GRAM-POSITIVE TOXIC SHOCK SYNDROMES

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Gram-Positive Toxic Shock Syndromes: A Pathophysiological Review

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Summary:

Toxic shock syndrome is an acute, multi-system, toxin-mediated illness, often resulting in multi-organ failure. It represents the most fulminant expression of a spectrum of diseases caused by toxin-producing strains of *Staphylococcus aureus* and *Streptococcus pyogenes* (Group A streptococcus, GAS). The importance of gram-positive organisms as pathogens is increasing, and it is likely that toxic shock syndrome is under diagnosed in patients with staphylococcal or GAS infection who present with shock. TSS results from the ability of bacterial toxins to act as superantigens, stimulating immune cell expansion and rampant cytokine expression in a manner that bypasses normal MHC restricted antigen processing. A repetitive cycle of cell stimulation and cytokine release results in a cytokine avalanche that causes tissue damage, DIC, and organ dysfunction. Specific therapy centres on early identification of the illness, source control, and administration on antimicrobial agents including those drugs capable of suppressing toxin production (e.g. clindamycin, linezolid). Intravenous immunoglobulin has the potential to neutralize superantigen and mitigate against the subsequent tissue damage.

Keywords: Toxic shock syndrome, superantigen, cytokine, septic shock, gram-positive shock, NF-κB, Toll-like receptor

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Search strategy and selection criteria

Data for this review were identified by a Medline search restricted to English language articles. The search terms used were “toxic shock”, “staphylococcal sepsis”, “streptococcal sepsis”, “superantigen”, “nuclear factor kappa B”, “toll-like receptor”, “immunity”, “enterotoxin”, “exotoxin”, “t-cell receptor”, “septic shock” and “immunoglobulin”. Further articles were identified through review of the references in selected papers. No limit was set on publication dates or types.

Introduction

Gram-positive infections are responsible for approximately 50% of sepsis cases in the United States.[1] In addition to "classical" sepsis syndromes, several gram-positive species are also capable of producing disease through toxin production. Toxic shock syndrome is an acute, multi-system, toxin-mediated illness, typically resulting in shock and multi-organ failure early in its clinical course. It represents the most fulminant expression of a spectrum of diseases caused by toxin-producing strains of *Staphylococcus aureus* and *Streptococcus pyogenes* (Group A streptococcus, GAS). Despite a mortality rate higher than meningococcal septicaemia, toxic shock syndrome has not achieved the same level of awareness amongst healthcare professionals, who will generally encounter very few recognised cases during their career. TSS may present anywhere within the healthcare system, from occupational health departments to specialist hospital units and may progress with a rapidity that, once seen, is never forgotten. It is therefore essential that all healthcare practitioners have a sound appreciation of the epidemiology, pathophysiology, clinical features and management.

Epidemiology

Staphylococcal Toxic Shock Syndrome

Staphylococcal Toxic Shock Syndrome (TSS) was reported in 1978 and came to prominence in the early 1980's in the United States in association with the use of "highly absorbent" tampons amongst young healthy women, with high percentages of vaginal cultures yielding *S. aureus*.[2] During this period, the peak incidence was reported to be between 6·2 and 12·3 cases per 100,000 inhabitants per year in active

surveillance programmes.[3] With changes in tampon manufacture and usage advise the incidence fell to around 1 case per 100,000 inhabitants per year in the US.[4] Data from a surveillance programme in Minneapolis-St. Paul for 2000 to 2003 suggest local increases, with a rise from 0·9 to 3·4 cases per 100,000 inhabitants per year over the 4-year period.[5] Currently, between 1 and 5% of healthy women have vaginal colonization with a toxin producing strain of S. aureus. This is unchanged from 1980-81 although overall staphylococcal colonization had increased.[6] A French surveillance study of 55 TSS cases over a 30-month period has suggested that non-menstrual staphylococcal TSS is more prevalent than menstrual TSS, accounting for 62% of the cases. There were no deaths in the menstrual TSS group compared with a mortality rate of 22% for non-menstrual cases.[7]

Non-menstrual TSS may result from any primary staphylococcal infection, or indeed from colonization with a toxin producing strain of *S. aureus* (including methicillin-resistant *S. aureus*, MRSA). It can arise following disruption of the skin or mucous membranes, in association with abscesses or burns, and after surgical procedures, although commonly no source of infection is confirmed.[8] In light of this, TSS should be considered in patients with shock and infection with S. aureus.

Streptococcal Toxic Shock Syndrome

A second toxic shock-like syndrome attributed to *S. pyogenes* was reported in 1987.[9] Streptococcal TSS (STSS) secondary to invasive GAS soft tissue infections had a mortality of approximately 30% in some early series.[10] Studies from Australia, Denmark and the USA cite the incidence of invasive GAS infection at between 1·5 and 5·2 cases per 100,000 inhabitants per year, higher rates being found at the extremes of age and amongst ethnic minorities.[11-13] Between 5 and 14·4% of cases

developed streptococcal TSS with an attendant case fatality rate ranging from 23 to 44%. Higher incidence was also observed in those with underlying chronic illness, following varicella infection, and with non-steroidal anti-inflammatory use. Recently published data from 11 European countries (Strep-EURO) gave an incidence of STSS of 13% in streptococcal infection from any source. This increased dramatically to 50% in patients with necrotising fasciitis. The 7-day mortality rate from STSS was 44%.[14]

Pathophysiology

Superantigens trigger a cytokine avalanche

Bacterial toxins are pivotal to the pathogenesis of TSS and STSS. They act as "superantigens", which are protein toxins that share the ability to trigger excessive and non-conventional T-cell activation with consequent downstream activation of other cell types, and cytokine/chemokine release.[15] In addition to gram-positive organisms, some gram-negative bacteria, Mycoplasma, and certain viruses are known to produce these proteins, and so-called "endogenous superantigens" are found coded within the human genome (generally within endogenous retroviral sequences). The staphylococcal and streptococcal superantigens identified to date are single chain proteins expressed as precursor molecules, which are then cleaved to release the functional extracellular toxin.[16] The structure and function of *S. aureus* and *S. pyogenes* superantigens are the best characterized.[17-18]

Superantigens bypass conventional mechanisms of major histocompatibility complex (MHC) limited antigen processing, where antigens are processed into peptide fragments within antigen presenting cells such as monocytes. These fragments are then presented to the T cell via a specific peptide-binding groove of the MHC class II molecule. T cells will only respond if they recognize the class II

molecule *and* specific antigen fragment being presented. In contrast, superantigens bind simultaneously as unprocessed intact proteins directly to the MHC class II molecule and to the T cell receptor.[18-19] They bind at sites distant to the conventional peptide binding area, primarily to the variable region on the T cell receptor (TCR) called the Vβ region, although a small number of superantigens bind to the TCR α chain.[20-21] The interaction of superantigen with specific TCR Vβ regions induces clonal expression of T cells possessing those specific Vβ TCR patterns. This allows for identification of a characteristic Vβ "signature" for the superantigen concerned and may be diagnostically useful.[22-24]

Binding activates up to 20-30% of host T cells whereas conventional antigen presentation activates only around 0·01% of the host T cell population.[18, 25-26] Interestingly, endogenous superantigen gene sequences appear to down-regulate the expression of T cells with the Vβ TCR appropriate to that superantigen. This may prevent subsequent expansion of that T cell population in response to exogenous superantigen challenge, offering a degree of protection to the host by limiting the inflammatory consequences of the exposure.[27]

When superantigen binds to TCR and MHC Class II there is a rapid increase in cytokine expression by T-cells (primarily TNF-β, IL-2, and IFN-γ) and by antigen presenting cells such as monocytes (primarily TNF-α, IL-1β, IL-6), likely linked to activation of the transcription factor known as Nuclear Factor Kappa B (NF-κB).[28] NF-κB plays a central role in the generation and expansion of the inflammatory response, activation of coagulation, and the development of organ dysfunction (Figure 1). The degree of NF-κB activation also correlates with mortality risk.[29-30] Recently, antioxidant agents such as N-acetyl cysteine have been shown to reduce T cell proliferation and cytokine expression through

inhibition of NF-κB in a superantigen stimulated cell line model, and other inhibitory approaches are under active investigation.[31-32]

T cell activation leads to recruitment of further T and B cells to the site of infection. Clonal T cell expansion continues, as does activation of antigen presenting cells, "winding up" the release of pro-inflammatory mediators and contributing to increased procoagulant activity.[33] There is a complex interplay between the cytokines released during this pro-inflammatory avalanche, with IFN-γ rapidly inducing TNF-α and IL-6 expression.

Superantigen structure-activity relationships

Superantigens have been grouped into five distinct populations (I-V) based on their phylogenetic relationships.[26] Superantigens take part in two key interactions, firstly with MHC II and secondly with the TCR, using mechanisms that are thought to differ across the five superantigen groups.[34]

Superantigens interact with the MHC-peptide antigen complex (pMHC) in four main ways.[35]

1) Binding to the MHC α-subunit at a site that extends over the peptide surface and contacts the β-subunit. This peptide-dependent interaction is exemplified by TSST-1.

2) Binding to MHC α-subunit without any interaction with the peptide. This peptide-independent interaction is seen with Group II superantigens such as SEB and SEC3.

3) Binding to the MHC β-subunit in a zinc-dependent manner and involving multiple sites of interaction with the peptide. This occurs at areas common to

multiple peptides and is seen with Group IV and V superantigens such as SpeC and SEK respectively.

4) Binding by a combination of methods 1 and 2 e.g. SEA.

The structural conformation of superantigen interaction with TCR Vβ has also been studied.[34-36] Although all superantigens appear to bind to the second complement determining region (CDR2), the Vβ region contains multiple hypervariable elements and superantigens vary in their binding specificity and cross-reactivity to these. Superantigens with low specificity such as SEB and SEC3 require only a few of these elements to complete binding e.g. CDR2 and HV4. As specificity increases (e.g. SpeA) more and more of these hypervariable components are required, and hydrogen bonds form between the superantigen and TCR. With even greater specificity (e.g. SpeC) the complete TCR hypervariable element series including CDR1-3 and HV4 is required. TSST-1 demonstrates the greatest degree of specificity, targeting a loop in the third framework region (FR3) rather than relying on interaction with multiple hypervariable elements. TSST-1 also requires the presence of a particular residue in a particular location within the FR3 loop (Lys62) in order to activate T cells. The Group V superantigen SEK possesses an extended α3-β8 loop with a specific residue that binds to Vβ 5.1, FR4 and FR3 regions and is critical for T cell activation.

T cell activation may vary between Groups based on the overall affinity and conformation of the MHC-superantigen-TCR complex.

1) TSST-1 (Group I) acts as a bridge between TCR and MHC molecules, with no direct MHC-TCR contact. The affinity of the TSST-1-TCR and TSST-1MHC interactions is comparable to that of conventional MHC-TCR interactions and is an effective T cell activator.

2) Group II superantigens such as SEB act as a wedge between MHC and TCR, preventing contact between TCR and peptide antigen. However, there is direct MHC-TCR contact. The SEB-MHC and SEB-TCR interactions are not sufficient to achieve effective T cell activation. However, the additional MHC-TCR interaction brings the total affinity to the point where T cell activation occurs.

3) With Group IV superantigens such as SpeC there is a bridging of MHC and TCR and again no direct MHC-TCR contact, as with TSST-1. However, the resulting conformational planes are different. The combined affinities of the zinc-dependent TCR interaction and the Vβ contact are sufficient for T cell activation

Specific superantigen-disease associations

In menstrual TSS, there is a clear picture of the superantigen-disease relationship with staphylococcal TSST-1 responsible for the vast majority (95%) of menstrual-related TSS cases.[37-38] This has traditionally been attributed to the ability of TSST-1 to cross mucosal barriers, although SEB is also able to cross nasal, conjunctival and vaginal mucosa[39] It should be noted that TSST-1 is also detectable in around 50% of non-menstrual related TSS (NMTSS), the remaining cases being due primarily to SEB and less often to other members of the family such as SEC, SEG and SEI.[40] Reports of TSST-1 in association with MRSA are becoming more frequent. Highly virulent clones of MRSA harboring the TSST-1 gene (*tst*) have been associated with TSS, a critical point to remember in managing patients with MRSA and shock.[41]

There are multiple associations between streptococcal superantigens and invasive diseases. One of the most intriguing is soluble streptococcal M protein type M1. It is well known that M1 streptococcal isolates are more virulent, and recent work suggests that soluble M1 proteins *may* be superantigenic, preferentially activating T cells with Vβ2 and Vβ4 TCR. M proteins also activate T-cells via Toll-like receptor-2.[42-43] The status of M protein as a superantigen remains contentious.

The expression of superantigen genes is also important. Four alleles of the streptococcal pyrogenic exotoxin A (spe A) gene, designated spe A1-A4, have been found in isolates from patients with severe invasive GAS disease.[44] There is a marked geographic distribution of genetic strains, with organisms expressing SPE A2 and SPE A3 being responsible for the majority (60-90%) of streptococcal TSS episodes in Europe and North America and Australia.[45] In the Danish data which contributed to the Strep-EURO study, either SpeA or SpeC was present in all cases of STSS.[12]

Superantigen acts synergistically with endotoxin

Critically ill patients may be exposed to both endotoxin from gram-negative organisms and superantigen from toxin producing gram-positive organisms, even if the organism is simply colonizing the patient. In animal models, co-administration of endotoxin and superantigen reduced the LD_{50} by a factor of up to 50,000 compared with either toxin given alone.[46] Immune effector cells recognise so-called "pathogen associated molecular patterns" (PAMP) such as lipopolysaccharide (LPS) from gram-negative and lipoteichoic acid (LTA) from

gram-positive organisms.[47] This recognition is intimately involved in the genesis of the endotoxin-superantigen "double-hit". Although there is a degree of overlap, the detection system for LPS mainly involves activation of a Toll-like receptor (TLR-4) and the co-receptor MD2, and that for gram-positive organisms mainly involves lipoteichoic acid or peptidoglycan activation of TLR-2.[48-49] The detailed biology of these receptors has been well reviewed elsewhere.[50-52] Activation of each of these recognition systems results in pro-inflammatory mediator release and further inflammatory stimulation via NF-κB. Superantigen-MHC binding up-regulates the TLR-4/MD2 receptor system, priming monocytes for endotoxin exposure, amplifying the expression of TNF-α, IL-6 and IL-1β, and inducing vasodilatation through type I interferon over-stimulation of inducible nitric oxide synthetase (iNOS).[53] In addition, streptococcal superantigens appear to up-regulate TLR-2, which may become diagnostically useful in identifying streptococcal toxin-mediated disease in a manner analogous to Vβ expansion.[54]

Superantigen genes are mobile within and across streptococcal strains

The genetic "plasticity" of the streptococcal genome results from the presence of bacteriophages within the genome (so-called prophages) and may contribute to the observed variability in virulence. [55] Prophage genetic material may account for up to 10% of the streptococcal genome.[56] The majority of GAS superantigen genes are found within these prophage sequences (also called pathogenicity islands or genomic islands), and these phages are capable of transferring superantigen genes between GAS strains, or indeed from GAS strains to group C and theoretically to group G

streptococci.[57] In so doing, they can convert a non-virulent or less virulent strain into a highly virulent one. Invasive GCS and GGS incidence also appears to be increasing along with the presence of superantigen genes within these organisms.[58] An Australian study has recently identified superantigen genes in GAS isolates and correlated the superantigen with *emm* gene type (the gene encoding M protein).[59] Twenty-six different superantigen profiles were present in 107 isolates, distributed amongst 22 different *emm* types. These results were similar to previous reports and support the hypothesis that conserved superantigen profiles result from surface M proteins influencing the entry of bacteriophages in a selective manner.

Host-pathogen Interaction

Not all patients colonized or infected with a toxin producing strain of *S. aureus* or *S. pyogenes* go on to develop TSS or STSS, and secondary infection rates are low. The interaction between the host immune system and the pathogen may play a major role in response to the bacterial and toxic challenge.

Deficient antibody titres predispose to TSS

The absence of antibodies to superantigens appears to be a major risk factor for the development of TSS.[25,60] More than 85% of women between 13 and 40 years of age have TSST-1 antibody titres considered protective.[38] Low or negative titres have been demonstrated in 90·5% of patients with menstrual TSS and less than 50% of these patients failed to sero-convert within 2 months of their illness.[61] This may predispose to repeated episodes of STSS and has been linked to the ability of TSST-1 to suppress the action of immunoglobulin secreting cells.[25] The superantigen-mediated cytokine

response is associated with minimal T-helper type 2 cell (Th2) response, resulting in failure to support B-cell proliferation and differentiation. In addition, high concentrations of TSST-1 induce B-cell apoptosis. Levels of antibody to streptococcal superantigens are lower in those with invasive disease than in healthy controls.

Immunogenetics - HLA haplotype variation modulates severity

The magnitude of the inflammatory response is closely linked to disease severity and may be governed by host genetic factors such as MHC class II haplotype.[62] The sites at which superantigens bind to HLA class II are polymorphic, and differences in binding are reflected in a varying T cell and cytokine response. As an example, the DRB1*15/DQB1*06 haplotype is associated with strong protection from streptococcal TSS and reduced cytokine levels during GAS infection, whereas the DRB1*14/DQB1*05 haplotype is associated with predisposition to TSS.[63-64]

Gender alters response to sepsis and superantigen shock differently

There is a complex relationship between gender and susceptibility to sepsis with 17β oestradiol having variable effects on immune function (low concentrations augmenting and high concentrations inhibiting IL-6 and TNF-α release), and disagreement over the applicability of animal studies to the human setting.[65] There is broad agreement that male sex increases the risk of post-injury bacterial sepsis, bacteraemia, referral to ICU, risk of septic shock, and mortality in conventional sepsis. Females have been shown to have a more pronounced and prolonged immune reaction to sepsis whereas males appear more prone to develop variable degrees of immunoparesis after the initial immune response.[66] However, there is a

female preponderance in superantigen-mediated shock that extends to NMTSS.[3] It appears that something different is going on in superantigen-mediated shock that alters the gender influence away from that found in septic shock. The exact nature of this difference is unclear, but seems in part related to oestrogen. In a transgenic mouse model, females were (a) more susceptible to *S. pyogenes* sepsis, (b) had a significantly more pronounced TNF-α response to superantigen (SEB) than males, (c) had lower levels of soluble TNF receptors (sTNF-R) I and II both at baseline and on superantigenic challenge, suggesting deficient TNF-α removal and (d) had a greater degree of TNF-α-induced hepatic apoptosis and hence liver damage than males.[67] In addition, the authors were able to demonstrate that pre-treatment with the oestrogen receptor modulator tamoxifen decreased both the early and late rise in TNF-α, reduced the level of hepatic apoptosis, and increased the levels of sTNF-R. This is an area that requires cautious interpretation and further study.

Clinical Features and diagnosis

Toxic shock syndrome is characterised by an acute, progressive illness associated with fever, rapid onset hypotension and accelerated multi-system failure. Multi-system involvement is frequently established by the time of presentation. Clinical case definitions for both syndromes have been proposed (Panels 1 and 2).[68-69]

Staphylococcal Toxic Shock Syndrome

Staphylococcal TSS presents abruptly with a flu-like prodromal illness consisting of fever, gastrointestinal upset and severe myalgia followed commonly by confusion, lethargy and agitation. Symptoms of hypovolaemia are frequent at presentation. If present, a focus of infection is more likely to be superficial, may complicate burns or

a surgical wound, or may result from a foreign body. Desquamation is a characteristic late feature of staphylococcal TSS, occurring 10-21 days following disease onset. It is important to note that blood cultures are positive in less than 5% of cases of staphylococcal TSS.[8]

The clinical features of menstrual and non-menstrual TSS are identical in the majority of cases. Up to 95% of patients diagnosed with menstrual TSS have an onset of illness during menstruation.[70] Patients with NMTSS are more likely to have acquired the condition nosocomially and to have had prior antibiotic treatment. Fever and rash are more prevalent in early illness and NMTSS is more frequently associated with CNS manifestations and renal complications.[8] Non-SEA and non-TSST-1 superantigens appear to have greater neurotoxic potential.[7] Post-operative NMTSS usually occurs within 48 hours of surgery and in many cases evidence of clinically significant surgical site infection is lacking at the time of presentation. Following the onset of symptoms, progression is rapid and multi-organ failure can be present in as little as 8 to 12 hours. Recurrence of menstrual TSS has been well documented but is rare in NMTSS. NMTSS must be considered in the aetiology of shock states in patients with definite or suspected staphylococcal infection.

Streptococcal Toxic Shock Syndrome

Streptococcal TSS more commonly arises from deep-seated invasive soft tissue infections such as necrotising fasciitis, cellulitis and myositis. Pain may be severe and relentless and is a common reason for seeking medical attention. A flu-like

illness is also common in the early stages with fever, sore throat, swollen lymph nodes and gastrointestinal upset. Those patients with a defined entry site may have early and visible signs of inflammation. In the absence of a defined portal of entry clinical evidence of a deep infection becomes more obvious as the illness progresses. The initiating injury may be blunt trauma, muscle strain, and haematoma or joint effusion and may appear trivial, so careful history taking is essential. Examination may reveal bruising, haemorrhagic bullae, skin sloughing and oedema. Hypotension and organ dysfunction are rapidly progressive.

The majority (60%) of patients with STSS have positive blood cultures.[71] Presence or absence of bacteraemia does not affect mortality. The diagnosis of STSS is confirmed when GAS are cultured from normally sterile body fluids in patients with shock and multi-organ failure. The mortality rate associated with streptococcal TSS is much higher than with staphylococcal TSS, and has been quoted at up to 80% in association with myositis.[26] A murine model of the disease suggests that an early initial infection may be followed up to 3 weeks later by bacteraemia, at which point symptoms and signs of the disease appear and that trivial injury such as bruising amplified the severity of the bacteraemia.[72]

Therapeutic Strategies

Supportive management and source control

Immediate intervention and resuscitation are required. In the early stages of illness, the causative organism will be unknown and the same basic therapeutic strategy should be applied as to any case of septic shock with active fluid resuscitation, early use of vasopressors and/or inotropes, and intubation and mechanical ventilation if

required. An appropriate antimicrobial regimen should begin immediately following culture samples being taken.

A thorough search for infective focus is essential. The presence of necrotizing fasciitis or myositis mandates immediate aggressive surgical debridement and is a true surgical emergency. The underlying tissue infection may be much more extensive than initially appreciated and the rate of spread may exceed the rate of debridement if a conservative approach is taken. Surgical wounds should be considered potential sources of infection, even in the absence of overt signs. Any infected wound should be reopened and widely debrided, and packs or infected devices removed. In females, a vaginal examination should be carried out and any tampon or foreign body removed.

Antimicrobial therapy to reduce toxin production as well as organism load

Inadequate initial antibiotic therapy increases mortality in intensive care patients with severe sepsis and septic shock.[73-75] Clinical trial data comparing antibiotic regimens in TSS is lacking. Recommendations are based on in vitro studies and theoretical principles and include the use of a β-lactam agent and a lincosamide pending culture results.[76] Therapy is focused on reducing both exotoxin production and organism load. In cases where the causative organism is unknown, the antibiotic regimen should cover both *S. aureus* (including MRSA if indicated) and *S. pyogenes*. There is an increasing range of antimicrobial agents active against gram-positive organisms, and definitive therapy decisions require knowledge of local drug availability, clinical preferences, and sensitivity pattern. Potential therapeutic agents for various causative organisms and strains are shown in Table I.

Therapeutic principles

Group A streptococci remain exquisitely sensitive to β lactam agents including penicillin G, an agent often considered as part of first line therapy. It is usually given with clindamycin, which has inhibitory actions on protein synthesis including superantigen production. Although Penicillin G is bactericidal, it has been shown to be less effective in the face of a higher organism load. This is perhaps due to the reduced expression of penicillin binding proteins by bacteria in the stationary phase of growth, which is reached more rapidly with large organism loads.[77] Streptococcal resistance to macrolides and fluoroquinolones appears to be increasing, especially in Europe and Asia. In addition, macrolide resistance is linked to lincosamide (clindamycin) resistance in so-called Macrolide-Lincosamide-Streptogramin B resistant *S. pyogenes* (MLS).[78-79] Therapy for MRSA has commonly included vancomycin, however *S. aureus* strains with intermediate sensitivity (GISA) or resistance (GRSA) to glycopeptides are increasing.[80] The newer agents such as linezolid, daptomycin and tigecycline are active against *S. pyogenes,* MRSA, GISA and GRSA and represent effective (if expensive) agents to fall back on.

The rationale for clindamycin in initial therapy regimens

Clindamycin is a bacteriostatic lincosamide with efficacy unaffected by bacterial growth phase or inoculum size. In a mouse model of *S. pyogenes*-induced myositis, penicillin was ineffective if treatment was delayed by greater than 2 hours following onset of infection, whereas mice receiving clindamycin had improved survival rates even if treatment was delayed.[81] Clindamycin has been shown to inhibit toxin production by both *S. aureus* and *S. pyogenes*. In vitro

models comparing the effects of clindamycin, linezolid and penicillin on SpeA release have shown a significant decrease in SpeA production in regimes containing clindamycin and linezolid as opposed to penicillin G alone, despite the theoretical ability of clindamycin to suppress synthesis of penicillin binding proteins.[82] This antagonistic effect does not seem to be clinically relevant with adequate drug dosages. Linezolid and clindamycin have both been shown to reduce TSST-1 production, and clindamycin significantly reduces SpeA expression by of *S. pyogenes* compared to ampicillin.[83] Linezolid has been used successfully to treat staphylococcal TSS and has demonstrated reduced TSST-1 production.[84]

Effects and Mechanism of Intravenous Immunoglobulin

Patients with a deficient antibody response against TSST-1 are at increased risk of primary or recurrent TSS, and patients with invasive GAS infections have significantly lower levels of superantigen-neutralising antibodies.[60] Case reports published in the mid-1990's suggested improved outcomes for patients with STSS treated with intravenous immunoglobulin (IVIG). [85-87] Administration of IVIG can block in vitro T cell activation by staphylococcal and streptococcal superantigens. Factors beyond the presence of neutralising antibodies may contribute to the efficacy of IVIG, at least in vitro, as the suppressive effect of whole IVIG on SEB-induced T cell proliferation and cytokine production remains significant even after removal of specific anti-SEB antibody from the IVIG.[88-89]

In a Canadian comparative observational study, the 21 patients receiving IVIG had

a 30-day survival rate of 67% compared to 34% in the 32 control cases.[90] Patients treated with IVIG were more likely to have had surgery and to have received clindamycin, and inclusion of historical controls may have introduced bias. Analysis of plasma from 10 cases and 10 controls in this study demonstrated a significant reduction in T cell triggered production of IL-6 and TNFα after a single dose of IVIG.

A subsequent multicentre, randomized, placebo-controlled trial studied the efficacy of IVIG as adjunctive therapy in STSS.[91] The trial was terminated due to slow patient recruitment after 21 patients were enrolled, 10 receiving IVIG and 11 receiving placebo. The primary end-point was 28-day mortality but despite a 3·6 fold higher mortality rate being found in the placebo group (36% vs. 10% in IVIG group) statistical significance was not reached. There was a greater improvement in sepsis-related organ failure assessment (SOFA) score on days 2 and 3 of the study in the IVIG group, and IVIG produced 87-100% inhibition of GAS strains on in vitro testing.

S. aureus was isolated from blood culture in one patient in this trial and was found to be inhibited to a lesser degree by IVIG. This prompted a comparison study to investigate differential effects of IVIG on staphylococcal and streptococcal superantigen production.[92] Culture supernatants of *S. pyogenes* were consistently inhibited to a greater degree than those of *S. aureus*. It was concluded that higher doses of IVIG might be required to provide protective titres and clinical efficacy in the treatment of staphylococcal TSS. In the original trial, the dose of IVIG used was

1g/kg body weight on day 1 followed by subsequent doses of 0·5g/kg on days 2 and 3, but a superior dose regimen for staphylococcal disease has not been confirmed. Different IVIG preparations may vary in their neutralizing capacity, likely due to differences (perhaps geographical) in organism exposure in the donor population.[93] The mortality risk and rapidity of decline in TSS and STSS are such that delays in effective therapy have significant potential to worsen outcome. On this basis we argue that immunoglobulin therapy should not be unreasonably delayed in these cases. There is no clear information from the literature on what constitutes a safe delay. The United Kingdom Department of Health has issued guidance on the use of immunoglobulin.[94] For the management of invasive streptococcal disease (presumably not just STSS) they advise that "IVIg may be added to adequate toxin-neutralising antimicrobials, source control, and sepsis management when these approaches have failed to elicit a response". In the absence of a recommendation relating to time delay, we advise that the same approach to timing be taken for STSS as is recommended for staphylococcal TSS. In this setting the guidance states that "IVIg may be used for TSS resulting from an infection refractory to several hours of aggressive therapy, in the presence of an undrainable focus, or when there is persistent oliguria with pulmonary oedema". It is our approach to consider IVIg in cases where there has been no clinical response within the first 6 hours of aggressive supportive therapy.

Conclusion

Toxic shock syndrome is a global disease entity caused by pathogens with the ability to evolve in terms of superantigen generation and avoidance of our immune system.

Despite intense research efforts, we do not yet have new clinically available therapies capable of neutralizing superantigen-mediated T cell activation. Further research is required addressing timing and components of therapy. In the real-world clinical arena, a sound understanding of the pathophysiology, a high index of suspicion, early diagnosis, and immediate intervention are the best ways to impact on the significant mortality and morbidity rate of toxic shock syndrome. Given the supportive background research and the severity of this syndrome, we recommend a therapeutic approach in both TSS and STSS that incorporates prompt use of toxin-neutralising antimicrobials such as clindamycin or linezolid, along with early IVIG where there is failure to improve with aggressive support and source control.

Conflict of Interest Statement

The authors confirm that they have no interests or relationships, financial or otherwise, that constitute a conflict of interest in the preparation or submission of this manuscript.

Panel 1: Staphylococcal Toxic Shock Syndrome Clinical Case Definition

1. Fever ≥ 38.9°C
2. Rash – diffuse macular erythroderma
3. Desquamation – 1-2 weeks after onset of illness, especially of palms and soles
4. Hypotension – SBP ≤90mmHg for adults
5. Multisystem involvement – 3 or more of the following
    a. Gastrointestinal: vomiting or diarrhoea at the onset of illness
    b. Muscular: severe myalgia or elevated creatine phosphokinase
    c. Mucous membranes: vaginal, oropharyngeal, conjunctival hyperaemia
    d. Renal: blood urea nitrogen or creatinine twice-upper limit of normal
    e. Hepatic: total bilirubin twice-upper limit of normal
    f. Haematological: platelets ≤100,000μL
    g. CNS: disorientation or alterations in consciousness without focal neurological signs
6. Negative results on the following tests
    a. Blood, throat or CSF culture (blood culture may be positive for S. aureus)
    b. Rise in titre to Rocky Mountain spotted fever, leptospirosis or measles

Case Classification

Probable: case with 5 of the 6 clinical findings described above

Confirmed: case with all six of the clinical findings

Panel 2: Streptococcal Toxic Shock Syndrome Clinical Case Definition

1. Isolation of Group A β-haemolytic streptococci
    a. From a normally sterile site – blood, CSF, peritoneal fluid, tissue biopsy
    b. From a non-sterile site – throat, vagina, sputum
2. Clinical signs of severity
    a. Hypotension – SBP≤90mmHg in adults
    b. ≥2 of the following signs
        i. Renal impairment: creatinine >2mg/dL (>177μmol/L)
        ii. Coagulopathy: platelets ≤100,000μL or DIC
        iii. Hepatic involvement: ALT, AST or total bilirubin twice the upper limit of normal
        iv. Adult respiratory distress syndrome
        v. Generalised, erythematous, macular rash that may desquamate
        vi. Soft tissue necrosis, including necrotising fasciitis, myositis or gangrene

Case classification

Probable: Case fulfils 1b and 2 (a and b) if no other cause for the illness is found

Definite: Case fulfils 1a and 2 (a and b)

Table I: Antimicrobial Options in TSS

Causative Organism	Option 1	Option2 β Lactam intolerant	Option 3	Comments
GAS	Penicillin G + Clindamycin	Macrolide/fluoroquinolone + Clindamycin	Linezolid/ Daptomycin/ Tigecycline	Macrolide and fluoroquinolone resistance increasing
GAS (MLS resistant)	Penicillin G + Vancomycin/Teicoplanin	Vancomycin/Teicoplanin		Macrolide resistance associated with clindamycin resistance
MSSA	Cloxacillin/nafcillin/cefazolin + Clindamycin	Clarithromycin + Clindamycin	Rifampicin/ Linezolid/ Daptomycin/ Tigecycline	
MRSA	Clindamycin/Linezolid ± Vancomycin/Teicoplanin			
GISA/GRSA	Linezolid ± Clindamycin (if sensitive)		Daptomycin/ Tigecycline	GRSA/GISA increasing. Geographical patterns highly variable

MLS: Macrolide, Lincosamide and Streptogramin B
MSSA: Methicillin sensitive *S. aureus*
GRSA/GISA: Glycopeptide resistance/intermediate S. aureus

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Figure 1: NF-κB plays a central role in the generation and propagation of the inflammatory response. Activation of toll-like receptor (TLR)-2 pathways by gram-positive components, TLR-4 pathways by gram-negative products, and superantigenic stimulation, all bring about a sequence of events that allow free NF-κB to pass into the nucleus and bind to DNA. This leads to 1) expression of inflammatory mediators and "wind-up" of the inflammatory cascade; 2) neutrophil adhesion and activation; 3) activation of tissue factor and plasminogen activator inhibitor 1 to reduce fibrinolysis and enhance coagulability; 4) inducible nitric oxide synthetase acceleration with consequent vasodilatation and hypotension; and 5) induction of cyclo-oxygenase 2 and 5-lipoxygenase systems elaborating pro-inflammatory prostanoids, leukotrienes and thromboxane A_2. This figure has been adapted from 29.

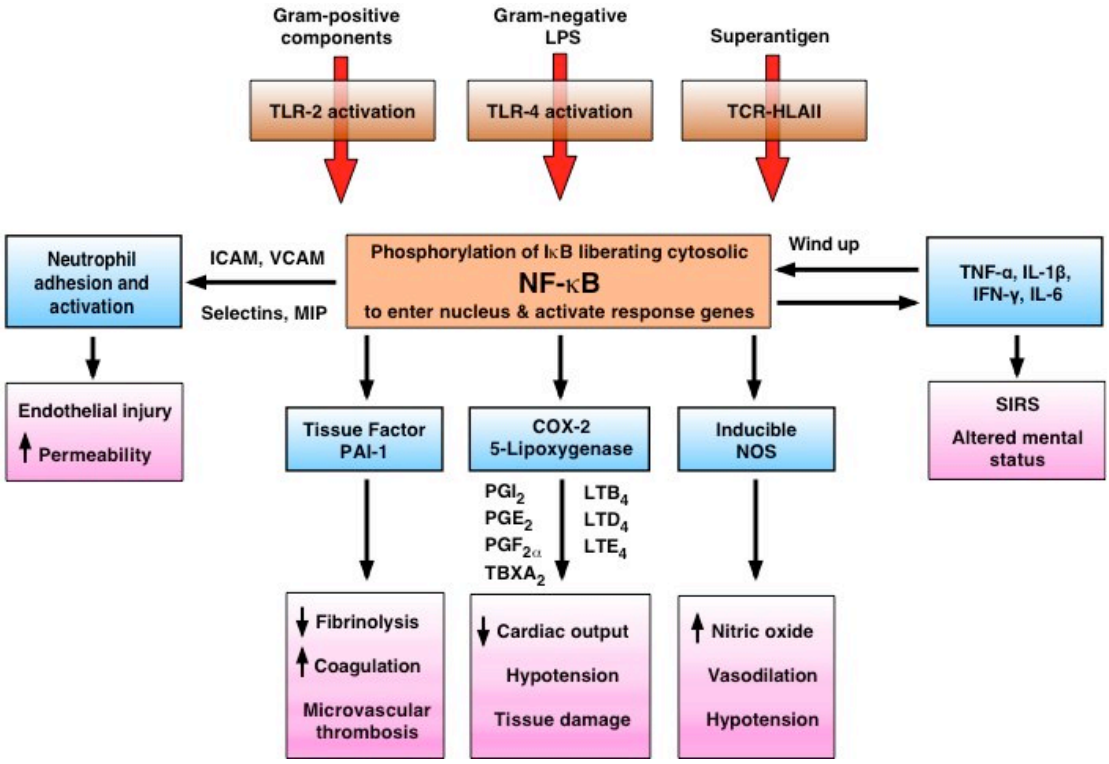
Gram-positive components
Gram-negative LPS
Superantigen
TLR-2 activation
TLR-4 activation
TCR-HLAII
Neutrophil adhesion and activation
ICAM, VCAM
Selectins, MIP
Phosphorylation of IκB liberating cytosolic
NF-κB
to enter nucleus & activate response genes
Wind up
TNF-α, IL-1β, IFN-γ, IL-6
Endothelial injury
Permeability
Tissue Factor PAI-1
COX-2 5-Lipoxygenase
Inducible NOS
SIRS
Altered mental status
PGI2
PGE2
PGF2α
TBXA2
LTB4
LTD4
LTE4
Fibrinolysis
Coagulation
Microvascular thrombosis
Cardiac output
Hypotension
Tissue damage
Nitric oxide
Vasodilation
Hypotension

Figure 1