Two-Component Signal Transduction System VraSR Contributes to Neuroinflammation in Streptococcus Suis Meningitis

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Research

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Abstract

Background: *Streptococcus suis* (*S. suis*) is an important zoonotic pathogen that can cause high morbidity and mortality in both humans and swine. As the most important life-threatening infection of the central nervous system (CNS), meningitis is an important symptom of *S. suis* infection. The VraSR is a critical two-component signal transduction system that affects *S. suis* ability to resist against host innate immune system and promotes the ability of *S. suis* to adhere to hBMEC. Whether and how VraSR contributes to the development of *S. suis* meningitis are currently unknown.

Methods: The *in vivo* colonization, *in vivo* BBB permeability, histopathological examination and immunohistochemistry were applied to compare and characterize the degree of destruction of brain tissue in response to wild type SC19 and mutant ΔvraSR. Western blotting and real-time PCR were combined to identify the breakdown of tight junction proteins (TJ proteins). The secretion of proinflammatory cytokines and chemokines in the serum were detected on a BD FACSVerse flow cytometer.

Results: We found an important role of VraSR regulatory system in *S. suis* SC19-induced meningitis. A mouse infection model demonstrated that ΔvraSR had significantly attenuated inflammatory lesions in the brain tissues compared with wild-type *S. suis*. *In vitro*, we characterized that SC19 could increase the blood-brain barrier (BBB) permeability through downregulating the TJ proteins compared with mutant ΔvraSR. Moreover, we found significant generation of proinflammatory cytokines and chemokines in the serum including IL-6, TNF-α, MCP-1, and IL-12p70 compared with ΔvraSR infected mice.

Conclusions: For the first time, our work investigated the VraSR regulatory system of *S. suis* played an important role in streptococcal meningitis and revealed VraSR to be an important contributor to the disruption of TJ proteins. Characterization of these BBB disruption will facilitate further study of meningitis mechanisms in humans, thereby offering the development of novel preventative and therapeutic strategies against infection with *S. suis*.

Background

*Streptococcus suis* is a major swine pathogen that can cause serious diseases including septicemia, arthritis, endocarditis, pneumonia, meningitis, endophthalmitis, as well as sudden death, and it results in serious economic losses in the porcine industry worldwide [1, 2]. *S. suis* can be transmitted to humans through direct contact with contaminated raw pork products or infected pigs, resulting in streptococcal toxic shock-like syndrome (STTLS) and meningitis [3-5]. So far, *S. suis* infections in humans have been reported in Asia, Europe, America, Oceania [2, 6, 7]. However, two large-scale human cases of *S. suis* infection in China, the one case was 14 deaths in Jiangsu in 1998, and the another was 204 cases with a fatality rate reaching 20% in Sichuan in 2005 [2, 5, 8]. Among the 29 *S. suis* serotypes, *S. suis* 2 is the most prevalent in pigs and humans [9-11]. In southern Vietnam and Thailand, *S. suis* was the most
frequent pathogen responsible for bacterial meningitis[12, 13]. However, the mechanisms that S. suis pass across the blood-brain barrier (BBB) to cause meningitis are poorly understood.

Bacterial meningitis, an inflammation of meninges, continues to be an important life-threatening infection with high mortality and morbidity throughout the world. It could affect the pia, arachnoid, and subarachnoid space, and most survivors sustain neurological sequelae such as permanent deafness [14, 15]. It is prerequisite that pathogens invade and traverse across the BBB for central nervous system (CNS) infection. The BBB, a structural and functional barrier, which can maintain CNS homeostasis by regulating the passage of molecules in and out of the brain tissue and protect the brain from pathogens and toxins into circulation. It is formed by brain microvascular endothelial cells (BMECs), astrocytes and pericytes [14, 16]. Pericytes and astrocytes are responsible for maintaining the BBB properties. As the indispensable structural component of BBB, BMECs are linked by cytoplasmic zonula-occludin family members (such as ZO-1, ZO-2, and ZO-3) and tight junction (TJ) proteins (such as β-catenin, Occludin, and Claudins) [17, 18]. Decreasing or destroying TJ proteins could increase the permeability of BBB, which is an indicator of BBB dysfunction[19].

Now, how the S. suis causes meningitis and STSLS and leads to high mortality and morbidity remains unclear. Previous literature has demonstrated that S. suis in humans could induce the generation of interleukin (IL)-1β, IL-6, IL-8, IL-12, tumor necrosis factor-alpha (TNF-α)[20]. An investigation showed high IL-6, IL-1α, monocyte chemoattractant protein-1 (MCP-1), MIP-2 and chemokine (C-X-C motif) ligand 1 (CXCL1/GRO-α) levels in the blood and brain of mice with meningitis [13]. The excessive production of proinflammatory cytokines was confirmed to be an important cause of septicemia, STSLS, and meningitis [21, 22]. S. suis virulence factors, such as SsPA, SLY, CPS, MRP, have been reported to mediate the release of proinflammatory cytokines, and contribute to the occurrence of meningitis [23-25]. Under the stimulation of S. suis, hBMEC can secrete arachidonic acid which would help pathogens enter brain tissue and regulate the local inflammation [26]. A report found that EGFR transactivation contributed to CNS infection in S. suis meningitis [13]. However, the mechanism of S. suis meningitis is as yet poorly understood.

Two-component signal transduction systems (TCSs) are an important mechanism that bacteria monitor, respond and adapt to environmental changes. TCSs typically consist of a membrane-bound sensor histidine kinase (HK) and a cytoplasmic response regulator (RR) [27, 28]. TCSs have been shown to be related to bacterial resistance, biofilm synthesis and virulence [29-31]. For example, SaeS, an important sensor, could detect α-defendin1 (HNP-1) and response to neutrophil-derived stimuli in staphylococcus aureus [32]. Among 15 TCSs of S. suis, CiaRH, VirR/VirR, Ihk/Irr, NisK/NisR, SalK/SalR, and CovR have been confirmed to regulations of virulence[11]. The 1910HK/RR signal transduction system can not only promote the adhesion of S. suis to HEp-2 cells, but also promote immune escape, and had an important impact on the pathogenic ability of mice and piglets [33]. Knockout of ltDR gene in group B streptococcus resulted in a significant increase in bacterial invasion in hBMEC, as well as disruption of BBB and meningitis in vivo [34]. Ihk/Irr had important effects on cell adhesion, anti-macrophage killing, oxidative stress, and pathogenicity [35]. Further studies had shown that Ihk/Irr directly regulated metal
endopeptidase SsPepO, and SsPepO is an important virulence factor for meningitis caused by *S. suis* [24]. We have found that disruption of VraSR resulted decrease in the ability to resist neutrophil killing and phagocytosis, as well as a significant decrease in cell adhesion and animal experiments also verified that the virulence of the missing strain decreased [11]. However, whether the VraSR TCS plays a role in the process of streptococcal meningitis is still unknown.

In this study, we provided evidences that VraSR is an essential TCS for *S. suis* meningitis. Moreover, *S. suis* infection led to disruption of TJ proteins, resulting in the increase of BBB permeability. Bacterial infection also leaded to production of chemokines and proinflammatory cytokines, which further accelerated BBB disruption. These findings provide evidences supporting the role of VraSR in *S. suis* mediated CNS dysfunction, which broaden our horizons on streptococcal meningitis.

**Methods**

**Bacterial strains and cell culture**

*S. suis* strain SC19 was originally isolated from a disease pig brain during the Sichuan Province *S. suis* outbreak in 2005 [36]. ΔvraSR mutant was a deletion of VraSR gene in SC19 through homologous recombination. And CΔvraSR was the complementary strain of ΔvraSR [11]. All strains were cultured in TSB broth (BD) or plated on TSA (BD) with 10 % (vol/vol) fetal bovine serum (FBS) at 37 °C. Spectinomycin (100 μg/mL) was incorporated into the growth medium when required.

The hBMEC cell line was provided by Prof. Kwang Sik Kim in Johns Hopkins University School of Medicine, and kindly shared by Dr. Xiangru Wang, Huazhong Agricultural University [16, 37]. The hBMEC was routinely cultured in RPMI1640 supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 10% Nu-Serum, 2mM L-glutamine, 1 mM sodium pyruvate, nonessential amino acids, vitamins, and penicillin and streptomycin (100 U/mL) in a 37 °C incubator under 5 % CO₂ [38]. Confluent cells were washed three times with Hanks’ Balanced Salt Solution (Corning Cellgro, Manassas, VA, USA) and starved in serum-free medium (1:1 mixture of Ham’s F-12 and M-199) for 16-18h before further treatment.

**Reagents and antibodies**

Evan’s blue dye was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd (Aladdin, Shanghai, China). Cytometric Bead Array (CBA) Mouse Inflammation Kit was purchased from Becton, Dickinson and Company (BD, New Jersey, USA). For the Western Blotting, Anti-ZO-1, anti-β-catenin and anti-Occludin antibodies (all rabbit) were from Cell Signaling Technology (Danvers, MA, USA). And the anti-Claudin-5 antibodies (mouse) was from Santa Cruz Technology (Dallas, TX, USA). The β-actin (mouse) was from Absin Bioscience Inc. (Shanghai, China). HRP-conjugated anti-rabbit IgG and HRP-conjugated anti-mouse IgG antibodies were all from Cell Signaling Technology (Danvers, MA, USA).

**In vivo colonization**
For the in vivo colonization assay, the five-week-old female CD1 mice purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China), were used for induction of hematogenous bacterial meningitis. Mice were injected intravenously with $2 \times 10^8$ CFUs in sterile PBS. At the indicated time points, mice were anesthetized and blood was collected for quantitative circulating bacterial cultures and serum collection. Mice were subsequently perfused as previously described [39]. The brains were homogenized and plated to determine the bacterial counts.

**Evaluation of BBB Permeability**

Evan’s blue was used to assess BBB permeability, which binds to serum albumin and not enter into the CNS when the BBB is integral. Briefly. Mice were injected with $2 \times 10^8$ CFUs or PBS, at 72 hours postinfection, 300 µL Evan’s blue (EB) solution (1 % in PBS) was injected intraperitoneally into the mice for 1 hour. Mice were anesthetized and perfused transcardially with sterile PBS. And then, brains were removed, and photographed[24].

**Histopathological examinations and IHC**

The brain samples were fixed in 4 % paraformaldehyde solution followed by embedding in paraffin. Sections were mounted on adhesive glass slides, dewaxed in xylene, and rehydrated in descending graded ethanol for the hematoxylin and eosin (H&E) histopathological staining [40]. For IHC, the sections were deparaffinized in xylene, rehydrated in ethanol, incubated in 3 % hydrogen peroxide to quench endogenous peroxidase and performed in 10 mM citrate buffer. Then the sections were blocked with 5 % BSA for 1 h at room temperature, and incubated with antibody at 4 °C overnight. After that, the sections were incubated with secondary antibodies and Diaminobenzidine (DAB) was utilized for color development [24].

**Western blotting**

Infected and uninfected hBMEC were collected and lysed in RIPA buffer supplemented with a protease inhibitor, sonicated and centrifuged at 10,000 g for 10 min at 4 °C to remove insoluble cell debris. The protein concentration in supernatant was measured using BCA protein assay kit (Beyotime, Shanghai, China). Cell lysates were then separated on 8 %-12 % SDS-PAGE, and transferred to PVDF membranes (Bio-Rad, CA, USA). The blots were blocked with 5 % BSA in Tris-buffered saline with Tween 20 at room temperature for 1-2h and incubated overnight at 4 °C with primary antibodies against β-actin, ZO-1, β-Catenin, Occludin, Claudin-5. After that, the blots were washed and incubated with HRP-conjugated anti-rabbit or anti-mouse IgG at 37 °C for 1h, and visualized with ECL reagents (Meilunbio, Dalian, China). The densitometric analysis was performed using Image J software (Bio-Rad).

**RNA extraction and quantitative real-time PCR**

Total RNA from cells were extracted using the TRIpure reagent (Aidlab biotechnologies CO. Ltd, Beijing, China). 1 µg of total RNA was used for cDNA synthesis using the HiScript II Q RT SuperMix for qPCR with
gDNA Eraser (Vazyme, Nanjing, China). Quantitative real-time PCR was performed with ViiATM 7 Real-Time PCR System (Applied BioSystems, Foster City, CA, USA) using Power SYBR Green PCR master mix (Vazyme, Nanjing, China) according to the manufacturers’ instructions. Primers for real-time PCR are listed in Table 1 [16]. Expression levels of target genes were normalized to GAPDH by $2^{-\Delta\Delta CT}$ method. Each assay was performed in triplicate.

**Table 1** Primers used in this study

| gene      | Forward (5’–3’)                          | Reverse (5’–3’)                          | species |
|-----------|------------------------------------------|------------------------------------------|---------|
| GAPDH     | TGCCTCCTCGCACACCACCAACT                  | CGCCTGCTTCCACACCTTC                     | human   |
| ZO-1      | TGTGGAAAGAGGATGAGATGAGAAGA               | GGTGAAGAGATGCTGTTGTC                    | human   |
| β-Catenin | ACAAGCCACAAGATTACAAG                     | ATCAGCAGTCTCATTCCAA                     | human   |
| Occludin  | TTAACTTCTGCTGTGGAT                      | TGTGTAGCTCTGTCTCATAGTG                  | human   |
| Claudin-5 | CGCCTTCTGGACCACACAAT                    | CCACGACGGAGTCTACACTT                    | human   |

**Cytokine measurement assays**

CD1 mice were injected with $2 \times 10^8$ CFUs of SC19, ΔvraSR or CΔvraSR as described above. At each indicated time. Serum was prepared from the blood samples and stored at -80 °C. The concentration of inflammatory cytokines in the serum was measured on a BD FACSVersa flow cytometer using a CBA mouse inflammation kit (BD, USA) according to the manufacturers’ instructions. The data were analyzed with FCAP Array software.

**Statistical analysis**

Data were expressed as the mean ± SD. Significance of the differences between each group was analyzed by Student’s t test and GraphPad Prism version 8.0 (GraphPad Software Inc., La Jolla, CA, USA). For all tests, $P < 0.05$ (*) was considered significant, and $p < 0.01$ (**), as well as $< 0.001$ (****) were all considered extremely significant.

**Results**

**VraSR contributes to development of meningitis in vivo**

We first analyzed *S. suis* SC19 and ΔvraSR infection of the brain in vivo. 5-week-old CD1 mice were intravenously injected at dosage of $2 \times 10^8$ CFUs SC19 or ΔvraSR for 48 hours to analyzed bacterial colonization in the brain and blood. The bacterial load in the blood and brains of SC19-infected and CΔVraSR-infected mice were significantly higher than of ΔvraSR-infected group (Fig. 1a), these results confirmed the weak ability of mutant ΔvraSR in forming bacteremia and colonizing brain. And the SC19-infected mice showed severe symptoms, such as trembling, circling, paddling and opisthotonos, but these
neurological symptoms were not observed in ΔvraSR infected mice (date not shown). Histologic examination of the brain tissue infected with SC19 showed classic histopathological changes of meningitis, such as meningeal thickening, inflammatory cell accumulation, and meningorrhagia, but this was not obvious in ΔvraSR infected mice. The presence of WT S. suis SC19 in the brains was observed by immunohistochemical analysis, but the ΔvraSR mutant was rarely found, meanwhile, we could observe the CΔvraSR in brain tissue section (Fig. 1b). In addition, the production of proinflammatory cytokines and chemokines in serum were detected at the indicated time points. As shown, the cytokines and chemokines (including IL-6, TNF-α, IL-12p70, MCP-1, IFN-γ) increased rapidly in infected with either SC19 or the mutant ΔvraSR compared with uninfected controls as early as 2h, and the average concentration of these cytokines (IL-6, MCP-1, IL-12p70, TNF-α) in SC19 infected mice were obviously higher than that of ΔvraSR-infected group. The production of IL-6 and TNF-α increased sharply to the highest at 2 hours postinfection (PI), and TNF-α rapidly decreased to nearly basal levels at 18h PI. We also measured the production of the anti-inflammatory cytokine IL-10, there was no significant difference in SC19-infected and ΔvraSR-infected mice, they all rapidly raised at 2h PI and decreased to the initial level at 18h PI (Fig. 1c). Taking together, these results demonstrate that VraSR contributes to the pathogenesis of meningitis.

**VraSR contributes to the increased BBB permeability**

We next investigated if mutant ΔvraSR could induce the disruption of BBB. Evan’s blue which binds to serum albumin to be protein tracer and permeate into the damaged tissues, was used to evaluate the change of BBB permeability after S. suis infection. It was obvious that Evan’s blue penetrated to the brains of SC19-infected mice more than the brains of ΔvraSR-infected mice (Fig. 2). Together, these observations directly indicated that VraSR might contribute to increase BBB permeability during the development of S. suis meningitis.

**VraSR contributes to the BBB permeability via downregulating and disrupting the TJ proteins**

As the most important components of BBB, the TJ proteins determined the paracellular permeability. Therefore, we determined the alteration of these TJ proteins (ZO-1, β-catenin, Occludin, and Claudin-5) in the hBMEC of SC19-infected or ΔvraSR-infected hBMEC by real-time PCR and western Blotting *in vitro*. It was found that the expression of these junction associated genes in SC19-infected hBMEC were obviously decreased compared with ΔvraSR-infected hBMEC (Fig. 3a-3d). Meanwhile, the translation of these TJ proteins in SC19-infected hBMEC also showed the trends of downregulation (Fig. 3e). Therefore, these *in vitro* findings suggest that S. suis VraSR induces BBB disruption via downregulating the expression of the TJ proteins.

**Discussion**

As an important zoonotic pathogen that causes public health problems and heavy economic losses in swine husbandry around the world, S. suis has been recognized as the fatal organism causing meningitis and streptococcal toxic shock-like syndrome [1, 41]. There many articles have reported the mechanism of streptococcal meningitis. Adenosine of S. suis was reported to contribute to the activation of A1
adenosine receptor signaling cascade and cytoskeleton remodeling, thus promoting \textit{S. suis} penetration across BBB [42]. Suilysin has been shown to remodel cytoskeleton of hBMEC by activating Rac1 GTPase and RhoA, therefore contributing to the breakdown of BBB [43]. In addition, recent study has demonstrated that \textit{S. suis} 2 Enolase could bind to RPSA promoting the expression of HSPD1 and leading to the destroy of BBB integrity [44]. Studies also have reported the important roles of host targets in development of streptococcal meningitis, for example, TRIM32 is the key role that positively regulated the production of proinflammatory cytokines and chemokines including IL-18, TNF-\(\alpha\), IL-6, MIP-1\(\alpha\), RANTES, and MCP-1 secretion in mice, and was found to upregulated hemorrhage and bacterial loads in the brains [45]. \textit{S. suis} infection could induce the transactivation of EGFR, thus triggered the MAPK-ERK1/2 and NF-\(\kappa\)B signaling pathway, which contributing to the development of streptococcal meningitis [13]. TCSs which present in all domains of life, are an important signal transduction protein for bacteria to monitor and respond to environmental stimuli [46, 47]. Some TCSs (\textit{e.g.}, 1910HK/RR, VirR/VirS, VraSR) have been identified to have important impacts on the ability to virulence and pathogenicity [11, 48]. And studies also found that some TCSs deletion strains had lower capability to adhere to hBMEC [11]. However, there are very few studies on meningitis caused by TCSs.

During the induction of streptococcal meningitis, bacterial adherence of hBMEC is an essential step for disruption of BBB [14, 24, 49]. Our previous study found that \(\Delta\text{VraSR}\) had lower adherence ability to hBMEC compared with wild \textit{S. suis} SC19, bacterial burden experiments also showed that there are fewer bacteria in the brain of \(\Delta\text{VraSR}\)-infected mice compared with SC19-infected mice [11]. Moreover, there are SC19-infected mice not \(\Delta\text{VraSR}\)-infected mice had obvious neurological symptoms in the process of animal experiments (date not shown). These results implied that VraSR had an important role in \textit{S. suis} induction of meningitis. In a mouse meningitis model, we found significant differences in the number of bacteria from blood and brains. Furthermore, in CD1 mice, infection with \(\Delta\text{VraSR}\) strain showed there was less bacterial invasion and neutrophil infiltration in the brain tissue.

As a pathogen characterized by STSLS and meningitis, \textit{S. suis} infection can stimulate inflammatory cytokines production (\textit{e.g.}, MCP-1, TNF-\(\alpha\), INF-\(\gamma\), IL-12p70, IL-6), which might be responsible for a strong inflammatory response, finally leading to sudden death or BBB breakdown [1, 5, 13]. Previous study had found that high levels of IL-6, IL-12, IFN-\(\gamma\), TNF-\(\alpha\), MCP-1, CXCL1, and CCL5 cytokines caused by \textit{S. suis} 2 might be responsible for the sudden death of animals[21]. Neutrophil can be activated by cytokines such as TNF-\(\alpha\) and IFN-\(\gamma\) [50]. Here, a similar situation was observed with SC19, the cytokine increased obviously in the blood at 2 h PI, indicating an acute inflammation response in mice. IL-6 and TNF-\(\alpha\) reached a peak after 2 h of infection. Meanwhile INF-\(\gamma\) and IL-12p70 reached a peak at 6 h PI. Likewise, we observed the high production of chemokines and proinflammatory in \(\Delta\text{VraSR}\)-infected mice. But there is significant difference between SC19-infected and \(\Delta\text{vraSR}\)-infected mice, the inflammatory response caused by \(\Delta\text{vraSR}\) was significantly lower than wild group. MCP-1 is a potent chemokine that recruits monocyte and exacerbate inflammation, and previous studies reported decreased BBB leakage, macrophage/microglia accumulation, and an increased neuronal density [51, 52]. MCP-1 had been reported to be able to alter expression of TJ proteins in brain microvascular endothelial cells [53]. We found that MCP-1 in blood caused by SC19 infection was higher than that of \(\Delta\text{VraSR}\)-infected group, this
finding implies MCP-1 might be an important role in streptococcal meningitis. Whether MCP-1 plays an important role in streptococcal meningitis remains to be further investigated. Taken together, our observations supported that VraSR TCS plays an important role in S. suis induced inflammation storm.

1. *suis* induction of BBB disruption is a complex process between the host and pathogen. Many studies have reported that as the most important junctional structure of BBB, the TJ proteins play important roles in maintain BBB integrity and stability of CNS microenvironment [17, 54, 55]. Recent study has demonstrated that Escherichia coli could aggravate BBB disruption by upregulating PDGF-B and ICAM-1[16]. Lipoproteins of GBS were important for BBB crossing and virulence which mediated GBS translocation through endocytosis via LpR2 [56]. Matrix metalloproteinases 8 (MMP-8) was associated with BBB damage and neurological sequelae in bacterial meningitis, studies had found that Neisseria meningitidis induced the downregulation of Occludin through MMP-8 [57, 58]. In addition, Study has showed that S. suis 2 STK mediated degradation of Claudin-5 by affecting expression of E3 ubiquitin ligase HECTD1, thus enabling S. suis to penetrate the BBB [59]. In our study, we further demonstrate that S. suis SC19 could enhance BBB permeability by downregulating the expression of TJ proteins. Compared with SC19, the ability of ΔVraSR to downregulate TJ proteins was significantly attenuated. Together, those observations imply that VraSR is an important regulatory system mediating regulation of S. suis on the disruption of TJ proteins.

**Conclusions**

Our results showed that VraSR regulatory system is vital for S. suis to downregulate TJ proteins, and to increasing BBB permeability. Moreover, SC19-induced high level of cytokines and chemokines are also important contributors in the disruption of BBB. Future studies will aim to understand the precise molecular regulated by VraSR which is critical for the development of meningitis induced by S. suis. Our study of the role of VraSR in S. suis meningitis indicates that therapies targeted at VraSR TCS could contribute to preventing the development of streptococcal meningitis.

**Abbreviations**

hBMEC: Human brain microvascular endothelial cell; *S. suis*: Streptococcus suis; CNS: Central nervous system; IL: Interleukin; TJ: Tight junction proteins; TNF-α: Tumor necrosis factor-alpha; MCP-1: Monocyte chemoattractant protein-1; STSLS: streptococcal toxic shock-like syndrome; ZO: Zonala occludin; MIP-2: Chemokine (C-X-C motif) ligand 2; CXCL1/GRO-α: Chemokine (C-X-C motif) ligand 1; TCSs: Two-component signal transduction systems; HK: histidine kinase; RR: response regulator; FBS: Fetal bovine serum; CBA: Cytometric bead array; EB: Evan’s blue; CFU: Colony forming unit; IFN-γ: Interferon-gamma; PI: postinfection; NF-κB: Nuclear factor kappa B; PDGF-B: Platelet-derived growth factor-B; ICAM-1: Intercellular adhesion molecule-1; MMP-8: Matrix metalloproteinases 8

**Declarations**
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Availability of data and materials

There is no data, software, databases, and application/tool available apart from the reported in the present study. All data is provided in manuscript.

Authors’ contributions

BBD performed all experiments, analyzed the data, and drafted the manuscript. XY, FMY, KY, WP and JT participated in animal experiments. XY, FMY and MZP participated in the western blot and immunohistochemistry. WCB conceived of the project, coordinated and supervised the experiments, and revised the manuscript. WCB, FYY and HCC provide technical and administrative support. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

Ethics approval and consent to participate

All study was carried out on the guidelines established by the China Regulations for the Administration of Affairs Concerning Experimental Animals (1998) and Regulation for the Administration of Affair Concerning Experimental Animals in Hubei province (2005) (project NO. 00270520 and Animal Welfare Assurance No. 180701). All procedures and handling techniques were approved by the Committee Protection, Supervision and Controls of Experiments on Animals guidelines, Huazhong Agriculture University (Permit NO. HZAUMO-2019-057).

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References

1. Lin L, Xu L, Lv W, Han L, Xiang Y, Fu L, Jin M, Zhou R, Chen H, Zhang A. An NLRP3 inflammasome-triggered cytokine storm contributes to Streptococcal toxic shock-like syndrome (STLS). PLoS Pathog. 2019;15:e1007795.

2. Goyette-Desjardins G, Auger J-P, Xu J, Segura M, Gottschalk M. Streptococcus suis, an important pig pathogen and emerging zoonotic agent—an update on the worldwide distribution based on serotyping and sequence typing. Emerging Microbes Infections. 2019;3:1–20.

3. Segura M, Fittipaldi N, Calzas C, Gottschalk M. Critical Streptococcus suis Virulence Factors: Are They All Really Critical? Trends Microbiol. 2017;25:585–99.

4. Yang C, Zhao J, Lin L, Pan S, Fu L, Han L, Jin M, Zhou R, Zhang A. Targeting TREM-1 Signaling in the Presence of Antibiotics is Effective Against Streptococcal Toxic-Shock-Like Syndrome (STLS) Caused by Streptococcus suis. Front Cell Infect Microbiol. 2015;5:79.

5. Lun Z-R, Wang Q-P, Chen X-G, Li A-X, Zhu X-Q. Streptococcus suis: an emerging zoonotic pathogen. Lancet Infect Dis. 2007;7:201–9.

6. Jiang F, Guo J, Cheng C, Gu B. Human infection caused by Streptococcus suis serotype 2 in China: report of two cases and epidemic distribution based on sequence type. BMC Infect Dis. 2020;20:223.

7. Yang WZ, Yu HJ, Jing HQ, Xu JG, Chen ZH, Zhu XP, Wang H, Liu XC, Wang SW, Liu LG, et al. [An outbreak of human Streptococcus suis serotype 2 infections presenting with toxic shock syndrome in Sichuan, China]. Zhonghua Liu Xing Bing Xue Za Zhi. 2006;27:185–91.

8. Tang J, Wang C, Feng Y, Yang W, Song H, Chen Z, Yu H, Pan X, Zhou X, Wang H, et al. Streptococcal toxic shock syndrome caused by Streptococcus suis serotype 2. PLoS Med. 2006;3:e151.

9. Takeuchi D, Akeda Y, Nakayama T, Kerdsin A, Sano Y, Kanda T, Hamada S, Dejsirilert S, Oishi K. The contribution of suilysin to the pathogenesis of Streptococcus suis meningitis. J Infect Dis. 2014;209:1509–19.

10. Okura M, Osaki M, Nomoto R, Arai S, Osawa R, Sekizaki T, Takamatsu D. Current Taxonomical Situation of Streptococcus suis. Pathogens 2016, 5.

11. Chang P, Li W, Shi G, Li H, Yang X, Xia Z, Ren Y, Li Z, Chen H, Bei W. The VraSR regulatory system contributes to virulence in Streptococcus suis via resistance to innate immune defenses. Virulence. 2018;9:771–82.

12. Huong VT, Thanh LV, Phu VD, Trinh DT, Inui K, Tung N, Oanh NT, Trung NV, Hoa NT, Bryant JE, et al. Temporal and spatial association of Streptococcus suis infection in humans and porcine
reproductive and respiratory syndrome outbreaks in pigs in northern Vietnam. Epidemiol Infect. 2016;144:35–44.

13. Yang XP, Fu JY, Yang RC, Liu WT, Zhang T, Yang B, Miao L, Dou BB, Tan C, Chen HC, Wang XR. EGFR transactivation contributes to neuroinflammation in Streptococcus suis meningitis. J Neuroinflammation. 2016;13:274.

14. Kim KS. Mechanisms of microbial traversal of the blood-brain barrier. Nat Rev Microbiol. 2008;6:625–34.

15. Kim KS. Human Meningitis-Associated Escherichia coli. EcoSal Plus 2016, 7.

16. Yang RC, Qu XY, Xiao SY, Li L, Xu BJ, Fu JY, Lv YJ, Amjad N, Tan C, Kim KS, et al. Meningitic Escherichia coli-induced upregulation of PDGF-B and ICAM-1 aggravates blood-brain barrier disruption and neuroinflammatory response. J Neuroinflammation. 2019;16:101.

17. Dejana E, Tournier-Lasserve E, Weinstein BM. The control of vascular integrity by endothelial cell junctions: molecular basis and pathological implications. Dev Cell. 2009;16:209–21.

18. Burkhart A, Thomsen LB, Thomsen MS, Lichota J, Fazakas C, Krizbai I, Moos T. Transfection of brain capillary endothelial cells in primary culture with defined blood-brain barrier properties. Fluids Barriers CNS. 2015;12:19.

19. Candelario-Jalil E, Yang Y, Rosenberg GA. Diverse roles of matrix metalloproteinases and tissue inhibitors of metalloproteinases in neuroinflammation and cerebral ischemia. Neuroscience. 2009;158:983–94.

20. Ye C, Zheng H, Zhang J, Jing H, Wang L, Xiong Y, Wang W, Zhou Z, Sun Q, Luo X, et al. Clinical, experimental, and genomic differences between intermediately pathogenic, highly pathogenic, and epidemic Streptococcus suis. J Infect Dis. 2009;199:97–107.

21. Dominguez-Punaro MC, Segura M, Plante MM, Lacouture S, Rivest S, Gottschalk M. Streptococcus suis serotype 2, an important swine and human pathogen, induces strong systemic and cerebral inflammatory responses in a mouse model of infection. J Immunol. 2007;179:1842–54.

22. Segura M, Vanier G, Al-Numani D, Lacouture S, Olivier M, Gottschalk M. Proinflammatory cytokine and chemokine modulation by Streptococcus suis in a whole-blood culture system. FEMS Immunol Med Microbiol. 2006;47:92–106.

23. Wang J, Kong D, Zhang S, Jiang H, Zheng Y, Zang Y, Hao H, Jiang Y. Interaction of fibrinogen and muramidase-released protein promotes the development of Streptococcus suis meningitis. Front Microbiol. 2015;6:1001.

24. Liu F, Li J, Yan K, Li H, Sun C, Zhang S, Yuan F, Wang X, Tan C, Chen H, Bei W. Binding of Fibronectin to SsPepO Facilitates the Development of Streptococcus suis Meningitis. J Infect Dis. 2018;217:973–82.

25. Bonifait L, Grenier D. The SspA subtilisin-like protease of Streptococcus suis triggers a pro-inflammatory response in macrophages through a non-proteolytic mechanism. BMC Microbiol. 2011;11:47.
26. Jobin MC, Fortin J, Willson PJ, Gottschalk M, Grenier D. Acquisition of plasmin activity and induction of arachidonic acid release by Streptococcus suis in contact with human brain microvascular endothelial cells. FEMS Microbiol Lett. 2005;252:105–11.

27. King AN, de Mets F, Brinsmade SR. Who's in control? Regulation of metabolism and pathogenesis in space and time. Curr Opin Microbiol. 2020;55:88–96.

28. Ferrand A, Vergalli J, Pages JM, Davin-Regli A. An Intertwined Network of Regulation Controls Membrane Permeability Including Drug Influx and Efflux in Enterobacteriaceae. Microorganisms 2020, 8.

29. Wang Q, Chen M, Zhang W. A two-component signal transduction system contributes to the virulence of Riemerella anatipestifer. J Vet Sci. 2018;19:260–70.

30. Lou Q, Ma Y, Qu D. Two-component signal transduction system SaeRS is involved in competence and penicillin susceptibility in Staphylococcus epidermidis. J Basic Microbiol. 2016;56:358–68.

31. Gao C, Dai Y, Chang W, Fang C, Wang Z, Ma X. VraSR has an important role in immune evasion of Staphylococcus aureus with low level vancomycin resistance. Microbes Infect. 2019;21:361–7.

32. Flack CE, Zurek OW, Meishery DD, Pallister KB, Malone CL, Horswill AR, Voyich JM. Differential regulation of staphylococcal virulence by the sensor kinase SaeS in response to neutrophil-derived stimuli. Proc Natl Acad Sci U S A. 2014;111:E2037–45.

33. Yuan F, Tan C, Liu Z, Yang K, Zhou D, Liu W, Duan Z, Guo R, Chen H, Tian Y, Bei W. The 1910HK/RR two-component system is essential for the virulence of Streptococcus suis serotype 2. Microb Pathog. 2017;104:137–45.

34. Deng L, Mu R, Weston TA, Spencer BL, Liles RP, Doran KS. Characterization of a Two-Component System Transcriptional Regulator, LtdR, That Impacts Group B Streptococcal Colonization and Disease. Infect Immun 2018, 86.

35. Han H, Liu C, Wang Q, Xuan C, Zheng B, Tang J, Yan J, Zhang J, Li M, Cheng H, et al. The two-component system Ihk/Irr contributes to the virulence of Streptococcus suis serotype 2 strain 05ZYH33 through alteration of the bacterial cell metabolism. Microbiology. 2012;158:1852–66.

36. Teng L, Dong X, Zhou Y, Li Z, Deng L, Chen H, Wang X, Li J. Draft Genome Sequence of Hypervirulent and Vaccine Candidate Streptococcus suis Strain SC19. Genome Announc 2017, 5.

37. Teng CH, Tseng YT, Maruvada R, Pearce D, Xie Y, Paul-Satyaseela M, Kim KS. NlpI contributes to Escherichia coli K1 strain RS218 interaction with human brain microvascular endothelial cells. Infect Immun. 2010;78:3090–6.

38. Zhao WD, Liu DX, Wei JY, Miao ZW, Zhang K, Su ZK, Zhang XW, Li Q, Fang WG, Qin XX, et al. Caspr1 is a host receptor for meningitis-causing Escherichia coli. Nat Commun. 2018;9:2296.

39. Zhu L, Maruvada R, Sapirstein A, Malik KU, Peters-Golden M, Kim KS. Arachidonic acid metabolism regulates Escherichia coli penetration of the blood-brain barrier. Infect Immun. 2010;78:4302–10.

40. Yan X, Prosniaik M, Curtis MT, Weiss ML, Faber M, Dietzschold B, Fu ZF. Silver-haired bat rabies virus variant does not induce apoptosis in the brain of experimentally infected mice. J Neurovirol. 2001;7:518–27.
41. Ouattara M, Tamboura M, Kambire D, Sanou M, Ouattara K, Congo M, Kabore A, Sanou S, Kabre E, Sharples S, et al. Identification of Streptococcus suis Meningitis by Direct Triplex Real-Time PCR, Burkina Faso. Emerg Infect Dis. 2020;26:2223–6.

42. Zhao Z, Shang X, Chen Y, Zheng Y, Huang W, Jiang H, Lv Q, Kong D, Jiang Y, Liu P. Bacteria elevate extracellular adenosine to exploit host signaling for blood-brain barrier disruption. Virulence. 2020;11:980–94.

43. Lv Q, Hao H, Bi L, Zheng Y, Zhou X, Jiang Y. Suilysin remodels the cytoskeletons of human brain microvascular endothelial cells by activating RhoA and Rac1 GTPase. Protein Cell. 2014;5:261–4.

44. Liu H, Lei S, Jia L, Xia X, Sun Y, Jiang H, Zhu R, Li S, Qu G, Gu J, et al. Streptococcus suis serotype 2 enolase interaction with host brain microvascular endothelial cells and RPSA-induced apoptosis lead to loss of BBB integrity. Vet Res. 2021;52:30.

45. OuYang X, Guo J, Lv Q, Jiang H, Zheng Y, Liu P, Zhao T, Kong D, Hao H, Jiang Y. TRIM32 Drives Pathogenesis in Streptococcal Toxic Shock-Like Syndrome and Streptococcus suis Meningitis by Regulating Innate Immune Responses. Infect Immun 2020, 88.

46. Groisman EA. Feedback Control of Two-Component Regulatory Systems. Annu Rev Microbiol. 2016;70:103–24.

47. Paterson GK, Blue CE, Mitchell TJ. Role of two-component systems in the virulence of Streptococcus pneumoniae. J Med Microbiol. 2006;55:355–63.

48. Zheng C, Li L, Ge H, Meng H, Li Y, Bei W, Zhou X. Role of two-component regulatory systems in the virulence of Streptococcus suis. Microbiol Res. 2018;214:123–8.

49. Charland N, Nizet V, Rubens CE, Kim KS, Lacouture S, Gottschalk M. Streptococcus suis serotype 2 interactions with human brain microvascular endothelial cells. Infect Immun. 2000;68:637–43.

50. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol. 2011;11:519–31.

51. Sawyer AJ, Tian W, Saucier-Sawyer JK, Rizk PJ, Saltzman WM, Bellamkonda RV, Kyriakides TR. The effect of inflammatory cell-derived MCP-1 loss on neuronal survival during chronic neuroinflammation. Biomaterials. 2014;35:6698–706.

52. Yoshimura T. The chemokine MCP-1 (CCL2) in the host interaction with cancer: a foe or ally? Cell Mol Immunol. 2018;15:335–45.

53. Song L, Pachter JS. Monocyte chemoattractant protein-1 alters expression of tight junction-associated proteins in brain microvascular endothelial cells. Microvasc Res. 2004;67:78–89.

54. Yang R, Chen J, Xu B, Yang B, Fu J, Xiao S, Tan C, Chen H, Wang X. circ_2858 Helps Blood-Brain Barrier Disruption by Increasing VEGFA via Sponging miR-93-5p during Escherichia coli Meningitis. Molecular Therapy - Nucleic Acids. 2020;22:708–21.

55. Doran KS, Fulde M, Gratz N, Kim BJ, Nau R, Prasadarao N, Schubert-Unkmeir A, Tuomanen EJ, Valentín-Weigand P. Host-pathogen interactions in bacterial meningitis. Acta Neuropathol. 2016;131:185–209.
56. Benmimoun B, Papastefanaki F, Périchon B, Segkia K, Roby N, Miriagou V, Schmitt C, Dramsi S, Matsas R, Spéder P. An original infection model identifies host lipoprotein import as a route for blood-brain barrier crossing. *Nature Communications* 2020, 11.

57. Schubert-Unkmeir A, Konrad C, Slanina H, Czapek F, Hebling S, Frosch M. Neisseria meningitidis induces brain microvascular endothelial cell detachment from the matrix and cleavage of occludin: a role for MMP-8. PLoS Pathog. 2010;6:e1000874.

58. Kumar H, Jo MJ, Choi H, Muttigi MS, Shon S, Kim BJ, Lee SH, Han IB. Matrix Metalloproteinase-8 Inhibition Prevents Disruption of Blood-Spinal Cord Barrier and Attenuates Inflammation in Rat Model of Spinal Cord Injury. *Mol Neurobiol.* 2018;55:2577–90.

59. Rui L, Weiyi L, Yu M, Hong Z, Jiao Y, Zhe M, Hongjie F. The serine/threonine protein kinase of Streptococcus suis serotype 2 affects the ability of the pathogen to penetrate the blood-brain barrier. *Cell Microbiol.* 2018;20:e12862.

**Figures**
SC19 infection induced a strong neuroinflammation compared with ΔvraSR. 

**Figure 1**

- **A**: Bacterial loads in the blood (CFU/ml of blood), and in the brain (CFU/g of tissue). 5-week-old CD1 mice were injected intravenously with 2×10⁸ CFUs SC19 strain, ΔvraSR or CΔvraSR. Brain histopathological changes in infected mice with neurological signs were examined by H&E staining (a, b, d, e, g, h, j, k). S. suis in the brains of mice was detected by IHC (c, f, i, l). Scale bar = 50 μm. 

- **C**: Serum was harvested at indicated time.
point, and the concentrations of cytokines were measured by a CBA Mouse inflammation Kit. Results were expressed as the mean ± SD from five infected CD1 mice at each time point. Statistical analysis was carried out between the SC19-infected group at each time point and the ΔvraSR-infected group.

Figure 2

S. suis infection increased the blood-brain barrier permeability of mice. CD1 mice were challenged by S. suis at 3 days postinfection, Evan’s blue was injected to evaluated the integrity of the BBB.
S. suis infection enhanced the blood-brain barrier permeability of mice via inducing downregulation of the TJ proteins. a-d Real-time PCR analysis of the TJ proteins transcription in RNAs from infected hBMEC. GAPDH was used as the internal reference for the cellular RNAs in vitro. Analyzed data are presented as mean ± SD from three independent assays. e Western blotting analysis of the TJ proteins in hBMEC in response to S. suis infection. β-actin was used as the loading control, and densitometry was performed to analyze the difference.

Figure 3