Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 132 (2008) 211–219
veterinary microbiology
www.elsevier.com/locate/vetmic

Short communication

Polysaccharide capsule and suilysin contribute to extracellular survival of *Streptococcus suis* co-cultivated with primary porcine phagocytes

Laurentiu Benga [1], Marcus Fulde [1], Christina Neis, Ralph Goethe, Peter Valentin-Weigand *

Institut für Mikrobiologie, Zentrum für Infektionsmedizin, Stiftung Tierärztliche Hochschule Hannover, Germany

Received 22 February 2008; received in revised form 29 April 2008; accepted 5 May 2008

Abstract

Streptococcus suis is a major cause of meningitis, sepsis and arthritis in piglets and a zoonotic agent. Survival in the blood circulation system represents a major step in pathogenesis of *S. suis* infections. To get further insights into the mechanisms of *S. suis* survival in the host, we compared a highly virulent *S. suis* serotype 2 strain with its non-encapsulated and suilysin-deficient mutants in their abilities to resist phagocytosis and killing by polymorphonuclear neutrophils (PMNs) and mononuclear cells. PMNs displayed a higher capacity to take up encapsulated bacteria than mononuclear cells, whereas both cell types internalized efficiently non-encapsulated *S. suis*. Differentiation of extracellular and intracellular survival of the WT strain revealed that in PMNs the majority of the cell-associated streptococci were intracellular, whereas in mononuclear cells the majority remained attached to the cell surface. *S. suis* survived mainly extracellularly, since both cells killed intracellular bacteria to a similar extent. As a consequence of different resistance to phagocytosis, only the encapsulated *S. suis* strains survived co-cultivation with PMNs. Comparison of the WT strain with its encapsulated suilysin-deficient mutant revealed reduced survival of the mutant after co-cultivation with PMNs. Involvement of suilysin in inhibition of phagocytosis was further confirmed by the use of anti-suilysin antibodies and recombinant suilysin. Kinetic experiments with PMNs suggested that reduced survival of the mutant strain was mainly associated with an increased uptake, whilst both strains adhered similarly. Concluding, our results indicate that the capsule and the suilysin play important roles in *S. suis* survival in the host by interfering with phagocytic uptake.

Keywords: *Streptococcus suis*; Capsule; Suilysin; Phagocytosis

1. Introduction

Streptococcus suis is a major cause of meningitis, sepsis and arthritis in piglets and a zoonotic agent. Bacteria are transmitted via the respiratory route and

* Corresponding author at: Stiftung Tierärztliche Hochschule Hannover, Zentrum für Infektionsmedizin, Institut für Mikrobiologie, Bischofsholer Damm 15, 30173 Hannover, Germany. Tel.: +49 511 9537362; fax: +49 511 9537697.
E-mail address: peter.valentin@tiho-hannover.de (P. Valentin-Weigand).
[1] Both authors contributed equally to this paper.

0378-1135/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2008.05.005

commonly colonize the palatine tonsils. Under certain, yet unknown, circumstances *S. suis* crosses the respiratory epithelial barrier and disseminates in the blood circulation system. A possible way how *S. suis* spreads into host tissues might be survival and ''travelling'' inside (Williams, 1990; Williams and Blakemore, 1990), or closely associated to monocytes and macrophages (Gottschalk and Segura, 2000). Alternatively, *S. suis* might migrate in blood as free bacteria protected by a thick capsule layer, since a high level of bacteremia usually precedes the onset of bacterial meningitis (Williams, 1990; Tunkel and Scheld, 1993). Nevertheless, experimental evidence supporting either of these theories is limited.

Pathogenicity of many bacteria seems to rely on the protective effect of the capsule against phagocytosis. For *S. suis* serotype 2 strains, the capsule represents a critical virulence factor, since it is involved in protection against phagocytosis by alveolar macrophages (Charland et al., 1998; Smith et al., 1999). On the other hand, interactions of *S. suis* with polymorphonuclear neutrophils (PMNs), is poorly described. These phagocytes participate in the first defense line against *S. suis* infection, however, it is not clear whether they efficiently take up and kill the bacteria (Wibawan and Lämmler, 1994; Busque et al., 1998).

Another streptococcal factor probably associated with resistance to phagocytosis is suilysin, a cholesterol-dependent cytolysin related to streptolysin O of *S. pyogenes* and pneumolysin of *S. pneumoniae* (Jacobs et al., 1994). These toxins seem to play a multifactorial role in pathogenesis by their cytolytic activities and their inhibition of bactericidal activity of phagocytes (Sierig et al., 2003; Orihuela et al., 2004). However, whether or not suilysin plays a similar role has not been reported yet.

To get new insights into *S. suis* survival in the host we compared a highly virulent *S. suis* serotype 2 strain with its non-encapsulated and suilysin-deficient mutants in their abilities to resist phagocytosis and killing by PMNs and mononuclear cells. We found evidence that CPS and suilysin prevent phagocytic uptake, thereby allowing the bacteria to survive extracellularly.

2. Materials and methods

2.1. Bacterial strains and growth conditions

S. suis capsular serotype 2 strain 10 and its non-capsulated isogenic mutant strain 10Δ*cps*EF (designated strain Δ*cps*) were kindly provided by H. Smith (Lelystad, NL) and have been described in an earlier study (Smith et al., 1999). In addition, a suilysin-deficient mutant, strain Δ*sly*, was constructed from strain 10 as described below. The hosts for molecular cloning and protein expression experiments were *Escherichia coli* strains DH5α and BL21 (DE 3), respectively. For co-cultivation experiments, *S. suis* was grown in Todd-Hewitt broth (THB, DIFCO Lab, Detroit, USA) over-night at 37 °C. The next day, an equal volume of fresh THB was then added to the cultures, which were further incubated for 1.5 h at 37 °C. Then the bacteria were harvested, suspended in PBS (pH 7.3) and adjusted photometrically (600 nm) to a concentration of 2×10^9 bacteria/ml, before use in further experiments. For some experiments, bacteria were opsonised using either fresh or complement-inactivated (30 min at 56 °C) porcine serum at 20% in RPMI (final concentration). Serum was obtained from an 8 weeks old piglet which had survived an experimental infection against *S. suis* (Beineke et al., 2008) and was tested positive for the presence of anti-*S. suis* antibodies by immunoblot as described earlier (Benga et al., 2004a).

2.2. Construction of the suilysin mutant strain 10Δ*sly*

Techniques were performed according to standard procedures (Sambroock and Russel, 2001). Restriction enzymes were purchased from NewEnglandBiolabs (Frankfurt/Main, Germany). To inactivate the *sly* gene in the strain 10, the gene was interrupted by insertion of an erythromycin cassette. For this, a 1510-bp fragment of the *sly* gene was amplified by PCR using the primer pair SL4-L (AATTCCATATGAGAAAAAGTTCGCACTTGATTTT) and SL4-R (CGGGATCCTTACTCTATCACCTCATCCGCATAC). Subsequently, the product was cloned into the plasmid pCRII-TOPO (Invitrogen), then the fragment was digested with SacI–PstI and ligated into the plasmid pBluescript II SK(+) (Stratagene, Amsterdam, The Netherlands) digested