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Assessment of the pathogenesis of *Streptococcus suis* type 2 infection in piglets for understanding streptococcal toxic shock-like syndrome, meningitis, and sequelae

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ABSTRACT

Streptococcus suis type 2 (SS2) is an zoonotic pathogen that had caused outbreaks in 1998 and 2005 in China. It is still not very clear how the disease progresses into the streptococcal toxic shock-like syndrome (STSLS) or meningitis, as well as the sequelae from the survivals. The present study used piglets as infection model to systematically investigate the pathogenesis of the infection caused by the SS2 strain 05ZYH33. The infected piglets showed joint swelling, lameness, and crouch at beginning, then developed into septic-like shock syndrome (SLSS) or prostration syndrome, at last the survivals showed physical activity impairment. The morbidity and mortality were 100% (71% for SLSS, 29% for prostration syndrome) and 29%, respectively. The pigs exhibiting SLSS had deep invasive infections in tissues and organs, and displayed more severe bacteremia and cytokine secretion in the bloodstream and organs than pigs with prostration syndrome. Moreover, the polymorphisms in the toll-like receptor 1 (TLR1) and TLR2 genes varied between the pigs affected with SLSS and prostration syndrome. Several lines of evidence indicated that SS2 infection progression into SLSS or relatively lighter prostration syndrome in pigs is closely related to the degrees of bacteremia and cytokine storm, which may be inherently determined by the diversity of innate immunity-associated genes. Furthermore, brain lesions, such as venous thrombosis, may directly contribute to the sequelae in human cases, were identified in the pigs. These results might help us to further understand the pathogenesis of SS2 in humans.

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1. Introduction

Streptococcus suis, a Gram-positive encapsulated coccus, is a causative agent of serious zoonotic diseases with clinical manifestations of meningitis, septicemia, arthritis, pneumonia, endocarditis, and even acute death in pigs (Gottschalk and Segura, 2000) and humans (Tang et al., 2006; Wertheim et al., 2009). *S. suis* infections have been reported in over 30 countries, and 35 different serotypes have been identified on the basis of capsular antigens (Feng et al., 2010). Among these serotypes, SS2 is the most

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prevalent and virulent in animals and humans, though SS2 includes pathogenic, weakly pathogenic, and nonpathogenic strains (Smith et al., 2001). Further, over 1600 human cases have been reported worldwide since the first case of meningitis was discovered in Denmark in 1968 (Tang et al., 2006; Wertheim et al., 2009; Yu et al., 2006).

Retrospective analysis shows that SS2 infections are mainly sporadic cases and cause meningitis and septicemia in humans through occupational exposure to infected pigs or raw pork products (Wertheim et al., 2009; Yu et al., 2006). However, two recent large-scale outbreaks of severe SS2 infection in China have raised serious concerns for global public health and have challenged the conventional conception of *S. suis* infections as sporadic in humans. During the 1998 outbreak, 14 deaths occurred among 25 reported human cases, and approximately 80,000 pigs were sick. The 2005 outbreak is associated with 39 deaths out of 215 identified human cases, and more than 600 pigs were confirmed to be infected (Feng et al., 2010; Tang et al., 2006; Yu et al., 2006). More importantly, the novel symptom of STSLS, which includes the hallmarks of acute high fever, blood spots, hypotension, shock, and dysfunction of multiple organs, as well as acute death occurring within a median of 25 h (range 8 h to 10.5 days) since the onset (Yu et al., 2006), was observed in fatal cases from the two outbreaks. The causative pathogen for the outbreaks was identified as SS2 sequence type 7 (ST7), which is thus far exclusively found in China (Ye et al., 2006, 2008). Therefore, the two Chinese outbreaks of STSLS imply that SS2 is another causative agent of streptococcal toxic shock syndrome (STSS), in addition to *Streptococcus pyogenes* (Feng et al., 2010).

Superantigens are the critical causative factors of STSS (Lappin and Ferguson, 2009). However, no superantigen candidates have been determined for SS2 strains; though some new putative virulence factors associated with the STSLS are described in recent studies (Chen et al., 2007; Ge et al., 2009; Pan et al., 2009; Wang et al., 2009; Zhao et al., 2011). Moreover, only a portion of patients progress into STSLS; the others simply display meningitis and/or sepsis during the outbreaks (Ye et al., 2009; Yu et al., 2006). All of these facts imply that a different mechanism is involved in the severe invasive infections caused by the SS2 variants. In addition, likely due to the lack of a suitable animal model, the pathogenesis of the disease progressing into STSLS or meningitis, as well as the sequelae, caused by the Chinese virulent ST7 strain has not been systematically elucidated. As the natural host, pigs are the best model to study *S. suis* infection and to reflect the symptoms of patients more exactly than other animal models (e.g., mouse) (Vecht et al., 1997).

Thus, to gain insight into the pathogenesis of the entire course of the disease, piglets were used as disease model for ST7 infection. The etiological factors of the disease progression into severe syndromes with septic-like shock or relatively lighter prostration syndromes were systematically analyzed. Our findings provide a new insight into the pathogenesis of the Chinese ST7 strain and provide clues for elucidating the mechanism of STSLS, meningitis, and sequelae.

2. Materials and methods

2.1. Animals

Seven-week-old Landrace piglets from the Pingdu Pig-Breeding Farm (Qingdao, Shandong Province) were screened for the presence of major pathogens (SS2, *Haemophilus parasuis* (HPS), *Actinobacillus pleuropneumoniae* (APP), reproductive and respiratory syndrome virus (PRRSV), porcine circovirus 2 (PCV2), and swine influenza virus (SIV). The herd did not have any episode of acute disease related to these pathogens, and their nasal-tonsillar swabs (analyzed for SS2, HPS, APP, and SIV) and anticoagulated blood samples (analyzed for PRRSV and PCV2) were PCR/RT-PCR negative when the animals were selected for this experiment.

2.2. SS2 isolates

The 05ZYH33 strain was isolated from a fatal human case with STSLS in Sichuan Province in 2005. The mildly virulent SD1 strain, isolated from a healthy pig in Shandong Province in 2005 and provided by Prof. Yanbo Yin, was used as an experimental control.

2.3. Animal infection

Twenty-four piglets were randomly divided into three groups and infected via posterior auricular muscle injection with 1×10^7 CFU 05ZYH33 (14 piglets), 1×10^7 CFU SD1 (five piglets), or an equal volume of THB media (mock-infection control, five piglets). The clinical symptoms (including body weight, body temperature, activity, and the course of disease) were monitored daily after infection for 14 days post infection (d.p.i.). Two recovered survivors were applied for another 3-month observation period with the animal ethics committee to study the sequelae. All experiments were conducted in a biosafety level 2^+ (BSL-2^+) facility.

2.4. Histopathology and bacteriology identification in tissues and organs

Gross organ lesions from four dead (1–3 d.p.i.) and four euthanized animals with SLSS (two at 3–4 d.p.i. and two at 14 d.p.i.), two euthanized animals with prostration syndrome (8 d.p.i.), and two euthanized SD-1-infected pigs (3–4 d.p.i.) and THB group pigs (3–4 d.p.i.) as controls were observed and photographed. Portions of the brain, heart, lung, spleen, kidney, liver, tonsil, lymph node, small intestine, and joint tissues were fixed in 10% phosphate-buffered formalin, embedded in paraffin, cut into 5-μm-thick serial sections, and Hematoxylin and Eosin (H&E) stained for histopathological evaluation.

The bacteriological distribution in the tissues and organs from the four dead and two euthanized animals with SLSS (3–4 d.p.i.), and two euthanized SD-1 infected pigs and THB group pigs (3–4 d.p.i.) were identified by bacterial culturing and qualitative PCR with primer pair SS2-CPS2J-675F (5′-CAAACGCAAGGAATTACGG-3′) and SS2-CPS2J-675R (5′-GAGTATCTAAAGAATGCCTATTG-3′) after the infection with 05ZYH33.

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