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Short communication

Characterization of IgA1 protease as a surface protective antigen of *Streptococcus suis* serotype 2

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Abstract

IgA1 protease of *Streptococcus suis* serotype 2 (SS2) has been proven to be relative with virulence and immunogenicity, however, its protective efficacy remained to be evaluated. The present study found evidence that immunization with purified recombinant IgA1 protease (600-1926aa) could induce high IgG antibody titers and could confer complete protection against a challenge with a lethal dose of SS2 in a mouse model. In addition, our findings confirmed that the IgA1 protease distributes on the surface of SS2. Therefore, the present study identified the virulence-associated protein, IgA1 protease, as a novel surface protective antigen of SS2.

Keywords: *Streptococcus suis*; IgA1 protease; Protective antigen

1. Introduction

Streptococcus suis (*S. suis*) is a major swine pathogen that is responsible for severe economic losses in the porcine industry and poses a significant threat to human health [5,15]. Among the 33 serotypes (types 1 to 31, 33, and 1/2), serotype 2 (SS2) is considered to be the most pathogenic and to prevalent capsular type [6]. Two large-scale, epidemic outbreaks of human SS2 infection in China have increased awareness of this public health threat, particularly because the cases presented with septic shock (streptococcal toxic shock-like syndrome, STSLS) and caused high mortality [15,19]. To date, more than 1500 cases of human SS2 infection have been reported in the world [7]. In addition, *S. suis* has been identified as the third most common cause of community-acquired bacterial meningitis in Hong Kong and as the leading and second leading cause of adult meningitis in Vietnam and Thailand, respectively [15].

Undoubtedly, development of an effective vaccine was a prominent strategy for preventing the diseases. Traditional inactivated vaccine was useful for homologous strains, but often invalid for heterologous strains. Therefore, identification of protective antigens was necessary for the development of novel and effective vaccines. SLY [8] or MRP and EF [18] were initially identified as vaccine candidates, but their application was hindered by a substantial number of virulent strains that did not express these proteins. Antigenic proteins with little sequence variation in diverse clinical isolates would be more effective. To date, a few protective antigens have been successfully identified, including 38-kDa protein [14], surface protein one (SAO) [11], Zinc bingding lipoprotein

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SsuiDRAFT 0103 [1], hypothetical protein HP0197 [20], RTX family exoprotein A, epidermal surface antigen, immunoglobulin G (IgG)-binding protein (IBP) [12], hypothetical protein HP0272 [2,13], pilus subunit PAPI-2b [3], secreted metalloendopeptidase SsPepO [9] and surface-anchored DNA-nuclease (SsnA) [4].

Apart from these antigens, there are a few potential candidates whose protective immune response remains to be evaluated. IgA1 protease was a such protein, This protein was found in most pathogenic clinical isolates, but sparsely in non-invasive isolates [23], and contributed to the virulence of *S. suis* [22]. Additionally, IgA1 protease could be recognized by convalescent sera [23], but the protective immune response remained to be evaluated. Based on an agreement made on the first international workshop on *S. suis,* it is urgent needed for the acceleration of efforts to better understand the protective immune responses of these identified protective candidates [16]. Therefore, in the present study, we aimed to evaluate the protective efficacy of recombinant IgA1 protease (rIgAP) against SS2 infection.

2. Materials and methods

2.1. Bacterial strains and culture conditions

SS2 strain ZYS (also named SC-19) was isolated from the brain of a diseased piglet collected during the 2005 Sichuan outbreak in China that expressed MRP, EF, and SLY [17]. *S. suis* was maintained on Tryptic Soy Agar (Difco Laboratories, Detroit) plus 10% bovine sera or cultured in Tryptic Soy Broth (Difco Laboratories, Detroit) plus 10% bovine sera to mid-log phase (OD at 600 nm of 0.4) at 37 °C under aerobic conditions.

2.2. Mouse immunization and challenge

The study was performed in strict accordance with the Guide for the Care and Use of Laboratory Animals Monitoring Committee of Hubei Province, China, and the protocol was approved by the Committee on the Ethics of Animal Experiments at the College of Veterinary Medicine, Huazhong Agricultural University.

Twenty-four BALB/c mice (4-week-old female) were randomly assigned to 3 groups of 8 each. Thirty micrograms of purified rIgAP was prepared as previously described [23] and emulsified with a Marcol 52 (ESSO, USA)-based adjuvant and then applied to immunizing mice in group 1 by i.p. followed by a booster dose on the 14th day. Mice were immunized according to the same procedure with commercially formaldehyde-inactivated SS2 vaccine (Keqian) or PBS emulsified in the same oil adjuvant and served as controls.

On the 24th day after the first immunization, blood samples were drawn by tail vein bleeding for the determination of antibody titers. Then, all remaining mice were challenged by i.p. with 2×10^9 CFU of SS2 ZYS. Infected mice were monitored three times per day for 7 days for clinical signs. And the clinical scores of mice were assigned as following: 0 = normal response to stimuli; 1 = ruffled hair coat and a slow response to stimuli; 2 = only response to repeated stimuli; 3 = nonresponsive or walking in circles; 4 = dead. Mice exhibiting extreme lethargy or neurological signs (score = 3) were considered to be moribund and were humanely killed. At the end of the experiment, the surviving animals were sacrificed via carbon dioxide inhalation.

2.3. Determination of antibody titers

Sera were examined by ELISA for IgG titers as previously described [21]. Microtitre plates were coated at 4 °C overnight with 200 ng/100 μl of purified rIgAP, and then saturated with 200 μl of 0.5% BSA for 1 h at 37 °C. After washing with washing buffer (PBS, pH 7.2, containing 0.05% Tween-20), the plates were incubated with 100 μl of serially diluted mice sera for 30 min at 37 °C. After washing, the plates were further incubated with 100 μl of IgG-HRP (Southern Biotech, USA), rabbit anti mouse IgG1-HRP or IgG2a-HRP (Sigma) for 30 min at 37 °C. After washing completely, the color was developed by incubation with 100 μl of an activated substrate solution (sodium citrate buffer, containing 1 mg/ml 3,3,5,5-tetramethylbenzidine and 0.03% H_2O_2) in the dark for 10 min and then stopped by adding 50 μl of 0.25% hydrofluoric acid to each well. The plates were read with a micro ELISA reader at 630 nm. Antibody titers were determined as the reciprocal of the dilution of serum yielding 50% of the maximum OD value above the background.

2.4. Relative neutrophilic killing assay with specific antibodies

Murine neutrophils were isolated as previously described [25], counted by trypan blue staining. More than 90% neutrophil purity was used for subsequent neutrophilic killing assay [25], and the density of neutrophils was adjusted to 2×10^6 cells/ml. To remove antibodies that reacted with the bacteria, the mouse serum was preabsorbed with a SS2 ZYS suspension by rotation at 4 °C for 30 min. After centrifugation and filter-sterilization, serum was used as a source of complement. SS2 ZYS was adjusted to 2×10^4 CFU/ml. Equal volumes (100 μl) of neutrophils, complement, bacteria, and immune sera were mixed and incubated at 37 °C for 90 min before dilution, agar plating, and bacterial enumeration. The percentage of relative bacterial killing was calculated as [1-(number of CFU recovered in the presence of phagocytes/number of CFU recovered in the absence of phagocytes)] × 100.

2.5. Surface staining

Immunofluorescence microscopy analysis of IgA1 protease on SS2 was performed according to procedures described previously [24]. SS2 strain was applied to slides and then fixed by 100% methanol for 10 min. The slides were incubated with mouse sera (1:20) against purified rIgAP or negative control sera. After washing, the slides were incubated with