Human Leukocyte Antigen Class II Haplotypes that Protect against or Predispose to Streptococcal Toxie Shock

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In the United States, 1.5–5.2/100,000 persons develop invasive Streptococcus pyogenes infections each year, and ~10%–20% of these patients go on to develop streptococcal toxic shock syndrome (STSS). Patients who develop STSS usually present with generalized erythema, conjunctivitis, and confusion. Fulminant cardiovascular shock develops over a period of a few hours, accompanied by multiorgan failure. Between 20% and 40% of patients with STSS die, compared with ~10% of patients with invasive streptococcal disease without STSS.

Streptococcal toxic shock syndrome (STSS) is triggered by exotoxins of Streptococcus pyogenes that function immunologically as superantigens. Microbial superantigens are a family of proteins with particular structural and sequence features that result in the shared ability to bypass the mechanisms of conventional, major histocompatibility complex (MHC)–restricted antigen processing [1]. Conventional antigens are processed within antigen-presenting cells, such as dendritic cells, into peptide fragments that are loaded into the peptide-binding groove of the MHC class II molecule for presentation at the cell surface to T cells. T cells will respond only if they recognize the class II molecule through CD4 and the specific peptide being presented. Thus, only a tiny fraction of the host’s T cell repertoire (<0.01%) will be activated. In contrast, superantigens bind, as intact proteins, directly to the MHC class II molecule and to the T cell receptor, extracellularly, at sites away from conventional peptide-binding sites (figure 1). On the T cell receptor, binding is to the variable region of the β chain (the Vβ region). Because ~30 different Vβ regions make up the total T cell repertoire in humans, and because most superantigens can bind to one region, up to 25% of a person’s T cells can be activated in this way [2]. For example, streptococcal pyrogenic exotoxin A (SPEA) principally targets Vβ12 and −14. Targeted T cell Vβ types are expanded and, subsequently, may be deleted from the T cell repertoire. Consequently, superantigen activity is characterized by Vβ-specific changes in the T cell repertoire.

When cases of STSS began to be reported worldwide in the 1980s, it seemed likely that novel virulent clones of S. pyogenes were probably responsible [3, 4]. Many of the superantigens associated with S. pyogenes are phage mediated. Strains carrying the spea gene were found to be closely linked with cases of STSS [5], and it was observed that contemporary strains carried spea genes (spea2 and spea3) that were subtly different from those carried by historical strains (spea1) [6, 7]. Although it is known that microbial factors play a central role in causing STSS, it is well recognized that toxigenic clones of S. pyogenes can be isolated from the whole spectrum of syndromes associated with S. pyogenes infection—from asymptomatic carriage, through trivial superficial infections, to life-threatening invasive disease and STSS [8, 9]. Were toxic shock syndrome solely the result of infection by novel virulent clones of bacteria, the epidemiology of the disease would be characterized by outbreaks and a high secondary attack rate. In fact, the epidemiology of severe streptococcal infection is characterized by isolated cases [10, 11]. The close household contacts of cases of STSS are frequently
found to be colonized with the organism that caused disease in the case patient but rarely develop more than mild disease [11]. Host predisposition to the toxic effects of superantigens must, therefore, play a significant part in determining the outcome of infection by toxigenic strains of \textit{S. pyogenes}.

During the early characterization of superantigenicity, it became clear that a hallmark of this effect was lack of classic MHC restriction [1]. This finding led to research focusing on the similarities, rather than the differences, in superantigen interactions with different MHC alleles. Crystal structures of several superantigens in complex with MHC class II are now published [12–14]. These demonstrate that although sites on the class II molecule involved in superantigen binding lie away from the most variable regions of the molecule, these sites are polymorphic between species, between isotypes, and, indeed, at a subisotype level. Interspecies differences in response to superantigens have been shown to be due to differences in MHC class II [15], and human leukocyte antigen (HLA) class II isotypes differ in their ability to support T cell activation by individual superantigens [16].

These observations raised the question of whether HLA class II polymorphisms could, at a subisotype level, influence the response to superantigens, because this would provide an explanation for interindividual differences in susceptibility to superantigen-producing strains of \textit{S. pyogenes}. We focused on SPEA partly because of its link to cases of STSS but also because sites on the HLA-DQ\(\alpha\) chain that interact with SPEA have been identified [12]. Using the crystal structure of the streptococcal superantigen SSA and a model based on the interaction between the staphylococcal superantigen SEB and HLA-DR, Sundberg and Jardetzky [12] made predictions about the precise sites of SPEA interaction with HLA-DQ. The interactions take place through the HLA-DQ\(\alpha\) chain. HLA-DQ\(\alpha\) chain is coded by the gene \textit{DQAI}. Twenty-four variants of \textit{DQA1} have been identified thus far. The HLA-DQ\(\alpha\) chain sites that make contact with SPEA during HLA-DQ–SPEA binding are nonpolymorphic in DQ\(\alpha\) chains coded by the \textit{DQA1*01} subgroup, which has a gene frequency of \(\sim 0.4\) in white populations. The \(\alpha\) chains encoded by \textit{DQA1*03} and \textit{DQA1*05} subgroups, which together have a gene frequency of \(\sim 0.36\) in white populations, all share 3 amino acid substitutions at sites of SPEA binding relative to the \textit{DQA1*01} sequence.

We demonstrated that polymorphisms in the superantigen-binding region of the HLA-DQA1 domain resulted in differential binding of SPEA and presentation to T cells [17]. The magnitude of the T cell response in terms of proliferation, and cytokine production was greater in response to SPEA presented by \textit{HLA-DQA1*01} than by \textit{HLA-DQA1*03} or *05. We also detected differences in the \(V\beta\)-specific response to SPEA, such that a broader range of T cell \(V\beta\) types are drawn into the response when SPEA is presented by \textit{HLA-DQA1*01}. It is likely that the greater response to SPEA seen in the context of \textit{HLA-DQA1*01} presentation is a result of a greater proportion of the T cell repertoire being involved. We have also detected differences between HLA-DR types in binding and presentation of staphylococcal superantigens [17] (authors’ unpublished data), suggesting that responses to many if not all microbial superantigens may be influenced by HLA class II polymorphisms.
Clinical studies designed to detect associations between HLA class II and susceptibility to superantigens are likely to be confounded in several important ways. First, the fulminating nature of STSS means that patients most severely affected may not survive to be enrolled in such a study. Second, multiple *S. pyogenes* strains may be isolated from cases of invasive disease, even in a single locale. Third, most pathogenic *S. pyogenes* strains carry multiple superantigen genes [18]. Nevertheless, in the only such study to be performed, Kotb et al. [19] studied patients with invasive *S. pyogenes* infections and sought differences in HLA class II haplotype in patients with and without features of superantigen toxicity. The majority of patients studied were infected with a clonal *speA* M1T1 strain. A clear association between HLA class II haplotype and susceptibility to superantigen toxicity was demonstrated. Intriguingly, the HLA associations these authors noted do not correspond with the different HLA-DQ types we found to influence response to SPEA. This finding is in keeping with data suggesting that other superantigens, such as streptococcal mitogenic exotoxin Z, are more important in the response to *S. pyogenes* [20]. Further studies exploring the influence of HLA class II on streptococcal mitogenic exotoxin Z are needed to resolve this question. Although different associations were revealed by the work of Kotb et al. and our own, taken together, these data clearly indicate that HLA class II polymorphisms provide a plausible explanation for observed differences in susceptibility to superantigen-mediated sepsis.

It is still not clear what selective advantage superantigens confer on *S. pyogenes* and *Staphylococcus aureus*. It is possible that they impair the development of specific immunity, thus prolonging superficial carriage. One previous dilemma has been why both of these organisms should make such a diversity of superantigens. If superantigen responses are determined by host HLA class II, it may be that HLA class II diversity has driven the evolution of superantigen diversity.

In addition to providing an explanation for the observed differences in susceptibility to superantigen-mediated shock, the influence of HLA class II on superantigenicity has several important implications for research into sepsis. Clinical trials of interventions in superantigen-mediated sepsis should be designed bearing in mind the influence of class II on the precise superantigens likely to be involved. The superantigen profile may need to be defined for each patient isolate, for example, by characterization of the T cell response to culture supernatant in vitro. Notwithstanding the failure of previous studies to show consistent Vβ repertoire changes in toxic shock [21, 22], the detection of Vβ-specific changes in T cell repertoire is sometimes used as a surrogate marker of superantigen activity in clinical studies. Such data need to be interpreted in the light of the superantigen profile of the organism involved and the class II influence over responses to those superantigens. Finally, the observation that minor changes in amino acid sequence at sites of superantigen binding can so markedly alter the superantigen response suggests that this may be a fruitful target for novel therapies. Monoclonal antibodies precisely targeted at the superantigen-binding regions of class II might well block superantigenicity without disrupting conventional antigen presentation.

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**References**