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Severe Invasive Group A Streptococcal Infections

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1. INTRODUCTION

Emerging infections can be defined as those diseases that have newly appeared in the population, or have existed but are rapidly increasing in incidence or geographic range. Reasons for disease emergence may include changes in the pathogen, the host, or the environment. The dynamics may be sufficiently complex that the occurrence appears inexplicable. The reemergence of severe group A streptococcal (GAS) infections is an excellent example of an emerging, or more correctly reemerging, infectious disease.

Severe GAS infections have long been recognized and the history of GAS disease has been characterized by periodic changes in severity of disease. Streptococcal infections were recognized by Greek physicians in the 5th century. Sydenham described the disease as "febris scarlatina" as early as 1664 (Katz & Morens, 1992). This description clearly differentiated this disease from measles and other rashes, which allowed outbreaks of scarlet fever to be documented throughout the world. The most severe forms of streptococcal disease were well known long before the discovery of the bacterium. Hippocrates recorded epidemic erysipelas and clinical descriptions of a disease that we would clinically refer to today as necrotizing fasciitis (NF) (Descamps et al., 1994).

In the United States during the mid part of the last century there was a dramatic decline in the occurrence and mortality of scarlet fever (Quinn, 1982). This occurred without an associated decrease in pharyngitis due to GAS and with continuing severe infections and their sequelae in developing countries (Kaplan, 1993). This decline in severity began in the 1930s, before the advent of antibiotics for the treatment of streptococcal disease, and continued up to and including the 1970s (Kaplan, 1996). The mortality declined from 72% in the pre-antibiotic era to 7–27% (Duma et al., 1969; Hable et al., 1973; Keefer et al., 1937). However in the early 1980s reports began to appear that described not only

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an increased mortality due to GAS bacteremia (35–48%), but also anecdotal reports emphasizing a rapidly fatal outcome in bacteremic patients presenting with shock (Goepel et al., 1980). This syndrome has been designated as the streptococcal toxic shock syndrome (STSS), and is commonly associated with specific M types (Gaworzewska & Colman, 1988; Hoge et al., 1993a; Martin & Hoiby, 1990; Schwartz et al., 1990; Stromberg et al., 1991).

In addition to the increase in STSS during the 1980s, there were increased reports of NF (Chelsom et al., 1994; Cone et al., 1987; Demers et al., 1993; Kaul et al., 1997; Stevens et al., 1989). The hallmark of NF is infection of the subcutaneous tissue and fascia that often results in necrosis with relative sparing of the underlying muscle. The diagnosis can be made if histopathology demonstrates both necrosis of superficial fascia and polymorphonuclear infiltrate and edema of the reticular dermis, subcutaneous fat, and superficial fascia. From 1992 to 1995, the annual incidence of NF increased 4-fold in Ontario, Canada from 0.85 per million population to 3.5 per million ($p < 0.001$) (Kaul et al., 1997).

2. MICROBIOLOGY

Streptococci are Gram-positive, catalase-negative facultatively anaerobic bacteria forming spherical or ovoid cells less than 2 μm in diameter. Streptococci are nutritionally fastidious, with variable nutritional requirements, and growth on complex media is enhanced by the addition of blood or serum. Glucose and other carbohydrates are metabolized fermentatively, and lactic acid is produced as the major metabolic end product. Gas is not produced as the result of glucose metabolism. Isolates of streptococci produce the enzyme leucine aminopeptidase, but production of pyrrolidonly arylamidase (PYR) is rare among streptococci, occurring only in isolates of group A streptococci (*Streptococcus pyogenes*) and some strains of *Streptococcus pneumoniae* (Ruoff, 1995). β-Hemolytic, bacitracin-susceptible, PYR-positive, large-colony-forming streptococci with Lancefield's group A antigen are included in the species *S. pyogenes.* The manifestations of GAS infections in humans are diverse in both clinical presentation and morbidity. Pharyngitis and impetigo are common childhood illnesses with few complications. Infrequently, GAS causes invasive disease, of which the most serious presentation is necrotizing soft-tissue infection with associated shock and multisystem organ failure.

3. PATHOGENESIS

GAS express numerous virulence factors, both surface-associated and secreted, which interact with immune cells and other factors of the host to promote colonization, growth, dissemination, and survival of the organism.

The surface-attached virulence factors, including among others hyaluronic acid capsule, M- and M-like proteins, Protein F1, C5a-peptidase, and α_2-macroglobulin binding protein, are pivotal in the primary stages of infection involving bacterial adherence, colonization, and evasion of phagocytosis (Boyle, 1995; Fischetti, 1989; Rasmussen et al.,

1999). Surface-associated streptococcal virulence factors interact with a variety of human proteins, such as immunoglobulins, fibronectin, fibrinogen, albumin, plasminogen, kininogen, complement factor C5a, and regulators of the complement system, thereby promoting attachment of the bacteria to host cells and tissue, as well as evasion of phagocytosis (Table 1.1).

The systemic effects seen in patients with severe invasive GAS infections, such as STSS and NF, are largely triggered by inflammatory mediators induced in response to microbial factors (Figure 1.1). The pro-inflammatory activity is mediated by both streptococcal cell wall components as well as secreted factors (Table 1.2). Peptidoglycan and

Table 1.1
Surface-Associated Virulence Factors of Group A Streptococci and their Major Human Ligands

Name	Human ligand(s)	Reference(s)
Hyaluronic acid capsule	CD44	Ashbaugh et al. (1998), Cywes et al. (2000), Dale et al. (1996), Husmann et al. (1997), Moses et al. (1997), Schrager et al. (1998), Wessels and Bronze (1994)
M-protein family: M/Emm Mrp/FcrA Enn	Albumin, Fibrinogen IgG, IgA Kininogen Fibronectin Factor H Plasminogen C4BP CD46 (MCP) N-CAM	Boyle (1995), Fischetti (1989), Navarre and Schneewind (1999)
Protein F1/SfbI	Fibronectin Fibrinogen	Boyle (1995), Cunningham (2000), Fischetti (1989), Navarre and Schneewind (1999)
Lipoteichoic acid	Fibronectin	Hasty et al. (1992), Simpson and Beachey (1983)
Fibronectin-binding protein FBP54	Fibronectin	Courtney et al. (1994, 1996)
Glyceraldehyde-3-phosphate dehydrogenase	Fibronectin Plasmin	Pancholi & Fischetti (1992) Winram & Lottenberg (1996)
Protein F2	Fibronectin	Jaffe et al. (1996)
Serum opacity factor/SfbII	Fibronectin	Courtney et al. (1999), Kreikemeyer et al. (1995), Rakonjac et al. (1995)
α-enolase	Plasminogen	Pancholi and Fischetti (1998)
C5a peptidase	Anaphylatoxin C5a	Ji et al. (1997), Wexler et al. (1985)
R28	Not defined	Stalhammar-Carlemalm et al. (1999)
Streptococcal protective antigen (Spa)	Not defined	Dale et al. (1999), McLellan et al. (2001)
Collagen-like protein (Scl) A and B	Not defined	Lukomski et al. (2000), Rasmussen and Bjorck (2001), Rasmussen et al. (2000)
α_2-macroglobulin-binding protein (GRAB)	α_2-macroglobulin	Rasmussen et al. (1999)
Streptococcal cystein protease/SpeB	Laminin Intergrins	Hytonen et al. (2001), Stockbauer et al. (1999)

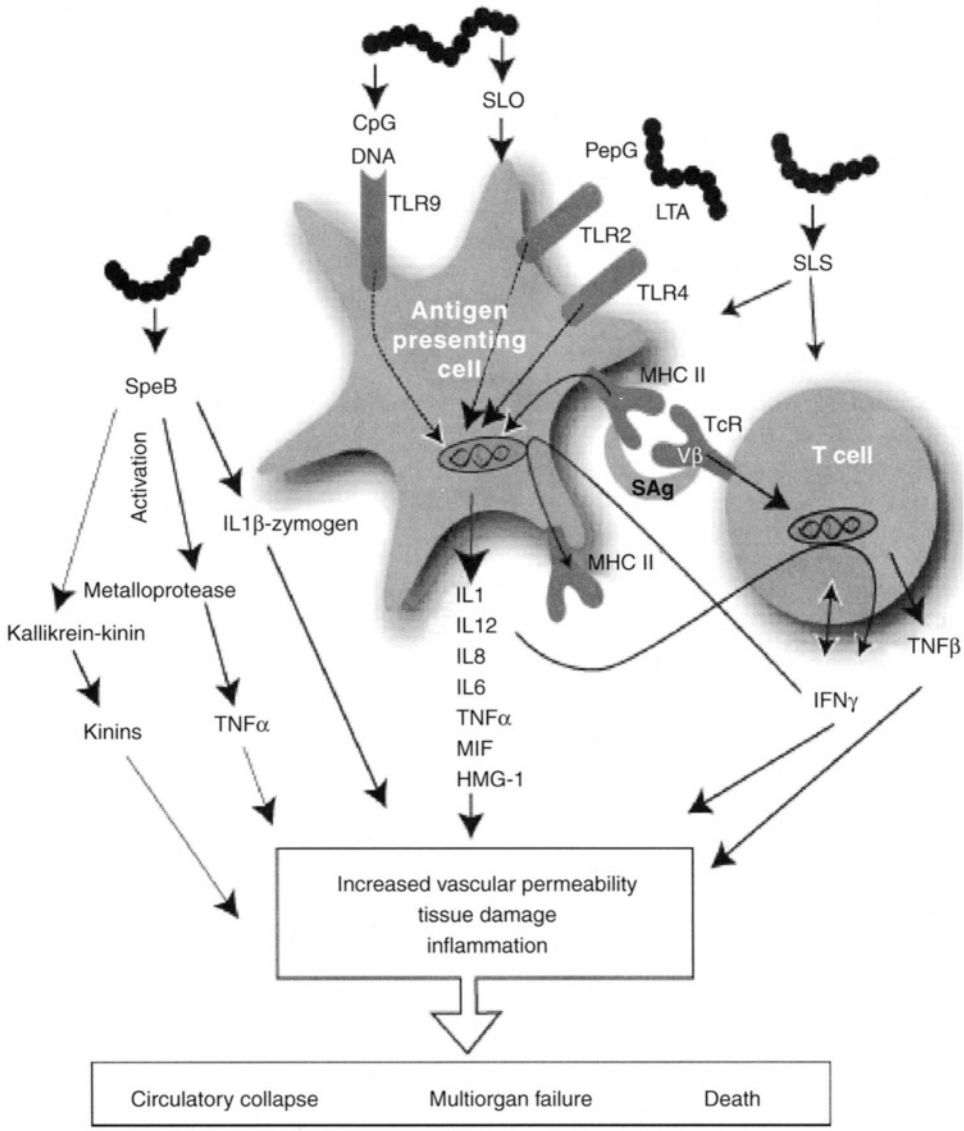

Figure 1.1. Induction of pro-inflammatory responses by group A streptococcal virulence factors. Lipoteichoic acid (LTA), peptidoglycan (PepG), and unmethylated CpG DNA activate antigen presenting cells through Toll-like receptors (TLR), to induce various cytokines, among others IL12 which promoted IFNγ production by T cells. Superantigens (SAg) interact with MHC class II molecules (MHC II) and the T cell receptor (TcR) to activate both cell types resulting in high production of cytokines. The cysteine protease, streptococcal pyrogenic exotoxin (SpeB), also promotes a pro-inflammatory response. Streptolysin O and S (SLO, SLS) are cytotoxic and pro-inflammatory.

lipoteichoic acid in the Gram-positive cell wall have been shown to activate leukocytes and trigger production of pro-inflammatory cytokines, including interleukin (IL)1β, IL6, IL8, IL12, tumour necrosis factor (TNF)α, and chemokines, as well as other inflammatory mediators, such as inducible nitric oxide synthetase (Bhakdi et al., 1991; Card et al., 1994;

Table 1.2
Streptococcal Virulence Factors with Pro-Inflammatory Activity

Name	Location	Proposed host receptor(s)	Reference(s)
Peptidoglycan	Cellbound	TLR2	Medzihtov and Janeway (2000), Sriskandan and Cohen (1999)
Lipoteichoic acid	Cellbound	TLR2, TLR4	Medzihtov and Janeway (2000), Sriskandan and Cohen (1999)
Superantigens	Secreted	TcR and MHC class II	Kotb (1995), McCormick et al. (2001)
Streptolysin O	Secreted	Not defined	Hackett and Stevens (1992), Shanley et al. (1996)
Unmethylated CpG DNA	Secreted	TLR9	Chatellier and Kotb (2000), Hemmi et al. (2000)
Cysteine Protease/SpeB	Secreted/cell bound	Cytokine-precursors and metalloproteases	Burns et al. (1996) Kapur et al. (1993a, 1993b)

Note: The table includes only receptors or substrates involved in the pro-inflammatory response.

Heumann et al., 1994; Keller et al., 1992; Mattsson et al., 1993; Morath et al., 2001; Riesenfeld-Orn et al., 1989; Standiford et al., 1994; Stylianos et al., 1991; Timmerman et al., 1993; Wang et al., 2000a, 2000b). Activation of innate immunity occurs via the Toll signalling pathway, in particular, Toll-like receptors (TLR) 2 and 4 (Figure 1.1 and Table 1.2) (Medzihtov & Janeway, 2000). Engagement of these TLRs triggers several intracellular signal transduction pathways, resulting in activation of the transcription nuclear factor (NF)-κβ, and subsequent induction of expression of pro-inflammatory genes (Medzihtov & Janeway, 2000).

The ability of purified peptidoglycan and lipoteichoic acid to trigger pathologic inflammatory conditions has been demonstrated in various animal models including experimental meningitis and septic shock (De Kimpe et al., 1995a, 1995b; Kengatharan et al., 1996; Spika et al., 1982; Tuomanen et al., 1985). In a rat model of septic shock, it was shown that although administration of lipoteichoic acid resulted in moderate hypotension, it did not trigger multiorgan failure and death (De Kimpe et al., 1995a, 1995b). However, when lipoteichoic acid was administered together with peptidoglycan a synergistic effect was achieved and the anaesthetized rats experienced shock and multiorgan failure (De Kimpe et al., 1995a, 1995b; Kengatharan et al., 1996).

Superantigens are by far the most potent streptococcal factors in induction of pro-inflammatory responses, and have therefore been implicated as crucial players in the pathogenesis of STSS and NF (Kotb, 1995; McCormick et al., 2001). There are several different superantigens produced by GAS including streptococcal pyrogenic exotoxins (Spe) A, B, C, F, G, H, J (Kotb, 1995; Proft et al., 1999), streptococcal superantigen (SSA) (Mollick et al., 1993), and the streptococcal mitogenic exotoxin Z and Z-2 (Kamezawa

et al., 1997; Proft et al., 1999). Most GAS strains express several different superantigens, although the repertoire of genes encoding superantigens varies between GAS strains (McCormick et al., 2001). It seems likely that several different superantigens can trigger invasive GAS diseases and that more than one superantigen are produced by the bacteria during the infection.

The "super" activity of superantigens is achieved by their ability to circumvent the normal rules for antigen processing and presentation, which results in excessive activation and release of inflammatory mediators. Superantigens interact, without prior cellular processing and outside the conventional antigen-binding cleft, with the relatively invariable Vβ-regions of the T cell receptor and MHC class II molecules on antigen presenting cells (APC) (Figure 1.1) (Marrack & Kappler, 1990). Each superantigen has a characteristic Vβ-specificity, and activates preferentially T cells expressing certain Vβ-elements, which may account for 5–20% of the naive T cell population. Interaction with MHC class II molecules differ between superantigens and preferential binding to certain alleles occur (reviewed in [Kotb, 1995; McCormick et al., 2001]). Interestingly, although there is significant difference in the primary sequence of the superantigens, they all share a common three-dimensional structure, which includes a folding of the superantigen into different domains, one amino-terminal hydrophobic β-barrel domain and a carboxy-terminal β-grasp domain (reviewed in Kotb, 1998). This common tertiary structure has been targeted in the development of superantigen antagonist (Arad et al., 2000; Visvanathan et al., 2001). The potential use of these antagonists as a novel therapeutic strategy is discussed in more detail below.

Another mediator of pro-inflammation is unmethylated oligonucleotides containing cytidine-phosphate-guanosine (CpG) motifs (Chatellier & Kotb, 2000; Klinman et al., 1996; Krieg, 1995). Unmethylated CpG DNA is exclusively found in prokaryotes, and has been shown to activate macrophages, dendritic cells, and B-cells, thereby triggering production of IL1, IL6, TNFα, IL12, IL10, and Thl type of cytokines (reviewed by Heeg et al., 1998). Activation of cellular responses by CpG DNA was recently reported to be mediated by TLR9 (Figure 1.1 and Table 1.2) (Hemmi et al., 2000). Although streptococcal DNA generally have a low GC-content, certain important virulence genes, such as the genes encoding M-proteins, have been reported to have relatively high frequencies of immunostimulatory CpG motifs (Chatellier & Kotb, 2000).

The streptococcal cysteine protease, also called SpeB, is an important virulence factor with multiple activities, including superantigenic as well as proteolytic activity. By virtue of its proteolytic activity, SpeB modulates several host defense systems including the cytokine, kallikrein-kinin, coagulation, and complement system, and has therefore been suggested to contribute to tissue destruction and the systemic effects seen in fulminant GAS infections (Figure 1.1). Numerous physiologically important host proteins, including IL1β-precursor (Kapur et al., 1993a), metalloproteases (Burns et al., 1996), the extracellular matrix proteins vitronectin and fibronectin (Kapur et al., 1993b), and kininogens (Herwald et al., 1996), are substrates for SpeB. Cleavage of human IL1β-precursor and activation of metalloproteases that cleaves the precursor form of TNFα, result in generation of bioactive pro-inflammatory IL1β and TNFα (Burns et al., 1996). The pro-inflammatory response can be further augmented by SpeB-cleavage of kininogen resulting in release of pro-inflammatory kinins and consequently increased vascular permeability and inflammation (Herwald et al., 1996). SpeB is not only active as a secreted factor, but

also in a cell-bound form with ability to function as an adhesin interacting with laminin and glycoproteins (Hytonen et al., 2001).

Several studies have supported a critical role of SpeB in the pathogenesis of GAS infections, including among others analyses of humoral immunity in patients with invasive GAS infections, where a lack of acute-phase antibodies were associated with invasive disease (Basma et al., 1999; Norrby-Teglund et al., 1994). Reduced morbidity and mortality of invasive GAS disease could be demonstrated in mice following immunization against SpeB (Kapur et al., 1994), administration of a protease inhibiting synthetic peptide (Bjorck et al., 1989), or genetic inactivation of the gene encoding SpeB (Hoe et al., 1999; Kuo et al., 1998; Lukomski et al., 1997; Svensson et al., 2000a). SpeB was also found to exacerbate the pro-inflammatory cytokine response and lung injury induced in rats by streptolysin O (SLO) and streptococcal cell wall fragments (Shanley et al., 1996). However, there are also several conflicting reports demonstrating either no effect of SpeB or even an association between SpeB expression and decreased virulence of the bacteria (Ashbaugh et al., 1998; Kansal et al., 2000; Raeder et al., 2000). One potential explanation for this could be that several important cell-associated virulence factors of GAS, including M-protein, protein H, and C5a peptidase, can be cleaved by SpeB resulting in either loss of, or altered properties of, essential virulence factors for the bacteria (Berge & Bjorck, 1995; Raeder et al., 1998). Thus, the role of SpeB in GAS pathogenesis is highly complex and remains to be defined.

Pro-inflammatory responses are also induced by SLO, which acts in synergy with both the superantigen SpeA (Hackett & Stevens, 1992) and as already mentioned above with SpeB (Shanley et al., 1996). In an experimental murine skin model, SLO-deficient strains were found to be attenuated in virulence, and although the bacteria disseminated in the animals, they were less likely to cause lethal infection than wild type strains (Limbago et al., 2000).

4. CLINICAL ENTITIES AND EPIDEMIOLOGY

The resurgence of severe disease was recognized not only by the increase in occurrence of STSS and NF, but also by an increase in severity and incidence of other clinical entities including GAS pneumonia and soft tissue infection (Low et al., 1997; Sharkawy et al., 2002).

4.1. STSS

STSS was defined by criteria established by The Working Group on Severe Streptococcal Infections (The Working Group on Severe Streptococcal Infections, 1993). Patients were considered to have STSS if they had hypotension in combination with two or more of the following: acute renal failure, coagulation abnormalities, liver abnormalities, acute respiratory distress syndrome, generalized rash and NF. Epidemiological studies

revealed that the majority of these outbreaks, although reported from different countries and on different continents, were caused predominantly by GAS strains of M1 and M3 serotypes (Cockerill et al., 1997; Eriksson et al., 1999; Holm et al., 1992; Kaul et al., 1999; Kiska et al., 1997; Martin & Hoiby, 1990; Nakashima et al., 1997; Stromberg et al., 1991; Svensson et al., 2000b; Upton et al., 1996). However, while serotypes M1 and M3 accounted for the vast majority of strains isolated from cases of STSS and NF during the recent outbreaks, many other M types, including some non-typable strains, are known to cause these disease (Cunningham, 2000; Stevens, 1992).

Davies et al. (1996) characterized 323 cases of invasive GAS disease from the Ontario streptococcal study group (OSSG), a prospective, population-based surveillance study of all invasive GAS infections in Ontario, Canada. Out of the 13% of the cases that were classified as having STSS (annual rate, 0.2 per 100,000 population), 31 fulfilled the consensus definition of the syndrome, 4 were dead on arrival to the hospital, and 7 died shortly after admission without having sufficient information available for classification. The patients who had the STSS were older than the other patients (median age, 61, as compared with 38; $p < 0.001$) and more likely to have an underlying chronic illness (71%, as compared with 51%; $p = 0.03$). The overall mortality rate was 81% among patients with the STSS (65% among those whose illness met the consensus definition), as compared with 5% among those without the syndrome ($p < 0.001$). The distribution of sites of infection were soft tissue (30%), bacteremia without focus (15%), arthritis (15%), upper respiratory tract (10%), adenitis (8%), NF (6%), pneumonia (5%), peritonitis (4%), and meningitis (2%). Laupland et al. (2000) described the incidence and clinical features of invasive GAS disease in children from the OSSG. There were 1.9 cases of invasive GAS disease per 100,000 children per year. STSS occurred in 7% of cases: six with bacteremia without focus, three with NF, two with pneumonia (one with cellulitis and one with peritonitis), two with cellulitis, and one each with pharyngitis and an infected iliac vein thrombus. Children under 10 years of age were less likely to be diagnosed with STSS than children 10 years of age and older ($p = 0.002$). They found that chickenpox infection was associated with a 58-fold increased risk of acquiring invasive GAS disease.

4.2. Necrotizing Fasciitis

In addition to the increase in STSS during the 1980s, there were increased reports of NF (Chelsom et al., 1994; Demers et al., 1993; Kaul et al., 1997; Stevens et al., 1989). The hallmark of NF is infection of the subcutaneous tissue and fascia that often results in necrosis with relative sparing of the underlying muscle. The diagnosis can be made if histopathology demonstrates both necrosis of superficial fascia and polymorphonuclear infiltrate and edema of the reticular dermis, subcutaneous fat, and superficial fascia. In the absence of examined specimens, the diagnosis requires the presence of gross fascial edema and necrosis detected at surgery (Kaul et al., 1997). Kaul et al. (1997) reported on 77 cases of streptococcal NF gleaned from the OSSG. From 1992 to 1995, the annual incidence of NF increased 4-fold from 0.85 per million population to 3.5 per million ($p < 0.001$). The majority (71%) of adult cases occurred in persons with at least one chronic underlying illness. Nine (12%) infections occurred in a chronically ischemic limb in patients with diabetes and/or peripheral vascular disease. None of the children affected had underlying

chronic medical conditions. However, in four of eight cases occurring in children (and in four of five cases in children less than 10 years of age), NF occurred as a complication of chickenpox. Use of nonsteroidal anti-inflammatory drugs (NSAIDs) prior to admission was reported by 26% of the cases where information was available. Eight patients had been taking NSAIDs chronically, and three had taken them because of pain associated with the acute illness.

The most common primary site of infection was the lower extremity (53%), followed by the upper extremity (29%), trunk (9%), groin/perineum (8%), and face (1%). Forty-nine percent of patients had significant hypotension. Forty-six percent of cases (35 of 75 from whom blood cultures were obtained) were bacteremic. The majority of patients (73%) had an elevated white blood cell count; however in 20% of patients white cell counts were within the normal range, and in 7% they were low. Acute renal failure was present in 35% of patients, coagulopathy in 29%, and 28% of patients had liver function test abnormalities. Overall, 49% of cases met the case definition for STSS.

In Laupland's study (Laupland et al., 2000) 10 children (4%) had NF, 3 of whom had STSS. In contrast to cellulitis, which was distributed across all body sites, 8 of 10 cases of NF occurred in the lower limb ($p = 0.001$), and 5 of 8 of these initially involved the thigh and/or groin. One patient (10%) with NF died at presentation to the emergency department. No other patients with NF alone or in combination with STSS died. In univariate analysis, only the presence of antecedent chickenpox was associated with the diagnosis of NF ($p = 0.007$). In multivariable analysis, both in all cases and in the subset with soft-tissue infections, only chickenpox was associated with the diagnosis of NF.

Haywood et al. (1998) reviewed 20 consecutive patients identified from the OSSG, that had been cared for in Toronto. The average age of all patients was 58 years (ranging from 33 to 89 years, with a median age of 55.5 years). A physician had seen seven (35%) patients in the 48 hr prior to admission. Their signs and symptoms were such that they were diagnosed with a condition other than NF. Fifty-five percent of patients had an underlying chronic illness. The body distribution of NF involved upper extremity (35%), lower extremity (30%), and trunk (35%). One patient developed a postoperative surgical wound infection that resulted in necrotizing fasciitis, which was found to be secondary to an asymptomatically colonized healthcare worker who was present during surgery. Clinical presentation of patients within the first 48 hr included hypotension (85%), use of vasopressors (55%), renal impairment (45%), coagulopathy (55%), liver involvement (25%), acute respiratory distress syndrome, ARDS (15%), and a rash (35%). Forty percent of all patients met the criteria of STSS.

Clinically it appears that STSS is a separate entity from NF. The studies from Britain and Sweden, which reported an increase in severe GAS infection, did not report the concomitant presence of NF (Colman et al., 1993; Gaworzewska & Colman, 1988; Stromberg et al., 1991). Martin and Hoiby (1990) in their report of an increase in incidence and severity of GAS disease in Norway, due primarily to M1 strains, reported only a small number of cases of necrotizing fasciitis. Hoge et al. (1993b) carried out a retrospective survey of the medical records from all 10 hospitals in Pima County, Arizona, to identify sterile site isolates of GAS between 1985 and 1990. They found significant changes in the clinical spectrum of invasive infections with an increase in patients with clinical features of STSS during the last three years of the study. Necrotizing fasciitis was not associated with shock or any of the other clinical features of STSS, suggesting that fasciitis was not a component of the syndrome. Kaul et al. (1997) found only 36 of 77 cases of GAS necrotizing fasciitis

associated with STSS. McGeer et al. (1998) reviewed all M3 disease identified in the OSSG data base between 1992 and 1998. There were 1,335 cases of invasive GAS disease (2/100,000 population) of which M3 serotypes represented 7.1% of isolates. Non-M3 isolates were more likely to be associated with soft-tissue infection without fasciitis ($p = 0.001$), whereas M3 isolates were more likely to be associated with necrotizing fasciitis ($p = 0.001$). M3 was also associated with increased severity of systemic disease, including STSS.

4.3. Soft-Tissue Infections

Soft-tissue infections are common diseases, which are rarely life threatening and can usually be managed without hospital admission. They are most commonly due to GAS. In order to better characterize patients with soft-tissue infections due to GAS in this era of emerging prevalence and severity of GAS infections, Sharkawy et al. (2002) characterized 474 cases of invasive GAS soft-tissue infections from the OSSG data base. The incidence of invasive disease with a soft tissue focus varied from 0.62 per 100,000 population per year in 1992 to 1.29 per 100,000 population per year in 1995; $p < 0.001$. Most of this variability was in the rate of NF, which ranged from 0.08 per 100,000 per year in 1992 to 0.49 per 100,000 per year in 1995. The incidence of all invasive soft-tissue disease including NF was highest in the elderly. Fifty percent of cases occurred in patients with at least one chronic underlying illness, of which diabetes mellitus and alcoholism were the most common. The overall case fatality rate was 13%. In multivariate analysis of factors identifiable at presentation hypotension, increased age, and underlying chronic illness were significantly associated with an increased case-fatality rate. None of 198 patients (including 92 who were bacteremic) without hypotension or chronic underlying illness died. Of the 158 patients with chronic underlying illness but no hypotension for whom age was known, 1 of 96 aged younger than 65 years died, compared to 9 of 62 aged 65 years or older ($p = 0.001$). Patients with positive blood cultures were more likely to die (61/315, 19%, vs. 7/205, 3.4%, $p < 0.001$) and to have severe systemic disease (37/301, 12%, vs. 10/203, 4.9%, $p = 0.008$). Patients with infection due to M1 and M3 strains were more likely to have NF than other patients. Patients infected with M3 strains were also more likely to die: 11/40 (28%) patients infected with M3 strains died, compared to 11/105 (10%) of those infected with Ml strains, and 36/293 (12%) of those infected with strains of other serotypes. Sharkaway's data, while limited to disease due to GAS, support the contention that cellulitis is seldom life threatening and can usually be managed in an outpatient setting. All patients in this series were admitted to hospitals, and had positive sterile site cultures, and 60% were bacteremic. Despite this, no patients without chronic underlying illness or hypotension died, and the case fatality rate in patients under the age of 65 with underlying illness was 1%.

4.4. Pneumonia

In the pre-antibiotic era GAS pneumonia was a common clinical entity accounting for 3–5% of community acquired pneumonia (CAP) (Keefer et al., 1941). Most cases

occurred following outbreaks of viral illness, commonly influenza or measles (Keefer et al., 1941; MacCallum, 1919; Parker, 1979). Cases also occurred following GAS pharyngitis or tonsillitis (Keefer et al., 1941). Underlying chronic lung disease was a predisposing factor. Typical clinical features included the sudden onset of high fever and pleuritic chest pain and the frequent development of pleural effusions and empyema (Basiliere et al., 1968; Keefer et al., 1941; MacCallum, 1919; Parker, 1979). Fatal outcomes were common and may have occurred in up to 50% of cases (MacCallum, 1919).

The incidence of GAS pneumonia declined significantly over the first half of the 20th century (Basiliere et al., 1968). Despite this, several large outbreaks of GAS pneumonia were described in military personnel in the 1960's (Basiliere et al., 1968; Welch et al., 1961). Unlike previously described outbreaks, these were not linked to preceding viral illnesses. Despite this the clinical presentation and the frequent occurrence of empyema were identical to previous descriptions of the disease.

Since the 1960s the incidence of GAS pneumonia has dramatically declined. Numerous case series examining the etiologic agents of CAP have failed to detect any contribution from the GAS (Bates et al., 1992; Fang et al., 1990; Lieberman et al., 1996; Lim et al., 1989). No further large outbreaks have been reported and only a handful of case reports exist describing the modern presentation of GAS pneumonia.

As noted previously, over the last 15 years the incidence of severe GAS infections has been rising. Whether these changes have lead to resurgence of GAS pneumonia or to a change in the clinical or epidemiological features of this illness has not been directly addressed.

Muller et al. (2000) described the clinical and epidemiological features of 222 patients with invasive GAS pneumonia identified through the OSSG between 1992 and 1999. The yearly incidence of GAS pneumonia rose from 0.16 per 100,000 per year in 1992 to 0.35 per 100,000 in 1999 paralleling an increase in the incidence of all invasive GAS infections in the same cohort. GAS pneumonia occurred predominantly during the winter months, with a striking nadir in infections in August/September of each year. The median age was 56 years and ranged from 1 day to 100 years. A significant chronic illness was identified in 61% patients. The majority of cases were community acquired (179, 81%). Four of these cases were subsequent to other nonpharyngeal, culture-confirmed GAS infections in household contacts. One patient's spouse had been admitted three days previously with GAS bacteremia, another patient's spouse had been admitted the previous day with epiglottitis due to GAS, a third patient's child had been treated for GAS vulvitis 1 week prior to his presentation, and one 3-week old child's mother had had GAS endometritis.

Blood cultures were positive in 178 patients (78%); five of these patients also had GAS isolated from cultures of pleural fluid. Of the 44 patients with negative blood cultures or where blood cultures had not been done, GAS was isolated from pleural fluid in 37, and cultures of autopsy lung tissue in 7. The predominant M-types were Ml (38%) followed by M3 (11%), M12 (8%), and M6 (5%).

The case fatality rate was 38% compared with 12% for remainder of the cohort of invasive GAS infection ($p < 0.001$) and 26% (58 of 221) of patients with NF ($p = 0.008$). The progression of fatal cases was rapid with a median time to death of 2 days. In multivariate analysis, only the presence of STSS and increasing age were associated with a higher case fatality rate. Although the case fatality rate increased significantly with age,

significant mortality occurred in young adults. The case fatality rate in previously healthy patients aged 1–65 years was 18% (9 of 49). The incidence of invasive GAS pneumonia during the period of our study ranged from 0.16 to 0.35 per 100,000 population with a trend toward increasing frequency. Data from the Centers for Disease Control and Prevention's (CDC) Active Bacterial Core (ABC) surveillance reports for 1997 and 1999 suggest that similar frequencies of invasive GAS disease and GAS pneumonia occur in the United States (Schuchat et al., 2001). Their data show an incidence of invasive GAS of 3.5 per 100,000 population with 11% representing pneumonia. Although the overall incidence of GAS pneumonia is low compared with common causes of community acquired pneumonia such as *S. pneumoniae*, it occurs with a frequency similar to that of other less common but well recognized causes of severe CAP such as *Staphylococcus aureus.* In a population based study by Marston et al. (1997) 0.3% cases in which a definite etiologic agent was identified were due to GAS compared with 0.4% due to *S. aureus.* The most striking findings of this study are the case fatality rate associated with GAS pneumonia, the high incidence of STSS, and the rapid progression to death that occurred in fatal cases. The overall case fatality rate of 38% is consistent with the 30–60% mortality found in bacteremic GAS pneumonia in other studies (Barnham & Anderson, 1997; Davies et al., 1996; Demers et al., 1993; Martin & Hoiby, 1990), and considerably higher than the case fatality rate of NF in our cohort of invasive GAS infection. The case fatality rate for GAS pneumonia is also higher than that reported for community acquired bacteremic pneumococcal pneumonia, estimated at 12–20% in recent studies (Metlay et al., 2000).

5. TREATMENT MODALITIES

5.1. Antimicrobials

Conventional therapy of invasive GAS infections has consisted of antimicrobials and, when necessary in severe invasive disease, support of vital functions for those patients with STSS and surgery for those patients with NF. Penicillin and cephalosporins are the antimicrobials most frequently used for treating GAS pharyngitis, cellulites, and impetigo. However, concern has been raised that in more severe infections the organism is less likely to respond to β-lactam antibiotics. Stevens et al. (1988) were able to show that penicillin was ineffective in a mouse model of myositis due to GAS if treatment was delayed ≥2 hr after initiation of treatment. The targets for the β-lactams are the penicillin binding proteins (PBPs), enzymes responsible for the formation of the peptidoglycan in the cell wall of the bacteria. The PBPs are expressed during the log-phase of growth, but in large inocula infections they are not expressed during the stationary phase of growth. Stevens et al. (1993) found that in addition to decreased binding of radiolabeled penicillin by all PBPs in stationary cells, PBPs 1 and 4 were undetectable at 36 hr. They speculated that the loss of certain PBPs during stationary-phase growth in vitro might account for the failure of penicillin in both experimental and human cases of severe streptococcal infection.

Stevens et al. (1988) found that antimicrobials that inhibit protein synthesis were able to improve survival over penicillin in a mouse model of myositis. Survival of

erythromycin-treated mice was greater than that of penicillin-treated mice and untreated controls, but only if treatment was begun within 24 hr. Mice receiving clindamycin had survival rates of 100%, 100%, 80%, and 70%, even if the treatment was delayed 0, 2, 6, and 16.5 hr, respectively (Stevens et al., 1988). There are several possible explanations for the greater efficacy of clindamycin in the treatment of severe GAS infections (Stevens, 1999). In vitro and in vivo data support the concept that clindamycin efficacy is not affected by inoculum size or stage of growth and in fact may suppress the synthesis of PBPs (Stevens et al., 1993; Yan et al., 1993, 1994). Other mechanistic actions of clindamycin in invasive GAS disease include enhanced opsonization of streptococci through suppression of bacterial M protein expression (Gemmell et al., 1981), reduced capsular expression, and suppression of bacterial toxin synthesis, including superantigens (Mascini et al., 2001; Sriskandan et al., 1997). Finally there is evidence that clindamycin can function as an immune modulator by suppressing synthesis of TNF-α from monocytes.

Although GAS have remained exquisitely sensitive to penicillin, resistance to the macrolides and clindamycin have emerged worldwide. Resistance to macrolides in GAS arises by two distinct mechanisms: (i) ribosomal modification resulting from the presence of an Erm methylase; and (ii) drug efflux conferred by a membrane protein encoded by the *mefA* gene. Presence of an Erm methylase confers cross-resistance to erythromycin, clindamycin, and streptogramin B-type compounds (MLS_B phenotype). MLS resistance in GAS is encoded by two types of methylase gene: the *erm* (AM) [*erm* (B)] and the recently described *erm* (TR). The latter has also been associated with inducible resistance to clindamycin when using a double disc diffusion test. Resistance resulting from efflux is encoded by the *mefA* gene. This resistance is specific for 14- and 15-member macrolides (erythromycin, azithromycin, and clarithromycin); 16-member macrolides (e.g., josamycin) are not affected and neither are clindamycin or streptogramin B-type compounds (M-phenotype). Therefore depending on the prevalence of resistance and the mechanism of resistance either erythromycin or clindamycin or both may not be active against the infecting GAS. Therefore, an approach for severe invasive GAS infection has been to utilize a combination of penicillin and clindamycin, since the penicillin provides coverage against 100% of GAS strains.

5.2. Novel Therapeutic Strategies

The conventional wisdom is that patients with severe GAS infections require antimicrobials, supportive therapy to manage their hypotension and multiorgan failure, and surgery for the removal of devitalized tissue. However, as with severe invasive *Streptococcus pneumoniae* disease, there has been a failure to influence mortality (Davies et al., 1996; Hook et al., 1983). Often patients succumb to their infection before antimicrobials can have any beneficial effect, despite supportive care of the intensive care unit and extensive debridement, emphasizing the importance of research in immune modulation therapy.

The finding that low levels of protective antibodies against the M-protein and superantigens correlated with invasive GAS disease highlighted the importance of antibodies in protection against these infections, and suggested that immunoglobulins might be a potential adjunctive therapy (Basma et al., 1999; Eriksson et al., 1999; Holm et al., 1992;

Mascini et al., 2000; Norrby-Teglund et al., 1994). In order for an immunoglobulin therapy to be efficacious, broad antibody specificity would be required to cover all the different serotypes of GAS and the whole spectrum of superantigens as well as other important virulence factors. Intravenous immunoglobulin (IVIG) exhibits high polyspecificity generated by antibodies pooled from several thousands of donors, and IVIG is commonly used as therapy in various autoimmune and immunodeficiency diseases, as well as in Kawasaki disease.

Several different modes of actions that contribute to the beneficial effect in autoimmune and systemic inflammatory diseases have been described for IVIG. These include blockade of Fc-receptors on reticuloendothelial cell system and phagocytic cells, modulation of Fc receptor expression, interference with activated complement, modulation of cytokine responses, modulation of immune cell functions, interaction with idiotype–antiidiotypic network, antigen-neutralization, and selection of immune repertoires (Ballow, 1997; Mouthon et al., 1996). A crucial pathway by which IVIG exerts its anti-inflammatory activity was recently identified in a murine model of immune thrombocytopenia and involved increased expression of the Fc inhibitory receptor for IgG, FcγRIIB, and consequently abrogated platelet destruction by macrophage phagocytosis (Samuelsson et al., 2001). Mechanistic actions directly related to the pathogenesis of invasive GAS infections, such as antigen neutralization, bacterial opsonization, as well as cytokine modulation, are discussed further below.

Opsonizing antibodies that promote phagocytosis and bacterial clearance of several pathogenic microorganisms, including GAS, have been demonstrated in IVIG preparations (Basma, 1998; Weisman et al., 1994; Yang et al., 1989). These opsonizing anti-Mi antibodies were found to be conferred on the patients upon IVIG therapy, since elevated opsonizing liters were demonstrated in post-therapy plasma (Basma, 1998). Thus, increased bacterial clearance through opsonizing antibodies against GAS might be a potential mechanistic action of IVIG contributing to clinical efficacy. However, an experimental murine model of necrotizing fasciitis that compared the efficacy of clindamycin, penicillin, and IVIG, alone or in combination, failed to support this hypothesis (Patel et al., 2000). Efficacy of the various treatment regimens was assessed based on quantitative bacterial clearance, and IVIG did not enhance killing of the M3 strain used. Thus, further studies are warranted to define the exact role of IVIG conferred opsonic antibodies in the efficacy of IVIG therapy in severe invasive GAS infections.

IVIG also contains neutralizing antibodies against several different GAS superantigens. These antibodies potently inhibit the proliferative and cytokine-inducing capacity of GAS superantigens in vitro at physiological concentrations of IVIG (Norrby-Teglund et al., 1996a, 1996b; Skansen-Saphir et al., 1994). IVIG has been shown to inhibit several SSAs, including SpeA, SpeB, and SpeC (Norrby-Teglund et al., 1996a, 1996b). In addition, culture supernatants prepared from GAS strains of different serotypes including M1, M3, M4, M6, and M28, were used as crude preparations of secreted virulence factors, and all tested supernatants were completely inhibited by IVIG (Norrby-Teglund et al., 1998). Thus, IVIG exhibits an extraordinary broad specificity against GAS virulence factors, which is transferred to the patients upon administration of IVIG with subsequent increased superantigen-neutralizing activity in patients' plasma (Norrby-Teglund et al., 1996a, 1996b). This inhibitory activity of IVIG is not exclusive for GAS superantigens, also superantigens produced by *S. aureus* are potently inhibited by IVIG (Darville et al., 1997;

Takei et al., 1993). Furthermore, antibodies against other important streptococcal virulence factors including DNaseB and SLO have also been found in IVIG preparations (Lissner et al., 1999; Stegmayr et al., 1992).

Analyses of different IVIG preparations containing varying concentrations of IgG, IgA, and/or IgM revealed that they varied in opsonizing and toxin-neutralizing capacity, and variation in inhibitory activity was even observed between lots of the same preparation (Hiemstra et al., 1994; Norrby-Teglund et al., 1998, 2000). IgA and IgM were found to be potent inhibitors of GAS superantigens, and in the case of SpeA the most efficient neutralization was achieved by a preparation containing a mixture of IgG, IgA, and IgM (Norrby-Teglund et al., 2000). These findings suggest that optimization of IVIG therapy may be achieved by changing the type or lot of IVIG preparation; however, this remains to be proven in a clinical setting.

IVIG is a powerful modulator of cytokine production, not only via direct antigen-neutralizing, but also through immunomodulatory activities that are mediated by pathways not yet completely understood. These pathways are believed to include Fc-interactions, soluble immune components, and induction of regulatory cytokines. A strong induction of IL1ra have been shown in human monocytes following culture on adherent IgG (Arend et al., 1991) or coculture with IVIG (Andersson et al., 1996; Poutsiaka et al., 1991). Also IL8 production is induced in human monocytes following coculture with IVIG (Andersson et al., 1996; Ruiz de Souza et al., 1995). IL1ra is well known to exert anti-inflammatory activity due to its interaction with IL1 signaling; however, the effect of IL8 as an anti-inflammatory agent is not as clearly defined but has been suggested to inhibit the accumulation of neutrophils at the sites of inflammation when induced systemically (Asano & Ogawa, 2000).

IVIG was shown in vitro to be a strong inhibitor of superantigen-induced lymphokine production, with the strongest suppression seen for the Th1 cytokines IFNχ and TNFβ, as their production was almost completely abolished (Andersson et al., 1996; Norrby-Teglund et al., 1996b, 2000; Skansen-Saphir et al., 1994). This inhibitory effect was seen, although to a lesser extent, even when addition of IVIG was delayed 24 hr poststimulation with superantigen. These findings suggest that additional mechanisms of IVIG, aside from antigen-neutralization, contribute to the inhibitory effect (Andersson et al., 1996; Skansen-Saphir et al., 1994). A differential effect of IVIG was noted on superantigen-induced monokine production with upregulated IL8 and decreased IL6 production (Andersson et al., 1994). Studies on the effect of IVIG on superantigen induced IL1 production have reported conflicting results, as one study demonstrated no effect on IL1 production (Skansen-Saphir et al., 1994), whereas the other reported a significant reduction of IL1 (Norrby-Teglund et al., 2000). Thus, superantigen-induced lymphokine production is potently suppressed by IVIG, and the monokine production may also be modulated by IVIG, but further studies are required to define the effect of IVIG on superantigen induced monokines. However, since the Th1 type of cytokines are the hallmark of a superantigen response, the powerful inhibition of these cytokines by IVIG most likely represents a major mechanistic action of IVIG contributing to clinical efficacy.

Cytokine modulation by IVIG have also been shown in vivo in several diseases, including among others severe invasive GAS infections where patients showed decreased levels of TNFα and IL6 (Kaul et al., 1999; Nadal et al., 1993); Guillain-Barré syndrome patients who showed a selective down-regulation of pro-inflammatory cytokines (Sharief

et al., 1999), and Kawasaki patients who demonstrated elevated IL1ra and IL8, as well as decreased pro-inflammatory cytokines following IVIG therapy (Leung et al., 1989).

Several case reports have demonstrated clinical improvement after IVIG therapy of patients with severe invasive GAS infections including STSS, NF, and necrotizing myositis (Barry et al., 1992; Cawley et al., 1999; Chiu et al., 1997; Lamothe et al., 1995; Mahieu et al., 1995; Nadal et al., 1993; Perez et al., 1997; Stegmayr et al., 1992; Yong, 1994).

There have been three studies conducted of IVIG therapy in patients with NF (Haywood et al., 1998; Kaul et al., 1997; Muller et al., 2001). In the study by Kaul et al. (1997), a reduction in mortality rate (10% compared to 37%) was noted between IVIG-treated patients as compared to the nontreated controls. However, only age, hypotension, and bacteremia were independently associated with mortality of streptococcal necrotizing fasciitis. Muller et al. (2001) described six patients with severe GAS disease and soft tissue involvement that were managed conservatively. Treatment in all cases included clindamycin, a β-lactam, and high-dose IVIG. All patients were hypotensive and three patients developed STSS. One patient had limited exploratory surgery without debridement. One patient had repeated bedside drainage of her olecranon bursa. No other patient had surgery. All patients survived.

An observational cohort study designed to evaluate the efficacy of IVIG therapy in patients with STSS was conducted in Canada (Kaul et al., 1999). The study included 21 cases that were treated with IVIG during 1994–1995, and 32 nontreated controls identified through OSSG's active surveillance during 1992–1995. Multivariate analysis revealed that IVIG therapy and a lower acute physiology and chronic health evaluation II (APACHE) score was significantly associated with survival. One confounding factor in the material was that IVIG-treated cases were more likely to have received clindamycin therapy than the controls. Therefore, a secondary multivariate analysis considering only cases and controls that had received clindamycin was performed, and APACHE II score and IVIG therapy remained the two variables associated with survival. Further support for the use of IVIG was provided by in vitro studies of blood samples collected pre- and post-IVIG therapy (Kaul et al., 1999). Neutralizing activity against culture supernatant prepared from the patient's own infecting isolate increased significantly post-therapy, and the majority of patient's plasma caused 80–100% inhibition of the bacterial supematants. Since the material included patients infected with GAS strains of varying serotype, this supports the in vitro findings that IVIG have a very broad spectrum of superantigen-neutralizing antibodies. IVIG therapy also resulted in a significantly reduced TNFa and IL6 production in peripheral blood mononuclear cells in four patients tested (Kaul et al., 1999). Thus, together these data suggest that the clinical improvement achieved by IVIG therapy is partly attributed to inhibition of the superantigens produced by the clinical isolates, and a reduction in the pro-inflammatory response.

The treatment of necrotizing fasciitis has emphasized the importance of early surgical intervention for diagnosis or surgical debridement and/or hyperbaric oxygen (Bisno & Stevens, 1996). Early aggressive surgery has been advocated in order to reduce the systemic inflammatory response and the spread of local infection (Stevens, 1999). Hyperbaric oxygen is recommended in order to administer oxygen at greater than normal pressure so as to lead to better wound healing. Unfortunately, both procedures usually occur at a time when the patient is most unstable and therefore interferes with monitoring and treatment. In addition, there is no evidence to support the use of hyperbaric oxygen and there is new

Table 1.3
Recommendations for the Treatment of Streptococcal Toxic Shock Syndrome

Penicillin 4 MU iv q6h
Plus
Clindamycin 900 mg iv q6h (discontinue at 72–96 hr, as long as the patient is hemodynamically stable and local disease is no longer progressing).
Plus
Intravenous immunoglobulin 2 g/kg iv for 1 dose (consider second dose of 1 g/kg at 72 hr only if patient remains hemodynamically unstable or local disease continues to progress).

information we may allow surgery to be delayed until the patient is stable and the degree of surgery required is better defined, thereby reducing unnecessary tissue debridement and/or amputation (Brown et al., 1994; Muller et al., 2001). IVIG has resulted in a reduction in the mortality associated with STSS and possibly a reduction in morbidity and mortality in patients with necrotizing fasciitis (Haywood et al., 1998; Kaul et al., 1997).

Antibiotic and IVIG treatment regimens are presented in Table 1.3.

6. PREVENTION

Prevention of streptococcal infections occurs naturally by acquisition of immunity to one or several streptococcal virulence factors. Considering that severe invasive GAS infections are such rare occurrences among the population despite widespread exposure to virulent GAS strains in communities, natural immunity most likely plays a very important role. However, the complexity of GAS pathogenesis involving several serotypes and toxins/superantigens, makes the development of a vaccine highly challenging. There are several strategies of vaccine development currently being pursued (Table 1.4).

The main target for a streptococcal vaccine has been the anti-phagocytic M-protein, since it is a major protective antigen of GAS. In 1962, Lancefield and colleagues (Lancefield, 1962) demonstrated that type-specific anti-M protein antibodies protected against infection in a murine model, and that recovery from infection in humans was related to the presence of type-specific anti-M antibodies. However, the protection was type-specific, and the individuals remained susceptible to infection by other serotypes. Considering that there exist over 100 different M-serotypes, type-specificity poses a major challenge in the design of M-protein vaccines. Another important issue with M-protein vaccines is that certain areas of the M-proteins have been shown to contain epitopes that evoke antibodies to human tissue including myocardium (Dale & Beachey, 1985), renal glomeruli (Kraus & Beachey, 1988), cartilage (Baird et al., 1991), and brain (Bronze & Dale, 1993). Hence, it is essential that these regions be avoided in a vaccine construct, and that the selected region only contains protective epitopes. One approach to overcome the problem of type-specificity and to increase the spectrum of protection has been to develop multivalent vaccines (Beachey et al., 1988; Dale, 1999a). Multivalent vaccines containing N-terminal fragments of several different M-proteins linked in tandem has been developed and demonstrated to be immunogenic. A recent study by Dale (1999b) reported a hexavalent M-protein vaccine, which was shown to be immunogenic and to evoke protective

Table 1.4
Vaccine Candidates in Group A Streptococcal Infections

Streptococal factor	Targeted region	Reference(s)
M-protein	N-terminal N- and C-terminal C-terminal	Bessen and Fischetti (1990), Brandt et al. (2000), Dale (1999a), Medaglini et al. (1995), Pruksakorn et al. (1994)
C5a peptidase	*scpA* gene with deleted signal sequence and cell wall anchor	Ji et al. (1997)
Cysteine protease/SpeB	The whole molecule	Kapur et al. (1994)
Group A carbohydrate	The whole molecule	Salvadori et al. (1995)
Streptococcal Protective Antigen (Spa)	N-terminal	Dale et al. (1999), McLellan et al. (2001)
Fibronectin-binding protein SfbI	Fibronectin-binding	Guzman et al. (1999), Schulze et al. (2001)
Fibronectin-binding protein FBP54	The whole molecule	Kawabata et al. (2001)

antibodies against all serotypes included in the construct. Brandt et al. (Brandt et al., 2000) recently reported a novel multi-epitope vaccine strategy, in which the construct included a minimum non-cross-reactive peptide from the conserved C-terminal half of the M-protein that was linked to seven serotypic peptides from the N-terminal region. The construct demonstrated good immunogenicity and protection in mice.

There are also other non-M-protein derived vaccine candidates, including C5a-peptidase, streptococcal cysteine protease (SpeB), streptococcal protective antigen (SPA), group A carbohydrate, and fibronectin-binding proteins (Table 1.4). One advantage with these candidates is that they provoke serotype-independent immunity, and can provide protection against heterologous GAS strains. With the recent advancements in multi-epitope vaccines, it seems plausible that future streptococcal vaccines may combine multiple streptococal antigens, thereby providing broad-spectrum immunity.

The occurrence of outbreaks of GAS in communities, especially clusters of STSS and/or NF, has raised concern about the transmissibility of the organism and the need for prophylaxis. DiPersio et al. (1996) described two clusters of GAS disease that occurred within separate family units. Davies et al. (1996) in their prospective study were able to estimate the incidence of invasive disease among contacts of persons with invasive GAS. They found that the risk to family members of patients' households was 2.9 per 1,000, almost 200 times the risk in the general population, supporting the use of chemoprophylaxis in patients' close contacts. The use of prophylaxis when severe disease occurs in such a setting is supported by the observation that subsequent cases are often also severe (Couper, 1997).

Numerous hospital-based case series have been reported on the clinical spectrum of pediatric invasive GAS disease and have identified that varicella-zoster virus (VZV) infection commonly precedes these infections, especially in cases of NF (Brogan et al., 1995; Davies et al., 1994; Doctor et al., 1995; Peterson et al., 1996a, 1996b; Vugia et al., 1996; Wheeler et al., 1991; Wilson et al., 1995).

Laupland et al. (2000) describe the incidence and clinical features of invasive GAS disease in children from the OSSG to better quantify the risk of this disease following chickenpox infection. There were 1.9 cases of invasive GAS disease per 100,000 children per year. Fifteen percent of children identified had preceding chickenpox infection, which significantly increased the risk for acquisition of invasive GAS disease (Relative Risk, RR: 58; 95% Confidence Interval, CI: 40–85). Children with invasive GAS and recent chickenpox were more likely to have NF (RR: 6.3; 95% CI: 1.8–22.3). The most striking finding of this study is the observation that VZV infection is associated with a 58-fold increased risk of acquiring invasive GAS disease in children. It is not clear why chickenpox infection increases the risk for GAS infection so dramatically.

Although the attack rate for invasive GAS disease following chickenpox is relatively low at 5.2 per 100,000, it is important that 15% of all pediatric invasive GAS infection in Laupland's study, including 50% of NF cases, followed VZV infection. Because there is an effective, safe vaccine for chickenpox available, many cases of invasive GAS disease, including NF, may potentially be preventable by chickenpox vaccination of young children. Laupland et al. (2000) estimated the impact of universal chickenpox vaccination effectiveness in preventing invasive GAS infection using the following assumptions: (1) vaccine coverage would be 80%; (2) vaccine efficacy for preventing chickenpox manifested by any skin lesions would be 85%; (3) vaccine would be administered at 1 year of age; and (4) the risk of invasive GAS infection would be equal in vaccinated and nonvaccinated children with skin lesions following wild type VZV infection. Based on their data and these assumptions, they estimated that universal vaccination of 1-year-old children would prevent at least 10% of all invasive GAS infections. Given that their estimates of vaccine efficacy (85%) and coverage (80%) are conservative, the effect of vaccination is likely greater than 10%. These data lend further support to the arguments in favor of introducing VZV vaccination into the routine childhood regimen.

7. FUTURE DIRECTIONS

At present the conventional therapy of severe invasive GAS infections includes clindamycin in combination with penicillin, supportive therapy to manage their hypotension and multiorgan failure, and surgery when indicated. However, the persistently high mortality rates demonstrate the need for adjunctive therapy in these diseases. The most promising therapies today seem to be agents that target several different streptococcal factors and/or host systems involved in sepsis. One such therapy is IVIG that has been shown to interact with GAS pathogenesis at several different stages (reviewed in Norrby-Teglund & Stevens, 1998), and in vitro data together with a case-control study strongly supports a clinical efficacy of IVIG as adjunctive therapy for STSS patients (Kaul et al., 1999).

By virtue of their pivotal role in the pathogenesis of the severe invasive GAS infections, superantigens are obvious targets for intervention. However, there are several different GAS superantigens with the potential for causing disease, hence, the intervention would need to be polyspecific. Despite significant difference in the primary sequence of the superantigens, they all share a common three-dimensional structure. Using this common tertiary structure, researchers have managed to produce peptides that inhibited the induction of cytokines and protected against lethal shock in experimental models of

superantigen-induced toxic shock (Arad et al., 2000; Visvanathan et al., 2001). Similarly, using site-directed mutagenesis of the conserved receptor-binding regions in superantigens, vaccines were generated that protected against lethal shock (Ulrich et al., 1998).

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