Group A Streptococcal Infections in Sweden:
A Comparative Study of Invasive and Noninvasive Infections and Analysis of Dominant T28 emm28 Isolates

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Surveillance of group A streptococcus (GAS) infections in Sweden during 1996–1997 indicated that T28 isolates were dominant, whereas T1M1 infections were uncommon. Circulating T28 isolates were nearly all emm28, MLST52, and these clones had also been prevalent 10 years earlier. Isolates from invasive and noninvasive infections were of similar types and prevalences. The average national incidence of invasive episodes was 2.9/100,000 population but varied between 0 and 8.3/100,000 population in different counties. It increased markedly with age, reaching 22.9 episodes/100,000 among people aged ≥90 years. The incidence of puerperal sepsis was higher than expected (22.4/100,000 of those at risk), with 1 death. Overall mortality was 16% and was associated with preexisting chronic disease (P = .002). Streptococcal toxic shock syndrome (STSS) developed in ~15% of patients with invasive episodes, with a mortality rate of 45%. The use of nonsteroidal anti-inflammatory drugs was not found to be associated with the development of STSS.

The past 2 decades have seen a change in group A streptococcus (GAS), epidemiology with an apparent increase of serious invasive infections in Europe and North America, as well as outbreaks of rheumatic fever in the United States [1–3]. The clinical presentation of recent invasive infections has often been that of bacteremia or serious soft-tissue infection, and a significant proportion of patients have developed shock and multiple organ failure, defined as streptococcal toxic shock syndrome (STSS) [4]. GAS isolates belonging to sero-
type M1 or M3 have caused a large proportion of severe infections, and outbreaks caused by M1 isolates have been reported elsewhere [2, 5, 6]. A majority of M1 and M3 GAS isolates appear to be genetically similar and are believed to constitute 2 recently emerged virulent clones [5]. However, recently found M1 GAS isolates have also been prevalent in noninvasive infections and have been found to be genetically similar to invasive isolates [7, 8]. Therefore, a high M1 prevalence among invasive episodes may reflect widespread transmission rather than increased virulence or invasiveness. Furthermore, host factors, such as preexisting chronic disease, advanced age, impaired immune status, and a lack of acquired immunity against GAS virulence factors (in particular, superantigen exotoxins) may all be important background factors in severe infections [9–11]. The present study was done to survey epidemiological and clinical characteristics of invasive GAS infections in Sweden during 1996–1997 and to study the spread of GAS clones among invasive and noninvasive infections.
MATERIALS AND METHODS

Surveillance of invasive and noninvasive GAS infections. Laboratory-based prospective surveillance of invasive GAS infections was done from 1 November 1996 through 31 October 1997. All 30 public bacteriological laboratories were asked to submit to the Swedish Institute of Infectious Disease Control all GAS isolates recovered from patients with invasive infections. Invasive infection was defined as GAS isolated from a normally sterile site or from a superficial site in a patient who developed a necrotizing infection or STSS. Also included were women with clinical signs of endometritis postpartum and for whom GAS was isolated from the cervix. Each month during the first 7 months of the surveillance period, 3 laboratories submitted the first 5 GAS isolates from throat and superficial skin infections, respectively, which we used as noninvasive control isolates. Patient age and sex were reported for these isolates. During the same period, a detailed questionnaire regarding patient characteristics and the outcome of invasive infections was sent to the physician in charge of the patient. The study was approved by the regional ethics committee at the Karolinska Hospital (Solna, Sweden).

Typing of isolates. T-agglutination was done according to standard procedures, using 5 polyvalent and 20 monovalent anti–T-agglutination sera (SEVAC). Pulsed-field gel electrophoresis (PFGE) was done as described elsewhere [12]. The emm sequence type was determined using universal oligonucleotide primers based on the 5′ end of the central emm gene within the chromosomal region [13]. An emm type was defined as having ≥95% sequence identity to another known emm type over 160 bp near the 5′ end, as specified by the Centers for Disease Control and Prevention [14]. The sequencing was done with ABI PRISM products (Perkin-Elmer Applied Biosystems).

For multilocus sequence typing (MLST), chromosomal DNA was prepared from freshly grown GAS isolates by the mutanolysin procedure, as described elsewhere [12]. Internal fragments (400–500 bp) of 7 housekeeping genes, encoding putative glucose kinase, glutamine transport protein, glutamate racemase, mismatch repair enzyme, transketolase, xanthine phosphoribosyltransferase, and acetylcoenzyme A acetyltransferase, were amplified by PCR that used primer pairs designed for GAS loci and sequenced as described elsewhere [15]. The sequences at the 7 loci were used to assign the sequence type of each isolate using the Streptococcus pyogenes MLST database (http://spyogenes.mlst.net).

Statistical analysis. Student’s t test and the χ2 test were used where appropriate.

RESULTS

Epidemiology of invasive infections. During the 12-month surveillance period, GAS isolates recovered from 255 patients with invasive infection were submitted, which corresponded to a national incidence of 2.9 cases/100,000 population. Incidence ranged between 0 (2 laboratories submitted no isolates) and 8.3 (mean, 3.2; 95% CI, 1.5–4.1)/100,000 population in the 24 different counties. There was no identifiable trend in relation to population density or geographic location. Incidence in male and female subjects was similar (2.6 and 3.2/100,000, respectively) but increased markedly with age, as shown in figure 1. In people aged 90–99 years, there were 13 invasive episodes in a population of 56,000, which corresponds to an incidence of 22.9/100,000 population, almost 46 times higher than for people aged 10–19 years (0.5/100,000 population) (figure 1). Puerperal sepsis was identified in 20 women. According to the National Board of Health and Welfare, Statistics Sweden (available at: http://www.scb.se), there were 89,208 women who gave birth in Sweden during the surveillance period, which results in an incidence of puerperal sepsis of 22.4/100,000 for the population at risk.

T-agglutination patterns of invasive versus noninvasive isolates. T-agglutination of the 255 invasive isolates demonstrated 25 patterns, most commonly T28 (31%), T12 (12%), and T8.25.imp19 (5%). There were only 12 T1M1 infections (4.7%). During the first 7 months, 181 invasive and 144 noninvasive isolates were submitted. The prevalence of different T-agglutination patterns was similar among invasive and noninvasive isolates, as shown in figure 2. Exceptions were T28 isolates, which were more prevalent among invasive isolates (31% vs. 17%; P = .009), and T8.25.imp19 isolates, which were more prevalent among noninvasive isolates (16% vs. 6%; P = .006). Ten (5.5%) of 181 invasive isolates were T1, compared with 1 (0.7%) of 144 noninvasive isolates (P = .03).

Molecular typing of T28 isolates. T28 isolates constituted 31% of all invasive isolates and 9 (45%) of 20 in the county (Kalmar county) with an increased incidence (8.3/100,000 pop-
Figure 2. Percentage distribution of the 15 most common T-agglutination patterns in isolates from invasive (black bars) and superficial (white bars) group A streptococcus infections.

ulation) of invasive GAS infection. All 83 invasive and noninvasive T28 isolates were further analyzed by PFGE. Using Smal, 22 different PFGE patterns were found.

We performed emm sequence typing on 1 representative T28 isolate for each of the Smal PFGE patterns obtained. Of the 22 isolates obtained, 17 were emm28, 3 were emm77, and 2 were emm2. Seventy-eight (94%) of 83 T28 isolates had a PFGE pattern consistent with a type emm28, including all 9 T28 isolates from Kalmar county. The PFGE pattern of the emm28 isolates differed by 1–4 bands.

MLST was done on 17 T28 isolates, 15 emm28 and 2 emm77. All emm28 isolates, including the 9 from Kalmar, belonged to a sequence type designated ST52, whereas the 2 emm77 isolates were both ST63.

Seven of 9 T28 isolates that had been subjected to PFGE in a previous study of invasive GAS infections in Stockholm during 1988–1995 [10] had a PFGE pattern identical to isolates from the present study; emm sequence typing of these 7 isolates showed that 6 were emm28 and 1 was emm87. Using MLST, all 6 emm28 isolates were ST52, whereas the emm87 isolate was ST62. Thus, all invasive T28, emm28 isolates were ST52 and had PFGE patterns differing by only 1–4 bands.

Clinical features of invasive infections. The age distributions differed considerably between patients with invasive and noninvasive GAS infections, as is seen in figure 3. Invasive infections were common in the higher age groups, whereas superficial infections were most commonly found among small children and young adults.

One hundred forty-four questionnaires (80%) were returned regarding the 181 patients with an invasive infection during the first 7 months. A preexisting chronic disease or local predisposing factor, most commonly cardiovascular disease, peripheral vascular disease, diabetes, or a malignancy was reported in 69%. Patients with a preexisting chronic illness were considerably older than those who were previously healthy (mean ages, 74 and 30 years, respectively) (figure 4). Skin and soft-tissue infection were the most common underlying foci of the infection (50%); a necrotizing infection was seen in only 3 patients. Puerperal infection was the underlying focus in 16 (11%) of 144 patients, with 1 fatal outcome. Puerperal sepsis was, in fact, the single most common focus of infection in previously healthy patients. These were sporadic episodes and were not related to an identified local outbreak.

Total mortality among the 144 patients was 16% (23 of 144 patients). Twenty-two of 100 patients with a preexisting chronic disease died, compared with 1 of 44 patients without a chronic disease (P = .002). Clinical and laboratory features consistent with STSS were reported in 22 (15%) of 144 patients, and 10 (45%) of these patients died.

Information about the intake of a nonsteroidal anti-inflammatory (NSAID) drugs initially during their illness was available for 118 (82%) of 144 patients. STSS developed in 1 (8%) of 13 patients who had taken an NSAID and in 18 (17%) of 105 with no history of NSAID intake. There was no statistical association between NSAID intake and development of STSS.

DISCUSSION

The present study provides an overview of GAS infections in Sweden during 1996–1997. Nearly one-third of the invasive episodes were caused by T28 isolates, whereas T1M1 infections were uncommon. However, the incidence of invasive infections, 2.9/100,000 population, was similar to that reported in previous
outbreaks in Sweden and North America during which T1M1 infections have been dominant [6, 9, 16, 17]. Large variations in incidence have been reported to be associated with ethnic factors and age rather than with GAS serotype [17]. The T-agglutination patterns of invasive and noninvasive isolates were generally similar, which indicates that most circulating GAS clones were potentially pathogenic and induced invasive episodes in proportion to their spread in the population. However, T1 and T28 isolates were somewhat more common among invasive than noninvasive infections, which perhaps suggests that newly emerging, widely transmitted clones may generate a higher proportion of invasive and severe infections.

During the T1M1 outbreaks in Sweden during 1988–1989 and 1994–1995, the T28 serotype was not predominant, and the increased spread of T28 isolates is a recent phenomenon [6, 10]. Ninety-four percent of the T28 isolates were *emm*28 and were of the same multilocus sequence type, ST52, whereas PFGE profiles differed by 1–4 bands. They are likely to have originated from a common ancestor and may be regarded as closely related clones [15, 18]. However, in contrast to recent T1M1 isolates [10], some genetic diversification detectable by PFGE has occurred among circulating T28, *emm*28 GAS serotypes.

Host factors—in particular, advanced age and a preexisting chronic illness—are of major importance in the progression of a GAS infection from harmless colonization or a superficial infection to a severe invasive episode. However, increased incidence was also found in patients aged 30–39 years. Asymptomatic carriage and throat infections are known to peak in school children, who seem to be the prime source of GAS transmission, with occasional secondary invasive episodes in the parental and grandparental generations [19, 20].

Preexisting chronic illness was associated with increased mortality \( (P = .002) \) but not with STSS. In previous studies, STSS has been associated with factors such as alcoholism, infection with a GAS serotype T1M1 isolate, or infection with an isolate carrying the *speA* gene [1, 10]. In addition, invasive GAS T1M1 infection was shown in a large study to be associated with increased mortality [17]. Infection with the dominant T28 isolates was not associated with increased risk of STSS or death in the present study. The overall case-fatality rate (16%), the proportion of patients developing STSS (15%), and STSS case-fatality rate (45%) were similar to results from previous studies performed during periods when T1M1 infections were more prevalent [6, 10]. The intake of NSAID drugs was not found to be associated with STSS [21].

Classic puerperal fever is generally regarded as uncommon in the antibiotic era, and 20 postpartum invasive GAS infections were reported during our study. However, this corresponded to a substantially increased risk of invasive GAS infection for this group of patients, compared with the population in general, and 1 death occurred. Thus, GAS infection should still be recognized as a possible cause of fever in postpartum women.

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