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The immunoglobulin M-degrading enzyme of *Streptococcus suis*, Ide_{Ssuis}, is a highly protective antigen against serotype 2

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ABSTRACT

Streptococcus suis (*S. suis*) is a major porcine pathogen causing meningitis, arthritis and several other pathologies. Recently, we identified a highly specific immunoglobulin M degrading enzyme of *S. suis*, designated Ide_{Ssuis}, which is expressed by various serotypes. The objective of this work was to access the immunogenicity and protective efficacy of a recombinant vaccine including Ide_{Ssuis}. Vaccination with $rIde_{Ssuis}$ elicited antibodies efficiently neutralizing the IgM protease activity. Importantly, 18 piglets vaccinated with $rIde_{Ssuis}$ alone or in combination with bacterin priming were completely protected against mortality and severe morbidity after *S. suis* serotype 2 challenge. In contrast, 12 of the 17 piglets either treated with the placebo or primed with the bacterin only, succumbed to *S. suis* disease. Immunity against Ide_{Ssuis} was associated with increased killing of *S. suis* wt in porcine blood *ex vivo* leading to a tenfold difference in the bacterial survival factor in blood of placebo-treated and $rIde_{Ssuis}$-vaccinated piglets. In conclusion, the results of this study indicate that $rIde_{Ssuis}$ is a highly protective antigen in pigs.

1. Introduction

Streptococcus suis (*S. suis*) is one of the most important porcine pathogens causing meningitis, arthritis and other pathologies [1]. In affected herds, problems typically augment between 7 and 10 weeks of age. Although *S. suis* is a very diverse species [2], at least 30% of *S. suis* diseases in Europe and China are caused by serotype 2 strains [3–5]. *S. suis* serotype 2 is also an important zoonotic agent causing meningitis and other diseases in humans [6,7].

In Europe no licensed vaccine is available and autologous bacterins are commonly used for prophylaxis [8]. However, there are major limitations associated with these bacterins, in particular, (i) most often only partial protection against the homologous serotype is observed, (ii) protection against heterologous serotypes is not elicited, (iii) differentiation of infected and vaccinated animals is generally not possible, and (iv) interference with maternal immunity is very common [9,10]. The latter makes it very difficult to prime piglets earlier than at an age of 4 to 6 weeks in porcine practice.

Several *S. suis* proteins have been tested as vaccine candidates, but substantial limitations are known. The hemolysin, suilysin (SLY), was found to elicit protection in mice but piglets with high titers of SLY neutralizing antibodies were found to be susceptible to infection [11,12]. A combination of muramidase-released protein (MRP) and extracellular factor (EF) is protective against serotype 2 [13], but other important pathotypes such as MRP* serotype 9 do not express EF.

Recently, we identified an immunoglobulin M-degrading enzyme of *S. suis*, designated Ide_{Ssuis}. The protein is homologous to the IgG protease IdeS of *S. pyogenes* [14], but cleaves solely class M antibodies. Ide_{Ssuis} is the first factor described reflecting *S. suis* adaptation to its main host as the protease exclusively cleaves porcine IgM [15]. It is expressed by all *S. suis* strains investigated and important for bacterial survival in blood of bacterin-primed piglets [15]. Thus, we designed this study to investigate the immunogenicities and protective efficacies of recombinant IdeSsuis ($rIde_{Ssuis}$) vaccination.

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2. Materials and methods

2.1. Bacterial strains and growth conditions

S. suis strain 10 (MRP$^+$ EF$^+$ SLY$^+$) is a virulent serotype 2 strain that has been used before for experimental infections [10,13,16]. The isogenic mutant 10Δ*ide*$_{Ssuis}$ deficient in IgM cleavage was included in the bactericidal assay to reveal effects mediated by antigen-specific immunity [15]. The unencapsulated mutant 10*cps*Δ*EF* was used as antigen in an ELISA to measure IgM-antibodies directed against other antigens but the capsule [17]. Strain I9841/1 is as strain 10 an MRP+ EF+ SLY+ CPS2 wt strain of clonal complex 1 used for bacterin generation [18]. *S. suis* was grown on Columbia agar plates supplemented with 6% sheep blood or in Bacto™ Todd Hewitt broth (THB). *Escherichia coli (E. coli)* strains were cultured in Luria-Bertani (LB) medium with the addition of 100 μg/ml ampicillin.

2.2. Expression and purification of recombinant Ide$_{Ssuis}$ protein

The expression and the purification of recombinant Ide$_{Ssuis}$ was performed as previously described [15].

2.3. Immunization of piglets

Piglets were prime and booster vaccinated with 0.25 mg rIde$_{Ssuis}$ containing 20% [vol/vol] Emulsigen as an adjuvant to generate reference hyperimmune sera independently of the vaccination trial. Vaccination of piglets at our institute is registered under 12A226 at the Lower Saxonian State Office for Consumer Protection and Food Safety (LAVES).

2.4. Animal experiments

German Landrace piglets from a herd known to be free of *sly*+ *mrp*+ *epf*+ *cps*2+ strains were infected experimentally and cared for in accordance with the principles outlined in the EU Directive 2010/63/EU (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm) and the German Animal Protection Law. The animal experiment was approved by the Committee on Animal Experiments of LAVES (permit no. 33.14-42502-04-12/0965).

At an age of 5 weeks, either a placebo (first and second group) or a vaccine containing rIde$_{Ssuis}$ as antigen (third and fourth group) were applied intramuscularly. Fourteen days later, the four groups were vaccinated as follows: placebo (first group); a serotype 2 bacterin (second group); rIde$_{Ssuis}$ and separately a serotype 2 bacterin (third group); rIde$_{Ssuis}$ (fourth group). One dose of prime and booster rIde$_{Ssuis}$ vaccination contained 0.4 mg and 0.25 mg rIde$_{Ssuis}$, respectively, supplemented with 20% [vol/vol] Emulsigen as adjuvant. The bacterin was generated with an overnight culture of strain I9841/1 inactivated in 0.2% formaldehyde (application of 1.6 ml with 1.8×10^9 inactivated bacteria/ml and 20% Emulsigen). The placebo consisted of PBS with 20% [vol/vol] Emulsigen.

Piglets were challenged intranasally at an age of 9 weeks (14 days after the second vaccination) with 1.2×10^9 CFU of *S. suis* strain 10 as described [19]. The health status of the animals was monitored every 8 h. A piglet was classified as morbid if a body temperature of ≥40.2 °C or/and severe clinical signs of an acute disease were observed. In case of high fever (≥40.5 °C), apathy and anorexia persisting over 36 h as well as in all cases of central nervous system dysfunction or clinical signs of acute polyarthritis animals were euthanized for reasons of animal welfare. All surviving piglets were sacrificed 14 days post infection (dpi). After euthanasia every animal went through the same procedure of necropsy to collect the following samples for histological (h) and semi-quantitative bacteriological (b) investigations as described previously [10,19,20]: cerebrospinal fluid (b); brain (b, h); tarsal and carpal joints (b, h), peritoneal, pleural and pericardial swabs (b), peritoneum, pleura and pericardium (h); cranial lobe of the left lung (b, h); liver (b, h); spleen (b, h); bicuspidalis (b, h) and tonsil (b, h). The histological screenings were scored as described [19] and briefly mentioned in the footnotes of Table 2. Isolation of the challenge strains was confirmed in a PCR for detection of *mrp*, *epf*, *sly*, *arc*A, *gdh*, *cps*1, *cps*2, *cps*7 and *cps*9 [4].

2.5. IgM cleavage neutralization assay

Sera of all piglets vaccinated with rIde$_{Ssuis}$ only or treated with the placebo were analyzed with regard to the presence of antibodies neutralizing the IgM cleavage activity of Ide$_{Ssuis}$. For this, sera were diluted 1:5 in PBS, spiked with the indicated concentrations of rIde$_{Ssuis}$ and incubated on a rotator for 45 min at 8 °C. Subsequently, samples were incubated for 2 h at 37 °C to allow cleavage of IgM by non-neutralized Ide$_{Ssuis}$. Serum proteins were separated under non-reducing conditions by SDS-PAGE [15] and analyzed in αpig IgM Western blots (Serotec, MCA637 was used as αpig IgM antibody as described [15]).

2.6. Bactericidal assay

Survival of *S. suis* in porcine blood *ex vivo* was determined as previously described [15]. Briefly, 500 μl of heparinized blood (16 I. U. heparin/ml) was mixed with 1.5×10^5 CFU of exponentially grown bacteria (OD_{600}: 0.5–0.6). The samples were incubated for 2 h at 37 °C on a rotator. Blood was drawn from all piglets of the vaccination trial three days before challenge for comparative analysis of survival of *S. suis* strain 10 and its isogenic mutant 10Δ*ide*$_{Ssuis}$ in the bactericidal assay. The specific bacterial content in CFU/ml were determined by plating serial dilutions at $t = 0$ min and $t = 120$ min and the survival factor of *S. suis* for each sample was calculated by dividing the two values.

2.7. Detection of anti-(α)Ide$_{Ssuis}$ IgG, αMRP IgG and αS. suis IgM antibodies

The detection of αMRP IgG was performed as previously described [10] αIde$_{Ssuis}$ antibodies were detected using the same protocol except that the plates were coated with rIde$_{Ssuis}$. Serum of piglets immunized with rIde$_{Ssuis}$ and a truncated derivative (rIde$_{Ssuis}$-homologue, [15]) served as reference serum and positive control in the αIde$_{Ssuis}$ ELISA, respectively.

α*S. suis* IgM titers were determined in an ELISA using a peroxidase-conjugated goat αpig IgM antibody (PA1-84625, Thermo Scientific, Schwerte, Germany) and Maxisorb® plates (Nunc, Rochester, USA) coated either with strain 10 or 10*cps*Δ*EF* (inactivated with 0.2% formaldehyde). Log linear regression analysis was conducted after background submission to calculate antibody concentrations. ELISA units were calculated as the mean of the calculated units for each of the four dilutions of two series. Quality was assessed through comparison to preset values for controls [10].

2.8. Suilysin-neutralization assay

Antibody titers neutralizing SLY were determined in a hemolysis assay as described [11].

2.9. Statistical analysis

Differences between groups were analyzed with the Mann–Whitney *U*-test. The Wilcoxon test was used for comparison