CASE REPORTS

Two Unusual Cases of Severe Soft Tissue Infection Caused by *Streptococcus dysgalactiae* subsp. *equisimilis*[▽]

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We present two cases of invasive infection caused by *Streptococcus dysgalactiae* subsp. *equisimilis*, one that showed rapidly developing necrotizing fasciitis in a previously healthy man and one that showed severe cellulitis and septic shock even though the bacterium possessed a mutated *emm* gene, predicted to encode a truncated M protein.

CASE REPORT

Patient 1 was a 48-year-old, previously healthy man admitted to Haukeland University Hospital in Western Norway with a fever and a rapidly spreading erythema of the skin overlying his right knee. The symptoms were preceded by a minor trauma toward the right knee 5 h prior to admission, leading to a disruption of the skin barrier. Blood pressure upon admission was 127/68 mm Hg, pulse rate 90/min, and temperature 39.6°C. Blood cultures were taken, and intravenous treatment with penicillin G at 5 million IU four times a day (q.i.d.) and clindamycin at 900 mg three times a day (t.i.d.) was started. Surgical exploration was performed 2 h later because of rapid deterioration of the patient's condition, and necrotic subcutaneous tissue and fascia of the lateral margin of the patella and the upper part of the calf were excised. Hypotension (blood pressure, 85/45 mm Hg) developed postoperatively, and normal blood pressure was reestablished after 5 to 6 h of intensive intravenous fluid therapy. Neither renal, hepatic, nor respiratory failure developed. The international normalized ratio (INR; normal range, <1.1) was temporarily elevated to 1.5, whereas the platelet count, activated partial thromboplastin time (APTT), and d-dimer, fibrinogen, serum alanin-aminotransferase (s-ALAT), s-creatinine kinase (s-CK), and s-creatinine levels were within the normal ranges. The initial values for C-reactive protein (CRP; normal range, <5 mg/liter) and white blood cell count (WBC count; normal range, 3.5×10^9 to 11×10^9 /liter) were 12 mg/liter and 13.8×10^9 /liter, respectively. Blood cultures were negative, but group G streptococci (GGS) grew in pure cultures from two biopsy specimens of excised fascia obtained during surgery. The bacterial isolate was sensitive to all tested antibiotics, with the following MIC values (mg/liter): for penicillin, 0.016; for clindamycin, 0.19; and for erythromycin, 0.19. Surgical exploration was repeated on day

three and revealed a small area of necrotic subcutaneous fat in the lateral margin of the patella, requiring further surgical debridement. On day seven, the wound was surgically closed. The patient received penicillin G and clindamycin intravenously for a total of 14 days. He was discharged 15 days after admission and eventually regained normal function of his left leg.

Patient 2 was a 54-year-old male patient admitted to Haukeland University Hospital with a 2-h history of fever, chills, and moderate pain in the left groin. The medical history and comorbid conditions included pulmonary irradiation fibrosis after treatment for Hodgkin's lymphoma in 1978, three previous coronary artery bypass operations, and chronic heart failure. The physical examination on admission revealed bilateral ankle edema and a saphenectomy scar in the left calf, paronychia of the first left toe, and moderate tenderness on palpation of the left groin but no lymphadenopathy and no obvious signs of skin or soft tissue infection in the leg or groin. Blood pressure was 145/113 mm Hg, pulse rate 100/min, and temperature 40.2°C. Blood cultures were taken, but treatment with antibiotics was not started on admission. The initial blood chemistry results were as follows, with normal range values in parentheses: CRP, 14 mg/liter (<5 mg/liter); WBC count, 19.5×10^9 /liter (3.5×10^9 to 11.0×10^9 /liter); platelet count, 389×10^9 /liter (140×10^9 to 400×10^9 /liter); s-ALAT, 41 U/liter (10 to 70 U/liter); s-creatinine, 89 μ mol/liter (60 to 105 μ mol/liter); s-CK, 89 U/liter (40 to 280 U/liter); INR, 1.3 (<1.1); APTT, 80 s (23 to 37 s); d-dimer, 1.07 mg/liter (0.00 to 0.50 mg/liter); and fibrinogen, 6.1 g/liter (2.0 to 4.0 g/liter). During the next 6 to 12 h, hypotension (blood pressure, 80/50 mm Hg), oliguria, erythema, swelling, and severe pain in the left calf developed. Intravenous treatment with penicillin G at 4 million IU q.i.d. and clindamycin at 600 mg q.i.d. was initiated approximately 10 h after admission. Necrotizing fasciitis (NF) was suspected, but surgical exploration of the left calf 20 h after admission revealed only marked edema of the subcutaneous soft tissue, without obvious signs of necrosis. On the first postoperative day, all four blood cultures grew GGS. The bacterial isolate was sensitive to all tested antibiotics, with

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FIG. 1. Segment of the *emm* gene *stG6.5* compared to a segment of allele *stG6.0* (GenBank accession numbers FJ531857 and FJ531851, respectively). The alleles are italicized in the left margin. Numerals representing the positions of the first nucleic acids shown are placed to the left of the nucleotide sequences. Encoded amino acid sequences are presented below the nucleotide sequences using single letter symbols. The asterisk indicates a stop codon, and the dash (in blue) indicates the single nucleotide deletion between positions 154 and 158 from the signal peptide cleavage site, resulting in a frameshift mutation and a stop codon downstream. Variation in the number of repeated nucleotide segments of *stG6.0* and *stG6.5*, probably representing A repeats, are highlighted in colors (red and green).

the following MIC values (mg/liter): for penicillin, 0.012; for cefuroxime, 0.016; for cefotaxime, 0.023; and for clindamycin, 0.19. Cultures of soft tissue aspirate from the left calf grew GGS, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, and a culture of wound secretion from the left toe grew *Staphylococcus epidermidis*. After 3 days of intensive medical treatment with antibiotics, vasopressors, and mechanical ventilation, the patient's condition stabilized. On the fourth postoperative day, he was transferred from the intensive care unit to the infectious disease section. He was discharged without sequelae 16 days after admission and received treatment with penicillin and clindamycin for a total of 28 days.

The GGS isolates were stored at -80°C in Greaves medium until further testing. Species identification was determined by partial sequencing of two genes, the superoxide dismutase gene (*sodA*), as described before (22), and the 16S rRNA gene, using the primers 5'-TTG-GAG-AGT-TTG-ATC-MTG-GCT-C-3' (forward) and 5'-TAC-CGC-GGC-TGC-TGG-CAC-3' (reverse) (21) and an annealing temperature of 65°C . The *sodA* nucleotide sequences of our two GGS isolates showed 99 to 100% similarity to the *sodA* sequences of *Streptococcus dysgalactiae* subsp. *equisimilis* and 99% similarity to the *sodA* sequences of *Streptococcus pyogenes* found in GenBank. A better discrimination was possible with 16S rRNA sequences, which identified both isolates as *Streptococcus dysgalactiae* subsp. *equisimilis*. *emm* typing was performed as previously described (15). In order to analyze the entire *emm* genes of the isolates, the primers used for *emm* amplification were also used for sequencing in both directions. The 5' ends of the *emm* genes were compared to sequences in the *emm* type database available at the website for the Centers for Disease Control and Prevention (CDC; <http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm>). The bacterial isolate associated with patient 1 (isolate 1; strain S19) was of *emm* type *stC74a.0*, and the sequenced region of this *emm* gene was predicted to encode a segment of the processed M protein consisting of 381 residues. A novel subtype, *stG6.5*, was assigned by the CDC for the isolate from patient 2 (isolate 2; strain S9). As shown in Fig. 1, this *emm* gene had a single nucleotide deletion in

the hypervariable 5' end compared to other alleles of sequence type *stG6* and was predicted to translate to a truncated M protein of only 56 residues, lacking the conserved domains and anchor region. A multiplex PCR with previously described primer pairs was conducted for detection of the 11 streptococcal superantigens *speA*, *speC*, *speG*, *speH*, *speI*, *speJ*, *speK*, *speL*, *speM*, *smeZ*, and *ssa* (17). In order to cover the allelic variation of the *smeZ* gene, we also used a single PCR with a previously reported primer pair (5). Single PCR amplifications of the *speG* gene homologue found in *Streptococcus dysgalactiae* subsp. *equisimilis* (*speG_{dys}*) and the genes encoding C5a peptidase (*scpA*), streptokinase (*ska*), streptolysin O (*slo*), streptolysin S (*sagA*), extracellular phospholipase A₂ (*slaA*), and cysteine protease (*speB*) were performed with primers previously described (13). The virulence gene profiles of the two GGS isolates are shown in Table 1.

Cellulitis is most often caused by *Streptococcus pyogenes* (group A streptococci [GAS]) or *Staphylococcus aureus* (2, 8) and is associated with bacteremia in approximately 2% of the cases (20). Risk factors for the development of cellulitis in the lower extremities include prior saphenectomy and the presence of *S. aureus* or beta-hemolytic streptococci in toe webs (1). Invasive GGS disease is most often associated with one or more predisposing factors, like skin lesions, cancer, chronic heart and lung disease, diabetes mellitus, and drug and alcohol abuse (4, 7, 16). There is growing evidence for *Streptococcus dysgalactiae* subsp. *equisimilis* possessing group G antigen as an important cause of cellulitis (27). However, severe systemic infections, like streptococcal toxic shock syndrome (STSS) and NF, are very rare clinical manifestations of GGS disease (9, 11, 13, 26).

Patient 1 illustrates that GGS, like GAS, may cause unusually fulminant NF in relatively young and previously healthy adults. The GGS strain causing this severe soft tissue infection was probably inoculated through a breach in the skin barrier after a minor trauma and caused NF only a few hours later. Previously published cases of NF caused by GGS have been

TABLE 1. Molecular characteristics of two isolates of *Streptococcus dysgalactiae* subsp. *equisimilis* associated with invasive soft tissue infection

Isolate	<i>emm</i> type	Size of predicted M protein (aa ^a)	Presence of:																		
			<i>speA</i>	<i>speC</i>	<i>speG</i>	<i>speH</i>	<i>speI</i>	<i>speJ</i>	<i>speK</i>	<i>speL</i>	<i>speM</i>	<i>ssa</i>	<i>smeZ</i>	<i>speG dys</i>	<i>speB</i>	<i>slaA</i>	<i>scpA</i>	<i>ska</i>	<i>sagA</i>	<i>slo</i>	
1	<i>stC74a.0</i>	381	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
2	<i>stG6.5</i>	56	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+

^a aa, amino acids.

associated with age of >70 years and/or predisposing disease or a considerably longer duration of symptoms before establishment of a diagnosis (11, 26). Patient 2 had risk factors for cellulitis and invasive GGS disease. Although NF did not develop and the criteria for STSS were not fulfilled, the disease took a fulminant course with rapid development of severe local pain, septic shock, and renal failure. In addition to GGS, *S. aureus* grew in a soft tissue aspirate from the left calf. Unfortunately, this isolate was not available for further analysis. That this staphylococcal strain could have contributed to the development of cellulitis and to the severe disease manifestations cannot be excluded. However, we find it likely that the systemic symptoms and organ failure were mainly attributable to GGS, the sole bacteria isolated from blood.

GGs share virulence factors with GAS, including the M protein (encoded by the *emm* gene). M proteins of GAS have a major antiphagocytic activity and have also been shown to influence other aspects of streptococcal pathogenesis, like adherence, invasion, and induction of inflammation (6, 18, 19). Recently, it was shown that complexes consisting of M1 protein and fibrinogen colocalized with IgG antibodies against M1 proteins in severely infected soft tissue induced platelet activation and thereby the formation of thrombi in the microvasculature (25). GGs causing human infections have *emm* genes with structural similarity to GAS *emm* genes (24), and it is conceivable that GGs M proteins are also important virulence factors. Homologues of other streptococcal virulence genes, like the phage-mediated superantigen genes *speA*, *speC*, *speM*, and *ssa* and the chromosomal superantigen gene *smeZ*, have been identified in human GGs (12, 14), implying lateral genetic transfers from GAS to GGs and thus possibly changing the virulence potential of GGs. Genes carrying the *speG* homologue *speG_{dys}* and encoding the complement inhibitor and chemotaxis C5a peptidase, the streptolysin O and S hemolysins, and the plasminogen activator streptokinase have been identified in GGs associated with invasive disease (3, 9, 13), and the expression of streptolysin S was essential in the development of NF caused by GGs in a mouse model (11). The facts that a GGs strain devoid of a functioning M protein was associated with severe soft tissue infection and grew avidly in human blood and that none of the 11 known GAS superantigens were detected in our two GGs isolates highlights the possibility and importance of other virulence proteins as well as host factors in the pathogenesis of severe streptococcal soft tissue infection. Both isolates carried *scpA*, *ska*, *slo*, and *sagA*, and one isolate harbored the *speG* homologue *speG_{dys}*. Whether or not the detected genes were expressed in our two isolates was not explored, and as the prevalence of them in GGs strains associated with carriage and noninvasive disease is unknown, their role in the pathogenesis of severe streptococcal soft tissue infection is unclear. Neither of our two patients developed disseminated intravascular coagulation (DIC) or fulfilled the criteria for STSS, but both showed coagulopathy and severe systemic manifestations. We might speculate that dysregulation of the coagulation system and microvascular thrombosis, as previously shown in association with severe GAS infection (10, 23, 25), are also crucial in the pathogenesis of severe soft tissue infection caused by GGs.

Streptococcus dysgalactiae subsp. *equisimilis* is increasingly recognized as a pathogen causing severe soft tissue infections.

This case report illustrates the fulminant course that soft tissue infections caused by this species can take and underlines the importance of early surgical intervention when soft tissue necrosis is suspected.

Nucleotide sequence accession numbers. The nucleotide sequences referred to in the text were deposited in GenBank under the following accession numbers: FJ531857 (strain S9 *emm* gene), GQ845001 (strain S19 *emm* gene), GQ390354 (strain S9 *sodA* gene), GQ390355 (strain S19 *sodA* gene), GQ390356 (strain S9 16S rRNA gene), and GQ390357 (strain S19 16S rRNA gene).

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