI 1640 medium containing 0.1% bovine serum albumin (BSA) and pelleted at 280 g for 15 min. The cells were incubated (45 min on ice) in the presence of fluorescein isothiocyanate–labeled goat anti–mouse IgG (ICN Pharmaceuticals). The fluorescent antibody-binding profile was analyzed by flow cytometer (FACSort; Becton Dickinson).

**Proliferation assay.** Splenic mononuclear cells were purified on a ficoll-hypaque gradient to enrich for mononuclear cells. The purified cells were resuspended in RPMI 1640 medium containing 5% fetal bovine serum (FBS), and the cell suspension (2 x 10⁶ cells) was added to triplicate wells of a 96-well, flat-bottom plate. The cells were cultured (37°C in 5% CO₂) in the presence of the bacterial toxin for 3 days. The cultures were then pulsed (12 h) with 1 μCi of [³H]thymidine (Amersham) per well, and incorporated radioactivity was measured by liquid scintillation.

**Cytokine detection for IFN-γ and interleukin (IL)–6.** Splenic mononuclear cells were isolated from transgenic and wild-type mice, as described above. We resuspended 10⁶ purified mononuclear cells in RPMI 1640 medium containing 5% FBS, which was exposed to bacterial SAg for 20 h. Supernatants were collected from the treated cells and assayed by ELISA for the presence of secreted IFN-γ and IL-6. The cytokine assays were done as instructed by the manufacturer (R & D Systems).

**Vaccination protocol.** Mice were injected intraperitoneally with 20 μg of recombinant SEB vaccine in 100 μL of adjuvant (Ribi Immunochem Research) or with adjuvant alone and boosted at 4 and 8 weeks in the same manner as that described for the primary injection. Mice were challenged 2 weeks after the second boost with 10 μg of wild-type SEB, and cytokine responses were measured as described above.

**Serum antibody titers.** Serum antibody titers against SEB were determined by ELISA, as described elsewhere [25]. The mean duplicate absorbance of each treatment group was obtained, and data are presented as the inverse of the highest dilution that produced an absorbance reading twice that of the negative control wells (antigen or serum was omitted from the negative control wells).

Results

**Potent T cell proliferation in HLA-DR3.CD4.Aβ0 transgenic mice by S. aureus SAgS.** Bacterial SAgS have much higher affinities for human MHC class II molecules than for mouse class II receptors. Unlike the HLA-DRα receptor, mouse MHC class II IDRα does not have a critical lysine 39 residue, one of the essential residues to form a high-affinity binding pocket for bacterial SAgS [11]. On the basis of this discrepancy between mouse and human MHC class II molecules, we postulated that mice transgenic for HLA-DRβ/IEα should behave like wild-type mice to the biologic effects of bacterial SAgS. Taub et al. [26] also showed that T cell responses and sensitivities of different strains of mice to bacterial SAgS varied substantially and differed by mouse strain; T cells obtained from BALB/c (H-2b) mice had the highest proliferative activity in response to bacterial SAgS, such as SEB and SEC [26]. Therefore, BALB/c mice should be the most sensitive to the biologic actions of these SAgS.

Table 1 shows the proliferative responses of splenic T cells obtained from HLA-DRβ/IEα and BALB/c mice to bacterial SAg SEB. Cells from both strains of mice proliferated similarly in
response to SEB at various doses. Cells from both the HLA-DR3.CD4.Ab0 transgenic and BALB/c mice reacted to 1–10 ng/mL SEB. These data suggested that no substantial increase in sensitivity was achieved by including DRβ and supports the notion that DRα mainly drives SAg binding. BALB/c mice have been used extensively to investigate the role of MHC class II and cytokines in the pathogenesis of bacterial SAg-induced immune dysregulation [27–29]. In the present study, we compared HLA-DRαβ transgenic mouse responses to bacterial SAg to those of BALB/c mice.

To determine whether HLA-DR3.CD4.Ab0 transgenic mice had enhanced sensitivity to bacterial SAg, we assessed proliferative responses to SEB, SEC, and SED (figure 1). Low amounts of SEB, SEC1, or SED (10–100 pg/mL) were sufficient to induce proliferation in the transgenic mice but had little effect on T cells from BALB/c mice (figure 1). T cells from the transgenic mice responded more potently to SEB, followed by SED and SEC1. At 1 ng/mL doses, SEB-induced proliferative activity was 3–5 times more than SEC1 and SED activity. Cells from BALB/c mice were resistant to low doses of SEs, and proliferation was observed when toxin concentrations reached 104 pg/mL.

Cytokine secretion profile of SAg-induced transgenic PBMC.

Because we found that HLA-DR3.CD4.Ab0 transgenic mice responded more vigorously to SEB than to other SEs, we used this SAg as a prototype toxin to further characterize these unique mice. Proinflammatory cytokines such as IL-6 and IFN-γ are critical components of SAg-induced toxicity [30, 31]. To further study the biologic responses of the HLA-DR3 mice to bacterial SAg, splenic mononuclear cells from the transgenic and BALB/c mice were cultured in the presence of SEB for differing times, and cytokine responses were measured. Mononuclear cells from HLA-DR3.CD4.Ab0 transgenic mice responded strongly to SEB with secretion of IL-6 and IFN-γ (figure 2). After SEB stimulation, cells from the transgenic mice produced ~5 times more IL-6 than cells from BALB/c mice. The level of IFN-γ produced by the transgenic mice was ~14 times higher than IFN-γ levels produced by BALB/c mice. These results plus the proliferation data show that HLA-DR3.CD4.Ab0 transgenic mice are extremely sensitive to SAg, especially SEB, compared with BALB/c responses.

Table 1. T cell responses of wild-type mice and HLA-DRβ/IEα transgenic mice responses to staphylococcal enterotoxin B (SEB).

<table>
<thead>
<tr>
<th>SEB concentration, ng/mL</th>
<th>BALB/c mice</th>
<th>HLA-DRβ/IEα mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>98,527 ± 5336</td>
<td>59,233 ± 6001</td>
</tr>
<tr>
<td>100</td>
<td>14,297 ± 2927</td>
<td>18,977 ± 1490</td>
</tr>
<tr>
<td>10</td>
<td>3775 ± 387</td>
<td>1992 ± 290</td>
</tr>
<tr>
<td>1</td>
<td>696 ± 125</td>
<td>589 ± 192</td>
</tr>
<tr>
<td>0</td>
<td>478 ± 195</td>
<td>398 ± 93</td>
</tr>
</tbody>
</table>

NOTE. Data are mean cpm ± SD for triplicate wells of splenic mononuclear cells incubated with various concentrations of SEB for 72 h and then pulsed-labeled for 12 h with [3H]thymidine.

Resistance to SEB toxicity in vaccinated transgenic mice.

The enhanced reactivity of HLA-DR3.CD4.Ab0 transgenic mice to SEs makes the transgenic model an excellent system in which to study humanlike immune responses in mice. To establish whether HLA-DR3.CD4.Ab0 transgenic mice can be used as a model to examine vaccines against bacterial SAg, we vaccinated the transgenic mice with a nonsuperantigenic SEB vaccine (SEB L45R/Y89A/Y94A) and tested their serum for antibodies against SEB [25]. The transgenic mice were vaccinated with 3 doses of the SEB vaccine mixed with adjuvant or with adjuvant only every 28 days. After the first vaccination, serum was obtained from all vaccinees, and cytokine responses to attenuated SEB vaccine were analyzed. Unlike wild-type SEB, the injection of the SEB vaccine failed to increase the cytokine responses (data not shown).

On the day of each vaccination and 4 days before challenge with wild-type SEB, serum antibody titers against SEB were determined for each transgenic mouse (figure 3). In 3 of 5 mice, the SEB vaccine elicited detectable antibody titers against SEB after the first vaccination. After the second vaccination, all mice had detectable titers, and 3 of 5 transgenic mice injected with the SEB vaccine had titers > 104. After the second boost, antibody titers in most of the vaccinees reached 105. Control mice, injected with adjuvant only, had no serum antibody responses against SEB. The high serum titers against SEB in vaccinated HLA-DR3 mice indicate that these transgenic mice can mount a significant response against the attenuated bacterial SAg vaccine.

Figure 1. Proliferative responses of HLA-DR3.CD4.Ab0 transgenic mice to staphylococcal enterotoxins (SEs). Results are shown as mean cpm (± SD) for triplicate wells incubated with various concentrations of SEB (circles), SEC1 (squares), and SED (triangles) for 72 h and then pulsed-labeled for 12 h with [3H]thymidine. Filled symbols, HLA-DR3.CD4.Ab0 mice; open symbols, BALB/c mice. Data are means of triplicate values for T cell proliferation, as measured by [3H]thymidine uptake.
To determine whether the vaccination elicited serum antibodies that were capable of deactivating SEB-induced T cell proliferation, we analyzed the neutralizing ability of the vaccinee’s serum in a T cell proliferation assay. Here, we incubated wild-type SEB with pooled serum samples obtained from SEB vaccine–injected transgenic mice or mice vaccinated with adjuvant control (table 2). The mixture of toxin and serum was added to splenic T cells obtained from naive HLA-DR3 transgenic mice, and SEB-induced T cell proliferation was measured. Serum from vaccinated mice neutralized the SAg activity of SEB by 96% and 89% at doses of 100 and 1000 pg/mL, respectively (table 2), indicating that the SEB vaccine elicits substantial amounts of neutralizing anti-SEB antibodies capable of protecting the cells from SAg toxicity.

Next, we wanted to determine whether the in vitro protection against SEB could be verified in vivo. Vaccinated transgenic mice were challenged with 10^2 g of SEB wild-type, and we evaluated serum proinflammatory cytokine secretion (figure 4). As expected, adjuvant-vaccinated mice produced large amounts of IFN–γ and IL–6 in response to wild-type SEB challenge. Transgenic mice that were vaccinated with the recombinant SEB vaccine were fully protected when challenged with the wild-type SEB. In this group of vaccinees, IFN–γ and IL–6 responses against SEB were substantially inhibited (~94% and 97%, respectively, vs. adjuvant-vaccinated mice). These results confirm the ability of recombinant SEB vaccine to produce high titers of protective antibodies against wild-type SEB in HLA-DR3.CD4.Ab^b transgenic animal model. These data suggest that this transgenic model can be used in the development and efficacy examination of vaccine against bacterial SAGs in the context of human MHC class II receptors.

**Discussion**

Compared with human MHC class II, mouse MHC class II receptors have much lower affinities for bacterial SAGs. Previous experiments in our laboratory and others show that wild-type mice have a lower sensitivity to the biologic effects of bacterial SAGs than primates [28, 29, 32, 33]. However, the sensitivity of mice to SAg could be substantially increased after a potentiating dose of lipopolysaccharide (LPS) [32]. The mechanism of toxicity was dependent on MHC class II expression and LPS injection in conjunction with SAGs substantially elevated serum cytokines. Other compounds, such as cyclohexamide or D-galactosamine, also exacerbate cytokine actions or increase biologic half-life of cytokines because of their effects on hepatic clearance of cytokines [28, 34]. Because both bacterial SAGs and potentiating components in these models alter the cytokine profiles, it is very difficult to interpret the data, greatly limiting the ability to assess anticytokine therapy or other therapeutic compounds against bacterial SAGs. Other potential problems with these models are the lack of clear relevance to human responses. For example, D-galactosamine–potentiated mice produce massive tumor necrosis factor–α, with substantial liver damage, presumably in response to SAGs [35, 36]; however, hepatocellular damage has not been observed in patients with fulminating toxic shock syndrome [14]. These data suggest that...
artificial models for toxic shock may have severe limitations and may not represent actual human disease.

Other models have been proposed to study pathogenesis of bacterial SAgs. Enhanced sensitivity of human CD4 and HLA-DQ6 transgenic mice deficient in both endogenous CD4 and bacterial SAgs. Enhanced sensitivity of human CD4 and HLA-DR3 transgenic mice expressing both human DQ6 transgenic mice deficient in both endogenous CD4 and CD8 receptors to SEB was demonstrated elsewhere [37]. However, in this model, in vivo supersensitivity of the transgenic mice was apparent only when biologic activities of SEB were enhanced with d-galactosamine.

Secondary bacterial infections that produce SAgs may also enhance viral pathogenesis in mice by causing superinfections and lethal synergism between viral infections and SAgs [38]. Zhang et al. [39] showed that it might be possible to use the lethal synergism between influenza infection and superantigen exposure in mice as a model to examine immunopathology of infections. Other investigators used lethally irradiated mice rescued with donor bone marrow from SCID mice and found them to be sensitive to SEB exposure [40]. More recently, a chimera of a mouse model was used to show that these mice respond to SAg treatment by releasing proinflammatory cytokines [41]. Although all these models are helpful in examining SAg-induced abnormality, they are not ideal because they use potentiating agents, do not address MHC class II affinity differences (the main cause of mouse resistance to bacterial SAgs), or are very cumbersome to set up.

Here, we showed that transgenic mice expressing both human HLA class II and human CD4 are highly sensitive to bacterial SAgs. T cells from HLA-DR3 transgenic mice responded vigorously to bacterial SAg SEB, suggesting that the SAg presentation by the transgenic cells is similar to human bacterial SAg presentation. The intense reaction to SEB was evident by massive production of proinflammatory cytokines by HLA-DR3 transgenic cells. We also tested the transgenic system for vaccine development against SAgs. A recombinant SEB vaccine (SEB L45R/Y89A/Y94A), which lacks SAg activities, was highly immunogenic in this system. The vaccine induced high levels of protective immune responses against wild-type SEB. The production of protective antibodies was evident because passive transfer of antibodies obtained from SEB vaccine–injected mice, but not the adjuvant control, were able to neutralize the in vitro effects of wild-type SEB. The protective effects of the vaccine were also highlighted by in vivo studies. When transgenic mice were challenged with high doses of wild-type SEB toxin, control mice responded by producing high levels of proinflammatory cytokines, whereas mice immunized with the SEB vaccine produced little IL-6 and IFN-γ.

These experiments were crucial because our future efforts will focus on identifying HLA-DR–restricted T cell determinants of SAgs. Because most humans have been exposed to bacterial SAgs and have T cell determinants bearing this experience, we can elucidate human T cell determinants of relevant SAgs and compare them with immunodominant T cell epitopes generated in HLA-transgenic mice [42]. In support of this hypothesis, Geluk et al. [43, 44] showed that T cell determinants identified in HLA-DR3 transgenic mice were similar to those recognized by human T cells. The MHC class II transgenic mice should help to identify well-defined immunodominant T cell determinants and may help in designing subunit and multipeptide conjugate vaccination strategies. Furthermore, high-affinity epitopes when bound to tetramerized MHC could serve as markers to detect specific T cells in exposed or vaccinated persons.

One of the hallmarks of bacterial SAg exposure is a massive expansion of cognate TCR Vβ subsets. Here, we showed that SEB induced the highest proliferative responses, compared with SEC1 and SED, in HLA-DR3 transgenic mice. It may be possible that HLA-DR3/human CD4 transgenic mice may have higher

<table>
<thead>
<tr>
<th>SEB concentration, pg/mL</th>
<th>Vaccination type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEB vaccine</td>
</tr>
<tr>
<td>100</td>
<td>544 ± 265</td>
</tr>
<tr>
<td>1000</td>
<td>1050 ± 603</td>
</tr>
</tbody>
</table>

NOTE. Data are mean cpm ± SD for triplicate wells incubated with various concentrations of SEB for 72 h and then pulsed-labeled for 12 h with [³H]thymidine. HLA-DR3/human CD4 transgenic mice injected with attenuated SEB vaccine (SEB L45R/Y89A/Y94A) or adjuvant only were bled 10 days after the last vaccination. Pooled serum samples from each group were tested for inhibition of SEB-induced proliferation of HLA-DR3 transgenic T cells. The control value (i.e., the incorporation of [³H]thymidine for cultures that contained no SEB) was 385 ± 209 cpm.

Table 2. Inhibition of T lymphocyte responses to staphylococcal enterotoxin B (SEB) by pooled serum samples from mice immunized with attenuated SEB vaccine.

Figure 4. Protection of immunized HLA-DR3 transgenic mice against surge of cytokine responses after wild-type staphylococcal enterotoxin B (SEB) challenge. Vaccinated transgenic mice were challenged 14 days after the last boost. Serum samples were collected 8 h after injection of the challenge dose of SEB. Interferon (IFN)–γ and interleukin (IL)–6 responses in the serum were measured. Filled bars, IFN-γ responses; open bars, IL-6 responses. Results are shown as percentages of control (age-matched) mice that were not vaccinated.
amounts of SEB-reactive TCR Vβ subsets [45]. Direct assessment of this hypothesis is currently under way. We observed that injecting 200 μg of SEB per mouse without potentiating agent caused lethal shock in 100% (5/5) of HLA-DR3 transgenic mice and caused no visible effects on HLA-DRβ1 Ia or BALB/c mice (authors’ unpublished data). The resistance of HLA-DRβ1 Ia was predicted because Ia lacks the critical lysine 39 residue that inserts into a binding pocket of SAgs. Although this dose of SEB was very high, we believe that we may be able to enhance the biologic activity of SAgs by using double transgenic mice expressing both DR and DQ. In fact, T cells from HLA-DR3/DQ8 mice responded with much higher intensity to mouse myelin and developed severe inflammatory demyelination, in contrast to those seen in single transgenic mice [46].

Associations between HLA class II alleles and susceptibility to certain autoimmune disorders have been proposed [46]. The presence of HLA-DR3 has been correlated with a relapsing form of multiple sclerosis, and mice transgenic for HLA-DR3 are extremely valuable as a predictive model of autoimmune disorder [46, 47]. Furthermore, SAgs have been associated with various forms of autoimmune disorders [6, 8, 48–51]. Examination of the in vivo role of SAgs in induction or exacerbation of autoimmune diseases has been severely hampered because of a lack of good predictive models. Therefore, human MHC class II transgenic mice may provide an ideal avenue in which to examine the role of SAgs in the induction of autoimmune diseases.

Appropriate animal models are clearly needed to better understand mechanistically how bacterial SAgs confer acute and chronic diseases in humans. Resistance to disease development and discrepancies in the symptoms of toxic shock syndrome in many animal models make a good case for the use of better animal models [14]. We found that HLA-DR3/human CD4 transgenic mice deficient in both endogenous class II and CD4 receptors have robust responses against bacterial SAgs. SEB was the most potent in inducing T cell proliferation and caused a massive cytokine release that was comparable to human responses. The transgenic mice showed a tremendous response to a mutationally silenced SEB vaccine by producing high-titer protective antibodies. These data should clear the way for preclinical examination of therapeutics and other SAg-associated or conventional antibodies. These data should clear the way for preclinical examination of therapeutics and other SAg-associated or conventional antibodies. These data should clear the way for preclinical examination of therapeutics and other SAg-associated or conventional antibodies. These data should clear the way for preclinical examination of therapeutics and other SAg-associated or conventional antibodies. These data should clear the way for preclinical examination of therapeutics and other SAg-associated or conventional antibodies. These data should clear the way for preclinical examination of therapeutics and other SAg-associated or conventional antibodies.

References


Acknowledgments

We thank Paul Zhou for producing the DR2 mice, Günther Hammerling for the DR3 mice, Chris Benoît and Diane Mathis for the Ab5 mice, Tak Mak for the CD44 mice, and Richard Flavell for the human CD4 transgenic mice.