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Individual Genetic Variations Directly Effect Polarization of Cytokine Responses to Superantigens Associated with Streptococcal Sepsis: Implications for Customized Patient Care

Mohammed M. Nooh,*† Suba Nookala,*‡§ Rita Kansal,*† and Malak Kotb*†§

Host immunogenetic variations strongly influence the severity of group A streptococcal sepsis by modulating responses to streptococcal superantigens (Strep-SAgs). Although HLA-II–DR15/DQ6 alleles strongly protect against severe sepsis, HLA-II–DR14/DR7/DQ5 alleles significantly increase the risk for toxic shock syndrome. We found that, regardless of individual variations in TCR-Vβ repertoires, the presentation of Strep-SAgs by the protective HLA-II–DR15/DQ6 alleles significantly attenuated proliferative responses to Strep-SAgs, whereas their presentation by the high-risk alleles augmented it. Importantly, HLA-II variations differentially polarized cytokine responses to Strep-SAgs: the presentation of Strep-SAgs by HLA-II–DR15/DQ6 alleles elicited significantly higher ratios of anti-inflammatory cytokines (e.g., IL-10) to proinflammatory cytokines (e.g., IFN-γ) than did their presentation by the high-risk HLA-II alleles. Adding exogenous rIL-10 significantly attenuated responses to Strep-SAgs presented by the high-risk HLA-II alleles but did not completely block the response; instead, it reduced it to a level comparable to that seen when these superantigens were presented by the protective HLA-II alleles. Furthermore, adding neutralizing anti–IL-10 Abs augmented Strep-Sag responses in the presence of protective HLA-II alleles to the same level as (but no higher than) that seen when the superantigens were presented by the high-risk alleles. Our findings provide a molecular basis for the role of HLA-II allelic variations in modulating streptococcal sepsis outcomes and suggest the presence of an internal control mechanism that maintains superantigen responses within a defined range, which helps to eradicate the infection while attenuating pathological inflammatory responses that can inflict more harm than the infection itself. *The Journal of Immunology, 2011, 186: 3156–3163.

H ost genetic variations can profoundly affect susceptibility to infectious diseases and can modulate disease manifestations and outcomes (1–9). This fact is particularly true of infections with group A streptococci (GAS), which are important human pathogens that rank among the top 10 killer pathogens (10). The bacteria can cause a wide spectrum of diseases, ranging from uncomplicated throat or skin infections to lifethreatening autoimmune diseases (11–13) or severe and often fatal invasive diseases, including streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis (2, 5, 7, 14–16), also known as the flesh-eating disease.

Severe suppurative sequelae of GAS infections were responsible for many deaths before the 1920s; thereafter, invasive infections (e.g., scarlet fever and erysipelas) were relatively mild and manageable. Suddenly, however, severe invasive cases of STSS and necrotizing fasciitis resurfaced in the 1980s, and this resurgence was associated with the emergence of hypervirulent strains, including a subclone of the M1T1 serotype, which has exhibited increased virulence and unprecedented persistence and global dissemination for the past 30 y (17, 18). However, despite its increased virulence, this M1T1 strain has been isolated from patients with a very wide spectrum of clinical manifestations (19, 20), a finding indicating that host factors must play a pivotal role in modulating the outcomes of GAS infections (2, 5, 18, 21). Indeed, our studies provided evidence suggesting that genetic variations in the pathogen and the host can contribute to the observed stark differences in the severity of invasive GAS disease (18, 22).

Although GAS possesses a large battery of virulence factors that engage a wide variety of host defenses (14–16, 18, 23, 24), the streptococcal superantigens (Strep-SAgs) play a pivotal role in triggering potent inflammatory responses which, if not regulated, can set off host-mediated pathogenesis that, in genetically susceptible individuals, can cause multiorgan dysfunction, vascular collapse, and death (2, 5, 25).

Superantigens (SAgs) bridge HLA-II molecules on APCs to TCRs on T cells; this bridging results in strong activation of both cell types, which then elaborate inflammatory cytokines and mediators (25). The magnitude of inflammatory responses triggered by the same Strep-SAg in different patients can vary drastically (26), and there is a direct correlation between the levels of inflammatory cytokine responses and the severity of GAS sepsis (2, 27–30). This finding suggests that host factors play a crucial role in dictating disease severity and outcomes.

In addition to inherent and environmental factors, host genetic variations play an important role in susceptibility and outcomes of virtually all infectious diseases (1, 6–9, 31–38). We were specifically interested in determining the role of HLA-II allelic variations in GAS sepsis, because these highly polymorphic HLA-II
molecules play a pivotal role in SAg presentation to T cells. Indeed, we found important associations between specific HLA-II haplotypes and the various manifestations of GAS sepsis. Specifically, the HLA-II–DRB1*14/DQB1*05 (DR14/DRQ5) and DRB1*07/DQB1*02 haplotypes were associated with severe forms of GAS sepsis, whereas the HLA-II–DRB1*15/DQB1*06 (DR15/DRQ6) haplotype conferred strong protection from STSS (2).

In vitro and in vivo biological validation studies using transgenic animal models of GAS sepsis demonstrated that disease-modulatory effects are largely mediated by HLA-II allelic variations (2, 39).

In the current study, we investigated the molecular basis of the role of the DR15/DRQ6 alleles in protecting GAS sepsis patients from progression to STSS. Our findings suggest that the presentation of the same SAg to the same T cells by HLA-II allelic variants results in starkly different ratios of proinflammatory cytokines to anti-inflammatory cytokines. Specifically, levels of the regulatory cytokine IL-10 and ratios of anti-inflammatory to proinflammatory cytokines were significantly higher when the Strept-SAgs were presented by the protective HLA-II alleles than when they were presented by the high-risk HLA-II alleles. We believe that this differential polarization in SAg cytokine response profiles, which is effected by HLA-II allelic variations, is likely to dictate disease severity and outcomes in genetically diverse GAS sepsis patients, and it may also provide a mechanistic explanation for the association of the DR15/DRQ6 alleles in conferring strong protection against STSS in sepsis patients. The findings may also inform the design of customized therapeutic intervention strategies for ameliorating disease severity in genetically high-risk patients (2).

Materials and Methods
Preparation of partially purified native, secreted Strept-SAgs

Bacterial culture supernatants were prepared from overnight cultures of M1T1 GAS bacteria by ethanol precipitation followed by extensive dialysis, as detailed elsewhere (39). These partially purified culture supernatants contain the native secreted SAg mixture produced by clinical M1T1 GAS isolates (19, 39).

PBMCs and B cell lines

We isolated PBMCs from various subjects, as previously described (40). APC-depleted T cells were purified by subjecting freshly isolated PBMCs to one cycle of erythrocyte rosetting; they were then isolated with the human pan T cell isolation kit and autoMACS, according to the manufacturer’s instructions (Miltenyi Biotec). To remove any residual APCs, we incubated the purified cells overnight at 37˚C in RPMI 1640 supplemented with 10% (v/v) heat-inactivated FBS, 50 mmol L-glutamine, 25 mM HEPES, 50 U/ml penicillin, and 50 mmol streptomycin (RPMI complete medium) and then carefully recovered the nonadherent cells. In each experiment, the purity of the APC-depleted T cells was confirmed phenotypically by flow cytometry and functionally by their lack of response to PHA or to M1T1 SAg. Experiments in which purified T cells (cultured alone without any APCs) exhibited a response to PHA or to the Strept-SAgs were discarded, inasmuch as this response indicated that the T cells were still contaminated by autologous APCs.

The erythrocyte rosette-negative fraction, enriched with B cells and monocytes, was used as APCs in cross-presentation assays, and their purity (T cell depletion) was determined in each experiment by their lack of response to PHA or to the M1T1 SAg.

Immortalized B cell lines (BCLs) homozygous for the high-risk DRB1*1401/DQB1*0503 haplotype (line 9061) or the protective DRB1*1501/DQB1*0602 haplotype (line 9014) were obtained from the DRB1*1401/DQB1*0503 haplotype (line 9061) or the protective response to PHA or the M1T1 SAgs.

APCs) exhibited a response to PHA or to the Strep-SAgs were discarded, and then carefully recovered the nonadherent cells. In each experiment, the indicated numbers of autologous or allogeneic APCs or with mitomycin C-treated BCLs (0.5 × 10^5/well). Cells were stimulated with PHA (1 μg/ml) or several dilutions of the mixture of native secreted M1T1 SAgs. The amount of M1T1 SAgs used was predetermined by titration assays so that the selected SAg dilutions were within the linear proliferation curve and not in the plateau area. Such predetermined is important because it allows detection of differences in responses that are due to HLA or TCR variations. In some experiments, exogenous human rIL-2, IL-6, IL-10, IFN-γ, and TNF-α were simultaneously measured by using the human cytokine MILLIPLEX kit (Millipore). TNF-β concentrations were measured with the ELISA kit from R&D Systems. The assays were performed according to the manufacturer’s instructions, and cytokine concentrations were calculated from standard curves obtained from experiments run in parallel with the test samples. All sample measurements were performed in triplicate or quadruplicate, and data are presented as mean ± SD. Each experiment was repeated at least three times.

Cytokine assays

Culture media were collected from individual wells at 24, 48, and 72 h after stimulation, cleared of cells by centrifugation, and stored at −80˚C for subsequent analysis. The concentrations of cytokines IL-2, IL-6, IL-10, IFN-γ, and TNF-α were simultaneously measured by using the human cytokine MILLIPLEX kit (Millipore). TNF-β concentrations were measured with the ELISA kit from R&D Systems. The assays were performed according to the manufacturer’s instructions, and cytokine concentrations were calculated from standard curves obtained from experiments run in parallel with the test samples. All sample measurements were performed in triplicate or quadruplicate, and data are presented as mean ± SD.

Flow cytometric assessment of HLA-II DQ and DR expression

We used mAb labeled with allopurinol-cyanin specific to HLA-DR and FITC-labeled mAb specific to HLA-DQ (Becton Dickinson). Analysis of HLA-II expression on the surface of APCs was conducted with a FACScalibur flow cytometer (Becton Dickinson).

Analysis of the TCR Vβ repertoire by flow cytometry

Analysis of the TCR Vβ repertoire was performed by flow cytometry using the IOTest β Mark TCR Vβ repertoire kit (Beckman Coulter). CD3-PC5 conjugate was used to enable proper gating on T cells only. Approximately 5 × 10^5 cells per 100 μL PBS containing 1% BSA in each tube were stained with mAbs for several Vβ elements, according to the manufacturer’s instructions. Using a FACScalibur flow cytometer (Becton

FIGURE 1. Individual variation in proliferative responses to the native mixture of Strept-SAgs secreted by M1T1 GAS isolates from severe and nonsevere invasive streptococcal cases. PBMCs (2 × 10^5/well) from subject A (expressing STSS-protective HLA-II alleles) and from subject B (expressing STSS high-risk HLA-II alleles) were stimulated in triplicate with the indicated serial dilutions of the native mixture of SAgs secreted by M1T1 isolate 1, recovered from a severe invasive STSS case (gray circle), by isolate 2, recovered from a nonsevere invasive case (black square), or by PHA (1 μg/ml). Experiments were repeated at least six times. Data shown are mean ± SD. **p < 0.01, ***p < 0.001. To ensure reproducibility, experiments were also repeated with two additional subjects: subject C (DR15/ DRQ6) and subject D (DR14/DRQ5). Data, shown in Supplemental Fig. 1, demonstrate the reproducibility of differences in SAg responses when presented by DR15/DRQ6 versus DR14/DRQ5 haplotypes.

T cell-proliferation assays

PBMCs (2 × 10^5/well) or pure T cells (10^5/well) were cultured in RPMI complete medium in 96-well plates. T cells were cultured with the indicated numbers of autologous or allogeneic APCs or with mitomycin C-treated BCLs (0.5 × 10^5/well). Cells were stimulated with PHA (1 μg/ml) or several dilutions of the mixture of native secreted M1T1 SAgs. The amount of M1T1 SAgs used was predetermined by titration assays so that the selected SAg dilutions were within the linear proliferation curve and not in the plateau area. Such predetermined is important because it allows detection of differences in responses that are due to HLA or TCR variations. In some experiments, exogenous human rIL-2, IL-6, IL-10, IFN-γ, and TNF-α were simultaneously measured by using the human cytokine MILLIPLEX kit (Millipore). TNF-β concentrations were measured with the ELISA kit from R&D Systems. The assays were performed according to the manufacturer’s instructions, and cytokine concentrations were calculated from standard curves obtained from experiments run in parallel with the test samples. All sample measurements were performed in triplicate or quadruplicate, and data are presented as mean ± SD.

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PBMCs from subject A (with the protective HLA-II alleles) or subject B (with the high-risk HLA-II alleles) were analyzed by flow cytometry for levels of expression of DR and DQ alleles. HLA-II analysis was performed for each study, and the data shown are mean fluorescence intensity (MFI) ± SD.

Statistical analyses

Two-tailed Student t tests were used to determine statistical significance, which was set at the level of p < 0.05.

Results

HLA-II allelic variations dictate the magnitude of response to Strep-SAgs and differentially polarize cytokine responses

We inspected the proliferative and cytokine response profiles of subjects who expressed HLA-II alleles associated with protection from or increased risk for STSS. Subject A expressed the protective DR15 and DQ6 alleles, and subject B expressed the high-risk DR7 and DQ5 alleles. Our goal was to understand the mechanism by which HLA-II allelic variations modulate responses to the native secreted mixture of M1T1 Strep-SAgs produced by the most prevalent and virulent M1T1 GAS strain. We selected two clinical isolates of this strain, one from a patient with STSS (isolate 1) and the other from a patient with mild GAS sepsis (isolate 2). By all accounts these two isolates were genetically identical (18, 19, 41). We compared the proliferative responses of PBMCs from subjects A and B with the SAgs produced by these isolates, and we found that each subject’s PBMCs responded equally to either isolate. However, the magnitude of the proliferative responses was significantly different. PBMCs from subject A, with the protective HLA-II haplotype, reproducibly mounted significantly lower proliferative responses to the M1T1 SAgs than did PBMCs from subject B, with the high-risk HLA-II haplotype (Fig. 1; p < 0.01). This difference was specific to the SAg response, inasmuch as PBMCs from both subjects exhibited quite comparable responses to the polyclonal mitogen PHA. Additionally, differences in SAg response levels between these subjects were not due to variations in the level of HLA-DQ or HLA-DR expression by APCs, because they both expressed comparable levels of these HLA-II molecules (Table I). To ensure reproducibility, experiments were repeated with two additional subjects: subject C (DR15/DQ6) and subject D (DR14/DQ5). Data shown in Supplemental Fig. 1 demonstrate the same difference in responses between DR15/DQ6 and DR14/DQ5 individuals.

To determine the relative contribution of variations in individual HLA-II type and T cell TCR Vß repertoire to SAg response levels, we performed experiments in which we separated the T cells and APCs from PBMCs obtained from each subject and used a crisscross presentation design to test the response of these cells to the same M1T1 SAgs. Pure, APC-depleted T cells from the high responder (subject B) with the high-risk HLA-II alleles were presented with the M1T1 SAgs by pure APCs from the low responder (subject A) with the protective HLA-II alleles; in the reverse design, the same SAgs were presented to pure T cells from subject A by APCs from subject B. As shown in Fig. 2A, in both cases the SAg response was dictated by the HLA-II allelic type expressed on the APCs that presented the SAgs. Little or no effect was due to

![Graph](image_url)
differences in their TCR Vβ repertoire, inasmuch as both subjects had comparable repertoires before and after SAgs stimulation and, as shown in Fig. 3, both showed comparable expansion of MIT1 SAgs signature Vβ elements (i.e., Vβ2, Vβ4, and Vβ8) (42–45). However, despite their similar TCR Vβ expansion profiles, the presentation of MIT1 SAgs by APCs from subject A consistently resulted in significantly lower proliferative responses regardless of the T cell donor, whereas their presentation by APCs from subject B elicited significantly higher responses (Fig. 2A).

To further challenge our deduction that the dominant role of HLA-II allelic variations is to act like a rheostat to adjust the magnitude of SAgs responses and to rule out possible peculiarities in the APCs and T cells from subjects A or B, we repeated the study with two additional subjects (C and D) who expressed STSS-protective or high-risk HLA-II alleles, respectively, and observed the same results seen with subjects A and B. Furthermore, we also analyzed responses of pure, APC-depleted T cells from both subjects to the MIT1 Strep-SAgs, presented by immortalized BCLs homozygous for the STSS-protective DR15/DQ6 haplotype or the high-risk DR14/DQ5 haplotype. Presentation of the MIT1 SAgs by BCLs expressing the protective DR15/DQ6 haplotype elicited significantly lower proliferative responses than did presentation of the same SAgs by BCLs expressing the DR14/DQ5 haplotype (Fig. 2B). In fact, the responses of pure T cells from both subjects were significantly diminished in the presence of BCLs expressing the protective DR15/DQ6 alleles (Fig. 2B). Thus, our findings indicated that T cells from subjects expressing the protective HLA-II alleles are perfectly capable of mounting potent responses to the MIT1 SAgs but that the magnitude of the response is primarily governed by the HLA-II allelic type, which can override or mask any possible effects that may be due to variability in the responder’s TCR Vβ repertoire.

In concordance with proliferation studies, cytokine responses to the MIT1 Strep-SAgs were also affected significantly by the type of HLA-II alleles expressed by the presenting APCs. Levels of the proinflammatory cytokines IL-2, IL-6, and TNF-α, as well as the signature SAgs-induced inflammatory cytokines IFN-γ and TNF-β were significantly lower in cultures of PBMCs from subject A (the low responder) than in cultures of PBMCs from subject B (p < 0.001 to < 0.05; Fig. 4A). Interestingly, however, subject A produced significantly higher levels of the anti-inflammatory cytokine IL-10 than did subject B (p < 0.01; Fig. 4A). Similarly, response levels of the proinflammatory cytokines IL-2, IL-6, TNF-α, TNF-β, and IFN-γ were significantly lower when MIT1 SAgs were presented to pure T cells by immortalized BCLs expressing the protective haplotype DR15/DQ6 than when they were presented by BCLs expressing the high-risk DR14/DQ5 haplotype (p < 0.001 to < 0.05; Fig. 4B).

Levels of the anti-inflammatory cytokine IL-10 were significantly higher, at all tested time points, when the Strep-SAgs were presented by the protective alleles than when they were presented by the high-risk alleles (p < 0.001 to < 0.05; Fig. 4B). Of particular clinical relevance, the ratios of IL-10/IFN-γ were ~2- to 5-fold higher in the presence of the protective alleles than in the presence of the high-risk HLA-II alleles (Fig. 5), and this finding was true whether Strep-SAgs were presented by untransformed

**FIGURE 3.** Individual variation in the TCR Vβ repertoire does not account for observed variations in responses to Strep-SAgs. PBMCs from subject A (expressing STSS-protective HLA-II alleles) and from subject B (expressing STSS high-risk HLA-II alleles) were stained with fluorescently labeled Abs to the CD3 Ag, plus a panel of Abs directed at the 24 indicated human TCR Vβ subfamilies, as described in Materials and Methods, and were analyzed by flow cytometry. Each bar represents the mean percentage of cells expressing a specific TCR Vβ subfamily within the CD3+ population.
APCs or by BCLs expressing disease-relevant HLA-II alleles. This result suggested that HLA-II allelic variations control the magnitude of proliferative responses to the M1T1 SAgs and affect the balance between proinflammatory and anti-inflammatory cytokines, with a clear polarization toward a net anti-inflammatory response in the presence of the protective HLA-II alleles.

Although IL-10 levels peaked at 24 h in cultures in which the SAgs were presented by the BCLs homozygous for the protective alleles, they continued to increase and peaked at 72 h after stimulation with the same Strep-SAgs that were presented by the autologous APCs (Fig. 5). This difference in kinetics could be related to differences in the metabolic status of untransformed and transformed APCs.

Exogenously added IL-10 diminishes SAg responses in the presence of the high-risk HLA-II alleles

The finding that proliferative responses are lower and levels of the anti-inflammatory cytokine IL-10 are higher in the presence of the protective HLA-II alleles than in the presence of high-risk alleles suggested that SAg presentation by the protective alleles actively suppresses the response to SAgs rather than merely eliciting a weak response. To test this possibility, we assessed the effect of human rIL-10 on the response of the high-risk responder (subject B) (Fig. 6A). On the basis of the amount of IL-10 produced by the low responder (subject A), we examined the effect of rIL-10 (0.5–5 ng/ml) on the proliferative responses of the high responder. As shown in Fig. 6A, the response level of the high-risk responder (subject B) to the M1T1 Strep-SAgs was reduced to the level of the low responder (subject A). The same trend was seen when pure T cells from either subject were stimulated by the same SAgs in the context of BCLs homozygous for DR14/DQ5: the responses to SAgs were comparably elevated when no rIL-10 was added and were similarly reduced in the presence of this regulatory, anti-inflammatory cytokine (Fig. 6B). Thus, in all possible combinations and regardless of the T cell donor, the higher proliferative responses seen in the presence of the high-risk alleles were significantly diminished by the addition of 1 ng/ml rIL-10 (Fig. 6).

Neutralization of secreted IL-10 by anti–IL-10 Abs increases SAg responses in the presence of the protective HLA-II alleles

To challenge the direct role of IL-10 in attenuating responses to the M1T1 Strep-SAgs, we tested the effect caused by neutralizing the IL-10 that was produced in the presence of the protective HLA-II alleles. As shown in Fig. 7, the addition of neutralizing anti–IL-10 Abs (1 mg/ml) to cultures of cells from subject A increased the

**FIGURE 4.** HLA allelic variations dictate the net cytokine profile in response to Strep-SAgs. A, Total PBMCs (2 × 10⁶/well) from subjects A and B (expressing protective or high-risk HLA-II alleles, respectively) were stimulated in triplicate with an optimal dilution of the native secreted mixture of M1T1 SAgs. B, Pure, APC-depleted T cells from subject A or B (10⁵/well) were stimulated with the same native secreted mixture of M1T1 SAgs, presented by BCLs (0.5 × 10⁵/well) homozygous for the STSS-protective or the STSS high-risk HLA-II haplotypes. Culture supernatants were collected at the indicated time points after stimulation of cultures and were analyzed for the production of TNF-β, IFN-γ, IL-6, TNF-α, IL-2, and IL-10. Data are presented as mean ± SD. Significant differences were determined as detailed in Materials and Methods. *p < 0.05, **p < 0.01, ***p < 0.001.

**FIGURE 5.** HLA allelic variations dictate the net ratio of anti-inflammatory cytokines to proinflammatory cytokines produced in response to Strep-SAgs.

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**FIGURE 6.** Exogenously added IL-10 diminishes SAg responses in the presence of the high-risk HLA-II alleles. A, Subject A: protective haplotype; Subject B: high-risk haplotype. B, APC: DRB1*15/DQB1*0602 (protective haplotype) or DRB1*16/DQB1*0605 (high-risk haplotype).

**FIGURE 7.** Neutralization of secreted IL-10 by anti–IL-10 Abs increases SAg responses in the presence of the protective HLA-II alleles. A, Ratio of IL-10 to IFN-γ. B, Ratio of IL-10 to IFN-γ.
response of these cells to the Strep-SAgs to the same level as that seen in cells from the high responder (subject B).

Taken together, the findings described above indicate that the relative ratio of anti-inflammatory cytokines to proinflammatory cytokines, elicited in response to SAgs, is dictated by the HLA-II variant that presents the SAgs and that the protective DR15/DQ6 HLA-II alleles exert their effect by their ability to elicit higher ratios of anti-inflammatory cytokines to proinflammatory cytokines. Ongoing studies in our laboratory, in which we are analyzing whether there are differences in SAg-binding affinities to distinct HLA-II alleles, suggest that HLA-II allelic variations affect early signaling events and that this is likely affected by the overall orientation of the TCR-SAg-HLA trimolecular complex, which seems to be affected by HLA-II allelic variations.

Discussion

Shortly after the discovery of the mode of action of SAgs (46), which showed that they bind to HLA-II molecules on APCs and are then presented to T cells where they interact with TCR Vβ elements that are specific to each SAg, several reports showed that various HLA-II alleles may bind or present certain SAgs differently (47–55). The clinical relevance of this phenomenon was demonstrated by our translational genetic-association studies of a cohort of previously healthy patients with invasive GAS infections who presented with starkly differing disease severity (2). Although patients with the HLA-II DRB1*14/DQB1*05 haplotype, the DRB1*07/DQB1*02 haplotype, or both experienced a severe and highly morbid clinical course, those with the DRB1*15/DQB1*06 haplotype were protected against severe sepsis (2). Evidence that this effect was directly related to HLA-II allelic variations was provided by our recent study of invasive GAS disease (39). Our focus in the current study was to investigate the basis of the protective effects of the DR15 and DQ6 alleles in clinical and experimental STSS.

HLA-II allelic variations potentiated the magnitude of Strep-SAg responses and effected differential polarization of the cytokine response profile. Although SAgs are known to elicit potent inflammatory cytokine responses, our findings showed that, depending on the types of HLA-II alleles presenting the SAgs, the same SAgs can also elicit high levels of anti-inflammatory cytokine production. Thus, HLA-II allelic variants controlled the magnitude of SAg responses and clearly dictated the net ratio of anti-inflammatory cytokines to proinflammatory cytokines. Of particular clinical relevance, higher ratios of anti-inflammatory cytokine responses to proinflammatory cytokine responses to the M1T1 SAgs were seen when the Strep-SAgs were presented by APCs expressing the DR15 and DQ6 alleles, which are associated with strong protection from STSS. This finding suggests a plausible mechanism of protection.

Proinflammatory cytokines, such as IFN-γ and TNF-α, play an important role in the host defense against infection; however, if unregulated they can cause severe pathology and inflict more harm upon the host than the infection itself. Thus, the relative ratios of anti-inflammatory cytokine responses to proinflammatory cytokine responses, particularly during the early phases of sepsis or systemic inflammatory response syndrome (SIRS), may be a strong prognostic marker of disease severity, progression, and outcomes. Of particular relevance to this discussion is an earlier phase 1 clinical trial by Suntharlalangam et al. (56), in which SIRS developed with starkly different degrees of severity in six healthy volunteers who were given a superagonist anti-CD28 mAb (TGN1412) that directly stimulates T cells. All volunteers experienced a cytokine storm within 90 min of a single i.v. administration of TGN1412, and all became critically ill and required critical care. Although four of the six subjects eventually

![Figure 6](http://www.jimmunol.org/DownloadedFrom/thejournalimimmunol.org) Active suppression of Strep-SAg-driven proliferative responses by rIL-10. PBMCs (2 × 10⁵/well) (A) or pure T cells (1 × 10⁵/well) (B) from subjects A and B (low and high responders, respectively) were stimulated with a native secreted mixture of M1T1 SAgs, presented by BCLs expressing the indicated HLA-II haplotypes. The cells were stimulated in the presence or absence of rIL-10 (0.5–5 ng/ml) Data shown are from cultures stimulated in the presence of 0 or 1 ng/ml rIL-10 and are presented as mean ± SD. *p < 0.05, **p < 0.01.

![Figure 7](http://www.jimmunol.org/DownloadedFrom/thejournalimimmunol.org) Addition of exogenous rIL-10 (1 ng/ml) suppresses Strep-SAg-driven proliferative responses of the high responder (subject B), and addition of anti–IL-10 Abs elevates the response of the low responder (subject A). PBMCs (2 × 10⁵/well) from subject A (low responder) or subject B (high responder) were stimulated with the native secreted mixture of M1T1 SAgs in the presence of rIL-10 (1 ng/ml) or anti–IL-10 Abs (1 µg/ml), as indicated. Proliferative responses were determined as described in Materials and Methods. The response of subject A (with the protective haplotype) was enhanced by the addition of anti–IL10 Ab, whereas that of subject B (high responder) was inhibited by the addition of rIL-10. Data are mean ± SD. **p < 0.01.
recovered, two experienced severe disease and required intubation and mechanical ventilation. Serum levels of TNF-α, IFN-γ, IL-2, IL-6, and IL-10 were significantly higher in all of the patients within 4 h after the infusion of TGN1412; however, of particular relevance to this study was the impact of the kinetics and relative ratios of IL-10 to proinflammatory cytokines on the patients’ clinical outcome at the earliest time points of analyses. In all four subjects who recovered early, IL-10 concentrations were elevated at the early time points, as were the concentrations of the proinflammatory cytokines (e.g., IFN-γ): the ratio of IL-10/IFN-γ was ~0.6:1. This was not the case for the two patients with the most severe manifestations of disease: one exhibited a delayed onset of IL-10 production relative to IFN-γ production, and the other exhibited a much reduced concentration of IL-10 during the entire observation period and substantially lower ratios of IL-10/IFN-γ than did those who recovered earlier. Thus, subjects who exhibited delayed or lower ratios of IL-10 to IFN-γ and other inflammatory cytokines were unable to resolve the cytokine storm and regress into multiple organ failure, requiring extended hospitalization and intubation (56). These findings are consistent with the notion that the net balance between proinflammatory and anti-inflammatory cytokines, rather than their absolute levels, plays a central role in shaping the systemic manifestation of SIRS or sepsis (57–59). This notion is supported by several studies in addition to our own.

For example, Bjerre et al. (60) compared plasma levels of IFN-γ and IL-10 in patients with systemic meningococcal disease with those of patients with severe systemic Gram-positive septic shock and found several interesting differences. The plasma of patients with severe Gram-positive septic shock exhibited significantly lower concentrations of IL-10 and higher concentrations of IFN-γ than did that of patients with fulminant meningococcal septicemia (p = 0.001). Patients with high concentrations of IFN-γ and low concentrations of IL-10 experienced severe disease, and all died. A similar scenario was seen in our previous studies of GAS sepsis patients: those who experienced severe systemic manifestation were found to have an inherent propensity to mount high levels of inflammatory cytokine responses during infection, as well as after complete recovery, as determined by their ex vivo response to stimulation with SAgs produced by their own clinical isolates (2, 27). That this effect is directly attributed to HLA-II allelic variation is supported by our cross-presentation experiments, where by swapping the APCs of the high and low responders, so that the same SAgs were presented to each other’s T cells, we showed that the response was dictated by the HLA-II allele type. Taken together, our findings suggest that patients expressing the DR15/DQ6 alleles may be protected from severe sepsis and from progression to STSS because of their ability to mount a balanced response to the Strep-SAgs, whereas their ability to mount a proinflammatory cytokine response helps them to fight the infection, and their propensity to produce higher levels of IL-10 allows them to counteract the pathological effects mediated by proinflammatory cytokines elicited in response to the Strep-SAgs. Our finding that the neutralization by anti–IL-10 Abs abolished the protective effect of IL-10, and thereby elevates the responses of the protected subject to the same level as those of subjects with high-risk alleles, is consistent with the notion that a balanced ratio of IL-10 to proinflammatory cytokines may be necessary for successfully resolving the infection while protecting patients against host-mediated pathogenesis.

IL-10 is produced by several cell types, including monocytes and lymphocytes, and exerts multiple effects in immunoregulation and inflammation (61, 62). IL-10 can downregulate the expression of several inflammatory cytokines and the inducible or constitutive expression of HLA-II and costimulatory molecules (61, 63). The administration of IL-10 in several experimental and clinical studies was beneficial (64, 65), but the effect can vary considerably depending on the type of infection, the timing of IL-10 administration, and the relative levels of other inflammatory and anti-inflammatory mediators. The initial production of inflammatory cytokines in infection helps to initiate protective immune responses; however, the unregulated overproduction of proinflammatory cytokines (e.g., IFN-γ and TNF-α) can be detrimental because of host-mediated pathogenesis, which leads to vascular collapse and organ dysfunction (66). In contrast, overexpression of regulatory cytokines, such as IL-10, may induce overt immunosuppression and thereby increase mortality (67, 68). Therefore, an imbalance in the relative production of proinflammatory or anti-inflammatory cytokines can lead to serious pathological consequences (69, 70).

The findings presented in this article suggest possible means by which management of GAS sepsis patients can be customized, taking into account their genetic type and the presence of early disease biomarkers, such as the relative abundance of proinflammatory and anti-inflammatory cytokines. We believe that therapeutic approaches aimed at balancing inflammatory responses during sepsis are likely to ameliorate disease and reduce morbidity and mortality. This would also shorten hospitalization time and accordingly reduce medical costs.

Disclosures
The authors have no financial conflicts of interest.

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