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Original article

In vitro transcriptome analysis of porcine choroid plexus epithelial cells in response to *Streptococcus suis*: release of pro-inflammatory cytokines and chemokines

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Abstract

The Gram-positive zoonotic bacterium *Streptococcus suis* (*S. suis*) is responsible for a wide range of diseases including meningitis in pigs and humans. The blood-cerebrospinal fluid (CSF) barrier is constituted by the epithelial cells of the choroid plexus, which execute barrier function also after bacteria have entered the central nervous system (CNS). We show that the bacterial capsule, a major virulence factor, strongly attenuates adhesion of *S. suis* to the apical side of porcine choroid plexus epithelial cells (PCPEC). Oligonucleotide microarray analysis and quantitative PCR surprisingly demonstrated that adherent wild-type and capsule-deficient *S. suis* influenced expression of a pronounced similar pattern of genes in PCPEC. Investigation of purified capsular material provided no evidence for a significant role of the capsule. Enriched among the regulated genes were those involved in "inflammatory response", "defense response" and "cytokine activity". These comprised several cytokines and chemokines including the interleukins 6 and 8, which could be detected on protein level. We show that after infection with *S. suis* the choroid plexus contributes to the immune response by actively producing cytokines and chemokines. Other virulence factors than the bacterial capsule may be relevant in inducing a strong inflammatory response in the CNS during *S. suis* meningitis.

Keywords: Choroid plexus; Blood-cerebrospinal fluid barrier; *Streptococcus suis*; Transcriptome; Immune response

1. Introduction

Streptococcus suis (*S. suis*), a Gram-positive bacterium, is a well characterized swine pathogen causing a wide range of infections including meningitis, septicaemia, arthritis and pneumonia [1]. *S. suis* capsular type 2 can cause meningitis in humans as well, which may be complicated by septicaemia and endocarditis and can ultimately result in death of the host [2]. The only factor proven to be critical to the pathogenesis of *S. suis* infections is the capsular polysaccharide. Whereas the presence of a capsule inhibits the activation of certain signal transduction pathways involved in phagocytosis, capsule-defective mutants of *S. suis* are highly sensitive to uptake by macrophages and are avirulent in mouse and pigs [3–5]. Also, unencapsulated *S. suis* mutants display higher adhesion and

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invasion properties to different epithelial and endothelial cells compared to the wild type [6–8].

The central nervous system (CNS) is protected against invasion by pathogens by specific barrier structures, which include the blood-brain barrier (BBB) at the endothelium of the brain parenchymal blood vessels and the blood-cerebrospinal fluid (CSF) barrier (BCSFB) constituted by the choroid plexus (CP) epithelium. Histopathological analyses have provided evidence that the CP can be a main entry site for pathogens during bacterial meningitis. In natural and experimentally induced cases of *S. suis* meningitis lesions have been observed at the CP [9–11] and *Escherichia coli* is associated with the CP in a rat model [12]. Also, association with the CP during meningitis has been shown for *Neisseria meningitidis* (*N. meningitidis*) [13,14].

The CP shares a large surface with the CSF volume and is highly metabolically active, which makes it suited for an active role against microorganisms in the ventricular space. In fact, in an *in vitro* model primary porcine choroid plexus epithelial cells (PCPEC) are capable of restricting growth of *S. suis* upon activation with pro-inflammatory cytokines by locally depleting the CSF of tryptophan, thereby creating an unfavourable environment for the bacteria [15]. On the other hand, after invasion of the CSF compartment *S. suis* encounters optimal growth conditions due to low immunoglobulin concentration and virtually absent complement compounds leading to high concentrations of the bacteria, which will engage with the epithelial cell lining via their apical CSF directed side. At this stage the main damage during bacterial meningitis occurs due to secondary inflammatory responses. In consequence *S. suis* causes disruption of the BCSFB and initiates cell death by apoptotic and necrotic mechanisms in PCPEC [16,17]. Disruption of the barrier function may lead to an excessive influx of activated phagocytes into the CSF volume.

During the course of meningitis the bacteria interact with several target cells including endothelial and epithelial cells as well as cells of the blood system. Previous studies have indicated that these interactions can cause different responses in the host cells, which can be operative at the posttranscriptional level, e.g. *S. suis* inhibits activation of the PI3K/akt and PKC pathways in a capsule-dependent manner in murine macrophages [4]. Additionally, interaction with bacterial pathogens results also in diverse transcriptional changes in the target cells. In this regard, *S. suis* can cause transcriptional regulation of cytokines and chemokines in murine macrophages, human monocytes and a porcine whole blood model as well as human brain microvascular endothelial cells (HBMEC) [18–21]. Furthermore, transcriptional activation by *S. suis* of several genes including TLRs, cytokines and chemokines in cerebral structures was demonstrated in an adult mouse model of infection. In this system early pro-inflammatory transcriptional responses could also be observed in the CP [22].

Microarray technologies are a powerful tool for genome wide analyses of the cellular response during pathogen–host interactions [23]. We have therefore sought to analyze the transcriptional response of PCPEC to apical interaction with wild-type and capsule-deficient *S. suis* to elucidate the role of the CP in the setting of an ongoing bacterial meningitis. Both mutant and wild-type *S. suis* induced expression of a surprisingly similar pattern of genes. We show that the CP contributes to the immune response by actively producing chemokines and cytokines putatively perpetuating the course of inflammation during meningitis.

2. Material and methods

2.1. Preparation and cultivation of porcine plexus epithelial cells

Epithelial cells from porcine choroid plexus were obtained by a modified preparation basically as described previously [24]. Briefly, brains from freshly slaughtered pigs were dissected and the choroid plexus tissue from the lateral and the fourth ventricles was removed and treated with consecutive cold and warm trypsinization (0.2% solution, Biochrom, Berlin, Germany, 45 min at 4 °C, 20 min at 37 °C). The cells were centrifuged at 20 × *g* for 10 min and resuspended in DMEM/HAM's F12 1:1 supplemented with 4 mM L-glutamine, 10% heat inactivated fetal calf serum (FCS), 5 μg/ml insulin and penicillin (100 U/ml)/streptomycin (100 μg/ml). In order to suppress the growth of contaminating fibroblast-like cells, 20 μM of cytosine-arabinoside were added. The cells were plated on 6-well or 12-well plates using a seeding density of 50 cm^2/g wet weight of choroid plexus tissue. Upon confluence, PCPEC were cultivated in serum-free medium and used for the experiments 2–5 days later.

2.2. Bacterial strains and growth conditions

S. suis serotype 2 virulent strain and its isogenic capsular mutant strain 10*cpsΔEF* were kindly provided by H. Smith (DLO-Institute for Animal Science and Health, Lelystad, The Netherlands) and have been described previously [25]. Bacteria were maintained as stock cultures in Todd-Hewitt broth (THB; Oxoid, Wesel, Germany) containing 20% glycerol at −80 °C. Working cultures for stimulation of cells were generated by inoculating 50 μl of the bacterial stock into 10 ml THB and subsequent incubation at 37 °C with mild agitation to mid log phase. Bacteria were washed twice in phosphate-buffered saline (PBS, pH 7,3) and adjusted to an optical density at 600 nm (OD_{600}) of 0.65. This stock solution with approximately 2×10^8 colony-forming units (CFU)/ml was further diluted in culture medium without antibiotics.

2.3. Bacterial cellular association assays

To analyze association (extracellular adhesion plus intracellular invasion) of *S. suis* to PCPEC cells were incubated in 12-well plates with bacteria for 2 h at an multiplicity of infection (MOI) of 20. Afterwards cells were washed four times with HBSS (Gibco, Invitrogen, Karlsruhe, Germany) containing 20 mM HEPES (BioWhittaker, Verviers, Belgium) and subsequently lysed by pulsed sonication with the homogenizer Sonopuls UW2070 (Bandelin, Berlin, Germany) for 20 s at 10%