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Pathogenesis and Toxins

Non-toxigenic *Clostridium sordellii*: Clinical and microbiological features of a case of cholangitis-associated bacteremia

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ABSTRACT

Toxigenic *Clostridium sordellii* strains are increasingly recognized to cause highly lethal infections in humans that are typified by a toxic shock syndrome (TSS). Two glucosylating toxins, lethal toxin (TcsL) and hemorrhagic toxin (TcsH) are believed to be important in the pathogenesis of TSS. While non-toxigenic strains of *C. sordellii* demonstrate reduced cytotoxicity *in vitro* and lower virulence in animal models of infection, there are few data regarding their behavior in humans. Here we report a non-TSS *C. sordellii* infection in the context of a polymicrobial bacterial cholangitis. The *C. sordellii* strain associated with this infection did not carry either the TcsL-encoding *tcsL* gene or the *tcsH* gene for TcsH. In addition, the strain was neither cytotoxic *in vitro* nor lethal in a murine sepsis model. These results provide additional correlative evidence that TcsL and TcsH increase the risk of mortality during *C. sordellii* infections.

1. Introduction

Clostridial species have long been identified as causative agents of human disease, but the importance of these toxigenic bacteria has gained recent attention due to the emergence and spread of pathogens like *Clostridium difficile* [1] and its close relative *Clostridium sordellii* [2,3]. *C. sordellii* is a sporulating, anaerobic, gram positive bacterium that is often isolated from soil samples and sometimes causes highly lethal human infections [4]. This bacterium is known to cause gynecological infections following childbirth or abortion [5,6], necrotizing soft tissue infections associated with the injection use of contaminated heroin [7–9], and post-operative infections complicating musculoskeletal transplants performed with contaminated graft material [10]. The incidence of *C. sordellii* infections is unclear, but an increasing number of cases has been reported over the past 10 years [11–15]. A recent report found that 1 in 200 deaths in women of reproductive age were associated with clostridial toxic shock, due to either *C. sordellii* or another clostridium, *C. perfringens* [3].

Perhaps two thirds of *C. sordellii* infections are associated with a clinically unique toxic shock syndrome (TSS), with mortalities exceeding 70% [4,16]. However, this percentage may be overestimated, since it is likely that the most dramatic clinical cases (especially those associated with TSS) are reported, while infections that run a more benign course or have a positive outcome remain unpublished.

The pathogenesis of *C. sordellii* TSS has been a focus of recent studies [16,17]. Though incompletely understood, the occurrence of TSS depends on the expression of one or both of the large glucosylating cytotoxins (TcsH and TcsL) of *C. sordellii*, which share structural and functional similarity to the large glucosylating cytotoxins of *C. difficile* (TcdA and TcdB, respectively). Both TcsH and TcsL intoxicate epithelial and endothelial cells by inactivating small GTPase proteins that are involved in maintaining cytoskeletal integrity [18–20]. Recent data suggest that *C. sordellii* TcsL is

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important for the development of TSS [17,19], though almost nothing is known about the participation of TcsH.

Herein we present a case of invasive *C. sordellii* infection that was associated with neither TSS nor death. Molecular analyses demonstrated that this clinical strain lacked the *tcsH* and *tcsL* genes encoding TcsH and TcsL, respectively. This strain also lacked virulence in a mouse model of peritonitis. These data provide correlative support for the hypothesis that hemorrhagic and lethal toxins are important virulence determinants of the highly lethal and treatment-refractory TSS caused by *C. sordellii*. Knowledge gained from non-lethal *C. sordellii* infections such as this one provides new information regarding the pathogenesis of severe infections caused by this organism.

2. Materials and methods

2.1. Institutional approval

This case report was reviewed and approved by the University of Michigan Institutional Review Board. Animals were treated according to National Institutes of Health guidelines for the use of experimental animals with the approval of the University of Michigan Committee for the Use and Care of Animals.

2.2. Animals

Eight-to-ten week old, female C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME).

2.3. Bacterial strains

C. sordellii strain HH-310 was isolated from anaerobic culture of a patient's blood sample (see below). The comparator strain ATCC9714 (*tcsL*$^+$, *tcsH*$^-$) was obtained from the American Type Culture Collection (Manassas, VA) and strain JGS6382 (*tcsL*$^+$, *tcsH*$^+$) was provided by Dr. J. Glenn Songer (Iowa State University).

2.4. PCR

DNA was extracted from *C. sordellii* strains HH-310, ATCC9714, and JGS6382 using an Easy-DNA™ extraction kit (Invitrogen, Carlsbad, CA). The taxonomic identity of HH-310 was verified using primers specific for a region of the *C. sordellii* 16S rRNA encoding gene [17]. Subsequent PCR for the *tcsL* gene encoding lethal toxin (TcsL) was performed using a previously reported primer pair (*tcsL* primer pair #3) and conditions [17]. New primers were designed to amplify an internal fragment of the *tcsH* gene encoding hemorrhagic toxin (TcsH) based on the genome sequence of the ATCC9714 strain which was obtained through Roche 454 Titanium genome sequencing (data not shown). Although this strain does not produce an active TcsH, it contains fragments of the *tcsH* gene at the appropriate genomic location downstream of (5′) the *tcsL* gene. These remnants were used to design PCR primers. Specifically, these primers were: *tcsH*_F1 (DLP37): GTAAATAAAACACATTTAAGAGCTTTGG and *tcsH*_R1 (DLP38): GGAATTTATATATGATAGGCAAAATAGG. Amplification conditions (94 °C denaturation for 10 min, then 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min 30 s, followed by a final extension at 72 °C for 5 min) were validated and optimized using DNA extracted from ATCC9714 and JGS6382 prior to assaying HH-310. All PCR amplicons were visualized using electrophoresis (0.8% agarose gels) and amplicon sizes were estimated using a 1 kb DNA ladder (Invitrogen, Carlsbad, CA).

2.5. Cytotoxicity assay

To assay for the production of toxins by strains JGS6382 and HH-310, the TechLab *C. difficile* Toxin/Antitoxin Kit (REF T5000, TechLab®, Blacksburg, VA) was used in conjunction with Vero cell monolayers according to the manufacturer's instructions (see package insert at http://www.techlab.com/product_details/t5000.shtml). This kit contains a toxin control reagent (positive control) and antitoxins that neutralize *C. sordellii* hemorrhagic and lethal toxins. Strains of *C. sordellii* were grown anaerobically in 10 ml of sterile Reinforced Clostridial Medium (RCM, BD, Franklin Lakes, NJ) for 20 h at 37 °C. Cultures were filter sterilized using 0.2 μm nylon syringe filters (Fisher, Waltham, MA) and the resulting supernatants were assayed for the presence of toxins. Vero cells were cultured as reported elsewhere [21], with the single exception of Dulbecco's Minimal Essential Medium (DMEM) with High Glucose (Invitrogen, Carlsbad, CA) in place of minimal essential media (MEM alpha medium). All assays were performed in triplicate using a final *C. sordellii* culture supernatant dilution of 1:10. Treated Vero cells were fixed with formalin and stained with Wright-Giemsa Stain Mixture (Ricca Chemical Co.). Cell morphology was observed by microscopy on an Olympus 1X71 inverted microscope (20× magnification). A positive cytotoxic reaction was noted by rounding of the Vero cells compared to wells containing toxin antibodies.

2.6. Virulence experiments

Virulence experiments in mice were performed as previously described [17]. Briefly, *C. sordellii* strains HH-310 or JGS6382 were grown overnight in RCM broth and washed with PBS. Five mice each were then injected intraperitoneally with 100 μl PBS containing approximately 1×10^{10} CFU and 1×10^{8} CFU of HH-310 or JGS6382, respectively. Infection was allowed to proceed for 7 d and survival was recorded daily.

3. Results

3.1. Case report

An 81 year-old female presented with the acute onset of stabbing abdominal pain that emanated from her epigastrium and radiated to her right upper abdominal quadrant and back. This was initially intermittent but became continuous and was exacerbated with inspiration. It was associated with fever and chills. The patient had a history of hypertension, diabetes mellitus, coronary artery disease, portal vein thrombosis and a congenital disorder causing non-obstructive dilation of intrahepatic bile ducts (Caroli disease).

As a result of the Caroli disease, the patient suffered repeated episodes of choledocholithiasis and cholangitis requiring multiple endoscopic retrograde cholangio-pancreaticogram (ERCP) procedures and stent placements. Ten months previously the patient was hospitalized with sepsis and cholangitis-associated with *Klebsiella pneumoniae* and *Escherichia coli* bacteremia. Two recent episodes of right upper quadrant abdominal pain radiating to her back associated with fever were managed successfully as an outpatient with empirical, 10-day courses of levofloxacin.

Physical examination revealed a temperature of 37.9° C, heart rate 137 beats per min, blood pressure 135/92 mm Hg and transcutaneous oxygen saturation of 92–94%. She had dry mucous membranes, and was tender to palpation in the epigastrium and right upper abdominal quadrant. Initial laboratory investigations showed a white blood cell count of 4.0 thou/mL (normal range 4.0–10.0 thou/mL) with 89.2% neutrophils (normal 36.0–75%), and a platelet count of 112 thou/mL (normal 150–450 thou/mL). Her