Spread of Serious Disease—Producing M3 Clones of Group A Streptococcus Among Family Members and Health Care Workers


Streptococcus pyogenes causes a variety of diseases ranging from mild pharyngitis to severe toxic shock syndrome (TSS) and acute rheumatic fever. Since 1987 there has been a resurgence of severe group A streptococcus infections including TSS, necrotizing fasciitis, and myositis. Using molecular and serotyping procedures, we recently studied two clusters of group A streptococcus disease that occurred within separate family units. The first cluster involved two family members (one with TSS and one with necrotizing fasciitis) and three health care workers who attended one of the index patients. The second cluster included a mother (with necrotizing fasciitis of the hand) and her three children. Group A streptococci isolated from individuals within both cluster groups were serotype M3; T3/13/B3264, and pulsed field gel electrophoresis revealed that all isolates except one had identical fingerprints of Sma I-digested chromosomal DNA. The findings demonstrate the potential for spread of serious group A streptococcus disease among individuals and the need for barrier protection when health care workers are exposed to secretions from infected individuals.

Group A streptococcus is a common pathogen of the throat and skin that can cause both mild and severe human disease. Serious group A streptococcus infections such as rheumatic fever and scarlet fever were common in the early part of this century, but a number of factors have led to their decreased frequency and severity since that time [1, 2]. During the 1970s and early 1980s, the morbidity and mortality rates associated with group A streptococcus infections and their supplicative and nonsuppurative sequelae were so low in the United States that reporting of group A streptococcus infection was arbitrarily stopped. This appeared to change during the late 1980s when cases of severe group A streptococcus infections were being reported with greater frequency [3, 4]. Outbreaks of necrotizing fasciitis due to group A streptococcus (“flesh-eating” bacterium) have attracted the most attention and have caused the greatest concern [5, 6].

A second life-threatening complication of group A streptococcus infection is toxic shock syndrome (TSS) [7, 8]. Both of these severe infections may arise during chicken pox, following surgical procedures, or following minor local injuries to the soft tissue, even without noticeable skin breaks. Mortality rates associated with necrotizing fasciitis sometimes reach 60%–70% despite antibiotic therapy, amputations, and modern supportive treatments.

The transmission of a group A streptococcus clone causing severe disease within two groups of family members was recently studied. The first cluster included two family members (one with TSS and one with necrotizing fasciitis); this cluster was of interest because three health care workers who treated one of the family members had group A streptococcus infections following exposure. The second cluster included a mother with necrotizing fasciitis and her three children. The spread of group A streptococcus infection among individuals within both groups was studied with use of both molecular and serotyping procedures. The isolates from both clusters were compared with other group A streptococci recovered from patients with severe disease who were seen at various hospitals in the Akron area between June 1994 and June 1995.

Clusters and Methods

Family Cluster 1

A 46-year-old woman (patient A) was admitted to hospital A on 11 June 1994 because of a 1-day history of fever, chills, vomiting, diarrhea, and severe right-elbow pain (table 1). Her blood pressure was 88/50 mm Hg; pulse rate, 140; respiration rate, 32; and temperature, 38.8°C. A diffuse erythematous rash was present, and the area surrounding the right elbow was erythematous, swollen, and very tender. There was a small amount of drainage over the area of the bursa of olecranon. Her WBC count was 26,000/mm³ (47% segmented neutrophils and 47% band forms). She was transferred to the intensive care unit. Percutaneous aspiration of the bursa of olecranon was performed; therapy with intravenous penicillin G was initiated,
Table 1. *Streptococcus pyogenes* infections in individuals in cluster 1.

<table>
<thead>
<tr>
<th>Patient age (y)/sex</th>
<th>Culture date</th>
<th>Infection</th>
<th>Serotype</th>
<th>Hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 46F</td>
<td>6/11/94</td>
<td>TSS, bursitis</td>
<td>M3;T3/13/B3264</td>
<td>A</td>
</tr>
<tr>
<td>B, 73M (family member of patient A)</td>
<td>6/30/94</td>
<td>TSS, necrotizing fasciitis</td>
<td>M3;T3/13/B3264</td>
<td>B</td>
</tr>
<tr>
<td>C, 35M (physician of patient B)</td>
<td>...*</td>
<td>Severe exudative pharyngitis</td>
<td>...*</td>
<td>B</td>
</tr>
<tr>
<td>D, 50M (paramedic of patient B)</td>
<td>7/2/94</td>
<td>Pharyngitis, rash</td>
<td>Group A streptococcus</td>
<td>C</td>
</tr>
<tr>
<td>E, 28M (physician of patient B)</td>
<td>7/6/94*</td>
<td>Mild nonexudative pharyngitis</td>
<td>M3;T3/13/B3264</td>
<td>B</td>
</tr>
</tbody>
</table>

NOTE. TSS = toxic shock syndrome.
* Culture not performed.
† Isolate not available for further study.
§ Surveillance culture date.

and one dose of ceftriaxone was administered. Hypotension resolved following administration of intravenous saline. Culture of the bursal drainage yielded *Streptococcus pyogenes*. The bursa was drained surgically. Her condition improved clinically, and she was discharged on 20 June 1994; her medication at the time of discharge was oral clindamycin.

A 73-year-old man (patient B; father-in-law of patient A) with no significant medical history presented to the emergency department of hospital B on 30 June 1994 because of large bullous lesions and rapidly progressive swelling on the dorsal surface of his right hand. He had been seen at an urgent care center 2 days before admission because of complaints of pain in his right arm of 4 days’ duration. At that time he was told he had a ligamentous tear, his arm was placed in a splint, and he was given prescriptions for hydrocodone bitartrate and acetaminophen (Vicodin, Knoll Pharmaceuticals, Whippany, NJ) and naproxen. The family stated that he and his daughter-in-law (patient A) had frequent casual contact, both before and after her hospitalization. His vital signs included the following: blood pressure, 98/60 mm Hg; pulse rate, 112; respiration rate, 24; and temperature (core), 35.9°C.

In the emergency department the discoloration of the skin rapidly spread proximally involving the right deltoid region. The patient was aggressively rehydrated and treated with intravenous vancomycin and clindamycin. He was taken to the operating room within 3 hours of presentation. Extensive necrosis of muscle including the right pectoral, latissimus dorsi, and deltoid was found, and the right upper extremity was amputated. He was treated with penicillin in the operating room. Postoperatively, he was admitted to the intensive care unit with sepsis syndrome requiring rehydration and treatment with pressors.

An expanding lesion involving the right flank was noted later that day, and he was again taken to the operating room where extensive necrosis of fascia, subcutaneous fat, and skin was noted. Cultures of blood and tissue specimens obtained at this time were positive for group A streptococcus. Patient B was readmitted to the intensive care unit where his condition continued to deteriorate, and he eventually died on 1 July 1994.

Forty-eight hours after patient B was treated, an emergency department physician (patient C) and a paramedic (patient D) involved in the direct management of patient B had symptomatic group A streptococcus infections. Patient C had exudative pharyngitis, and patient D had scarlet fever syndrome. Patient C treated himself empirically with oral antibiotics before specimens for cultures could be obtained. Patient D presented at a neighboring hospital with upper respiratory tract complaints, and a throat specimen was obtained; culture of the specimen was positive for group A streptococcus. However, the isolate was not available for further study. All other health care workers involved with the care of patient B were screened by standard throat culture. Culture of a throat specimen from one of the orthopedic house staff (patient E), who had had close contact with the patient during the initial period in the emergency department, was positive for group A streptococcus. Group A streptococcus isolates from patients A, B, and E were available for further study.

Family Cluster 2

A 29-year-old woman with no significant medical history presented to the emergency department on 8 February 1995 because of swelling and erythema of the right index finger that extended into the palm. Three days earlier she had sustained a puncture wound on the right index finger that had become increasingly painful; she had had no other systemic signs or symptoms of infection. The patient was taken to the operating room for debridement where purulence was noted. Cultures of specimens obtained at that time yielded group A streptococcus. Postoperatively, the patient’s condition initially improved, but over the next 24 hours, erythema and swelling of the right hand increased and extended to the palm and wrist. Additional debridement on 10 February 1995 revealed necrosis. Therapy with intravenous penicillin G was administered, and her condition subsequently improved; she was discharged on 14 February 1995.

During hospitalization the patient’s children (2, 4, and 6 years old) were screened for group A streptococcus infection by throat culture. All three children were found to be colonized with group A streptococcus, and symptomatic pharyngitis developed in the oldest child.
Bacteriologic Methods

All organisms were recovered from specimens submitted to the clinical microbiology laboratory for culture. Group A streptococci were identified by means of standard methods [9]. Serotyping of M and T proteins was performed at the World Health Organization Streptococcal Reference Laboratory or the Centers for Disease Control and Prevention (CDC) [10].

DNA Extraction and Restriction Analysis

Organisms were grown overnight at 35°C in 5 mL of Todd-Hewitt broth, and cells in a 60–360 μL aliquot were harvested by centrifugation. Cells were resuspended in 100 μL of buffer (10 mM Tris [pH 7.2], 20 mM sodium chloride, and 50 mM EDTA). Samples were incubated at 50°C, and 4 μL of lysostaphin (2 mg/mL)/lysozyme (25 mg/mL) and 100 U of mutanolysin (Sigma Chemical, St. Louis)/mL were added followed by 100 μL of 2% clear-cut agarose. Solidified plugs were placed in 1 mL of lysis buffer (10 mM Tris [pH 7.2], 50 mM sodium chloride, 0.2% sodium lauryl sarcosine, and 0.5% sodium deoxycholate) together with 40 μL of lysostaphin/lysozyme and 100 U of mutanolysin/mL for 1 hour at 37°C without agitation. Plugs were washed with buffer (20 mM Tris [pH 8.0] and 50 mM EDTA) and treated with 40 μL of proteinase K and 1 mL of proteinase K buffer (100 mM EDTA [pH 8.0], 1% sodium lauryl sarcosine, and 0.2% sodium deoxycholate) for 16–20 hours at 50°C without agitation. Plugs were treated with 20 μL of 100 mM phenylmethylsulfonyl fluoride for 30–60 minutes at room temperature with gentle agitation, washed two times with buffer for 30–60 minutes at room temperature, and stored at 4°C in buffer for further use.

Each plug was washed with buffer to remove EDTA, and the DNA was digested overnight at 25°C with the restriction enzyme Sma I (25 U per plug) in 300 μL of buffer. Restriction fragments were separated by means of a-contoured-clamped homogeneous electrical field instrument (GenePath; Bio-Rad, Hercules, CA). The pulse time was ramped from 5.3 to 34.9 seconds over 20 hours at 200 V at a temperature of 13°C. Gels were stained with ethidium bromide and photographed under ultraviolet light. Analysis of fingerprint patterns of chromosomal DNA were performed by visual inspection. Group A streptococcus isolates showing identical DNA banding patterns were considered a group. Those isolates with patterns that differed by one or two DNA bands (the result of a single genetic event) were considered subgroups. Gel patterns with three or more different DNA bands were considered different groups.

Results

Figure 1 shows the patterns of Sma I-digested chromosomal DNA from the three group A streptococcus isolates available from cluster 1 (patients A, B, and E) together with three epidemiologically unrelated group A streptococcus isolates (patients X, Y, and Z) that were recovered from wound specimens submitted for culture around the same time. The outbreak isolates (lanes 2–4) from patients E, A, and B, respectively (designated clone A), demonstrated identical DNA fingerprints that were distinct from the DNA banding patterns of the other group A streptococcus isolates tested. Subsequent analysis showed that the three outbreak isolates belonged to serotype M3;T3/13/B3264 (table 1).

The DNA fingerprints of group A streptococcus isolates from cluster 2 are shown in figure 2 (the mother’s isolate, lane 1; isolates from her three children, lanes 2, 3, and 4, respectively). Two of the children were colonized with a group A streptococcus strain identical to the mother’s (lanes 2 and 3); the fingerprints of these isolates matched the fingerprint pattern seen in cluster 1. The oldest child who was symptomatic with pharyngitis was infected with a strain of group A streptococcus (lane 4) that exhibited a different DNA banding pattern (designated clone B). Subsequent serotyping, however, showed that this strain was also serotype M3;T3/13/B3264. Although there are similarities between the banding patterns of the clone B isolate and the clone A isolates recovered from other family members, we placed this isolate in a distinct clone group since there were more than three different DNA bands. However, the possibility exists that clone B is a subtype of clone A because of the high number of matching DNA bands.

Patterns of Sma I digests of isolates from other cases of serious group A streptococcus disease seen after the cluster 1 outbreak are also shown in figure 2 (an M nontypeable;T4 isolate from a case of group A streptococcus cellulitis with TSS, lane 7; M3;T3/13/B3264, M nontypeable;T11, and M1;T1 isolates from three additional cases of necrotizing fasciitis, lanes 5, 6, and 8, respectively). The M1;T1 isolate (lane 8) revealed a DNA banding pattern seen in all M1 strains we have tested to date. The banding pattern of the M3;T3/13/B3264 isolate (lane 5) is identical to the pattern of clone B in cluster 2 (lane 4). Another M3;T3/13/B3264 isolate (lane 9) was recovered from a wound specimen from a patient not epidemiologically linked to any known cases of severe disease. These findings demonstrate that DNA polymorphism exists within the M3 serotype. At least two clones have been identified as causing severe group A streptococcus disease to date in the Akron area.

Discussion

Group A streptococcus has long been recognized as a serious human pathogen that causes life-threatening illnesses, including bacteremia, pneumonia, and rheumatic fever. Dramatic declines in the morbidity and mortality rates associated with group A streptococcus infections and their nonsuppurative sequelae occurred in the United States after the mid 1950s. Since the late 1980s, however, there has been an as yet unexplained resurgence of invasive group A streptococcus disease in the United States and Europe. Bacteremia, severe soft-tissue infec-
Figure 1. Pulsed field gel electrophoresis patterns of SmaI-digested chromosomal DNA from six group A streptococcus isolates (lanes 2–7). Lanes 2–4, isolates from patients E, A, and B, respectively (cluster 1); lanes 5–7, three epidemiologically unrelated group A streptococci isolated from wound cultures; lanes 1 and 8, molecular size standards (Saccharomyces cerevisiae and λ oligomers, respectively); and lane 9, a Staphylococcus aureus control. Molecular sizes (in kb) are indicated to the right and left of the figure.

Figure 2. Pulsed field gel electrophoresis patterns of SmaI-digested chromosomal DNA from group A streptococcus isolates from cluster 2 (lanes 1–4), isolates from additional cases of invasive group A streptococcus disease (lanes 5–8), and a Staphylococcus aureus control (lane 10).
tions (including necrotizing fasciitis and myositis), and TSS are seemingly more common. Although group A streptococcus infections have not been reportable diseases in most states, the CDC, public health officials, and other researchers have estimated that the number of serious group A streptococcus infections in the United States is 10,000 to 15,000 per year. Cases usually arise sporadically, but secondary cases of severe infection have been described, albeit rarely.

Some medical researchers suggest that the increase in the number of cases of invasive group A streptococcus disease is the result of an emergence of a highly virulent clone of an M1 strain [11]. Other investigators argue that the low attack rate in the face of a high prevalence of the clone in the community suggests that host factors are what determine the development of severe infection [12]. In the United States 64% of the cases of group A streptococcus bacteremia seen between 1989 and 1990 were caused by the M1 serotype. Before 1979 only 18% of the bacteremic cases were caused by M1 strains [3]. More than 80% of the group A streptococcus isolated from patients with severe disease produce certain virulence factors such as streptococcal pyrogenic exotoxin, as opposed to <20% of strains causing less serious disease [8]. Thus, virulence factors together with the immunologic state of the host seem to be responsible for the clinical presentation observed.

Previous studies have documented the spread of group A streptococcus infection to close contacts of infected individuals [13, 14]. The transmission of group A streptococcus from patients to health care workers, primarily in the nursing home setting, has also been reported [15–17]. In these studies traditional M and T serotyping procedures were used to document transmission of strains between patients, patients and health care workers, and family members. Infections within clusters have ranged from asymptomatic carriage to pharyngitis and severe invasive disease. In our cluster 1, each of the five individuals presented with different symptoms, ranging from those of mild pharyngitis to those of severe TSS with necrotizing fasciitis. In cluster 2, only the mother had invasive disease. Two children were asymptomatic carriers of the same group A streptococcus strain, while the third child had symptoms of mild pharyngitis caused by a group A streptococcus isolate of the same serotype but with a different DNA banding pattern.

Group A streptococcus serotypes M1 and M3 are the most common isolates associated with invasive infection. However, many different M serotypes as well as nontypeable strains have been linked to severe disease [18]. Serotyping and genetic analysis have suggested a shift in the prevalence of M protein type or the appearance of group A streptococcus clones possessing virulence factors that can cause disease and/or trigger shock as possible reasons for the increased number of infections. In 1992 Cleary et al. [11] studied a group of M1 strains from patients with serious and uncomplicated group A streptococcus infections using restriction enzyme digestion and agarose gel electrophoresis. The isolates tested showed two main profiles of restriction fragments. Ninety percent of the serious disease–producing isolates had a characteristic “invasive” restriction fragment profile. Only 44% of the uncomplicated disease–producing isolates demonstrated this profile. Uncomplicated disease–producing isolates had four restriction fragment patterns. In addition, 90% of the serious disease–producing isolates possessed the speA gene, as opposed to 54% of the uncomplicated disease–producing isolates.

Earlier studies by Cleary et al. [19] and other investigators led to a conclusion that members of the same group A streptococcus serotype may have identical restriction fragment profiles, although individual strains may show minor differences. It was also suggested that fingerprints could distinguish epidemiologically distinct strains of group A streptococcus within the same serotype. In addition, strains of the same serotype isolated from different continents had similar but distinguishable restriction fragment profiles. These earlier studies used frequent cutting restriction enzymes together with conventional electrophoresis to produce 40–80 DNA fragments. Many of the fragments, however, were difficult to resolve.

DNA fingerprinting with pulsed field gel electrophoresis (PFGE) has become an accepted method for studying the clonal relationship between strains of epidemiologic interest. In recent studies Sfi I digests and PFGE were used to study polymorphism among isolates within M serotypes [20, 21]. The results demonstrated observable differences between group A streptococcus isolates within the same M type and between isolates of different M types. Musser et al. [21] recently showed 16 DNA banding patterns among 126 M1 isolates collected from patients with group A streptococcus infections around the world. However, PFGE with Sfi I revealed that 66% of the isolates had a single DNA banding pattern.

In the present investigation PFGE was used to separate Smal–digested chromosomal DNA from group A streptococcus. The three group A streptococcus isolates available from cluster 1 and three of four group A streptococcus isolates from cluster 2 all showed identical fingerprints and were serotype M3. A fourth group A streptococcus isolate recovered from one child with pharyngitis in cluster 2 demonstrated a different DNA banding pattern but also belonged to serotype M3; T3/13/B3264. This DNA banding pattern was seen in one case of necrotizing fasciitis that was not epidemiologically related to cluster 2. Subsequent molecular studies of group A streptococcus isolates from cultures of wound, blood, and throat specimens have not shown that the M3 serotype was predominant or very common in the Akron area during the last half of 1994 or early 1995.

This study demonstrates that group A streptococcus is a highly transmissible microorganism, an observation that is supported by epidemiologic studies of different types of group A streptococcus infection (including scarlet fever, pharyngitis, rheumatic fever, impetigo, myonecrosis, and childbed fever). This study also demonstrates that a strain of group A streptococcus may cause different disease (necrotizing fasciitis, pharyngitis, scarlet fever, or pharyngeal carriage) in different hosts.
The host factors that determine the clinical manifestations (or lack of them) are undefined but likely include the presence or absence of antibody to streptococcal virulence factors such as specific M protein or pyrogenic exotoxins [12].

Our observations document the ability of group A streptococcus strains associated with invasive disease to spread to close contacts (both family members as well as health care providers). The results support previous recommendations that health care providers should be aware of the risk of secondary spread and that appropriate infection control measures should be instituted when caring for patients with suspected invasive group A streptococcus infections [13]. CDC guidelines recommend that gloves and gowns be worn during contact with patients with “major” group A streptococcus wound infection [22]. It is also possible that patients with overwhelming infection (such as necrotizing fasciitis) may be capable of spreading the infection via the airborne route. We speculate that the health care providers who had pharyngeal infection in association with our patient B may have acquired their infections in this manner. Assuming this to be true, appropriate infection control measures should perhaps include the wearing of masks during the care of a patient with suspected rapidly spreading group A streptococcus necrotizing fasciitis.

In summary, close contacts and hospital workers exposed to patients with invasive group A streptococcus infections are at risk for colonization with the identical strain. Most of these individuals will be asymptomatic or have mild infection. Rarely, secondary cases of invasive group A streptococcus infection may occur. PFGE is a powerful tool for tracing the transmission of group A streptococcus in contacts. The use and efficacy of prophylactic antibiotics in this setting have not been established or recommended but should be investigated.

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References