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The contribution of group A streptococcal virulence determinants to the pathogenesis of sepsis

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Abbreviations: GAS, group A streptococcus; TSST-1, toxic shock syndrome toxin 1; STSS, streptococcal toxic shock syndrome; TLR, toll like receptor; SAg, superantigen; ABD, antigen binding domain; TCR, T-cell receptor; DIC, disseminated intravascular coagulation; TF, tissue factor

Streptococcus pyogenes (group A streptococcus, GAS) is responsible for a wide range of pathologies ranging from mild pharyngitis and impetigo to severe invasive soft tissue infections. Despite the continuing susceptibility of the bacterium to β-lactam antibiotics there has been an unexplained resurgence in the prevalence of invasive GAS infection over the past 30 years. Of particular importance was the emergence of a GAS-associated sepsis syndrome that is analogous to the systemic toxicosis associated with TSST-1 producing strains of *Staphylococcus aureus*. Despite being recognized for over 20 years, the etiology of GAS associated sepsis and the streptococcal toxic shock syndrome remains poorly understood. Here we review the virulence factors that contribute to the etiology of GAS associated sepsis with a particular focus on coagulation system interactions and the role of the superantigens in the development of streptococcal toxic shock syndrome.

Streptococcal Sepsis, Septic Shock, and Toxic Shock Syndrome

Sepsis is a progressively injurious systemic immunopathology that is triggered in response to a range of bacterial and fungal pathogens. The features of sepsis and septic shock were first described by Jacobs and Bone and have since been refined several times, along with the published guidelines for management of the condition.[1-4] The initial features of sepsis are mild and largely non-specific however, severe sepsis is characterized by impaired organ function and may be associated with coagulation defects such as disseminated intravascular coagulation.[1,2] The onset of septic shock is associated with a profound drop in arterial blood pressure that is refractory to adequate volume resuscitation, and precedes eventual multisystem failure and death.[1,2]

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Streptococcal toxic shock syndrome (STSS) is defined by a number of criteria that largely mirror those defining septic shock, coupled with evidence of an invasive GAS infection (**Table 1**).[3,5] While staphylococcal toxic shock syndrome presents as a discrete entity in association with otherwise mild or even occult infections (e.g., menstrual-related TSS in tampon users), STSS is commonly associated with systemic GAS bacteremia or pathological soft tissue necrosis. The defining criteria of STSS include some specific features that are not associated with toxic shock per se, but instead indicate the presence of the underlying GAS pathology (**Table 1**). As there is considerable overlap between the criteria for septic shock and STSS, and it is common for patients to satisfy the conditions for both, herein the term STSS is used interchangeably with streptococcal septic shock.

The Epidemiology of Invasive GAS Disease and STSS

STSS reportedly complicates approximately 10–16% of invasive GAS infections; however, the true incidence of toxic shock may well be higher as ICU admission is recorded in around 20% of invasive GAS cases.[6] STSS is associated with a case fatality rate of 35–45% which is almost twice as high as that reported for invasive GAS cases lacking a shock manifestation.[6,7] There is no correlation between STSS and any specific invasive pathology, and the condition is frequently encountered in association with bacteremia, necrotizing fasciitis, pneumonia, and puerperal sepsis.[8]

GAS strains are routinely divided into serotypes based upon the variable antigenic properties of the major surface M protein. While STSS can be caused by a large number of different GAS M serotypes, the condition is particularly associated with M1 and M3 strains, which together account for approximately 50% of STSS cases in Europe, and over 30% of all invasive disease cases in the United States.[6,7] The recent resurgence of serious streptococcal disease has coincided with the emergence of a highly virulent clone of serotype M1T1 GAS which is frequently recovered from invasive infections and STSS cases (discussed in detail below).[9,10] M1T1 clones can be distinguished from related serotype M1 isolates by the presence of the phage-encoded virulence

Table 1. The diagnostic criteria for septic shock and streptococcal toxic shock syndrome

Septic shock	Streptococcal toxic shock syndrome
Presumed or confirmed infection	**Isolation of GAS from normally sterile site**
Plus at least **ONE** of the following	Plus at least **TWO** of the following
Renal dysfunction	Renal dysfunction
Respiratory distress	Respiratory distress
Hepatic dysfunction	Hepatic dysfunction
Hematological abnormalities	Coagulopathy
Altered mental status	Erythroderma ± desquamation
Unexplained metabolic acidosis	Soft tissue necrosis
Tachycardia	Pain
	Tissue destruction
	Skin discoloration
AND	**AND**
Hypotension that is refractory to adequate volume resuscitation	Hypotension that is refractory to adequate volume resuscitation

factors *speA* (encoding a superantigen) and *sdaI* (encoding a DNase) within the accessory genome.[11] Furthermore, M1T1 isolates are associated with heightened production of the cytolytic toxins streptolysin O and NAD^+ glycohydrolase, which have been shown to contribute to the epithelial inflammation during streptococcal sepsis (discussed below).[12] The enhanced virulence that is associated with these genetic alterations, coupled with the ability of M1T1 to switch from a superficial to an invasive disease phenotype in vivo has facilitated global dissemination of this clone over the past 30 years.[9,10]

While the presence of a single superantigen gene (or superantigen gene repertoire) cannot be uniquely associated with the development of STSS, the phage encoded *speA* and *speC* genes have received particular attention with regard to invasive infection.[8,13,14] A high rate of *speA* and/or *speC* gene carriage has frequently been reported among STSS-associated isolates compared with those recovered from superficial infections however, the relevance of this association (if any) is yet to be elucidated.[8,13,15]

The Control of Virulence (Cov) System and Invasive GAS Infection

The CovR/S system (also known as CsrR/S) is a two component transcriptional regulator that modulates expression of 10–15% of the GAS transcriptome.[16-18] Mutation of the CovR/S system results in transcriptional upregulation of an aggressive repertoire of virulence associated genes, and thus triggers a phenotypic switch from a superficial to an invasive disease phenotype.[12,19-21] Indeed such mutations have been shown to account for the prolific phenotypic switching of M1T1 GAS in vivo. Of particular relevance is the reported upregulation of the *speA* and *speJ* genes which contribute to the inflammatory pathogenesis of STSS through non-specific T-cell activation (discussed in detail below).[11] CovR/S mutation also results in derepression of a multitude of virulence factors that facilitate resistance to opsonophagocytosis, including the hyaluronic acid capsule, streptococcal inhibitor of complement (Sic) and the chemokine protease SpyCEP.[10] Such mutations may help to perpetuate the symptoms of streptococcal sepsis by facilitating persistence of GAS at the nidus of infection.

Interestingly, the cysteine protease SpeB undergoes reciprocal regulation by CovR/S, yet is also implicated in the pathogenesis of necrotizing fasciitis and STSS.[22] SpeB can augment inflammation through activation of the kallikrein-kinin system (discussed in detail below) and by cleaving interleukin 1β precursor to form biologically active IL-1β.[23,24] While SpeB is therefore predicted to enhance the classical symptoms of shock, the precise role of the molecule during STSS remains unclear. Recently Ikebe et al. have reported that the frequency of CovR/S mutation is higher among strains recovered from STSS patients than those isolated from superficial infections although the significance of this finding remains the subject of some debate.[25,26]

GAS Interactions with the Coagulation System during Severe Sepsis

The pathophysiology of sepsis-associated coagulopathy

Blood coagulation (thrombogenesis) is an essential process that maintains the integrity of the circulatory system and provides innate protection against systemic infection through the isolation of invading pathogens.[27,28] Thrombogenesis is initiated following vascular injury and involves a stepwise series of proteolytic reactions that culminate in the formation of a fibrinous clot.[29] Vascular injury also facilitates adhesion and activation of circulating platelets which subsequently become incorporated into the growing clot.[29] Streptococcal sepsis is often associated with aberrant thrombogenesis resulting in the consumption of clotting factors and the formation of circulating microthrombi.[28,30] The pathological effect of this disseminated intravascular coagulation (DIC) is 2-fold. The formation of circulating microthrombi has been shown to result in venous thrombosis and infarction of the subcutaneous tissues in a murine model, while trafficking

of microthrombi to the organs is thought to contribute to the pathogenesis of organ dysfunction.[31-33] In addition, the depletion of platelets that results from microthrombus formation has been shown to impair normal clot formation resulting in severe secondary bleeding when vascular injury occurs.[28,32]

M1 protein interactions with fibrinogen

GAS has been shown to facilitate platelet aggregation through a series of stepwise, immune mediated reactions (**Fig. 1**). The initial interaction may be facilitated by a fibrinogen intermediate which simultaneously binds to the GAS M1 protein and the $\alpha_{IIb}\beta_3$ integrins present on the surface of platelets.[34] Alternatively GAS may colocalize with circulating platelets at sites of vascular damage where the components of the subendothelial matrix have become exposed. Subendothelial collagen in particular provides a platform for the multimerization of circulating von Willebrand factor, which has been shown to facilitate platelet immobilization upon the vascular endothelium under sheer force conditions.[35,36] Regardless of how the initial interaction occurs, the adherent platelets are subsequently activated by the anti-GAS IgG response which engages with platelet Fc receptors (FcγRII) and initiates clot formation.[34] Soluble M1 protein that has been released from the bacterial cell surface is similarly capable of activating and aggregating platelets in the presence of anti-M1 IgG.[37] M1 activated platelets may also interact with neutrophils and monocytes resulting in the activation of both cell types and the generation of more tissue factor.[37]

In addition to platelet activation, binding of fibrinogen to soluble M1 has been shown to result in the formation of large aggregates (M1/Fg complexes) that are capable of activating neutrophil β2 integrins.[38,39] β2 integrin activation triggers a release of heparin binding protein, a soluble inflammatory mediator that has been shown to induce localized vascular leakage. The crystal structure of these M1/Fg complexes has been resolved and the irregular coiled coil structure of the M1 protein has been shown to cross link four fibrinogen molecules leading to the construction of a supramolecular network.[38] Intravenous administration of M1 protein into mice induces colocalization of activated neutrophils and protein aggregates within the tissues of the lung, resulting in localized inflammation and pulmonary vascular leakage.[39] M1/Fg complexes have been detected in necrotic tissue biopsies recovered from STSS patients supporting a role for these complexes in the induction of vascular leakage during human infection.[39]

GAS and the extrinsic pathway of coagulation

One of the most important early initiators of the coagulation cascade is tissue factor (TF). TF is produced by the subendothelial tissues and monocytes, and forms an active complex with the serine protease FVIIa on contact with the blood.[29] The TF-driven (or extrinsic) coagulation pathway is known to be activated during sepsis, and several studies have reported increased plasma TF levels in patients manifesting trauma-associated SIRS and severe sepsis.[40-43] While TF upregulation in GAS sepsis patients has never explicitly been examined, serotype M1 and M3 (but not serotype M6) GAS have been shown to elicit TF synthesis from freshly isolated human monocytes.[44] A similar effect was achieved using purified soluble M protein indicating that this virulence factor may be partly responsible for GAS-mediated DIC.[30] This hypothesis is supported by recent data demonstrating that purified soluble M protein stimulates the release of TF-rich microparticles from activated monocytes, and that these microparticles can be identified in the blood of septic patients.[30,45] TF/factor VII complexes have also been shown to stimulate production of the proinflammatory cytokines IL-1β and TNF-α from macrophages and neutrophils via protease-activated receptor 1 activation.[46]

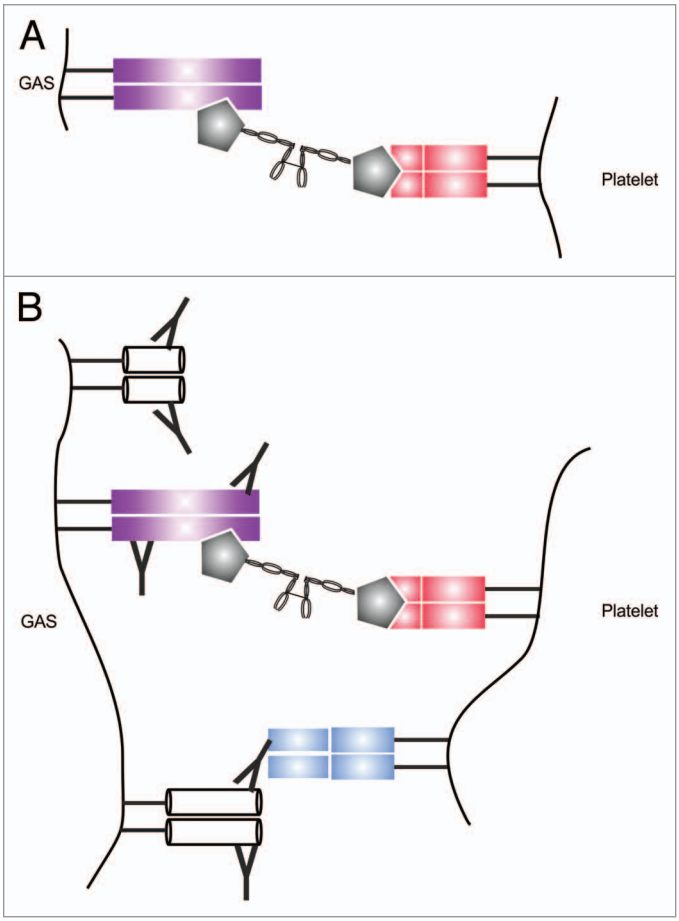

Figure 1. GAS-mediated platelet aggregation and activation. The initial cross linking interaction occurs via simultaneous binding of circulating fibrinogen (gray) by the M protein (purple) and the platelet $\alpha_{IIb}\beta_3$ integrin (red) (**A**). Platelet activation occurs when the Fc receptor (blue) comes into contact with surface associated IgG (**B**).

GAS and the intrinsic pathway of coagulation

In addition to the extrinsic arm of the coagulation cascade, GAS may also trigger coagulation via the intrinsic pathway through a specific interaction with the contact (or kallikrein–kinin) system. The contact pathway helps to stabilize the formed clot through activation of factor XII, and stimulates the generation of small proinflammatory peptides known as kinins from high molecular weight kininogen.[28] Activation of the contact system at the GAS cell surface triggers the release of bradykinin, a potent vasodilator that induces vascular leakage, localized edema, hypotension, and pain.[47] Bradykinin release results from the proteolytic processing of the soluble zymogen H-kininogen,

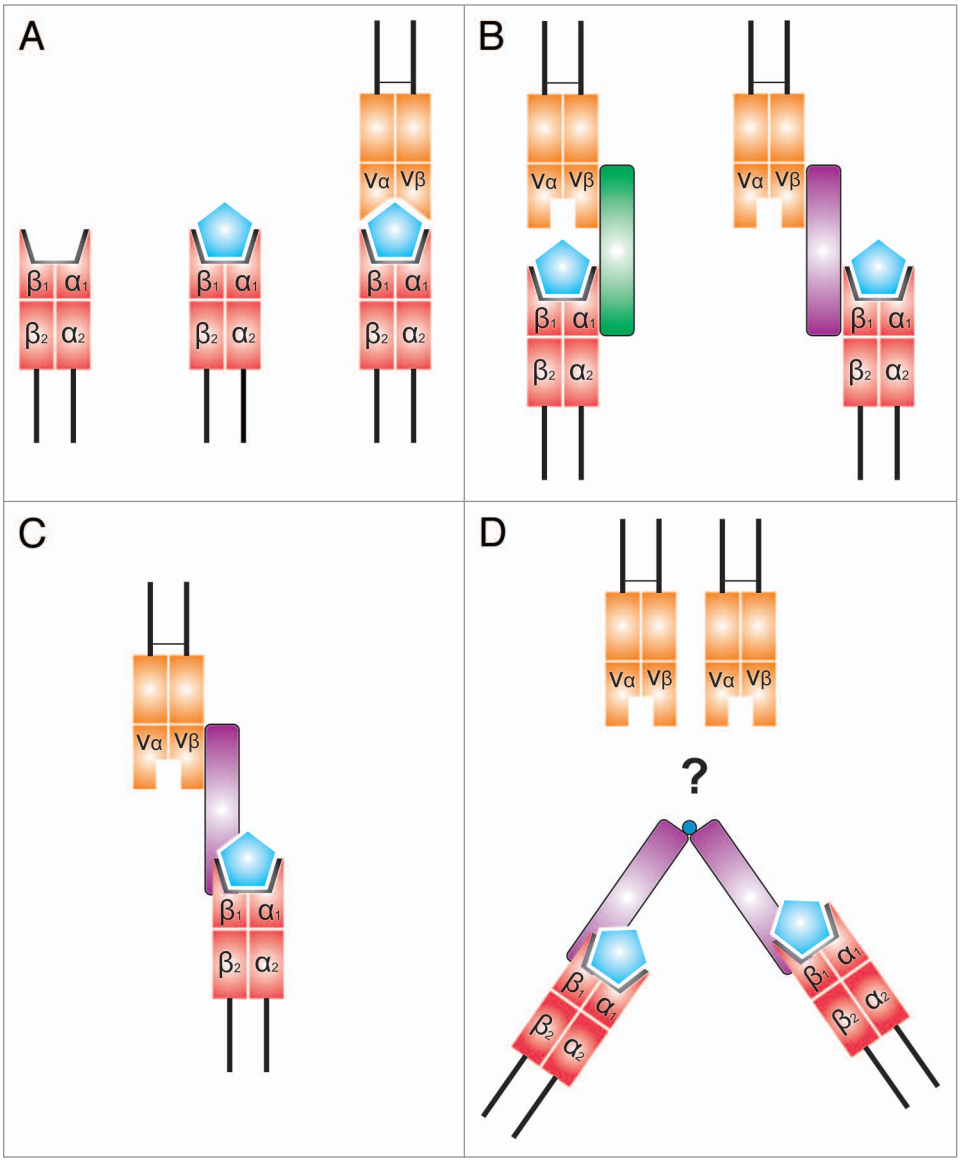

Figure 2. The different modes of SAg binding to MHC class II molecules (red) and the T-cell receptor (yellow). The mitogenicity of a conventional antigen (blue) is limited by its ability to cross link the hypervariable ABD with the TCR (**A**). SAgs are capable of activating many more T cells by binding to a specific repertoire of Vβ subsets. SAg presentation is facilitated by non-specific binding of the MHC α chain (e.g., SpeA in green) or β chain (e.g., SpeC in purple) (**B**) or by zinc-dependent β chain binding and engagement of the bound peptide antigen (**C**). Zinc-dependent β chain binding may also occur following dimerization of SpeC (**D**); however, the significance of this with regards T-cell activation is yet to be elucidated.

which can bind to the GAS cell surface via the M protein. Subsequent recruitment of plasma kallikrein results in a massive release of active bradykinin and thus promotes localized inflammation and vasodilation.[48,49] The secreted GAS cysteine protease SpeB has also been shown to promote cleavage of H-kininogen, suggesting a role for this protease during in vivo kinin activation and inflammation.[23] Systemic cleavage of H-kininogen has been demonstrated in vivo using a murine model of streptococcal sepsis and ex vivo using inflamed tissue from invasive disease cases.[50,51] Patients with severe GAS infections and STSS often demonstrate evidence of isolated activation of the intrinsic pathway of coagulation, as a surrogate of contact system activation.[52] Taken together, these studies suggest that activation of the contact pathway at the GAS cell surface may be responsible for much of the abnormal vascular physiology observed during STSS.

Superantigen-Mediated Modulation of the Host Immune Response

The superantigens

The superantigens (SAgs) are a family of toxins that are capable of activating a large set of human T cells, resulting in a massive production of proinflammatory cytokines. Conventional antigens are processed into short fragments by antigen presenting cells prior to translocation to the antigen binding domain (ABD, also known as the epitope groove) of the MHC class II molecule.[53] The amino acids that line the ABD are highly polymorphic and as a result each MHC class II isoform is only capable of presenting a finite number of antigens, and activating a very small subset circulating T cells (**Fig. 2**).

In contrast, SAgs are capable of non-specifically cross linking loaded MHC class II molecules with the variable N-terminal domain of the T-cell receptor (TCR) β chain (denoted the Vβ region) without prior processing (**Fig. 2**).[54,55] Subsequent ligation with the dimer interface of CD28 results in a massive release of proinflammatory cytokines (most notably IL-2, IFN-γ, and TNF-α) which in turn potentiates the acute shock and systemic vascular leakage that is associated with STSS.[54,56-59]

The majority of GAS SAgs are also capable of binding to MHC class II receptors via high affinity zinc binding (**Table 2**). Zinc binding is facilitated by the formation of a tetravalent metal complex that incorporates a conserved zinc atom located within the MHC class II β-domain.[59,60] Subsequent interactions with the N-terminus of the bound peptide antigen facilitates promiscuous MHC II engagement. Zinc-dependent binding thus facilitates deposition of SAg molecules upon the antigen presenting cell surface, leading to further T-cell activation.[59,61]

In addition to these monovalent binding strategies, SpeA and SpeC are capable of forming monomeric dimers that are

Table 2. The Vβ specificities and binding preferences of the GAS SAgs

SAg	MHC II chain bound	Zinc binding	Vβ specificity
SpeA	α	Y	2.1, 12.2, 14.1, 15.1
SpeC	β	Y	2.1, 3.2, 12.5, 15.1
SpeG*	β	Y	2.1, 4.1, 6.9, 9.1, 12.3
SpeH	β	Y	2.1, 7.3, 9.1, 23.1
SpeI	β	Y	6.9, 9.1, 18.1, 22
SpeJ*	β	Y	2.1
SpeK/L	β	Y	1.1, 5.1, 23.1
SpeL/M	β	Y	1.1, 5.1, 23.1
SpeM	?	Y	1.1, 5.1, 23.1
SSA	α	N	1.1, 3, 15
SMEZ-1*	β	Y	2.1, 4.1, 7.3, 8.1
SMEZ-2*	β	Y	4.1, 8.1

*SMEZ and SpeG are chromosomally encoded and encoded ubiquitously within the GAS metagenome. SpeJ is also chromosomally encoded; however, *speJ* negative clones of GAS are extremely common. While SpeB and SpeF were initially identified as SAgs, they have since been reclassified as a cysteine protease and a DNase (DNaseB) respectively. Table adapted from reference 59.

potentially capable of interacting with two MHC II molecules (and/or TCRs) simultaneously.[62,63] While the physiological relevance of this dimerization remains the subject of some debate, the current evidence suggests that it is not essential for the mitogenic activity of either SAg.[62-65]

Each SAg is specific for a distinct repertoire of Vβ gene products, 20–30 of which exist within the human genome (**Table 2**) and as such a single SAg is capable of activating up to 20% of all circulating naive T cells.[53,66] Cytokine production in response to secreted SAg is extremely rapid, as evidenced by animal models where gene transcription and systemic cytokine release can be detected within one hour of toxin exposure.[67,68] While the benefits of SAg production remain incompletely understood, the ubiquitous presence of SAg genes within the GAS metagenome suggests that SAg-mediated T-cell activation imposes a significant selective advantage upon toxigenic GAS isolates.[8,55,69]

Superantigen-mediated TLR upregulation

Further to direct TCR binding, streptococcal SAgs may augment cytokine release via a number of additional pathways. It has been reported that SAg exposure can enhance the TLR response to gram-negative lipopolysaccharide, thus enhancing TNF cytokine responses during natural coinfection.[70] This effect is particularly marked where there is hypoperfusion of the gastrointestinal tract that affects mucosal integrity. While such synergy has been hard to demonstrate in vivo, the effect has been replicated in vitro using primary human monocytes. In vitro priming of monocytes with physiological concentrations of streptococcal SAgs increases membrane expression of TLR4 via MHC class II ligation, and thus enhances the response to endotoxin and other TLR4 ligands.[71] MHC class II recognition of streptococcal SAgs may therefore contributes to the pathophysiology of sepsis through upregulation of the proinflammatory cytokines TNF-α, IL-1β, and IL-6.[71]

In vitro SAg exposure also upregulates monocyte TLR2 expression, enhancing the potential for further synergistic interactions.[72] TLR2 signaling in response to peptidoglycan and lipoteichoic acid results in activation of the transcription factors NFκB and AP1, stimulating the release of proinflammatory cytokines and a downstream induction of the adaptive immune response.[73,74] TLR2 transcription and expression has been shown to be upregulated in neutrophils and monocytes from streptococcal and non-streptococcal sepsis patients compared with healthy controls.[72,75,76] This suggests that TLR signaling may drive the immunopathological symptoms of sepsis prior to activation of the adaptive immune response.

Superantigen-mediated epithelial inflammation

The ability of SAgs to interact with epithelial cells has been demonstrated using a variety of cell lines however, most studies focus mainly on the staphylococcal superantigens.[77-79] Stimulation of vaginal epithelial cells with SpeA reportedly triggers production of the proinflammatory cytokines IL-6, IL-8, and MIP-3α in vitro; however, the specific receptor binding interactions that elicit this effect remain uncharacterized.[77] A concomitant production of streptolysin O, a hemolytic exotoxin that promotes cytolysis through the formation of transmembrane pores, is thought to enhance mucosal inflammation through localized tissue destruction and the exposure of further epithelial cells.[77] This "outside-in" signaling may result in SAg mediated T-cell activation at the submucosa and could therefore facilitate early activation of proinflammatory cytokine cascade during streptococcal septic shock.

Experimental Studies of the Effect of the GAS Superantigens

Laboratory studies

In 1989, Lee and Schlievert reported that the administration of purified SpeA stimulated an STSS-like pathology in a rabbit model, providing early evidence that this family of toxins could elicit features of profound inflammation and shock.[80] However,

this study was conducted prior to the identification of several additional GAS SAgs, one of which (SMEZ) is a highly potent activator of rabbit Vβ T cells, and is a recognized contaminant of crudely purified SpeA preparations.[81,82] Indeed targeted mutagenesis of the *speA* gene has little impact on the overall mitogenic activity of toxigenic GAS in vitro despite the presence of high SpeA concentrations (>500 ng/ml) within the tested culture supernatants.[83] It should be noted that SAg production in vitro is markedly reduced compared with in vivo synthesis, and that this effect is particularly apparent when phage encoded SAgs are considered (potentially as a result of in vivo phage induction).[84-86] Nevertheless, the targeted mutagenesis data suggests that a large amount of functional redundancy exists among the GAS SAgs, and that the differential Vβ specificities allow the repertoire of each isolate to act in concert to stimulate a potent T-cell mitogenesis.

The interpretation of experiments using bolus SAg administration is complicated by the rapid clearance of injected SAg toxins and the natural resistance of mice to bacterial SAgs. Rabbits, pigs, and non-human primates demonstrate greater SAg responsiveness than mice and are more amenable to SAg infusion; however, reagents to study such models are limited and present obvious ethical and cost implications. To circumvent these issues, humanized transgenic mouse models of streptococcal sepsis that express human MHC class II molecules have been used to demonstrate the impact of SAgs produced during GAS soft tissue infection.[85,87] Using a high SpeA-producing scarlet fever isolate and an isogenic *speA* knockout mutant, it was possible to show that Vβ-specific T-cell expansion did indeed occur in response to SAg exposure, and that T-cell activation takes place in a number of tissues during invasive infection.[83] Using a similar pair of isogenic GAS clones that differed in ability to produce SMEZ, a specific release of cytokines was attributed to SAg synthesis during sepsis, consistent with the widely held view that SAgs can and do trigger a cytokine storm.[88] Importantly this also indicates that STSS can occur in the absence of phage-encoded SAgs suggesting that carriage of one or more chromosomal SAg genes is sufficient to stimulate cytokine release.[69,89]

SMEZ elicits detectable T-cell proliferation at sub-picomolar concentrations and exhibits a high degree of antigenic variation that does not affect the potency or Vβ specificity of the molecule.[90] This pattern of antigenic variation suggests that SMEZ is uniquely important for survival of GAS during invasive pathogenesis, and that the need to escape antibody neutralization has driven the progressive variation within the *smez* locus, without affecting the mitogenicity of the molecule. Despite these observations, a highly conserved naturally occurring *smez* mutation has been shown to abrogate production of SMEZ by M3 GAS isolates, including those recovered from active STSS cases.[91] Taken together these data suggest that a combination of GAS virulence factors that include, but are not limited to, the SAgs are required to stimulate the signs of STSS.

Clinical studies

Despite the advances made through the study of SAgs under experimental conditions, definitive clinical evidence of SAg induced T-cell proliferation is lacking. Most clinicians report that lymphocyte levels are too low to conduct such studies at the time of presentation; however, a few case studies reporting T-cell repertoire changes during STSS have been published.[92,93] In addition several studies describe a sequential release of cytokines during STSS and invasive soft tissue infection that is consistent with SAg mediated T-cell activation.[94,95] SAg production and immune recognition is also evidenced by a handful of studies that successfully detected circulating SAg and anti-SAg antibodies within the blood of STSS patients by bioassay and ELISA.[94,96]

Several clinical studies have suggested that the SAgs may suppress phagocyte recruitment during active disease and therefore promote survival of bacteria at the nidus of infection.[97-99] However, the observed reduction in neutrophil recruitment during severe GAS infection most likely results from chemokine cleavage by SpyCEP and C5a peptidase.[100,101] Furthermore, conflicting data recovered from experimental analysis has suggested that the SAgs may enhance inflammation and subsequent phagocyte recruitment when produced in isolation. These concomitant effects are thought to result from the rapid induction of endothelium-activating cytokines such as TNF, and CXC chemokines.[83,102-104] Given that SAgs undergo a specific interaction with the components of the adaptive immune response, collateral interference with T-follicular helper cell function may be expected. Consequent interference with memory B cell activation may impact on clearance of GAS at the nidus of infection if the generation of de novo anti-GAS antibody is impaired.[57,96,105] The current data therefore suggests that SAg production is strongly immunostimulatory, but with an as yet unclear impact on anti-GAS immunity and bacterial clearance.

Inherited and Acquired Host Susceptibilities to Streptococcal Toxic Shock Syndrome

The lack of association between a specific SAg profile and the provocation of STSS, coupled with the observation that highly toxigenic GAS clones can be isolated from a spectrum of disease manifestation suggests that the immunogenetics of the host may be partly responsible for the outcome of toxigenic GAS infection.[106-108] The human MHC genes are highly polymorphic and three pairs of linked α and β chains (denoted HLA-DP, HLA-DQ, and HLA-DR) may give rise to four isoforms of MHC class II molecule. With the exception of the monomeric DRα locus, a multitude of different alleles has been described for each locus.[53] Structural characterization of several SAg-MHC complexes has established that the structure of the SAg binding domain is highly variable between different MHC class II isoforms.[109-111] Polymorphism within the $DQ\alpha_1$ locus has a direct effect on SpeA binding, and alleles that facilitate a higher affinity interaction have been shown to trigger a more prolific T-cell response in vitro.[108] MHC class II polymorphism has also been shown to influence the outcome of SAg mediated disease in vivo. Certain "protective" haplotypes have been shown to confer strong protection against STSS through the elicitation of a polarized, anti-inflammatory cytokine response.[107,112] Interestingly, SAg cytokine responses in experimental rodents are doubled in females compared with males, underlining the

importance of conducting such experiments in study groups controlled carefully for gender.[113] Whether such sexual dimorphism influences the incidence and outcome of STSS is unclear from existing human epidemiological studies, and may be offset by the generally enhanced male predisposition to acquisition of bacterial infection. Pre-existing immunity to streptococcal virulence factors such as the SAgs and the M protein may also influence disease severity, notwithstanding prevention of superficial and invasive infection acquisition, providing a rationale for the use of pooled intravenous immunoglobulin as an adjunct therapy for STSS.[57,114]

A number of large epidemiological studies have attempted to identify additional preventable risk factors for severe streptococcal disease. However, as invasive GAS is rare (~3 cases per 100 000 in the UK) and STSS somewhat rarer, clear patterns are hard to identify and most risk factors relate to acquisition of infection rather than susceptibility to severe disease or death. One study of invasive GAS infection has reported that the risk of death was highest among those with a prior diagnosis of malignancy, those admitted from long-term residential care and those with alcoholism listed as a comorbidity.[115] Mortality from GAS invasive infection was greatest during the winter months (December–April) when acquisition of invasive disease is also common, suggesting that the factors that influence immunity can also affect severity. Cases of invasive disease diagnosed in October were 80% less likely to have a fatal outcome than those in January, although surges in specific, highly virulent M types could explain this seasonal pattern.[115]

Concluding Remarks

The resurgence of invasive GAS infection over the past 30 years is a matter of great concern especially when one considers the rapid onset and high mortality rates associated with sterile site infections manifesting STSS. While SAg production is responsible for the most recognizable symptoms of toxic shock, there are many other facets that must be considered if novel treatment options are to be explored. Importantly the question of how and why the most highly virulent clones of GAS can produce such profoundly different disease pathologies between cases remains unanswered.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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