

# Getting under the Skin: The Immunopathogenesis of *Streptococcus pyogenes* Deep Tissue Infections

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***Streptococcus pyogenes* can cause a variety of diseases in immunocompetent individuals, from pharyngotonsillitis to life-threatening invasive diseases, such as streptococcal toxic shock syndrome, and rapidly progressing deep-tissue infections, such as necrotizing fasciitis. Necrotizing fasciitis is often seen in combination with streptococcal toxic shock syndrome, which further increases morbidity and mortality. We review here the host-pathogen interactions in the tissue milieu and discuss the use of intravenous immunoglobulin as potential adjunctive therapy in these life-threatening infections.**

Since the late 1980s, a resurgence of severe invasive infections due to *Streptococcus pyogenes* (also known as group A streptococci) has been reported world wide [1, 2]. The 2 most severe invasive manifestations are streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis, both of which are associated with high morbidity and mortality (Figure 1). A prospective population-based surveillance for invasive *S. pyogenes* infections in Ontario, Canada, during 1991–1995 identified 323 patients, corresponding to an annual incidence of 1.4 cases per 100,000 population [3]. The most common clinical presentations were soft-tissue infection (48% of cases), bacteremia with no septic focus (14%), and pneumonia (11%). Necrotizing fasciitis occurred in 6% of patients, and STSS occurred in 13%. The mortality rate was 15% overall and was 81% among those with STSS ( $P < .001$ ). Seventy-seven patients met clinical and/or histopathological criteria for necrotizing fasciitis; of these patients, 47% (36) also presented with STSS [4]. Among these patients, the overall case fatality

rate was 34% (26 of 77); patients who met the criteria for STSS had a mortality of 67% (24 of 36), compared with 4.9% (2 of 41) among those who did not have STSS.

## **S. PYOGENES: COLONIZATION TO RAPIDLY PROGRESSIVE SOFT-TISSUE INFECTIONS**

The throat and skin of the human host are the principal reservoirs for *S. pyogenes*. The M proteins of group A streptococci form elongated structures on the bacterial surface and provide the basis of widely used epidemiological typing schemes that employ serological methods (M type) or nucleotide sequence analysis of the M protein gene (*emm* type). Although >150 distinct *emm* and *emm*-like genes are now recognized, their evolutionary history can be traced to 4 major phylogenetic lineages, designated as subfamilies [5, 6]. The content and relative chromosomal arrangements of the 4 *emm* subfamily genes are found to exist in only 5 basic patterns, A–E. The *emm* pattern A–C isolates are found to be disproportionately associated with the nasopharynx, whereas *emm* pattern D strains are most often isolated from skin and impetigo lesions [7]. Organisms of a third pattern group, *emm* pattern E, are found at both tissue sites.

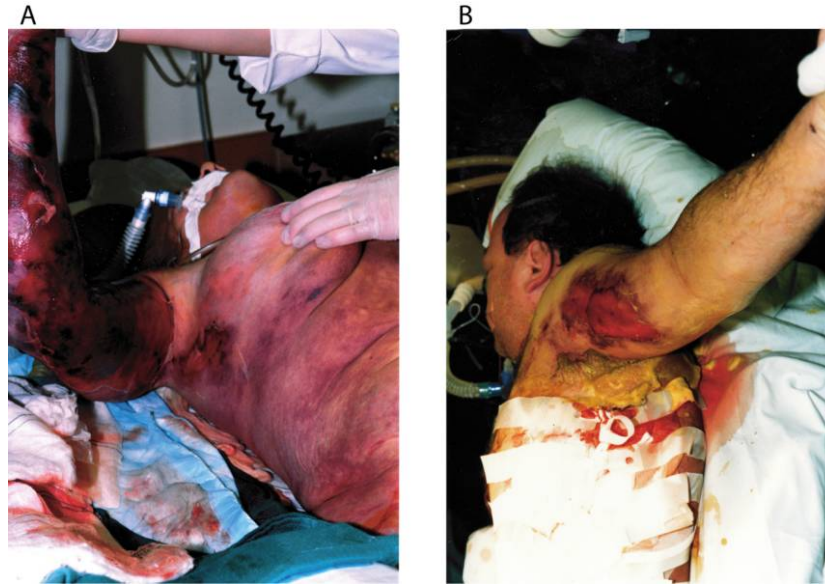
Epidemiologic studies have revealed that certain disease manifestations are commonly associated with par-

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**Figure 1.** Patients with group A streptococcal toxic shock syndrome and necrotizing fasciitis. *A*, An 85-year-old woman admitted to the hospital with a 2-day history of diarrhea, confusion, and the rapid onset of swelling, pain, and discoloration of the right arm. There was a history of blunt trauma to the arm 1 day before the onset of the patient's symptoms. The patient had type II diabetes. *B*, A 38-year-old man 12 h after admission from the emergency department, where he had presented with a complaint of left-sided chest pain thought to be possibly due to a pulmonary embolism. The patient's history was unremarkable except for an uncomplicated laceration of the patient's left second finger 6 days before admission while lacing up his son's skates. During the subsequent 6 days, the patient developed worsening symptoms of fever, malaise, and left-sided chest and arm pain.

ticular M types, such as M1 and M3 types, which are associated with the severe invasive manifestations STSS and necrotizing fasciitis [1, 2]. Although studies have shown that this tissue preference or disease manifestation may be linked to particular pathogenic traits of the strains, the associations are far from exclusive, which likely reflects the fact that the outcome of infection depends not only on bacterial factors but also on host factors [8].

It is noteworthy that a substantial number of invasive streptococcal infections have no known portal of entry [9, 10]. Transient bacteremia originating from the oropharynx has been suggested as the source in such cases. *S. pyogenes* cause a variety of skin and soft-tissue infections, including frequent and less complicated manifestations of impetigo, erysipelas, and mild cellulitis and rare but life-threatening infections of deep tissue or muscle (eg, necrotizing fasciitis and myositis). Several studies have reported that patients with necrotizing fasciitis and myositis often have a history of recent blunt trauma [4, 10]. A case-control study confirmed that patients with necrotizing fasciitis were 6 times more likely than control subjects to have had a recent blunt trauma [11]. A potential mechanism underlying the association of blunt trauma and severe streptococcal tissue infection was provided by Bryant et al [12], who reported that skeletal muscle injury resulted in increased cellular vimentin

expression, which enhanced binding of *S. pyogenes* to skeletal muscle cells.

### **MASSIVE BACTERIAL LOAD AND INTRACELLULAR PERSISTENCE AT THE INFECTED TISSUE SITE**

*S. pyogenes* is readily cultured from tissue samples from patients with necrotizing fasciitis, myositis, or severe cellulitis, in contrast to erysipelas biopsy specimens, from which streptococci only rarely can be cultured [13]. A similar association between severity of tissue infection and bacterial load was demonstrated by Thulin et al [14], who analyzed snap-frozen tissue biopsy specimens collected from patients with necrotizing fasciitis or severe cellulitis caused by *S. pyogenes* of varying serotypes. Bacteria were detected in all biopsy specimens, even those collected from distal areas, and the bacterial load was positively correlated to severity of tissue infection. Strikingly, biopsy specimens obtained as late as 20 days after diagnosis of infection and initiation of intravenous antibiotics still contained bacteria [14]. Bacterial viability assessment confirmed the presence of viable bacteria in the biopsy specimens, despite the fact that the patients had been receiving intravenous antibiotics, many of them for a prolonged time. The bacteria were tested and were found to be susceptible to the antibiotics used (in most cases, penicillin

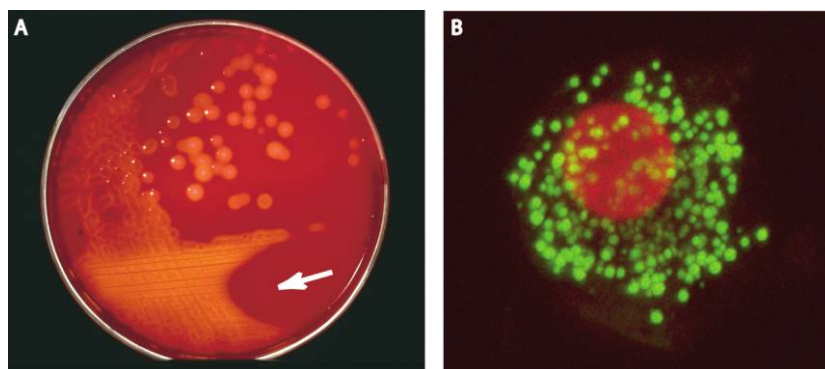
and clindamycin). *S. pyogenes* remain exclusively susceptible to  $\beta$ -lactam agents, but in severe invasive infections, a combination therapy with clindamycin is recommended, because the efficacy of clindamycin is unaffected by bacterial growth phase and also has inhibitory actions on protein synthesis, including the important superantigens [15]. The streptococcal tissue infections are commonly associated with poor tissue perfusion as a result of microvascular thrombosis [16], and it has been questioned whether the antibiotic concentration at the tissue site is sufficient. Culture of a tissue biopsy on a blood agar plate revealed a clear inhibitory zone around the biopsy specimen, and bacteria only grew at a distance from the biopsy specimen (Figure 2A) [14]. Hence, the biopsy contained toxic substances, which were likely antibiotics and/or other bacteriostatic substances, such as host antimicrobial peptides (discussed below in Host Antimicrobial Peptides and Bacterial Counter Strategies). However, despite the presence of these toxic substances within the biopsy specimen, the bacteria still resisted killing.

Subsequent studies provided an explanation to this persistence, because viable *S. pyogenes* was found intracellularly in host cells at the local site of tissue infection during the acute phase of infection (Figure 2B) [14]. The ability of *S. pyogenes* to invade and persist in human cells has previously been reported in epithelial cells [17, 18] and in neutrophils [19, 20]. In the tissue biopsy specimens, the main host cells were phagocytic cells and were predominantly macrophages [14]. In vitro cultures confirmed that *S. pyogenes* could survive intracellularly in macrophages for an extended period of time, and once the antibiotic was removed, a striking extracellular bacterial growth could be observed [14]. Antibiotic eradication failure as the result of an intracellular streptococcal reservoir has previously been reported for recurrent tonsillitis cases [21, 22]. The results emphasize that alternate approaches to antimicrobial therapy

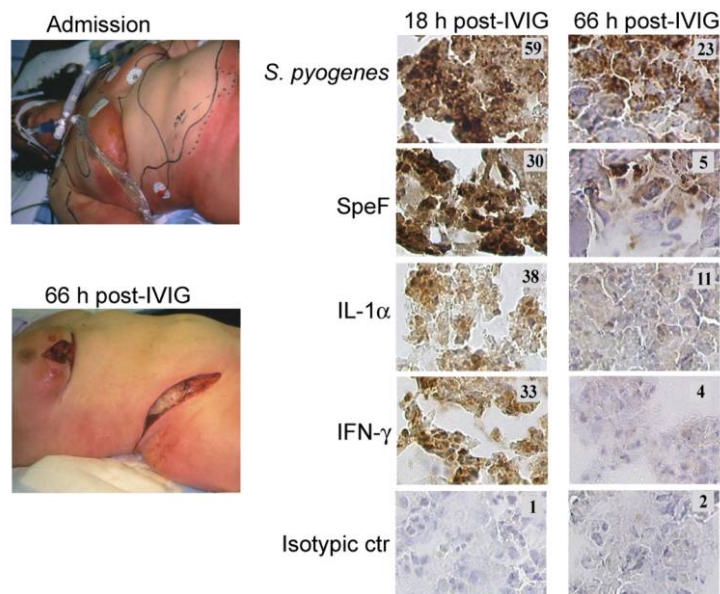
may be required to improve the morbidity and mortality associated with severe *S. pyogenes* soft-tissue infections.

### **STREPTOCOCCAL FACTORS PRESENT AT THE INFECTED TISSUE SITE: THE STREPTOCOCCAL CYSTEINE PROTEASE**

*S. pyogenes* express an array of virulence factors that are crucial for adherence, colonization, dissemination of infection, and immune evasion [23, 24]. The expression and function of many virulence factors may differ depending on the site of infection and the infection stages. One such factor is the cysteine protease SpeB, which is highly expressed at the tissue site of infection in patients with necrotizing fasciitis [14, 25], whereas it is down-regulated in blood [26]. SpeB has been recognized as a critical factor in promoting tissue site of infection at the skin [27], and genetic inactivation results in significantly reduced skin and soft-tissue injury in experimental models [28, 29]. There is also epidemiologic data linking a single nucleotide mutation in the *mtsR* gene with a decreased prevalence of necrotizing fasciitis cases [30]. A subsequent study proposed that this is likely attributable to a dysregulated multiple gene virulence axis, which in turn leads to reduced enzymatic activity of SpeB and, thus, attenuated virulence at the tissue site [31]. SpeB is a protease with numerous substrates, including many physiologically important human proteins, as well as its own virulence factors [32]. It was proposed that SpeB-mediated degradation of virulence factors and host proteins, such as extracellular matrix proteins, LL-37, and immunoglobulins, are required during the initial stages of infection, whereas, at later points and during systemic infection, down-regulation of SpeB occurs to ensure that the bacteria are equipped with the essential virulence factors required to survive in a hostile hyperinflammatory environment [26, 33]. Another way of regulating the proteolytic activity of SpeB is to retain human proteinase in-



**Figure 2.** Persistence of *Streptococcus pyogenes* during severe infections. A, Blood agar culture of a tissue biopsy specimen obtained on day 7 after onset of necrotizing fasciitis caused by *S. pyogenes*. The arrow indicates the inhibitory zone where the tissue biopsy specimen was initially placed. B, Viable *S. pyogenes* intracellularly (green) within a human macrophage (cellular nuclei is shown in red).



**Figure 3.** Bacterial factors and inflammatory responses in same site tissue biopsy specimens after administration of intravenous immunoglobulin (IVIG). Photographs illustrate the extent of tissue infection at hospital admission and after 66 h in a patient with necrotizing fasciitis. Tissue biopsy specimens taken from the same surgical site at 18 and 66 h, respectively, after IVIG therapy were immunostained for specific factors as indicated in the figure. The stains were quantified by acquired computerized image analysis, and image analysis data are indicated in each image. ctr, control; IFN, interferon; IL, interleukin; *S. pyogenes*, *Streptococcus pyogenes*. Used with permission from Norrby-Teglund et al [71].

hibitors, in particular  $\alpha$ 2-macroglobulin, at the bacterial surface through binding to the streptococcal surface protein G-related  $\alpha$ 2-M-binding protein (GRAB) [34]. Once secreted, SpeB becomes trapped in the GRAB/ $\alpha$ 2-macroglobulin cage, where it remains proteolytically active only against factors small enough to penetrate the cage [35]. Patient tissue biopsy data support that this regulation occurs in vivo and contributes to bacterial resistance towards antimicrobial peptides, discussed below [25].

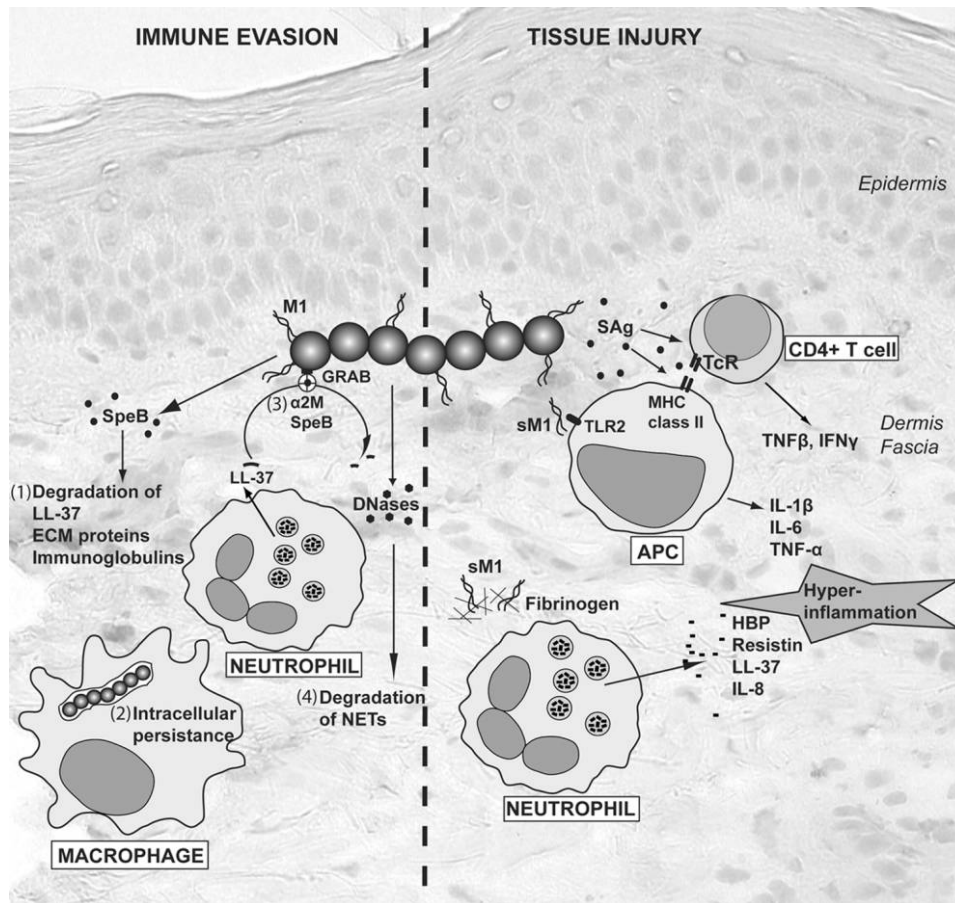
### SUPERANTIGENS AND INFLAMMATORY RESPONSES IN THE TISSUE

Similar to the link between proinflammatory cytokine responses in circulation and the severity of invasive streptococcal infections [8, 36], a significant correlation between in vivo inflammatory responses at the infected tissue site and the severity of streptococcal tissue infection was demonstrated [37]. Increasing levels of interleukin (IL) 1 and significantly higher frequencies of Th1 cytokines (eg, tumor necrosis factor [TNF]  $\beta$ - and interferon [IFN]  $\gamma$ -producing cells) were associated with more-severe tissue infection. Detection of streptococcal superantigens in these tissue biopsy specimens, together with a typical superantigen cytokine response, provided strong support for the direct action of superantigens at the tissue site [37]. The high prevalence of TNF- $\beta$ - or IFN- $\gamma$ -producing cells in the tissue was an interesting finding, because several studies have failed to detect significant numbers of these cells in the peripheral circulation of patients with invasive *S. pyogenes* in-

fection [37–39]. Assessment of homing receptor expression on cells in patient tissue biopsy specimens revealed a strong correlation between the magnitude of Th1 cytokines and certain homing receptors, in particular CCR5, CD44, and cutaneous lymphocyte antigen [37]. Taken together with previous reports on superantigens and these particular homing receptors [40–44], this suggested that superantigen-mediated activation of peripheral T cells may induce up-regulation of skin-homing receptors and thereby promote the migration of activated T cells to the skin and, subsequently, exacerbated inflammation at the local site of infection.

### NEUTROPHILS AT THE TISSUE SITE OF INFECTION

Infiltration of polymorphonuclear cells in superficial fascia and dermis was one of the histopathological criteria for diagnosis of necrotizing fasciitis proposed in 1984 by Stamenkovic and Lew [45]. This was also evident in the patient tissue material described above, in which neutrophils represented one of the dominant cell populations and the degree of infiltration correlated significantly with bacterial load [14]. However, there have also been several elegant studies that describe a paucity of neutrophil influx at the tissue site of streptococcal infection resulting from degradation of IL-8 by a streptococcal trypsin-like protease, SpyCEP/ScpC [46–48]. The reports also demonstrated a dramatic effect of SpyCEP/ScpC, because protease-deficient strains were attenuated in virulence when tested in



**Figure 4.** Host-microbe interactions at the tissue site of infection during severe deep-tissue infections caused by *Streptococcus pyogenes*. *S. pyogenes* have evolved several immune evasion mechanisms that contribute to the massive bacterial persistence that characterizes deep-tissue infections. Such mechanisms include (1) proteolytic degradation of host immune effector molecules, including LL-37 and immunoglobulins; (2) intracellular persistence within phagocytic cells; (3) protection against antimicrobial peptides by SpeB entrapped in protein G-related  $\alpha$ 2-M-binding protein (GRAB)/ $\alpha$ 2-macroglobulin ( $\alpha$ 2m) complexes on the bacterial surface; and (4) degradation of neutrophil extracellular traps (NETs) by bacterial DNases. Dissemination of infection and tissue injury are contributed by proteolytic events, such as SpeB-mediated degradation of extracellular matrix (ECM) proteins, as well as induction of excessive inflammatory responses mediated largely by superantigens (Sag) and soluble M1 protein (sM1) that activate T cells, antigen presenting cells (APC), and neutrophils. This activation results in release of heparin-binding protein (HBP), an inducer of vascular leakage and shock, as well as release of pathologic levels of proinflammatory cytokines. IL, interleukin.

murine experimental models. The fact that heavy infiltration is detected in patient biopsy specimens suggests that the murine models fail to mimic the complexity of the clinical setting, in which there is a plethora of chemotactic signals that may mask an effect of SpyCEP/ScpC.

In fact, the concept of neutrophil activation and degranulation as important contributors to disease pathology in invasive group A streptococcal infections has recently been emphasized [49–52]. The classical streptococcal virulence factor M-protein has been implicated as a major trigger of these responses through its ability to form complexes with fibrinogen [49]. The complexes bind to  $\beta$ 2-integrins on the neutrophil surface, resulting in activation and release of massive amounts of the granule protein, which is directly responsible for induction of vascular leakage and acute lung damage [49, 51, 52].

Soluble M1-protein and M1-protein/fibrinogen complexes have been demonstrated in patient biopsy specimens, which underline the potential pathophysiological significance of these complexes generated during infection [49, 51]. This is further substantiated by the presence of neutrophil proteins at the infected tissue site, including heparin-binding protein, IL-8, resistin, and LL-37, all of which are likely to contribute to the hyperinflammatory state that characterizes these infections [25, 49–52].

## HOST ANTIMICROBIAL PEPTIDES AND BACTERIAL COUNTER STRATEGIES

Other important host factors are the antimicrobial peptides that are essential components of the first line of defence against pathogens [53]. The cathelicidin LL-37 was shown to provide

protection against murine necrotic skin infections caused by *S. pyogenes* [54]. However, several pathogenic bacteria secrete factors that can degrade and inactivate antimicrobial peptides, such as the streptococcal cysteine protease SpeB [35, 55], and the streptococcal inhibitor of complement [56].

Analyses of patient tissue biopsy specimens revealed that the active LL-37 peptide was present in all infected biopsy specimens, and its expression was positively correlated with bacterial load [25]. Although an up-regulation of LL-37 is expected in response to streptococcal infection [54, 57], such a positive correlation to bacterial load, together with the fact that there are viable bacteria in the tissue during a prolonged time, strongly implied that LL-37 in the infected tissue did not efficiently contribute to bacterial killing. Further studies revealed that this lack of antimicrobial activity was likely attributable to SpeB inactivation of LL-37 at the bacterial surface [25], according to the model proposed by Nyberg et al [35]. In this model, SpeB is entrapped by the  $\alpha$ 2-macroglobulin-GRAB complex, thereby achieving an accumulation of SpeB around the bacteria, where the biological significance of an inactivation of LL-37 will be the greatest.

It is becoming increasingly evident that many of the antimicrobial peptides act not only as antimicrobial agents, but also as significant mediators of other biological effects, including immunomodulatory and chemotactic activities [53]. Considering the hyperinflammatory state of these severe tissue infections, such effects would likely exacerbate the pathological responses and worsen the disease progression. In addition, a potential effect on bacterial virulence was suggested by Gryllos et al [58], who reported that subinhibitory concentrations of LL-37 resulted in enhanced expression of several streptococcal virulence factors, including capsule, SpyCEP/ScpC, and IdeS.

### **INTRAVENOUS POLYSPECIFIC IMMUNOGLOBULIN (IVIG) AS ADJUNCTIVE THERAPY FOR SEVERE *S. PYOGENES* INFECTION**

The finding that lack of protective antibodies against streptococcal M-protein and superantigens correlated with risk to develop invasive streptococcal diseases [59–61] highlighted the importance of antibodies in protection against these infections and suggested that IVIG might be a potential adjunctive therapy. IVIG exhibits high polyspecificity generated by antibodies pooled from several thousands of donors and has been shown to contain broad-spectrum antibodies against streptococcal superantigens and M-proteins [62–65]. In addition, IVIG has a general anti-inflammatory effect that is attributable, in large part, to Fc-receptor mediated mechanisms [66, 67].

The documentation of clinical efficacy of IVIG in STSS includes several case reports, as well as 2 observational cohort studies, 1 case-control study, and 1 multicenter placebo-con-

trolled trial [68]. The case-control study was designed to evaluate the efficacy of IVIG therapy in patients with STSS and included 21 case patients that were treated with IVIG during 1994–1995 and 32 nontreated control subjects identified through active surveillance of invasive *S. pyogenes* infections during 1992–1995 [69]. Multivariate analysis revealed that IVIG therapy and a lower acute physiology and chronic health evaluation (APACHE) II score was significantly associated with survival. Although the results of this study demonstrated a significant benefit of IVIG, the study had 2 confounding factors—the use of historical control subjects and differences in antibiotic therapy—that could potentially affect the mortality rate. To further document the safety and efficacy of this adjunctive therapy, a multicenter placebo-controlled trial of IVIG in STSS was initiated in Europe [70]. The trial was prematurely terminated because of a low incidence of disease in the participating countries and, consequently, a slow patient recruitment. Results were obtained from 21 enrolled patients (10 IVIG recipients and 11 placebo recipients). The primary end point was mortality at 28 days, and a 3.6-fold higher mortality rate was found in the placebo group. This trend to improved survival was strengthened by the significant improvement in organ function revealed by the reduction in the sepsis-related organ failure assessment score after treatment, which was evident in the IVIG group but not in the placebo group. Furthermore, a significant increase in plasma-neutralizing activity against superantigens expressed by autologous isolates was noted in the IVIG group after treatment.

In an observational case study involving patients with severe *S. pyogenes* soft-tissue infections [71], the use of an aggressive medical regimen that included high-dose IVIG together with a conservative surgical approach was studied. The report describes 7 patients with severe soft-tissue infection caused by *S. pyogenes* who did not undergo surgery or for whom only limited exploration was performed. Six of the patients had STSS, and they all received effective antimicrobial therapy and high-dose IVIG. Impressively, all patients survived. One of the main mechanisms of action of IVIG in STSS is neutralization of bacterial virulence factors, in particular superantigens, as well as a general anti-inflammatory effect. Tissue biopsy specimens collected from the same surgical site at different time points after IVIG administration were available from 1 patient. Analyses of bacterial load, superantigens, and inflammatory cytokines in the biopsy specimens revealed dramatic improvement in all markers at the later time point (Figure 3). This observational study, although limited in numbers, suggests that an initial conservative surgical approach combined with the use of immune modulators, such as IVIG, may reduce the morbidity associated with extensive surgical exploration in hemodynamically unstable patients without increasing mortality.

## CONCLUSIONS

*S. pyogenes* is an important human pathogen by virtue of its many immunomodulatory properties. Analyses of host-microbe interactions at the tissue site of infection have provided in vivo evidence for many of the immune evasion strategies previously described in vitro (Figure 4). The studies have revealed that severe soft-tissue infections are characterized by massive bacterial load; the presence of important streptococcal virulence factors, including soluble M1-protein, the cysteine protease SpeB, and superantigens; DNAses; and heavy infiltration of inflammatory cells and inflammatory mediators. Important bacterial resistance mechanisms at the tissue site include exploitation of human phagocytic cells as host cells, thereby allowing persistence intracellularly, as well as protection against antimicrobial peptides by SpeB retained at the bacterial surface through GRAB- $\alpha$ 2-macroglobulin complexes. It is clear that the pathogenesis of severe streptococcal tissue infections is multifactorial in nature. This complexity is important to consider in the design of novel therapeutic strategies, in which IVIG represents an immunomodulatory therapy that should be evaluated further.

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