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MiniReview

Evolving superantigens of *Staphylococcus aureus*

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Abstract

Staphylococcus aureus bacteria utilize an extensive array of molecular countermeasures to manipulate the defensive microenvironment of the infected host and colonize potentially any tissue. The secreted polypeptides referred to as superantigens are unique among these countermeasures, because they target the multireceptor communication between T cells and antigen-presenting cells that is fundamental to initiating pathogen-specific immune clearance. Superantigens play a critical role in toxic-shock syndrome and food poisoning, yet their function in routine infections is not well understood. While an association of superantigens with cases of human autoimmune disease seems tantalizing, convincing data are not yet available. Blocking antigen-specific T-cell recognition is the primary evolutionary driving force behind superantigen selection, whereas superantigen-specific pathologies are by-products that are apparent only under select conditions.

Keywords: Superantigen; *Staphylococcus aureus*; Autoimmunity; Toxic-shock syndrome; Virulence factor; HLA-DR; T-cell receptor

1. The superantigen and disease paradigm

The common human pathogen, *Staphylococcus aureus*, is the most frequent cause of hospital-acquired infections [1]. These opportunistic bacteria colonize virtually any human tissue site, and survival under these highly variable conditions of growth and immune pressure is accomplished by the selective expression of factors that facilitate attachment, tissue invasion or immune escape. Bacterial superantigens (SAgs) are 23–29 000 M_r secreted polypeptides that aid in immune escape and cause severe physiological responses in the host. While SAgs are expressed by several medically important microorganisms, those produced by *S. aureus* are the most diverse and have genetic origins that are linked with SAgs of another common human pathogen, group A streptococci (GAS). Severe SAg-associated diseases caused by *S. aureus* and GAS also have many similarities [2].

The cellular receptors for SAgs are human major histocompatibility complex (MHC) class II molecules, primarily HLA-DR, and T-cell antigen receptors (TCRs) [3–6] (Fig. 1). The SAg binds to the TCR principally by contacts with the complementarity-determining region 2, the hypervariable region 4, and framework regions 2 and 3 of the variable domain (Vβ) of the TCR β subunit [7]. These TCR determinants form contacts with protein surfaces of a cleft between the two structural domains of the SAg [8]. Additionally, each SAg has the highest affinity for distinct Vβ subsets of TCRs. Binding to MHC class II molecules is sensitive to interspecies differences in protein structure. As a consequence, mice are 100–10 000 times less sensitive to SAg than humans [9]. Affinities of SAgs toward different HLA-DR allotypes also vary [10], suggesting that expression of the most favorable MHC receptor may increase host susceptibility to SAg-associated disease. No indications of species-dependent TCR affinities have been reported.

The normal antigen-specific signal transduction of T cells is disengaged by the SAg [11], which acts as a wedge to prevent contacts of MHC-bound, antigenic peptides with specific combining site elements of the TCR [7]. The magnitude of the T-cell response to SAgs is significantly greater than antigen-specific activation and results in pathological levels of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interferon-γ. In experimental models, the SAg-activated T cells are eliminated by a normal process of apoptosis or become nonresponsive to antigenic stimuli. However, the biological effects of SAg are not solely the result of the mass activation of T cells. Recent studies suggest that levels of antigen-specific T cells that are activated in a normal immune response have been greatly underestimated [12] and

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Fig. 1. Bacterial superantigens target receptors on T lymphocytes and antigen-presenting cells. Staphylococcal enterotoxin A (green) cross-links class II molecules of the major histocompatibility complex, such as HLA-DR (purple and cyan subunits), on the surface of monocytes, dendritic and other antigen-presenting cells (larger cell). This cell-bound complex is recognized by antigen receptors (magenta and red subunits) of T lymphocytes (smaller cell), leading to activation and cytokine release. The sizes of receptors and superantigen, relative to the cells, have been exaggerated to illustrate the binding interactions.

may in some cases approach quantities equivalent to SAg exposure. The primary target of SAgs in humans are $CD4^+$ T cells [13], and their activation results in T-helper type 1 (Th1) cytokine release, without evidence of any significant Th2 response [14]. Among the possible consequences of dominant Th1 responses are suppression of antibody expression and reduced clearance of the invading microbe [15]. The T-cell activation signal induced by SAgs may mimic the effect of high antigen dose and affinity in determining the Th1 bias in subsequent cytokine release [16].

Exposure to SAgs can occur by contact with environmental sources that are essentially cell-free, such as through ingestion of contaminated foods, or by release from proliferating bacteria residing on or within human tissues. Acute effects in humans range from self-limiting gastrointestinal distress to life-threatening toxic-shock syndrome (TSS). Enteric pathology is T-cell-dependent [17] and presumably results from a cytokine toxicity that is similar to TSS but localized. In addition, bacterial SAgs activate experimental autoimmune diseases [18]. Indirect evidence suggests that SAg may have some involvement in human autoimmune diseases. For example, the skin lesions of atopic dermatitis are frequently colonized with SAg-producing strains of *S. aureus* [19]. The expansion of T-cell subsets that express specific Vβ gene families of TCRs has been used as an indicator of possible SAg involvement in human autoimmune disease. Patients with acute Kawasaki disease had significantly elevated levels of circulating Vβ2+ and Vβ8.1+ T cells compared to the other control groups and a preponderance of SAg-expressing *S. aureus* or GAS [20]. On the basis of these observations, the authors of this report concluded that TSST-1 or another SAg activated an autoimmune response [20]. Yet, more extensive studies of bacterial isolates from clinical cases that originated from a wider geographic base could find no correlation between SAg expression and Kawasaki disease [21,22]. Based on knowledge gained from comprehensive studies of staphylococcal and streptococcal TSS [2,23], extensive epidemiological data that also incorporate molecular analyses of bacterial clonal types are needed to confirm a link between SAgs and human autoimmunity.

Bacterial strains that express SAgs are commonly isolated and are more virulent than nonproducing strains [24]. Approximately 40% of isolates from both healthy carriers and clinical cases are capable of expressing one or more SAg [25,26]. Yet, pathologies that are directly attributable to SAg alone probably occur only under exceptional conditions, despite the likelihood of frequent exposures to toxigenic bacteria. Therefore, colonization does not necessarily predispose one to the severe physiological effects of SAgs. Host, bacterial, and environmental factors are all likely to contribute to susceptibility.

Bacterial housekeeping genes of *S. aureus*, such as peptide and amino acid transporters, are necessary for growth during infection of most tissue sites [27] and are probably essential for expression of virulence factors. In contrast, virulence factors are nonessential gene products that are

selectively expressed and result in lower host immunity, release of host nutrients, facilitated bacterial adhesion to host tissues, or other related functions. The virulence factor itself can at times become the primary cause of disease. The menstruation-associated TSS epidemic was a result of changes in host mucosal surfaces, brought about by the composition of specific brands of tampons that encouraged expression of the SAg, TSST-1. Several outbreaks of staphylococcal TSS were traceable, by genetic characterization of unique combinations of virulence genes or alleles of virulence genes, to perhaps a single bacterial clone [23]. The clonal nature of *S. aureus* strains that exploited the altered mucosal niche suggested that these cases of TSS might have been the result of an unusual adaptation to environmental stimuli.

Expression of most SAgs is subjected to tightly regulated genetic controls that respond to extracellular feedback. Culture observations indicate that input from multiple environmental stimuli, such as nutrient depletion, pH fluxes, and cell density, affect the activation status of SAg genes within the bacterial population [28–30]. Expression is controlled by quorum-sensing regulatory mechanisms, consisting of a peptide pheromone, sensor, and response-regulator proteins [30]. Central to expression are the global regulatory loci *agr, sar*, and *xpr* [31–33] that control levels of the regulatory transcript RNAIII. A secreted peptide factor, which is a processed product of the *agr* locus, appears to autoregulate the *agr* operon. Three overlapping transcripts within *sar* may be differentially expressed in separate zones within the same nidus of infection, perhaps reflecting the physiological response of the microbe to distinct host microenvironments [34]. Collectively, these complex mechanisms have evolved to control secretion of SAgs.

The introduction of defects or deletions of genetic regulatory elements that control environmental feedback could conceivably result in elevated SAg levels in vivo. The emergence of bacterial strains that harbor this type of destabilizing mutation is likely to be uncommon, because of an evolutionary tendency towards regulated expression of acquired virulence genes. Thus toxigenic isolates that were associated with epidemic-like cases of TSS [23] probably originated from the same bacterial progenitor. In another study, staphylococcal food poisoning was most frequently associated with strains producing staphylococcal enterotoxin A (SEA) alone or in combination with other SAg [35]. Transcription of SEA, unlike most other SAgs, generally occurs independently of *agr* [36]. However, the clonal nature of bacterial isolates from these food poisoning outbreaks is not known. It remains a possibility that SEA-associated food poisoning and TSS are caused by strains that harbor unique combinations of genes or regulatory elements.

Additional determinants of the host environment can also contribute to SAg susceptibility. Most adults have antibody titers to common SAgs [37,38], probably as a result of repeated subclinical exposures. Therefore, normal immune responses probably prevent the occurrence of SAg toxicity while an immunocompromised host will be more susceptible to infection and the effects of SAgs. Anti-SAg immunoglobulin titers are lower in TSS patients [39], are slow to recover in patients recuperating from TSS, and low antibody levels are associated with recurrent disease [37,40]. Other bacterial septic factors can potentiate the physiological effects of SAgs. For example, lipopolysaccharides from Gram-negative bacteria dramatically increased mouse sensitivity [9], and anti-lipopolysaccharide increased survival in an experimental rabbit model of lethal TSS induced by TSST-1 [41].

2. Polypeptide evolution

The absolute number of SAgs is unknown and new genetic variants are frequently described. Amino acid sequence comparisons suggest that SAgs can be loosely compiled into three major subgroups and numerous sequence variations [42], while genetically they are all likely derived from common ancestral genes. Most remarkable is the observation that despite significant sequence divergence, with homologies as low as 14%, overall protein folds are similar among staphylococcal and streptococcal SAgs. The SAgs have evolved by strong selective pressures that preserved protein three-dimensional structure to maintain receptor-binding surfaces. HLA-DR receptor contact surfaces of SAgs involve variations of conserved structural elements [43]. These include a ubiquitous hydrophobic surface loop, a polar-binding pocket present in most SAgs, and one or more zinc-binding sites found in a select number of SAgs. The TCR-binding surfaces of SAgs, while more variable than HLA-DR contacts, are as tightly packed as antigen-antibody interfaces [8]. Comparison of antibody recognition between grouped SAgs [44] suggests that antigenic variation is maximized while three-dimensional structures, and hence receptor-binding surfaces, are conserved (Fig. 2). Evolutionary conservation of protein surfaces of SAgs that interact with HLA-DR is essential for immune escape because MHC molecules are the primary natural ligands of TCRs. Although it is presumed that binding to cell receptors of the immune system provides the selective pressure, it is also possible that bacterial ligands may be essential. As an illustration, streptococcal pyrogenic exotoxin C (SpeC) spontaneously forms homodimers. The dimer interface between the two SAgs closely mimics the molecular surface complementarity provided by the HLA-DR receptor [45]. The molecular surfaces of SpeC that are necessary for dimer and HLA-DR binding appear to have coevolved.

The great diversity of SAgs and the highly mobile nature of their genetic elements suggest an accelerated rate of evolution. In general, phage, plasmids and the mobile genetic units of pathogenicity islands [46] facilitate these

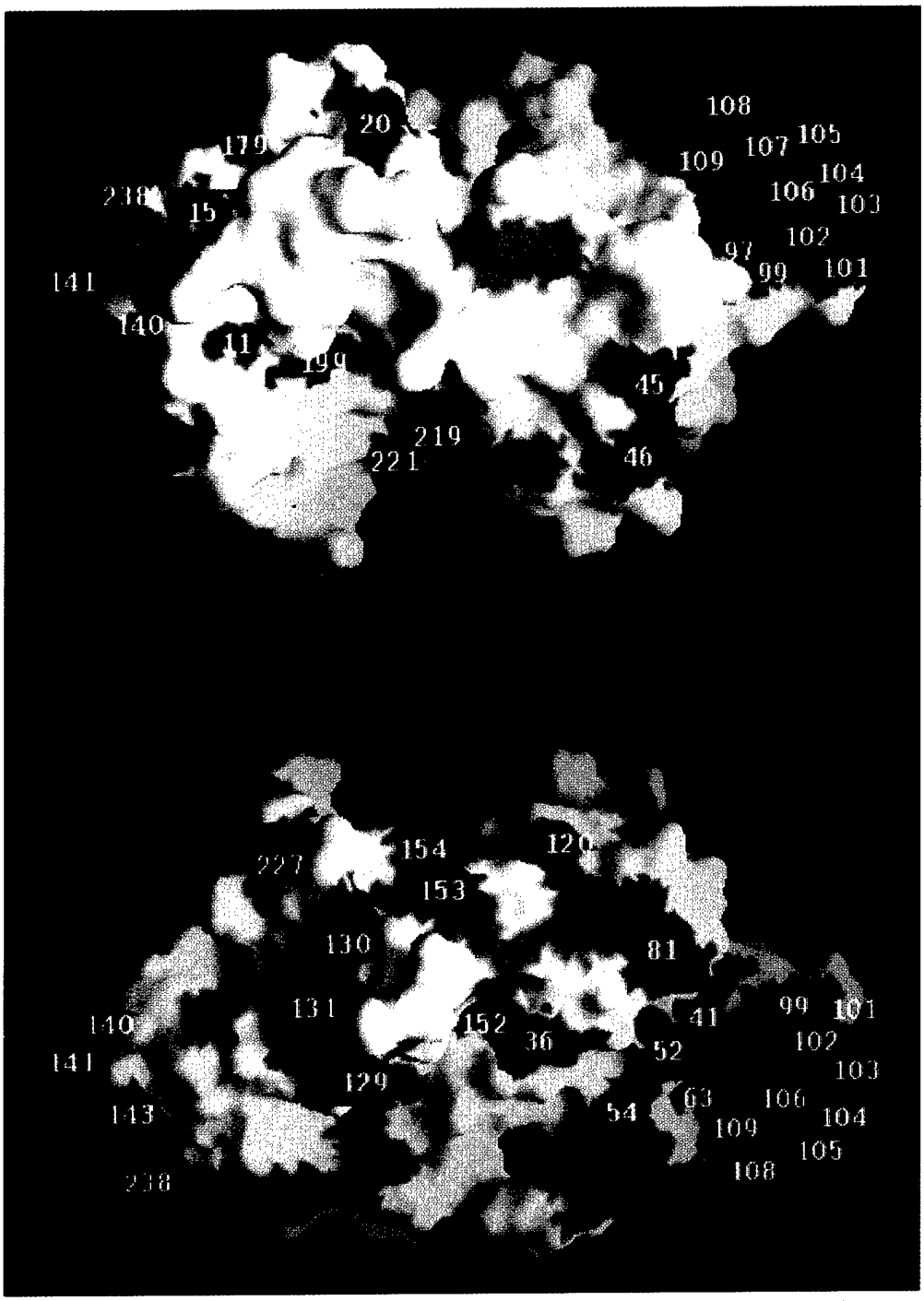

Fig. 2. Sites of potential antigenic diversity on the molecular surface of bacterial superantigens. A data base of 25 reported streptococcal and staphylococcal superantigens were aligned by amino acid sequence and this alignment was then adjusted to fit known or predicted secondary structure. Based on this final alignment, amino acid side chains that were ≥30% exposed to solvent on the surface of staphylococcal enterotoxin B were colored according to the extent of substitutions at each site that were observed among all superantigens. Color code for substitutions: 0–28%, red; 32–76%, gray; 80–88%, light blue; ≥92%, dark blue. Opposite faces of the molecule are shown. The disulfide-bonded loop (residues 97–109) is highly variable, while the HLA-DR receptor-binding residue L45 is one of the most conserved positions.

processes of fast evolutionary movement. For example, the spread of the TSST-1 gene *tst* is facilitated by specific interaction with select staphylococcal phages that transfer the encoding pathogenicity island [47]. Beneficial assimilation of newly acquired genes is usually accompanied by genetic stabilization and host control of expression [46]. Mutational silencing of potentially competing SAg genes and competition for preferred integration sites often reduces expression to one type of SAg polypeptide [48]. Weakly regulated gene expression, noted for SEA, may

represent an intermediate, unstable genotype that is more frequently associated with severe disease. Genetically inactivated pseudogenes, potential refuse of genetic stabilization, are often found in tandem with transcriptionally active SAgs, and may conceivably aid gene diversification by serving as partners in homologous recombination events. In addition, more speculative mechanisms may promote the evolution of virulence traits, such as defects in DNA-repair proficiency [49]. Also, SAg genes may be acquired by direct transformation by DNA from heterologous bacterial species [50], although regulated genetic competence, typical of *Streptococcus pneumoniae* [51,52], has yet to be demonstrated in *S. aureus*. Exchange of genetic elements between GAS and *S. aureus* is highly likely, considering the close homology between SAg genes carried by each respective species. Finally, staphylococcal strains that colonize domestic animals are potential genetic reservoirs for new SAg genes, and the transfer of these sequences may contribute to hybrid polypeptides. Many SAg genes isolated from domestic animals differ by only a few DNA bases from homologs that are found in human isolates [53].

3. Future directions

The growing threat from antibiotic-resistant *S. aureus* has heightened efforts to develop new means to control diseases caused by these organisms. Measures that target SAgs alone or in combination with other virulence factors should be considered as viable alternatives to antibiotics. The profuse amount of available protein structural data will facilitate the design of inhibitor molecules that block receptor binding. These data have been exploited to design recombinant SAg vaccines that have proven efficacious for the prevention of TSS in nonhuman primates [54]. Further studies are needed in animal sepsis models. Other promising approaches involve combinatorial chemistry or rational design in the discovery of new pharmacological agents to inhibit critical biochemical events that are initiated by SAg exposure. Moreover, inhibitors that are engineered to target the activation of key cytokines, such as TNF-α, may prevent or diminish the shock cascade.

The role of SAgs in emerging bacterial diseases should be considered from an epidemiological, molecular, and evolutionary perspective. Acquisition of methicillin resistance by *S. aureus* was a clonal event that eventually led to the global distribution of resistant isolates [55]. Reported increases in the frequency of toxigenic strains [56] suggest that widespread dissemination of SAg genes is also likely. However, there are insufficient survey data available to ascertain current trends in dispersal of genetic elements. In addition, the contributions of antibiotic-resistance determinants [57], or other genetically linked factors, to dispersal of SAg genes are poorly understood. Finally, future studies should carefully examine the relationship between bacterial virulence and SAg gene regulation in vivo to understand the molecular nature of events that cause the progression to TSS or invasive infections.

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13. ABSTRACT *(Maximum 200 words)*

Staphylococcus aureus bacteria utilize an extensive array of molecular countermeasures to manipulate the defensive microenvironment of the infected host and colonize potentially any tissue. The secreted polypeptides referred to as superantigens are unique among these countermeasures, because they target the multireceptor communication between T cells and antigen-presenting cells that is fundamental to initiating pathogen-specific immune clearance. Superantigens play a critical role in toxic-shock syndrome and food poisoning, yet their function in routine infections is not well understood. While an association of superantigens with cases of human autoimmune disease seems tantalizing, convincing data are not yet available. Blocking antigen-specific T-cell recognition is the primary evolutionary driving force behind superantigen selection, whereas superantigen-specific pathologies are by-products that are apparent only under select conditions.

14. SUBJECT TERMS
Superantigen, Staphylococcus aureus, Autoimmunity, Toxic-shock syndrome, Virulence factor, HLA-DR, T-cell receptor

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