Molecular Characterization of a Strain of Group A Streptococcus Isolated from a Patient with a Psoas Abscess

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We report the first case of a primary group A streptococcus (GAS) psoas abscess in a 31-year-old woman. The psoas abscess was preceded by an episode of acute pharyngitis. The M-protein gene (emm) and streptolysin S structural gene (sagA) were present in the isolate, with no significant amino acid differences from previously described sequences of M1 GAS isolates. Multilocus sequence typing (MLST) showed that the isolate belonged to MLST sequence type (MLST-ST) 28, the predominant MLST-ST associated with invasive disease caused by M1 isolates.

CASE REPORT

A 31-year-old housewife was admitted to the hospital in December 2002 because of left lower quadrant abdominal pain lasting for 2 weeks. The pain was aggravated by walking and relieved by rest. She also experienced episodes of fever, chills, and rigor, associated with nausea and change of bowel habit. One week before this episode, she recalled having flu symptoms, including fever and sore throat, which had resolved spontaneously. Apart from having depressive illness requiring regular antidepressant treatment, she was otherwise well before this episode. On admission, she was afebrile, and the pharynx was not inflamed. Examination of the abdomen showed a vague, tender mass in the left lower quadrant of the abdomen, with local guarding and rebound tenderness. Bowel sounds were normal. The left lower quadrant pain was provoked by resisted flexion of the left hip joint. Her blood sample was examined and revealed the following: 34.2 × 10⁹ white blood cells per liter, 29.7 × 10⁹ neutrophils per liter, 2.1 × 10⁹ lymphocytes per liter, 2.4 × 10⁹ monocytes per liter, 12.9 g of hemoglobin per dl, and 426 × 10⁹ platelets per liter. Fasting blood glucose levels and liver and renal function tests were within normal limits. Erythrocyte sedimentation rate (ESR) was 39 mm/h, and the level of C-reactive protein (CRP) was 1.3 mg/dl. Blood culture was performed. Urgent computed tomography of the abdomen and pelvis with contrast revealed a vague, tender mass in the left lower quadrant of the abdomen, with local guarding and rebound tenderness. Blood cells and gram-positive cocci arranged in chains. Culture of the pus recovered pure growth of beta-hemolytic gram-positive cocci arranged in chains. Blood culture was negative.

Histology of the trucut biopsy of the abscess capsule showed the presence of acutely inflamed granulation tissue. Stain and culture for acid-fast bacilli on both the pus and biopsy tissue specimens were negative. Intravenous penicillin G was administered, and the patient’s symptoms gradually improved. Her white cell count, ESR, and CRP returned to normal levels, and the abscess subsided on follow-up computed tomography scans. The patient developed generalized skin rash and eosinophilia after 2 weeks of penicillin. She was discharged with oral erythromycin for 3 more weeks and remained well up to the time of writing.

Certain Streptococcus species, including Streptococcus agalactiae, Streptococcus pneumoniae, and Streptococcus milleri group, have also been reported to cause psoas abscesses, either as a result of bacteremia or secondary spread from vertebral osteomyelitis, epidural abscess, and colonic carcinoma (1). In contrast, Streptococcus pyogenes or Lancefield group A beta-hemolytic streptococcus (GAS), a well-known invasive streptococcus that commonly causes skin and soft tissue infections, has not been recognized to cause psoas abscesses. In the past 15 years, there has been a global resurgence of invasive GAS infections, such as necrotizing fasciitis and streptococcal toxic shock syndrome. It is now estimated that 9,600 to 9,700 cases of invasive GAS infections occur in the United States each year, resulting in 1,100 to 1,300 deaths (13). The phenomenon has been correlated with the resurgence of “virulent” clones (5, 14). Despite this increase in severe GAS infections, there has been no reported case of a primary psoas abscess associated with GAS in the literature. In this report, we describe the first case of a primary GAS psoas abscess in an immunocompetent host. In view of the doubtful association between GAS and psoas abscess, the 16S rRNA gene of the isolate was sequenced. To investigate the pathogenesis in this case, the M-protein gene (emm) and streptolysin S structural gene (sagA) were sequenced, and multilocus sequence typing (MLST) was also performed.

Clinical and microbiological data. All clinical data were collected prospectively as described in our previous publication.
The bacterium was identified by standard conventional biochemical methods (12). The isolate grew on sheep blood agar as beta-hemolytic, grayish white colonies of 0.5 to 1 mm in diameter after 24 h of incubation at 37°C in ambient air. No enhancement of growth was observed in 5% CO₂. It did not grow in 10 or 40% bile, on MacConkey agar or bile esculin agar, or in 6% NaCl. It was catalase negative, L-pyrrolidonyl-β-naphthylamide hydrolysis positive and bacitracin susceptible. Lancefield grouping of the strain performed using Streptex (Murex Biotech Ltd., Dartford, United Kingdom) showed that it belonged to Lancefield group A. The Vitek system (GPI) showed that it was 99% S. pyogenes. It was sensitive to penicillin, erythromycin, and clindamycin by the Kirby Bauer disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) criteria.

16S rRNA gene sequencing. PCR amplification and DNA sequencing of the 16S rRNA gene of the isolate were performed according to methods described in previous publications (10, 20, 21). LPW200 (5′-GAGTTGCGAAGGGGTGA G-3′) and LPW205 (5′-CTTGTACGACTTCACCC-3′) (Gibco BRL, Rockville, Md.) were used as the PCR primers, and LPW200, LPW205, LPW99 (5′-TTATTGGGCCTAAAG CGA-3′), and LPW273 (5′-TTGCGGGACTTAAACCCAC-3′) were used as the sequencing primers. The sequences of all the PCR products were compared with known corresponding gene sequences in the GenBank database by multiple sequence alignment using the CLUSTAL W program (16). PCR showed a band at about 1,400 bp. There were 2 base differences between the 16S rRNA gene sequence of the isolate and that of a Lancefield group A beta-hemolytic S. pyogenes (GenBank accession number AE014168), indicating that the isolate was a strain of S. pyogenes.

M-protein gene (emm) sequencing. PCR amplification and DNA sequencing of the emm gene of the isolate were performed according to the methods described in previous publications (15, 22), using LPW616 (5′-ATAAGGAGCATAAAA ATGGCT-3′) and LPW617 (5′-AGCTTAGTTTTCTTCTTT GCG-3′) as the PCR and sequencing primers. PCR showed a band at about 1,400 bp. There were 3 nucleotide and 2 amino acid differences, respectively, between the nucleotide and predicted amino acid sequences of the emm gene of the isolate and those of a previously described M1 protein of S. pyogenes (GenBank accession number AE006624).

Streptolysin S structural gene (sagA) sequencing. PCR amplification and DNA sequencing of the sagA gene of the isolate were performed according to the methods described in previous publications (7, 22), using LPW614 (5′-ATAKAAAAAGAAAGGTTTACAT-3′) and LPW615 (5′-CATATAGTAAATT AGCAGGTAC-3′) as the PCR and sequencing primers. PCR showed a band at about 500 bp. There was 1 nucleotide difference but no amino acid difference between the nucleotide and predicted amino acid sequences of the sagA gene of the isolate and those of a previously described M1 S. pyogenes (GenBank accession number AE006526).
**MLST.** MLST was performed by PCR amplification and DNA sequencing of the internal fragments of seven housekeeping genes, including glucose kinase (gki), glutamine transporter protein (gtr), glutamate racemase (murL), DNA mismatch repair protein (mutS), transketolase (recP), xanthine phosphoribosyltransferase (xpt), and acetyl coenzyme A acetyltransferase (yqIL), using primers and PCR conditions described in a previous publication (4). The sequences of all the PCR products were analyzed using the MLST website for *S. pyogenes* (www.mlst.net). Results showed that the isolate possessed alleles 4, 3, 4, 4, 2, and 4 at loci gki, gtr, mutL, mutS, recP, xpt, and yqIL, respectively, which corresponded to MLST sequence type 28.

This report represents the first case of primary psoas abscess caused by GAS. GAS infections involving muscles most often occur in the form of necrotizing fasciitis and necrotizing myositis, with rapid and extensive soft tissue and muscle necrosis. Pyomyositis is a less common manifestation of GAS infections. In such circumstances, diffuse inflammation and necrosis in the involved muscles in the absence of abscess formation are characteristic features. As a result, abscess formation in muscles is an uncommon phenomenon in GAS infections. Moreover, involvement of the psoas muscle is rarely reported. In the English literature (MEDLINE search of 1966 to 2002 data), only two cases of GAS infections involving the psoas muscle have been reported, including a case of psoas myositis and flank fasciitis in a 62-year-old man and a case of bacteremia with multiple small abscesses in the psoas, obturator internus, and iliac muscles in an 8-year-old girl (17, 19). However, in neither of the two cases was a large, solitary abscess with thick capsule formation encountered, as in the present case. Furthermore, both patients in the two previous reports were near the two extremes of age, which may have rendered them less immunocompetent. Therefore, the present report represents the first case of a primary psoas abscess caused by GAS in an immunocompetent adult host.

The psoas abscess in this patient may have been a complication of GAS acute pharyngitis. It has been suggested that GAS pharyngeal infections may serve as a reservoir for invasive clones. An epidemiological study on GAS infections in North Carolina has shown that serotypes M1 and M3 accounted for 50% of invasive isolates and 58% of pharyngeal isolates, with the latter being genotypically identical to the former by pulsed-field gel electrophoresis (9). Similar findings were also obtained in a recent study based on our local GAS isolates (6). In a report of pyomyositis in a 7-year-old boy, the same strain of GAS has also been isolated from the pharynx and muscle of the child and the pharynx of the child’s asymptomatic sibling (23). In the present report, the isolate was also GAS type M1 by emm gene sequencing. Moreover, the patient recalled having a preceding episode of acute pharyngitis. Although there is no microbiological proof, it is possible that her psoas abscess has occurred as a result of transient bacteremia complicating acute pharyngitis.

The underlying reasons for the development of psoas abscess in this patient remain elusive. The rare occurrence of muscle abscess in GAS infections is best explained by its extreme tissue invasiveness. Once GAS infections develop in skin, soft tissue, or muscles, it is believed to rapidly spread and invade adjacent tissues, causing local inflammation, necrosis, and subsequent systemic dissemination. This also explains the strong association of GAS with necrotizing fasciitis. Moreover, GAS is known to possess antiphagocytic properties, thus inhibiting localization of infective foci and abscess formation.

Tissue invasiveness of GAS has been attributed to several virulence factors. Among these, the cell wall M protein, encoded by the *emm* gene, is the most important antiphagocytic factor. Serotypes M1 and M3, in particular, have recently been associated with many sporadic cases and outbreaks of invasive infections (8). In a recent study on the pathogenesis of invasive GAS soft tissue infections, animals challenged with the wild-type isolate recovered from a patient with necrotizing fasciitis developed spreading tissue necrosis at the site of inoculation, became bacteremical, and subsequently died, whereas the animals challenged with either hyaluronic acid capsule- or M-protein-deficient mutants developed focal area of tissue swelling at the site of inoculation without necrosis or systemic disease. This suggested that the hyaluronic acid capsule and M protein are important factors in the pathogenesis of tissue necrosis, invasion, and systemic disease of *S. pyogenes* (2). It has also been shown that certain amino acid substitutions within different regions of the M1 proteins may also affect opsonization by sera (18). However, the M1 protein in the present GAS isolate does not possess important amino acid substitutions from known M1 proteins. The *sagA* gene is necessary for the production of streptolysin S, another GAS virulence factor, and for the surface localization and hence the antiphagocytic ability of M protein (3). The *sagA* gene of the present isolate also did not differ from known *sagA* sequences. We speculate that there may be microbial factors, other than M protein and *sagA* locus, in the present GAS isolate that have led to the formation of a psoas abscess in this patient. By MLST, the present isolate seems to have originated from the same clone predominantly associated with invasive disease among M1 isolates recovered from different geographical areas (4). Although the pathogenesis in the present report is uncertain, isolates of the same GAS clone present in other countries may have the potential of causing a similar clinical syndrome.

**Nucleotide sequence accession number.** The 16S rRNA, *emm*, and *sagA* gene sequences of the isolate have been deposited in the GenBank sequence database under accession no. AY273147, AY273148, and AY273149, respectively.

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**REFERENCES**


